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Biological activity of peptides purified from fish skin hydrolysates

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Abstract

Fish skin waste accounts for part of the solid waste generated from seafood processing. Utilization of fish skin by bioconversion into high-grade products would potentially reduce pollution and economic cost associated with treating fish processing waste. Fish skin is an abundant supply of gelatin and collagen which can be hydrolyzed to produce bioactive peptides of 2–20 amino acid sequences. Bioactivity of peptides purified from fish skin includes a range of activities such as antihypertensive, anti-oxidative, antimicrobial, neuroprotection, antihyperglycemic, and anti-aging. Fish skin acts as a physical barrier and chemical barrier through antimicrobial peptide innate immune action and other functional peptides. Small peptides have been demonstrated to possess biological activities which are based on their amino acid composition and sequence. Fish skin-derived peptides contain a high content of hydrophobic amino acids which contribute to the antioxidant and angiotensin-converting enzyme inhibitory activity. The peptide-specific composition and sequence discussed in this review can be potentially utilized in the development of pharmaceutical and nutraceutical products.

Keywords: Fish skin, Peptides, Bioactivity, Antioxidant, Antihypertensive, Antimicrobial, Anti-Alzheimer's

Background

The marine environment is a source of functional biomaterials such as polyunsaturated fatty acids (PUFAs), polysaccharides, minerals and vitamins, antioxidants, enzymes, and bioactive peptides (Kim and Wijesekara 2010; Kim et al. 2008; Pomponi 1999). By-products of marine processing industries, i.e., skin, trimmings, viscera, and blood, contain a good amount of proteins which can be used as a source for bioactive peptides. These by-products are utilized as additives in animal husbandry as animal feed or in agriculture as fertilizers (Chalamaiah et al. 2012). Fish by-products like skin and frame need to be processed into fish hydrolysates either by fermentation or by hydrolysis techniques before they can be effectively utilized. By so doing this increases production costs (Chalamaiah et al. 2012). Therefore, the utilization of fish processing waste through bioconversion into high-grade products like bioactive peptides would be a better alternative. This not only would increase the economic value of the catch but also would reduce the amount of marine processing waste. Thus, reducing pollution and economic cost associated with treating the generated waste (Fang et al. 2017).

Biological activity of peptides is based on their amino acid composition and sequence (Pihlanto-Leppälä 2000). This biological activity ranges from antioxidant, antihypertensive, immunomodulatory, and antimicrobial activity as demonstrated by several studies using different fish species like Pollack, skate, Nile tilapia, sea beam, yellow fish, and skipjack (Chalamaiah et al. 2012). Bioactive peptides are specific protein fragments derived from plants or animal sources which possess nutritional benefits and positively influence health (Hartmann and Meisel 2007; Korhonen and Pihlanto 2003). Bioactive peptides are inactive in their parent protein sequence but can be released by enzymatic hydrolysis; however, for effective use, bioactive peptides must reach the target organ or receptors in the intestinal lumen intact and must survive enzymatic degradation (Adessi and Soto 2002). This review will highlight and discuss the different preparation methods of bioactive peptides from fish skin, their biological activity, and associated mode of action in regard to specific peptide composition, sequence, and cell signaling pathways.

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Preparation of bioactive peptides from fish skin

Fish protein hydrolysates contain peptides of 2–20 amino acid sequences after hydrolysis, and these peptides usually have biological activity. Several extraction methods are utilized to liberate bioactive peptides from the parent protein, and these include acid-alkaline hydrolysis: extracting collagen by using acidic or alkaline reagent; enzymatic hydrolysis: using enzymes to hydrolyze fish skin; and fermentation method: using microorganisms as a source of the enzymes (Huang et al. 2015).

Enzymatic hydrolysis

Enzymatic hydrolysis is the best way to hydrolyze fish skin without losing nutritional value (Huang et al. 2015). The method is preferred especially in the food and pharmaceutical industries because the hydrolysis process does not leave residual organic solvents or toxic chemicals in its products (Kim and Wijesekara 2010). Steps in enzymatic hydrolysis involve substrate preparation, choice of the right enzyme, measuring the extent of enzymatic hydrolysis, homogenization, and heating to inactivate endogenous enzymes, hydrolysis, and termination of the enzymatic reaction. Commercial enzymes such as alcalase, trypsin, pepsin, papain, pancreatin, and thermolysin are employed in the enzymatic hydrolysis (Bernardini et al. 2011). Conditions like enzyme concentration, pH, time, and temperature have to be well monitored and maintained during hydrolysis. Enzyme concentrations, pH, and temperature vary with the type of enzyme used. Enzyme concentrations of 0.01–5.00% (*w*/*w*) and pH range of 1.5–11 have been documented (Halim et al. 2016). Black-barred halfbeak gelatin was dissolved in DW and subjected to enzymatic hydrolysis with an enzyme/substrate ratio of 30:1, pH 10.0 and 50 °C. The enzymatic activity was evaluated by a method described by (Kembhavi et al. 1993) using casein as a substrate. The gelatin solution was equilibrated for 30 min before the enzyme addition. The pH was maintained by addition of 2 N NaOH, and after 3 h, the enzymes were inactivated by heating the solution at 95 °C for 20 min (Abdelhedi et al. 2017). Extraction of pepsin soluble collagen (PSC) from fish skin was performed by (Mahboob 2014). Undissolved residue obtained after acid soluble collagen (ASC) extraction was utilized for the PSC extraction as described by (Singh et al. 2011).

Acid-alkaline hydrolysis

During fish skin hydrolysis by acid-alkaline hydrolysis, certain amino acids, i.e., tryptophan, serine, and threonine, can be destroyed at high pH. Therefore, the pH and temperature of the hydrolysates must be closely observed during the hydrolysis process. Collagen extraction from fish skin by acid-alkaline hydrolysis involves treatment of pre-cleaned skin samples with an alkali (NaOH) as an initial extraction step. The step is followed by continuous stirring at a controlled temperature for a set time. The procedure is repeated about 3 times, and it is carried out with an aim to remove non-collagenous proteins and pigments (Jongjareonrak et al. 2005; Wang et al. 2008; Wang et al. 2015). The skin is alternatively treated with an acid (HCl) (Wu et al. 2017). After acid-alkali treatment, the skin was washed to neutralize the pH and further extraction carried out with distilled water at 65 °C for 4 h. Some extraction procedures include a defatting step (Mahboob 2014). Jongjareonrak et al. 2005 removed fat using butyl alcohol for 24–48 h with gentle stirring and a change of solution every 8 h. The resultant matter was then subjected to acid treatment with acetic acid for 24 h with gentle stirring. Collagen was extracted from fish skin, scale, and bone using a procedure described as follows (Wang et al. 2008). The collagen was extracted with 0.5 M acetic acid at a sample/solution ratio of 1:100 (*w*/*v*) for 24 h with continuous stirring. The extracts were centrifuged at 20,000*g* for 1 h at 4 °C, and the extraction step was repeated using the obtained residue, followed by centrifugation under the same conditions. The supernatants of the two extracts were combined and precipitated by the addition of NaCl to a final concentration of 0.9 M and centrifuged at 2500*g* for 0.5 h to obtain a precipitate that was dissolved in 0.5 M acetic acid. The precipitate was dialyzed for 48 h against 10 volumes of 0.1 M acetic acid and distilled water, respectively, which were changed every 8 h, before being lyophilized. Antimicrobial peptides were purified from winter flounder epidermis and mucus extracts (Cole et al. 1997). The mucus was obtained from the skin by scraping and further subjected to homogenization in a solution of 50 ml of 0.2 M sodium acetate, 0.2% Triton X-100, and 1 mM phenyl methyl sulfonyl fluoride. The homogenate was centrifuged for 20 min at 20,000*g*, and the resultant supernatant was further purified.

Fermentation

Fermentation is considered a more natural method of protein hydrolysis. The technique has been employed for centuries especially in East Asian countries as a traditional preservation method. Fermentation not only enhances the flavor and taste of food but also increases its nutraceutical value. During the fermentation process, bioactive peptides are released by the action of both microorganisms and endogenous proteolytic enzymes. Several studies have demonstrated the bioactivity of various marine products like Thai fermented shrimp paste, shrimp by-products, squid *miso*, and a variety of traditional fermented fish products (Bueno-Solano et al. 2009; Giri et al. 2011; Kleekayai et al. 2015). Majumdar et al. 2016 examined the chemical and microbial properties of *shidal*, a traditional fermented fish product of Northeast India. A combination of both fatty acids

(eicosapentaenoic, docosahexaenoic, arachidonic, linolenic, and linoleic acid) and proteins or peptides of MW (molecular weight) range between 45 and 29 kDa and 45 and 6 kDa respectively were reported to be present in the fermented fish product. Hydrolysates were prepared from turbot skin by utilization of the fermentation method using 3 microorganisms, i.e., *Saccharomyces cerevisiae*, *Aspergillus oryzae*, and *Streptococcus thermophiles* (Fang et al. 2017).

Purification of peptides

The biological activity of peptides is determined by properties like molecular weight, charge, and hydrophobicity. Therefore, peptides are purified through a multi-step purification process based on such properties. Purification based on molecular weight employs methods like ultrafiltration (UF), nanofiltration (NF), and gel filtration (GF) (Halim et al. 2016). Ion exchange chromatography (IEC) is used to fractionate peptides basing on their net charge. Fractioned peptides are then further purified using technologies like reversed-phase HPLC which separates compounds based on hydrophobicity and hydrophilicity (Conlon 2007). Peptide sequences of the most active fractions from HPLC analysis are then analyzed and identified using mass spectrometry methods like matrix-assisted laser deionization time-of-flight (MALDI-TOF), electrospray ionization mass (ESI), matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS), etc. (Bernardini et al. 2011).

Biological activities

Antihypertensive activity

Hypertension is a chronic condition affecting millions of people around the world. According to a report by World Health Organization 2011, the global prevalence of hypertension among adults ≥ 25 years stands at 40% and it is estimated that 1.56 billion people will have the condition by 2025 (Kearney et al. 2005). Blood pressure is regulated by the renin–angiotensin–aldosterone system (RAS). Low renal blood flow or low plasma sodium concentration initiates the conversion of pro-renin into renin in juxtaglomerular cells in the kidneys. Renin in circulation catalyzes the conversion of angiotensinogen to angiotensin I which is then subsequently converted to angiotensin II by the enzyme angiotensin-converting enzyme (ACE) (Paul 2006). ACE is mainly produced in the lungs and other sites including the endothelial lining of vascular tissues, heart, brain, kidney, placenta, bone marrow, pancreas, and testis. Angiotensin II is a potent vasoconstrictor that causes blood vessels to narrow resulting in increased blood pressure (Rogerson et al. 1992). Simultaneously, it stimulates the secretion of the hormone aldosterone from the adrenal cortex causing the renal tubules to increase the reabsorption of sodium and water into the blood, while at the same time causing the excretion of potassium. Inhibition of ACE diminishes angiotensin II-mediated aldosterone secretion from the adrenal cortex, leading to a decrease in water and sodium reabsorption and a reduction in extracellular volume sequentially leading to vasodilation and eventually reduced arterial blood pressure. Hypertension is manageable with various synthetic ACE inhibitors such as captopril (Dezsi 2000). Hypertension can also be counteracted by endothelial-dependent vasodilation through the upregulation of nitric oxide (NO) expression, phosphorylation of eNOS, and downregulation of endothelin (ET-1) expression. Endothelial smooth muscle cell relaxation is achieved via the nitric oxide/cyclic guanosine monophosphate (cGMP)-mediated intracellular signaling pathway. This pathway involves the upregulation of cGMP-dependent protein kinase I (cGK-I) and a decrease in intracellular calcium levels via downregulation of the inositol-1,4,5-trisphosphate (IP 3) receptor (Daiber and Münzel 2015).

Conventional antihypertensive drugs cause several adverse effects; therefore, the search for safer natural alternatives is in progress. Among the natural alternatives, interest has grown in the utilization of bioactive peptides in the prevention of hypertension and in the initial treatment of mild hypertension (Guang and Phillips 2009). The antihypertensive activity of fish skin peptides is discussed and summarized below in Table 1.

Ultrafiltration of steelhead/rainbow hydrolysates yielded fractions of less than 3 kDa with ACE inhibitory activity higher than the activity of the whole hydrolysates (Cheung and Li-Chan 2017). The low MW fractions were prepared from whole hydrolysates using commercial enzyme kits. Two fractions showed ACE inhibition activity of 54% and 63%. The ACE inhibitory activity was affected by different conditions suggesting that the additional hydrolysis achieved with higher enzyme concentration and longer duration effectively generated shorter peptides with higher activity. The highest ACE inhibition was demonstrated in samples produced after 6 h hydrolysis with 4% protease. Similarly, fish skin peptide bioactivity has been shown to be associated with low MW peptides (Iwaniak et al. 2014; Power et al. 2014).

Black-barred halfbeak (*Hemiramphus far*) skin showed high protein content in the gelatin extracts, estimated at 91.36% (Abdelhedi et al. 2017). The protein quantity in the gelatin was closely similar to that obtained in other fish skin species such as splendid squid, cuttlefish, thornback ray, and cobia (Jridi et al. 2013; Lassoued et al. 2014; Nagarajan et al. 2012; Silva et al. 2014). The ACE inhibitory activity was 36.51% for 1 mg/mL of whole gelatin while 1 mg/mL gelatin hydrolysate showed a significantly greater activity of 80.76%. However, these values were significantly lower than the positive control

Table 1 ACE inhibitory activity peptides purified from fish skin hydrolysates

Species	IC_{50} (μM)	Peptide sequence	Reference
Nile tilapia (*Oreochromis niloticus*)	760–1490	GIV, GAP*GF, GFA*GPA, SGNIGFP*GPK, GIPGPIGPP*GRP	Thuanthong et al. (2017)
Pacific cod skin	6.9	GASSGMPG	Ngo et al. (2016)
	14.5	LAYA	
Thornback ray skin	27.9	GIPGAP	Lassoued et al. (2015)
	170	APGAP	
Skate	3.09	MVGSAPGVL	Ngo et al. (2014b)
	4.22	LGPLGHQ	
Salmon (*Oncorhynchus keta*)	8.7	GLPLNLP	Lee et al. (2014)
Skate	95	PGPLGLTGP	Lee et al. (2011)
	148	QLGFLGPR	
Alaska pollock	2.6	GPL	Byun and Kim (2002)
	17.13	GPM	
Cod (*Gadus microcephalus*)	–	TCSP, TGGGNV	Ngo et al. (2011)

captopril. The hydrolysate had an ACE inhibitory IC_{50} value of 332.66 ± 16.57 mg/mL. Higher values of ACE inhibitory activity of hydrolysate in comparison to those of the gelatin are an indicator that bioactive peptides are released from the protein molecules upon enzymatic hydrolysis (Abdelhedi et al. 2017). In another study, five novel ACE inhibitory peptides GIV, GAP*GF, GFP*GPA, SGNIGFP*GPK, GIPGPIGPP*GPR were identified from the most active fractions of Nile tilapia skin gelatin. The IC_{50} value of the active peptides ranged between 760 and 1490 μM (Thuanthong et al. 2017).

Pacific cod skin gelatin was hydrolyzed using several enzymes and the pepsin hydrolysate showed the highest ACE inhibitory effect of about 91% (Ngo et al. 2016). ACE inhibitory active peptides were identified as GASSGMPG, IC_{50} 6.9 μM, and LAYA, IC_{50} 14.5 μM. The MW of peptides GASSGMPG and LAYA was less than 1 kDa which indicates they can cross the intestinal barrier and exert biological effects. The ACE inhibitory activity of GASSGMPG was higher than that of ASL, IC_{50} 102.15 μM from silkworm pupa (*Bombyx mori*) protein; PVNNPQIH, IC_{50} 206.7 μM from small red bean *Phaseolus vulgaris*; GDLGK-TTTVSNWSPPKYKDTP, IC_{50} 11.28 μM from tuna frame protein; and AHEPVK, IC_{50} 63 μM from edible mushroom *Agaricus bisporus* (Lau et al. 2014; Lee et al. 2010; Rui et al. 2013; Wu et al. 2015). A docking simulation of the ACE–ligand complexes between ACE/peptides and ACE/captopril demonstrated the potential of these peptides as ACE inhibitors. The binding sites of GASSGMPG and captopril on the ACE molecule were observed to be the same at the Asn72 residue while LAYA and captopril shared two binding sites on the ACE molecule at Asn72 and Arg348 residues. Overall molecular docking simulation demonstrated good protein–drug interaction which can be attributed to factors such as Van der Waals force and hydrogen bonds of molecules that stabilize the ligand–protein.

Peptides IGPAG, FGYGG, GIPGAP, IGAPGATGPAG, AKGDS, GASGPRGPA, GQDGRPGPAG, and GEAGNPGPAGP were purified from Thornback ray skin gelatin neutrase hydrolysate (Lassoued et al. 2015). Peptide GIPGAP, IC_{50} 27.9 μM, was the most potent ACE inhibitor among the purified peptides. A hydrolysate TRGH-A26 was prepared using crude proteases from *Bacillus subtilis* A26, and peptides AVGAT, GGVGR, APGAP, GEPGAPGPA, and GPRGAPGPA were purified. The peptide APGAP, IC_{50} 170 μM, was the most potent ACE inhibitor from this hydrolysate. The two most potent peptides GIPGAP and APGAP from Thornback ray skin gelatin hydrolysates had a strikingly similar sequence of PGAP at the C-terminus. Another peptide FGYGG with a high ACE inhibitory activity with an IC_{50} value of 231 μM contained the aromatic residue phenylalanine.

The antihypertensive effect of skate skin hydrolysates in an animal model experiment using spontaneously hypertensive rats (SHRs) was evaluated (Ngo et al. 2014b). Purified peptides were orally administered to SHRs, and changes in heart rate and blood pressure were monitored over a period of 20 days. Systolic blood pressure readings were monitored, and the maximal decrements in systolic blood pressure observed were 127.2 mmHg at 20 days and 118.8 mmHg at 10 days in the purified peptide (1000 mg/kg of BW) and captopril treatment groups respectively. The peptides were identified as MVGSAPGVL, IC_{50} 3.09 μM, and LGPLGHQ, IC_{50} 4.22 μM. Docking simulation of the ACE molecule and the purified peptide on the Docking Server revealed almost similar binding on the ACE molecule as captopril. The binding site between the ACE molecule and the

purified peptide had many residues including Trp67, Asn68, Thr71, Asn72, and Arg348. This suggested that blockades by the purified peptides on the ACE molecule may contribute to the ACE inhibitory potency of the purified peptides for preventing hypertension.

Furthermore, the protective effect of the same peptides MVGSAPGVL and LGPLGHQ from skate skin peptides was investigated against angiotensin II-induced endothelial dysfunction using human endothelial cells (Ngo et al. 2014a). Increased production of endothelial nitric oxide synthase (eNOS) and inhibition of endothelin-1 ET-1 production through upregulation of the PPAR-γ pathway was observed. The enzyme eNOS upregulates the generation of nitric oxide (NO) in the vascular endothelium while NO maintains endothelial integrity and proper function through regulation of vascular tone, local blood flow, platelet aggregation and adhesion, and leukocyte–endothelial cell interactions (Dessy and Feron 2004). Thus, endothelial dysfunction, a precursor of hypertension and other health conditions like diabetes, aging, and atherosclerosis, results from abnormalities in NO production by the vascular endothelium. Purified peptides from skate skin not only showed ACE inhibitory activity but also provided protection against endothelial dysfunction in endothelial cells. Other peptides with ACE inhibitory activity purified from similar studies include PGPLGLTGP, IC_{50} 95 μM, and QLGFLGPR, IC_{50} 148 μM, from skate skin; GLPLNLP, IC_{50} 18.7 μM, from salmon skin; and GPL, IC_{50} 2.6 μM, and GPM, IC_{50} 17.13 μM, from Alaska Pollock skin (Byun and Kim 2002; Lee et al. 2014; Lee et al. 2011).

Typically, ACE inhibitory has been attributed to small-sized peptide residues with 2–12 amino acids (Yamamoto et al. 1994). Peptides with tryptophan, proline, or phenylalanine at the C-terminus or branched-chain aliphatic amino acids at the N-terminus are suitable to act as competitive inhibitors of ACE (Cushman and Cheung 1971). Many of the peptides identified in ACE inhibitory fractions contain proline at one of the three C-terminal positions. Hydrophobic amino acids in the N-terminus may also contribute to the ACE inhibitory activity (Rho et al. 2009). Therefore, in addition to the presence of proline in the C-terminal position, the presence of alanine could also be a contributing factor to ACE inhibitory activity (Yamamoto et al. 1994). Natural ACE inhibitory peptides and ACE substrates such as bradykinin and angiotensin I have been shown to contain aromatic amino acid residues such as phenylalanine (Camargo et al. 2012; Cheung et al. 1980; Hara et al. 1984). Peptides inhibiting ACE activity can potentially be used as nutraceuticals to lower elevated blood pressure. As described, ACE inhibitory peptides have been successfully purified from fish skin and their biological activity has been demonstrated by both in vitro and in vivo studies.

Antioxidant activity

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced as a result of the metabolism of oxygen and nitrogen. ROS and RNS can cause damage to cellular components in the body. During metabolism and respiration, ROS are constantly produced. These include superoxide anion radials O_2^-, hydroxyl radical OH^-, and non-free radical species like hydrogen peroxide and singlet oxygen $^1O_2$. Excess amounts of ROS as in oxidative stress exert oxidative damage to cellular macromolecules like proteins, lipids, and DNA by subtracting electrons. This starts a series of reactions which eventually leads to new radicals attacking and damaging other cellular macromolecule components (Kaur and Kapoor 2001). Peptides are considered to be more potent antioxidants than free amino acids because of the increased stability of the resultant peptide radical (Elias et al. 2008). The exact structure–antioxidant activity relationship of peptides has not been established. However, the type, position, and hydrophobicity of amino acids in the peptides are considered to play an essential role. The most reactive amino acids in proteins are usually those with nucleophilic sulfur-containing side chains like taurine, cysteine, and methionine or aromatic side chains like tryptophan, tyrosine, and phenylalanine (Elias et al. 2008). Fish skin is an abundant supply of gelatin and collagen. Gelatin contains an abundance of hydrophobic amino acids such as glycine, valine, alanine, proline, and hydroxyproline and could potentially contain a range of peptides with potent lipid–peroxidation inhibitory activity (Kim and Mendis 2006). Several methods are used for assessing antioxidant activity, and these include oxygen radical absorbance capacity (ORAC), Ferric-reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH). Low MW peptides have higher ORAC values and metal-chelating activities while high MW peptides have higher FRAP and DPPH radical scavenging (Theodore et al. 2008). The antioxidant activity of fish skin peptides is summarized in Table 2.

Abdelhedi et al. 2017 investigated the antioxidant activity of gelatin extract from black-barred halfbeak (*Hemiramphus far*) skin. The DPPH radical scavenging activities of the gelatin extract (5 mg/mL) and the positive control vitamin C were 43.39% and 70.0% respectively. The antioxidant activity was lower than that of the positive control. However, lower MW fish skin protein hydrolysates of cobia skin and *Raja clavata* skin have been demonstrated to show higher antioxidant activity than their protein molecule precursors (Lassoued et al. 2015). The ferric-reducing antioxidant power for whole gelatin, gelatin hydrolysate, and vitamin C was determined to be 0.47, 1.03, and 2.01 respectively. Whole gelatin and gelatin hydrolysate exhibited similar antioxidant activity using the β-carotene bleaching assay with activities of 53.73% and 78.47% respectively.

Table 2 Anti-oxidative activity of peptides purified from fish skin

Species	Radical scavenging activity (μg/mL)			Peptide sequence	Reference
	DPPH	Hydroxyl (HO•)	ABTS		
Thornback ray	1980	–	–	AVGAT	Lassoued et al. (2015)
Nile tilapia (*Oreochromis niloticus*)	8.82 μM	7.56 μM	–	DPALATEPDMPF	Ngo et al. (2010)
Horse Mackerel (*Magalaspis cordyla*)	72.3%	51.2%	–	NHRYDR	Sampath Kumar et al. (2012)
Crocker (*Otolithes ruber*)	79.6%	56.8%	–	GNRGFACRHA	Sampath Kumar et al. (2012)
Hoki (*Johnius belengerii*)	156.2 μM	–	–	HGPLGPL	Mendis et al. (2005b)
Blue leatherjacket (*Navodon septentrionalis*)	405	179	–	GSGGL	Chi et al. (2015)
	194	89	–	GPGGFI	
	118	73	–	FIGP	
Seabass (*Lates calcarifer*)	–	–	81.41*	GLFGPR	Sae-Leaw et al. (2017)
	–	–	10.4*	GATGPQGPLGPR	
	–	–	2.59*	VLGPF	
	–	–	0.50*	QLGLGPV	
Jumbo squid (*Dosidicus gigas*)	–	–	–	FDSGPAGVL	Mendis et al. (2005a)
	–	–	–	DGPLQAGQPGER	
Amur sturgeon	5380	890	8	PAGT	Nikoo et al. (2015)
Cod (*Gadus microcephalus*)	–	–	–	TCSP, TGGGNV	Ngo et al. (2011)
Nile tilapia (*Oreochromis niloticus*)	–	4.61	–	EGL	Zhang et al. (2012)
	–	6.45	–	YGDEY	
Pacific cod	Iron-chelating activity			GPAGPHGPPGKDGR, AGPHGPPGKDGR, AGPAGPAGAR	Wu et al. (2017)

*Units in millimole TE per millimole peptide

Similar results have been recorded for thornback ray skin by (Lassoued et al. 2015). Whole gelatin, gelatin hydrolysates, and vitamin C inhibited the peroxidation of linoleic acid by 15.91%, 34.78%, and 70.22% respectively after 3 days and 39.25%, 74.88%, and 99.2% respectively after 9 days. Similarly, the gelatin hydrolysate from Nile tilapia skin exhibited 59.74% of lipid peroxidation inhibition after a 5-day incubation period while whole gelatin had activity of only 7.12% (Choonpicharn et al. 2015). The authors noted that antioxidant activity observed could be due to the presence of hydrophobic amino acids. Other studies using black-barred and *Acipenser schrenckii* skin gelatin hydrolysates observed that glycine and proline had high antioxidant activity (Ngo et al. 2011; Nikoo et al. 2015).

The antioxidant activity of thornback ray gelatin hydrolysates was assayed using various in vitro tests (Lassoued et al. 2015). Hydrolysis was carried out using alcalase, neutrase, and *Bacillus subtilis* A26 proteases. A hydrolysate obtained by treatment with *Bacillus subtilis* A26 proteases (TRGH-A26) had a high DPPH scavenging activity with an IC_{50} value of 1.98 mg/mL and β-carotene bleaching inhibition activity of 70%. The high content of positively charged amino acids lysine and histidine (16.83%) in TRGH-A26 may be responsible for the high antioxidant activity (Carrasco-Castilla et al. 2012). The anti-oxidative efficacy of 180 mol/ml α-tocopherol equivalents at 5 mg/mL in the phosphomolybdenum assay was also evaluated and TRGH-A26 exhibited the highest anti-oxidative efficacy. TRGH-alcalase gelatin hydrolysate was the most potent inhibitor of DNA oxidation by hydroxyl radicals. Likewise, it also demonstrated DNA protective effect as no degradations were observed for the two forms of plasmid DNA utilized in the test. DNA oxidation inhibition activity may be attributed to hydrophobic amino acids which were present in TRGH-alcalase and TRGH-neutrase peptide hydrolysates. Amino acids histidine, tyrosine, methionine, and phenylalanine were high in TRGH-A26 and TRGH-Crude at total percentages of 13.22 and 13.09%, respectively.

Nile tilapia (*Oreochromis. niloticus*) scale gelatin protein was hydrolyzed using alcalase, pronase E, trypsin, and pepsin (Ngo et al. 2010). A peptide purified from the alcalase hydrolysate provided significant protection against the DNA oxidative damage when exposed to *OH generated by Fe^{2+}/H_2O_2. The DNA damage was inhibited by about 70%. The cell viability tests using mouse macrophages (RAW 264.7) and human lung fibroblasts

(MRC-5) confirmed that gelatin alcalase hydrolysate was non-cytotoxic. The purified peptide was identified as DPALATEPDMPF. The ROS scavenging activity was determined using a fluorescence probe, 2′,7′-dichlorofluorescin diacetate (DCFH-DA). DCFH reacts with ROS to form a highly fluorescent compound DCF. Pre-treatment with the purified peptide decreased the DCF fluoresce in a time-dependent manner. The antioxidant activity of the purified peptide could be attributed to the presence of several amino acids, such as tyrosine, methionine, lysine, and tryptophan. The peptide sequence analysis showed high hydrophobic amino acid content (> 69%). The activity of the purified Nile tilapia (*O. niloticus*) scale gelatin peptide could be attributed to the presence of non-aromatic amino acids such as alanine, proline, valine, and leucine (Mendis et al. 2005a; Mendis et al. 2005b). Other peptides with anti-oxidative activity purified from fish skin have been purified from blue leatherjacket (*Navodon septentrionalis*), seabass (*Lates calcarifer*), horse mackerel (*Magalaspis cordyla*), crocker (*Otolithes ruber*), and Nile tilapia (*Oreochromis niloticus*) (Chi et al. 2015; Sae-Leaw et al. 2017; Sampath Kumar et al. 2012; Zhang et al. 2012).

Chelation of pre-oxidative transition metals like $Fe2^{+}$, Cu^{2+}, and Pb^{2+} is another antioxidant mechanism. Therefore, peptides exhibiting metal-chelating activity are considered as potential antioxidants. Three novel iron-chelating peptides were purified from Pacific cod skin gelatin (Wu et al. 2017). The sequences of the purified peptides were identified as GPAGPHGPPGKDGR, AGPHGPPGKDGR, and AGPAGPAGAR. The iron-chelating ability was evaluated using ESI-MS and FTIR spectroscopy. The analysis showed that the amino and carboxylate terminal groups, peptide bonds from peptide backbone, amino, and imine from arginine side chain were involved in the formation of a complex with iron. Amino acid side chain groups of GPAG PHGPPGKDGR and AGPHGPPGKDGR, including amino (lysine), imine (histidine), and carboxylate (aspartic acid), provided additional iron-binding sites.

Scales of *Lates calcarifer*, *Mugil cephalus*, *Chanos chanos*, and *Oreochromis* spp. were hydrolyzed by papain and flavourzyme, and the $Fe2^{+}$-binding activity for the different species was compared (Huang et al. 2015). Ferrous ion together with collagen peptides from four fish scales was placed in a dialysis bag (MW cut off 500 Da) for 3–4 days of dialysis. A fraction from *Chanos chanos* had the highest iron-binding capacity at approximately 22.1 ppm/mg based on $Fe2^{+}$ binding activity/peptide concentration. MW distributions of the collagen peptides from the scales of the four fish were all less than 10 kDa, with an average MW of 1.3 kDa. It is suggested that the iron-binding ability of peptides is related to the net charge and the exposure of glycine residues. Glycine is important for ferrous ions and peptides to form stable complexes (Lee and Song 2009; Wu et al. 2012). The $Fe2^{+}$ binding activity of fish scales is probably derived from the exposure of glycine during hydrolysis since fish scale collage is rich in glycine (Chaud et al. 2002; Fahmi et al. 2004). However, to fully understand the actual mechanism, more study is needed. Peptides purified from Alaska Pollock skin gelatin and jumbo squid (*Dosidicus gigas*) skin gelatin demonstrated a protective effect against oxidative stress in rat liver cells and human fibroblasts respectively (Byun and Kim 2002; Mendis et al. 2005b). While peptides from hoki skin (*Johnius belengerii*) gelatin showed protection against oxidative stress by upregulating the expression of antioxidant enzymes, i.e., glutathione peroxidase, catalase, and superoxide dismutase, in human hepatoma cells in vitro (Mendis et al. 2005b).

Peptides with antioxidant activities from marine sources with high radical scavenging antioxidant activity contained amino acids histidine, tyrosine and methionine (Saiga et al. 2003). While peptides containing amino acids histidine, glutamic acid, aspartic acid, phosphorylated serine, and threonine have been demonstrated to be active metal chelators. Amino acid methionine is considered as central in antioxidant activity, and its antioxidant mechanism is attributed to the action of two-electron transfer from the sulfide of methionine's thioester group (Garner et al. 1998). Last but not least, peptides with antioxidant activity also have potential anti-inflammatory activity, neuroprotective activity, and anti-allergy activity which in some cases it has been tested as with the neuroprotective effect of grass carp skin hydrolysates observed in MES 23.5 cells (Cai et al. 2015).

Antimicrobial activity

Fish live in an environment where a myriad of saprophytic and pathogenic microbes flourish putting them in constant direct contact with potential pathogens. Therefore, the fish skin acts a physical barrier by providing immediate protection from the environment and as a chemical barrier through several innate immune factors such as antimicrobial peptides (AMPs) (Bergsson et al. 2005). AMPS are low MW peptides that have a net positive charge and are amphiphilic. They are involved in the natural defense mechanism against pathogens (innate immunity); however, their main role is modulation of mammalian cell functions. AMPS can be majorly divided into different families which include defensin, parasin, cathelicidin and hepcidin, and piscidin. These AMP families are species-specific, with piscidin being unique to teleost fish (Campoverde et al. 2017). Table 3 outlines the specific MEC and MIC values for fish skin peptides with antimicrobial activity along with their sequences.

The antibacterial activity of black-barred halfbeak gelatin and its hydrolysate was evaluated against three Gram negative (*Klebsiella pneumonia*, *Salmonella enterica*, and *Salmonella typhi*) and three Gram positive (*Micrococcus*

Table 3 Antimicrobial activity of peptides purified from fish skin

Species	MECs (μg/mL)	MIC (μg/mL)	Microorganism	Peptide sequence	Reference
Skipjack tuna (*Katsuwonus pelamis*)	3	–	*B. subtilis*	SJGAP	Seo et al. (2014)
	26	–	*M. luteus*		
	4.8	–	*S. iniae*		
	25	–	*A. hydrophila*		
	2.7	–	*E. coli*		
	9	–	*V. parahaemolytics*		
	16	–	*C. albicans*		
Yellowfin tuna (*Thunnus albacares*)	1.2	–	*B. subtilis*	YFGAP	Seo et al. (2012)
	6.5	–	*M. luteus*		
	17	–	*S. iniae*		
	8	–	*A. hydrophila*		
	3	–	*E. coli*		
	3.2	–	*V. parahaemolytics*		
Yellow catfish (*Pelteobagrus fulvidraco*)	–	2	*B. subtilis*	GKLNLFLSRLEILKLFVGA	Su (2011)
	–	4	*S. aureus*		
	–	16	*E. coli*		
	–	64	*C. albicans*		
Winter flounder (*Pleuronectes americanus*)	–	1.1–2.2 *	*B. subtilis*	GWGSFFKKAAHVGKHVGKAALTHYL	Cole et al. (1997)
	–	4.4–8.8 *	*P. haemolytica*		
	–	17.7–2.2 *	*S. aureus*		
	–	2.2–3.3 *	*E. coli*		
	–	8.8–17.7 *	*S. typhimurium* (I and II)		
	–	17.7–35.0 *	*A. salmonicida*		

*Units in micromolar

luteus, *Staphylococcus aureus*, and *Bacillus cereus*) bacteria (Abdelhedi et al. 2017). Black-barred halfbeak gelatin at 10 mg/mL exhibited a slight inhibitory activity against Gram positive *M. luteus* and *B. cereus*, with inhibitor diameter zones of 6.5 mm and 7.0 mm, respectively. Increasing concentration from 10 to 25 mg/mL slightly increased activity with inhibition zone diameter reaching 8.5 mm and 9.0 mm against *M. luteus* and *B. cereus*, respectively. The gelatin hydrolysate showed higher activity and was able to inhibit all the tested bacteria strains with different potentialities with *S. aureus* and *B. cereus* being the most sensitive. The positive control gentamicin was more potent against *S. aureus* and *B. cereus*, and it showed effective inhibition against the growth of all tested bacteria strains than the black-barred gelatin hydrolysate.

The antimicrobial activity of peptides purified from yellowfin tuna (*Thunnus albacares*) and skipjack tuna (*Katsuwonus pelamis)* skin was investigated (Seo et al. 2014; Seo et al. 2012). Two glyceraldehyde-3-phosphate dehydrogenase (GAPDH)-related AMPs, YFGAP and SJGAP, were identified. Both peptides showed broad-spectrum activity against Gram-positive and Gram-negative bacteria including three fish pathogens, *Aeromonas hydrophila*, *Streptococcus iniae*, and *Vibrio parahaemolyticus*. Antimicrobial activity of SJGAP and YFGAP showed MECs value of 1.2–17.0 μg/mL against Gram-positive bacteria while the MEC value against Gram-negative bacteria was 3.1–12.0 μg/mL. The SJGAP peptide purified from skipjack tuna had a higher antimicrobial activity as it showed activity against *Candida. Albicans* with MEC value of 16.0 μg/mL unlike the peptide YFGAP purified from yellowfin tuna. These results suggest that these peptides might be related to the innate defense in tuna. Based on the secondary structure prediction and the homology modeling, the peptides formed an amphipathic structure and consisted of a β-α-β motif with three secondary structural motifs including one α-helix, two parallel β-strands, and two loop regions. Sequence analysis results showed that both peptides, YFGAP and SJGAP, had high similarities with the N-terminus of GAPDH from other fish species by 81–91% and 91–97% respectively. GAPDH is a multifunctional protein that regulates the sixth step of glycolysis and mediates cell death under oxidative stress as well. Involvement of GAPDH in nuclear translocation and its aggregation under oxidative

stress have been proposed as processes leading to GAPDH-mediated cell death. Furthermore, oxidative stressors initiate amyloid-like GAPDH aggregation via intermolecular disulfide bonds at Cys-152 (Nakajima et al. 2017). However, the antimicrobial activity observed could be as a result of the peptides, YFGAP and SJGAP, acting as analogs of GAPDH in the sixth step of glycolysis due to high similarities with its N-terminus.

Aside from hydrolysates, AMPs have also been successfully isolated from fish skin mucus as well. The antimicrobial activity of yellow catfish (*Pelteobagrus fulvidraco*) skin mucus was investigated, and a novel peptide GKLNL FLSRLEILKLFVGAL was identified and named pelteobagrin (Su 2011). Structural analysis using Schiffer–Edmundson helical wheel modeling revealed that pelteobagrin forms an amphipathic alpha-helix composed of 10 out of 12 hydrophobic residues on the surface and 4 out of 6 hydrophilic residues on the opposing side. The peptide had a positive charge + 2 and was made up of 60% hydrophobic amino acids. It displayed a broad-spectrum antimicrobial activity against Gram-positive bacteria, Gram-negative bacteria, and fungi. However, Gram-positive bacteria *B. subtilis* was the most sensitive to the peptide at a minimal inhibition concentration (MIC) of 2 μg/mL. The peptide demonstrated no hemolytic activity against rabbit red blood cells, and it was relatively salt tolerant to concentrations of NaCl up to 137 mM. Similarly, a novel peptide from skin mucous secretions of the winter flounder (*Pleuronectes americanus*) was purified and characterized (Cole et al. 1997). The peptide was named pleurocidin, and it had an amino acid sequence of GWGSFFKKAAHVGKHVGKAALTHYL. It exhibited a broad-spectrum activity against a wide range of Gram-positive and Gram-negative bacteria. Gram-positive bacteria *B. subtilis* was the most sensitive to the peptide with a MIC value of 1.1–2.2 μM.

A study by Bergsson investigated antimicrobial components from the skin mucus of healthy Atlantic cod (*Gadus morhua*) (Bergsson et al. 2005). Results revealed acidic extracts were active against both Gram-positive and Gram-negative bacteria in conditions that likely mimicked the natural environment of cod. This suggests that the skin mucus layer of the Atlantic cod is an important tissue in surface defenses of cod and most likely protects the fish from infections caused by pathogenic microbes. Antimicrobial test results revealed *Bacillus megaterium* as the most sensitive to the extract at all concentrations of NaCl. Antimicrobial peptides were identified as histone H2B and ribosomal proteins L40, L36A, and L35. Histone-derived peptides originate from both the N-terminus and C-terminus of H1, H2A, H2B, and H6 histones. These histone peptides are found in the skin, skin mucus, and other tissues, including gills, the spleen, and the gut. They are produced in response to epidermal damage, LPS, or certain Gram-negative bacteria. Their antimicrobial activity is broad spectrum against both human and fish pathogens Gram-positive and Gram-negative bacteria, parasites, and fungi (Katzenback 2015).

Anti-Alzheimer's and neuroprotective activity

Alzheimer's disease is a kind of neurodegenerative disease characterized by progressive loss of neurons. The prevalence of such degenerative neuro-diseases has increased with an increase in life expectancy especially as seen in developed countries (Choi and Choi 2015). Anti-Alzheimer's disease activity is profiled using β-secretase inhibitory activity. The enzyme β-secretase along with another enzyme ϒ-secretase generate a peptide amyloid-β (Aβ) through endo-proteolytic reactions of the amyloid precursor protein (APP) (Choi and Choi 2015). Apolipoprotein enhances the breakdown of beta-amyloid; however, an isoform of apolipoprotein, APOE4, ineffectively breakdowns beta-amyloid and leads to an excess amyloid buildup in the brain. The peptide Aβ molecules can aggregate to form flexible soluble oligomers, some of which turn out misfolded. These misfolded oligomers can induce other Aβ molecules to also take the misfolded oligomeric form (Haass and Selkoe 2007; Nussbaum et al. 2013; Pulawski et al. 2012). Anti-Alzheimer's and neuroprotective activity of fish skin hydrolysates is summarized in Table 4. A β-secretase inhibitor peptide was purified from skate skin hydrolysate (Lee et al. 2015). The peptide was purified from a neutrase hydrolysate of skate skin on a Sephadex G-25 column and with reversed-phase HPLC. The peptide sequence was determined to be QGYRPLRGPEFL and showed β-secretase inhibitory activity with an IC_{50} value of 24.26 μM. The neuroprotective effect of protein hydrolysates with antioxidant activity from grass carp (*Ctenopharyngodon idella*) skin was demonstrated (Cai et al. 2015). The hydrolysates at the degree of hydrolysis DH5, DH10, and DH15 showed the most significant neuroprotective effect on 6-OHDA-induced neurotoxicity in MES 23.5. Salmon (*Oncorhynchus keta*) skin enzymatic hydrolysate showed learning and memory enhancement in mice (Pei et al. 2010). Oxidative stress was alleviated, apoptotic neurons reduced, and brain-derived neurotrophic factor (BDNF) expression was upregulated in treatment groups compared with the control group. Similarly, another study showed that salmon skin collagen peptides reduced oxidative damage and acetylcholinesterase (AChE) while it increased phosphorylated cAMP-response element binding protein (p-CREB) and BDNF expression in mice (Xu et al. 2015).

Other biological activities

Other biological activities including antihyperglycemic, MMP inhibitory activity, and adipogenic regulatory have been demonstrated using fish skin as shown in Table 5. Antihyperglycemic activity of fish skin was evaluated

Table 4 Anti-Alzheimer's and neuroprotective activity of peptides purified from fish skin

Activity or mechanism	Species	Peptide sequence	Reference
β-Secretase inhibitory	Skate (*Raja kenojei*)	QGYRPLRGPEFL	Lee et al. (2015)
Anti-acetylcholinesterase	Salmon (*Oncorhynchus keta*)	–	Xu et al. (2015)
Neuroprotection	Grass carp (*Ctenopharyngodon idella*)	–	Cai et al. (2015)
Learning and memory	Salmon (*Oncorhynchus keta*)	–	Pei et al. (2010)

using dipeptidyl peptidase IV (DPP-IV) inhibitory assay. Steelhead (*Oncorhynchus mykiss*) skin gelatin hydrolysates were prepared, and the hydrolysate of 4% papain had the highest DPP-IV inhibitory activity 40–45% (Cheung and Li-Chan 2017). The hydrolysates were purified with ultrafiltration to obtain fraction of less than 3 kDa. Two fractions showed 42% and 44% DPP-IV inhibitory activity showing that the activity was not influenced by ultrafiltration as the values of the fractions and the whole hydrolysates were similar.

In a similar study, the DPP-IV inhibitory and glucagon-like peptide-1 (GLP-1) stimulating activity of fish skin gelatin from various warm- and cold-water fish skins were evaluated and compared (Wang et al. 2015). Results revealed that the DPP-IV inhibitory activity of gelatin hydrolysates from warm-water fish was greater than that from cold-water fish. Halibut and tilapia skin gelatin hydrolysate (HSGH and TSGH) fractions at a cutoff of < 1.5 kDa UF were used for peptide sequence identification and to compare the in vivo antihyperglycemic effect. MS/MS spectra analysis revealed amino acid sequences of 6 active peptides as SPGSSGPQGFTG, GPVGPAGNPGANGLN, PPGPTGP RGQPGNIGF, IPGDPGPPGPPGP, LPGERGRPGAPGP, and GPKGDRGLPGPPGRDGM. All these peptides possessed the amino acid proline as the second N-terminal residue. Moreover, it has been reported that peptides with DPP-IV inhibitory activity have amino acids proline, tryptophan, alanine, valine, lysine, and aspartate as the second N-terminal residues in their sequences (Lacroix and Li-Chan 2012). The IC_{50} values against DPP-IV of the purified peptides ranged from 65.4 to 146.7 μM, and these were comparable to the peptides from other proteins with the IC_{50} values between 41.9 and 174 μM (Huang et al. 2012; Lacroix and Li-Chan 2014; Silveira et al. 2013). Halibut and tilapia skin gelatin hydrolysates (HSGH and TSGH) lowered blood glucose levels of diabetic rats after 28-day administration. The normal rats and diabetic rats treated with HSGH showed similar plasma DPP-IV activity range of 86.6–94.6% while the diabetic rats treated with TSGH had a significantly lower DPP-IV activity of 71.6%. The effect of peptides on GLP-1 levels was also evaluated. TSGH exhibited the highest increase in the total GLP-1 level (27.81 pM) while HSGH and sitagliptin exhibited a comparable effect on GLP-1 secretion (23.46–23.81 pM) in diabetic rats after 30-day treatment. However, the underlying mechanism of peptide GLP-1 stimulatory activity is not well defined. However, it is has been proposed that the presence of amino acids leucine and glutamic acid can induce GLP-1 secretion (Chen and Reimer 2009; Reimer 2006). Gelatin hydrolysates of warm-water fish skins (TSGH) exhibited greater in vitro and in vivo DPP-IV inhibitory activity in comparison to cold-water fish skins (HSGH). TSGH contained higher imino acid contents which resulted in increased antihyperglycemic activity in STZ-induced diabetic rats.

Fish skin hydrolysates have also been demonstrated to show MMP-1 inhibitory activity and thus have great potential use as cosmeceuticals. Two active peptides from cod skin gelatin hydrolysates (CGH) with anti-photoaging activity were identified (Lu et al. 2017). The peptides were purified from CGH by ion exchange chromatography and RP-HPLC. The peptide sequences were determined using QTOF mass spectrometer as EIGPSGGRGKPGKDG-DAGPK and GFSGLDGAKGD. The purified peptides had a MMP-1 inhibitory activity of 16% and 15% respectively. The activity of the peptide GFSGLDGAKGD was achieved through the downregulation of MMP-1, p-ERK, and p-p38 while GEIGPSGGRGKPGKDGDAGPK activity was by the downregulation of p-JNK in MAPK signaling pathways. A study by Chen et al. 2016 purified 23 polypeptides

Table 5 Antihyperglycemic and MMP inhibitory activity of peptides purified from fish skin

Activity or mechanism	Species	Peptide sequence	Reference
Antihyperglycemic	Halibut (*Hippoglossus stenolepis*)	SPGSSGPQGFTG, GPVGPAGNPGANGLN, PPGPTGPRGQPGNIGF	Wang et al. (2015)
	Tilapia (*Oreochromis niloticus*)	IPGDPGPPGPPGP, LPGERGRPGAPGP, GPKGDRGLPGPPGRDGM	
MMP inhibitory activity	Cod	EIGPSGGRGKPGKDGDAGPK, GFSGLDGAKGD	Lu et al. (2017)
	Tilapia (*Oreochromis niloticus*)	LSGYGP	Sun et al. (2013)
	Sutchi catfish (*Pangasius hypophthalmus*)	LMWCP	Pyun et al. (2012)

from cod skin hydrolysates. Amino acid sequences of Gly-Po and Gly-Leu and the amino acid arginine predominated at the C-terminus of the polypeptides. The hydrolysates showed a protective effect against UV-induced photo-damage to collagen. Expression and activities of matrix metalloproteinases (MMP-1, MMP-3, MMP-9) were downregulated through the elevation of tissue inhibitor of matrix metalloproteinases (TIMPs) and suppression of the activation of mitogen-activated protein kinase (MAPK) signaling pathway in the skin of mice treated with the hydrolysate. In another study, the anti-photoaging effect of a peptide LSGYGP purified from tilapia skin (*Oreochromis niloticus*) was evaluated by Sun et al. 2013. In vivo experiments showed that the peptide improved the skin condition of UV irradiation-induced photoaging mice through its antioxidant activity. Furthermore, the mechanism of action of the same peptide LSGYGP was studied using ultraviolet B (UVB)-induced mouse embryonic fibroblasts (MEFs) (Ma et al. 2018). The peptide reduced the intercellular ROS generation and decreased superoxide dismutase (SOD) activity as well as reduced MMP-1 and MMP-9 activities. Molecular docking simulation analysis showed that the peptide inhibited MMP activities by docking the active sites of MMP-1 and MMP-9. The anti-photoaging effect of a peptide LMWCP purified from catfish skin (*Pangasius hypophthalmus*) was evaluated both in animal models and in a clinical trial. The peptide downregulated the expression of MMP-3 and MMP-13, while it upregulated the expression of MMP-2 and MMP-9 (Pyun et al. 2012). In the clinical trial results, treatment groups receiving a daily oral dosage of 1000 mg of LMWCP for 12 weeks showed significantly improved skin and less wrinkling in comparison with the placebo group (Kim et al. 2018).

Last but not least, fish skin has also been demonstrated to have adipogenic regulatory activity. The effect of subcritical water-hydrolyzed fish collagen peptide (SWFCP) from tuna skin on the protein levels of the master adipogenic transcription factors C/EBP and PPAR was investigated (Lee et al. 2017). This was done with the aim of evaluating the underlying inhibitory mechanism of SWFCP in the adipogenic differentiation of 3T3-L1 pre-adipocytes. Results revealed that SWFCP downregulated the expression of the key adipogenic target gene and transcription factors in 3T3-L1 pre-adipocytes exposed to MDI. After 8 days of incubation of 3T3-L1 cells with 1 μM dexamethasone and 1 μg/ml insulin (MDI) and SWFCP, the expression levels of C/EBP and PPAR protein were greatly reduced compared with cells stimulated with MDI alone. SWFCP was also shown to downregulate the expression of aP2 an adipogenic target gene, hence inhibiting adipogenic differentiation. Furthermore, SWFCP reduced lipogenesis in hepatocytes. This was demonstrated by the use of palmitate-induced intracellular lipid vacuole accumulation visualized by Nile red staining. The palmitate-induced intracellular lipid vacuole accumulation was greatly reduced in the presence of 1 mg/ml SWFCP. SWFCP significantly affected other obesity-related factors like low serum cholesterol, low serum triglyceride, and low-density lipoprotein; high serum high-density lipoprotein levels; and reduced size of epididymal adipocytes.

Conclusion

As elaborately discussed in this review, biologically active peptides have been effectively produced through the bioconversion of fish skin. These different peptides with a range of bioactivities such as antihypertensive, antioxidants, antimicrobial, neuroprotection, antihyperglycemic, and anti-aging have been demonstrated in vitro experiments and to an extent in vivo as well. However, further study is required in the preparation of fish skin hydrolysates using the fermentation method for the production of bioactive peptides. In addition, further study is also required, to determine the potential immunomodulation activity of antioxidant peptides, i.e., anti-inflammatory, and anti-allergy and the corresponding cell signaling pathways. More investigations are also required to determine the neuroprotective effect of fish skin hydrolysates especially with protection against amyloid-β-associated neurotoxicity as observed in Alzheimer's disease. Nevertheless, bioactive peptides purified from fish skin can potentially be utilized in the development of pharmaceutical and nutraceutical products.

Abbreviations

ABTS: 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid); ACE: Angiotensin-converting enzyme; AMPs: Antimicrobial peptides; APOE4: Apolipoprotein; APP: Amyloid precursor protein; Aβ: Amyloid-β; BDNF: Brain-derived neurotrophic factor; C/EBP: Ccaat (cytosine-cytosine-adenosine-adenosine-thymidine)-enhancer-binding proteins; cGK-I: cGMP-dependent protein kinase I; Cgmp: Cyclic guanosine 3′5′monophosphate; DCFH-DA: Dichlorofluorescin diacetate; DMI: 1 μM: Dexamethasone and 1 μg/ml insulin; DMPO: 5,5-Dimethyl-1-pyrroline-N-oxide; DPPH: Diphenyl-2-picrylhydrazyl; DPP-IV: Dipeptidyl peptidase IV; EMR: Enzymatic membrane reactor; eNOS: Endothelial nitric oxide synthase; ESI-MS: Electrospray ionization mass spectrometry; ET-1: Endothelin-1; FRAP: Ferric-reducing antioxidant power; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; HPLC: High-performance liquid chromatography; IP 3: Inositol-1,4,5-trisphosphate; LPS: Lipopolysaccharide; MAPK: Mitogen-activated protein kinase; MMP: Matrix metalloproteinase (MMP-1, MMP-3, MMP-9); NO: Nitric oxide; ORAC: Oxygen radical absorbance activity; PPAR-γ: Peroxisome proliferator-activated receptor gamma; PUFAs: Polyunsaturated fatty acids; RAS: Renin–angiotensin–aldosterone system; RNS: Reactive nitrogen species; ROS: Reactive oxygen species; RP-HPLC: Reverse phase high-performance liquid chromatography; SDS/PAGE: Sodium dodecyl sulfate polyacrylamide gel electrophoresis; SHR: Spontaneously hypertensive rats; TIMPs: Tissue inhibitor of matrix metalloproteinase

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HGB conceived, designed, and also revised the manuscript. RA designed and drafted the manuscript. AUR revised and edited the manuscript. All authors read and approved the final manuscript.

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