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Short Review

Angiotensin-I converting enzyme (ACE) Inhibitory and Antioxidant peptides from Seafood Processing Waste

D. Pavan Kumar[1,2], K. Elavarasan[2], B. A. Shamasundar[3], R. Anandan[2]*, Suseela Mathew[2], C. N. Ravishankar[2]

[1] *Mangalore University, Mangalore - 574 199, India*
[2] *ICAR- Central Institute of Fisheries Technology, Cochin - 682 029, India*
[3] *KVAFSU, College of Fisheries, Mangalore - 575 002, India*

Abstract

Bioactive peptides are oligopeptides that contain 2–20 amino acid residues that can exhibit beneficial nutritional and physiological effects on human health. Protein hydrolysates or peptides derived from sea food processing waste with bioactive properties have been reported from different waste such as skin, fin, viscera, bone and air bladder. *In vitro* and controlled enzymatic hydrolysis of sea food processing waste using commercial enzymes as well as digestive proteases are the most employed for hydrolysis. The antihypertensive and antioxidant activities of bioactive peptides are influenced by several factors such as amino acid composition, hydrophobic amino acid residues, sequence and size. Most of the antihypertensive and antioxidant bioactive peptides from seafood have been investigated using *in vitro* and *in vivo* cell culture and animal models. This short review provides a comprehensive information of antihypertensive and antioxidant bioactive peptides from fish and fishery waste.

Keywords: Fish and Fishery waste; Gelatin; Proteases; Bioactive peptides

Introduction

The health benefits of fish and fishery products are well recognized due to its protein, lipid, and minerals. With the increase in demand for fish and fishery products, the supply is becoming a limiting factor. Global fish production during the year 2018 has been estimated to be 170.9 million tons (FAO, 2018). The perishable nature of fish has compounded the problem in the supply of nutritious and safe fish. Capture fish and production from the marine sector has almost become stagnant and contributes about 93.4 million tons. However, the fish production from culture practices both in marine and freshwater has shown rapid development and contributes more than 56% of total fish production (Dara et al., 2020a). In the agriculture sector, aquaculture is considered to be the fastest growing sector at the rate of 6–8% per annum (FAO, 2018). With the increase in urbanization in developing countries, the population, at large, depends on ready-to-cook/processed food. This necessitates more and more fish processing units to meet the demand.

The perishable nature of fish and shellfish coupled with insufficient infrastructure to handle the catch has led to huge post-harvest losses. A conservative estimate of post-harvest losses is to the extent of 25% of total production (Dara et al., 2020b). Hence, there is a need to develop effective post-harvest technologies to minimize post-harvest losses and at the same time evolve technologies for efficient utilization of fish including processing waste. Processing sector generates a huge amount of fish processing waste (e.g., head, skin, viscera, bones, fins and scales) which is around 50–75% of the fish and is generally discarded (Karim & Bhat, 2009). The gastrointestinal tract or the viscera constitutes 5–8% of the fish weight. In 2010, the volume of global waste released was approximately 24 M.T (16.5%) of the total

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**E-mail: kranandan@rediffmail.com*

harvest (Suleria et al., 2016). Seafood processing operations in India generates over 300,000 tons of waste including visceral mass (Mahendrakar, 2000). Utilization of fish processing waste for the production of high-value compounds is an important industrial strategy and an active area of research. Among the different components of fish processing waste, the skin and visceral mass accounts for more than 40% of total waste. The fish skin is essentially made up of collagen and which can be used either in the biomedical application or converted to gelatin as a food ingredient (Dara et al., 2020c).

The food proteins from the aquatic environment are unique, in that it comprises of less connective tissue and easily digestible protein fractions (Aluko, 2015). Food proteins, when subjected to controlled hydrolysis yield peptides of different size and these peptides are known to possess different biological activity. The peptides that possess biological activity are termed as 'bioactive peptides. Bioactive peptides are specific protein fragments that has a positive impact on physiological function which influence human health (Elavarasan et al., 2016). The discovery of the bio-regulatory role of derived peptides in understanding the molecular mechanism of action has given biological peptides, a promising lead as bioactive material in regenerative medicine as well as food and pharmaceutical industries. The molecules with benefits beyond basic nutrition are considered as nutraceuticals. Nutraceuticals are medicinal or nutritional components that are used for the improvement of health, by preventing or treating a disease (DeFelice, 1995). The development of nutraceutical products has been driven by the developed countries, primarily as dietary supplement in their regular diet. The bioactive molecules naturally present in fish and shellfish like carotenoids, chitin, peptides, collagen and ω-3 fatty acids are known to improve the health of humans and well-being (Je et al., 2005). The health benefits of fish lipids are well documented in controlling cardiovascular, immunomodulatory and anti-inflammatory responses (Kris-Etherton et al., 2002).

Collagen is the fibrous protein and abundantly found in connective tissue of vertebrates and marine invertebrates. Collagen constitutes approximately 25-35% of total body protein in all vertebrates (Dara et al., 2020b). The collagen content in fish varies from 3-10% and Elasmobranchs are known to have higher collagen content. The yield of collagen varies from species to species, conditions of raw material and extraction procedure. The physiological functions of collagen are diverse depending on the function of organ and collagen molecule is known to associate with the toughness and tenderness of muscle and, also provides strength and support to the muscle (Kumar et al., 2017). Commercially, the collagens are derived from bovine/porcine hides and bones. The seafood processing waste which comprises of substantial quantity of collagen has been found to be good source of raw material for collagen and gelatin production (Pavan Kumar et al., 2017). The conversion of collagen to gelatin process and yield of gelatin depend on several factors such as temperature, duration of heating and nature of the raw material (Elavarasan et al., 2017). Gelatin is a derived protein from collagen and this molecule is one of the important industrial biopolymers used as food ingredients in functional food formulations due to its versatile functionality (Kumar et al., 2017; Pavan Kumar et al., 2017). The amino acid composition of collagen and gelatin is distinct from other proteins. Glycine accounts for more than 30% of total amino acid residues followed by hydroxyproline (Hyp) and proline (Pro). Amino acids such as tryptophan, tyrosine and cysteine are negligible or mostly absent. As collagen and gelatin molecules are devoid of tryptophan, it is considered as nutritionally incomplete. The collagen and gelatin from fish skin, bone and scale are different from bovine and porcine sources. Due to the difference in amino acid composition, the molecular properties will vary and hence the source of collagen and/gelatin is critical for a particular application. The applications of gelatin include thickening, water holding, colloid stabilization, crystallization control, film formation, whipping and emulsification (Pangestuti & Kim, 2014). The gelatin extracted from different sources of fish and fishery waste are given in Table 1.

Protein hydrolysis can be achieved by different methods to produce fish protein hydrolysate or gelatin protein hydrolysates. The common methods such as enzymatic hydrolysis, thermal hydrolysis, and bacterial fermentation are used (Godinho et al., 2015). The physicochemical and techno-functional properties of proteins are affected by hydrolysis. Protein hydrolysis yields the peptides with different molecular size, surface-active and bioactive properties (Benjakul et al., 2014). Enzymatic hydrolysis of fish protein can be performed using specific proteolytic enzymes/digestives proteases like trypsin, chymotrypsin, pepsin as well as commercially available proteases like papain, bromelin, neutrase

Table 1. Extraction of gelatin from fish processing waste*

	Raw material	Reference
Skin	Bigeye Tuna (*Thunnus obesus*)	Dara et al., 2020
Skin	Rainbow trout (*Oncorhynchus mykiss*)	Kamer et al., 2019
Skin	Croaker fish	Kumar et al., 2018
Scales	Bighead carp (*Hypophthalmichthys nobilis*)	Huang et al., 2017
Skin	Squid (*Loligo vulgaris*)	Abdelmalek et al., 2016
Swim Bladders	*Catla catla*	Chandra & Shamasundar, 2015
Skin	Seabass (*Lates calcarifer*)	Sinthusamran et al., 2014
Skin	Cuttlefish (*Sepia officinalis*)	Jridi et al., 2013
Skin	Splendid squid (*Loligo formosana*)	Nagarajan et al., 2012
Skin Scales	Leatherjacket (*Aluterus monoceros*)	Ahmad & Benjakul, 2011
	Grass carp fish (*Ctenopharyngodon idella*)	Zhang et al., 2011
Skin	Rainbow trout (*Onchorhynchusmykiss*)	Tabarestani et al., 2010
Skin Bones	Bigeye snapper (*Priacanthus hamrur*)	Binsi et al., 2009
	Channel Catfish (*Ictalurus Punctatus*)	Liu et al., 2009
Fish offal	Baltic cod, Salmon	Kolodziejska et al., 2008
Skin	Atlantic salmon (*Salmo salar*)	Arnesen and Gildberg, 2007
Skin	Bigeye snapper and Brownstripe red snapper	Jongjareonrak et al., 2006

*Source: Pavan Kumar Dara, 2018

and alcalase (Elavarasan & Shamasundar, 2014). Enzymatic hydrolysis is one of the most preferred controlled techniques for providing bioactive peptides to improve the techno-functional properties which add value to a by-product or a low value fish. (De Castro et al., 2015). Different types of digestive proteases from sea food processing waste used for the preparation of bioactive peptides are given in Table 2.

Enzymatic hydrolysis

Generally, no specific proteins or enzymes are needed to obtain antihypertensive peptides. However the proteins with digestibility which tend to generate high peptide yield is the key factor for potential commercialization. Further, proteins from various sources such as plant, animal and marine organisms have been used extensively for the production of bioactive peptides preferably, ACE inhibitory peptides. Nevertheless, generation of bioactive peptides from aquatic food waste is cost effective and eco-friendly. Commercially available food grade proteases such as alcalase, protamax and flavourzyme derived from microorganisms, plant derived proteases papain and bromelain, animal derived proteases trypsin, pepsin and chymotrypsin have been widely used for the preparation of bioactive peptides from marine sources. Proteases from different sources like microbial and plant sources are obtained so as to have selective hydrolysis specific to peptide bonds originating from specific residues (Peterson and Johnson, 1978). The visceral mass from fish and fishery waste comprises of liver, pancreas, pyloric-cecae, and stomach can be a source of different enzymes, especially proteolytic enzymes. The proteolytic enzymes associated with visceral mass include pepsin, trypsin, chymotrypsin, and pancreatin (Simpson, 2000; Pavan Kumar et al., 2017). The utilization of fish processing waste especially skins and visceral mass for the production of gelatin protein hydrolysate (GPH) has received desired attention (Arvanitoyannis & Tserkezou, 2010). The properties of peptides obtained from GPH have been evaluated and are compared to protein hydrolysate from other sources. The nature of proteolytic enzymes used for the production of protein hydrolysates will determine its bioactive properties. The duration of enzymatic hydrolysis is found to have inverse relationship with peptides.

Table 2. Extraction of digestive proteases from gastro-intestinal tract of different fish*

Proteases	References
Brownstripe red snapper Bigeye snapper and Threadfin bream proteases	Khantaphant et al., 2008
Proteolytic and collagenolytic enzymes	Murado et al., 2009
Bigeye snapper protease	Phanturat et al., 2010
Smooth hound crude acid proteases	Balti et al., 2011
Zebra blenny proteases	Ktari et al., 2014
Cuttlefish proteases	Balti et al., 2015
Giant catfish protease	Ketnawa et al., 2016
Mekong giant catfish protease	Ketnawa et al., 2017
Silver Catfish protease	Ismail & Jaafar, 2018
Rainbow trout alkaline protease	Andevari et al., 2019
Labeo rohita and *Catla catla* proteases	Dara et al., 2020c

*Source: Pavan Kumar Dara, 2018

Further the increase in time of hydrolysis will not affect the biological activity or size of peptide. Though there are many proteolytic enzymes available commercially which are well characterized, there is a search for finding different sources of proteolytic enzymes for the preparation of protein hydrolysates. In this context, the use of crude proteases from the visceral mass of fish processing waste will be an ideal choice both from the point of economics and to minimize environmental pollution. Hence, it will be of interesting in preparing the gelatin protein hydrolysates (GPH) from fish processing waste like skin using visceral enzymes. The advantages of promoting GPH production will enable utilization of fish processing waste into a high-value product and also reduce environmental pollution. The methods for the preparation of gelatin protein hydrolysates by the application of proteases extracted from sea food processing waste are given in Table 3 and Fig. 1.

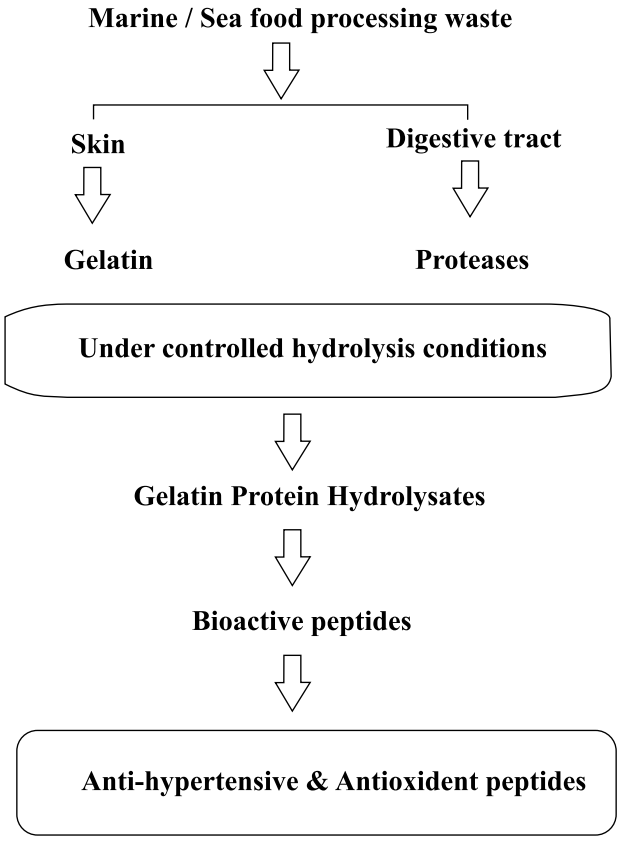

Fig. 1. Procedure for the extraction of gelatin protein hydrolysates (GPH)

Bioactive Properties

A growing body of scientific evidence shows that bioactive peptides in protein hydrolysates from different proteins including fish and fish processing waste may promote human health (Kim & Wijesekara, 2010). Nutraceuticals and food supplements with bioactive peptides especially derived from natural sources are considered to be safe and healthy

compounds. It shows impact on human healthcare and nutrition due to its low molecular weight, simple structure, low cost, more stability, high activity and easy absorption (Noomhorm et al., 2014). Epidemiological investigations demonstrated that the oral ingestion of collagen/gelatin hydrolysates attenuates the pain in osteoarthritis patients (Moskowitz et al., 2000). Based on the amino acid sequence of peptides, they may be involved in various biological functions such as antioxidant, antihypertensive, antimicrobial, anticancer, antiproliferative and immunomodulatory properties (Guaadaoui et al., 2014). Gelatin protein hydrolysates exhibit various bioactive properties such as antioxidant, angiotensin-converting enzyme (ACE) inhibitory activity, anticoagulant, and antimicrobial activities (Dara et al., 2020c). All the bioactive properties of hydrolysates arise from small peptides which form a major part of hydrolysates.

Angiotensin-I converting enzyme (ACE) inhibitory properties

Hypertension or high blood pressure (BP) is one of the major risk factors of cardiovascular diseases (CVDs) (Staessen et al., 2003). The renin-angiotensin-aldosterone system (RAAS) is a major cardiovascular regulatory system in human body which plays a crucial role in regulating hypertension. Angiotensinogen (AGT), a hormone released from the liver is primarily cleaved by renin to provide angiotensin-I (AG-I), a N-terminal decapeptide (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu). AG-I is biologically inactive and is then converted to potent vasoconstrictor octapeptide angiotensin-II (AG-II) (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe) by the action of Angiotensin-I-converting enzyme (ACE, E.C. 3.4.15.1) or peptidyl-dipeptidase A or kininase II to, by cleaving the dipeptide (His-Leu) from the C - terminal of AG-I (Lu et al., 2016).

Several synthetic ACE inhibitors such as Captopril, Enalapril, Lisinopril are commercially available for clinical use, among which, Captopril 1-[2(S)-3-mercapto-2-methyl propionyl]-L-proline, a sulfhydryl-containing agent, was the first antihypertensive compounds, potent and specific orally active competitive inhibitor of ACE with certain adverse effects. ACE-inhibitory peptides were categorised into three groups, the first group comprises of true inhibitors, the activity of which cannot be changed by pre-incubation with ACE, the second group comprises of substrates for ACE, which converts them to inactive peptides, the third group comprises of pro-drug peptides, which are converted into true inhibitors by ACE or gastrointestinal proteases, resulting in increased activity (Elavarasan et al., 2016). Most of the ACE inhibitory peptides are of moderately short sequences containing 2 - 12 amino acids residues. It is believed that ACE inhibitory peptides with certain amino acids have ability to modulate the RAS (renin-angiotensin system) regulation by decreasing renin and or ACE enzymatic activities which in turn regulates hypertension. The ACE inhibitory peptides promote vasodilation and enhances the endothelial nitric oxide synthase (eNOS) pathway to increase nitric oxide (NO) levels within vascular walls (Aluko, 2015).

Over the past decades, it was observed that the ACE-inhibitory peptides from fish waste especially skin gelatin and collagen, have the ability to inhibit *in vitro* and *in vivo* activities. ACE-inhibitory peptides from fish sources were first identified in sardine meat (Suetsuna & Osajima, 1986). ACE inhibitory peptides have been isolated and characterized from fish sources and fish processing waste, either from gelatin or visceral discards. The protein hydrolysates with highest DH are tend to have shorter peptides and thus exhibits the highest ACE inhibitory activity. This indicates the bioactivity of proteins or peptides depends on their molecular weight and/ peptide length (Je et al., 2005). Further, the peptide sequence, position of amino acids in the sequence and nature of peptides plays a crucial role in inhibiting the ACE enzyme (Dara et al., 2020c; Elavarasan et al., 2016; Elavarasan & Shamasundar, 2014).

Spontaneously hypertensive rats (SHR) are found to be one of the suitable animal experimental models used in testing the capability of ACE-inhibitory peptides. Most of the tests were performed by administrating the bioactive peptides either orally or by intravenous injection. Recent studies of bioactive peptides from fish waste have shown the reduction of blood pressure and induction of endothelium relaxation by increasing the NO production (Aluko, 2015). The bioactive peptides define its ability in renin inhibition by reducing the α-helix and β-sheet fractions of the enzyme through hydrogen bond interactions with active site and peptide residues (Elavarasan et al., 2016). The absorption of peptides from gastrointestinal tract into the blood stream influences the efficacy of bioactive peptides. The treatment of peptides with

digestive proteolytic enzymes such as pancreatin, trypsin and pepsin at ambient conditions confirms the *in vitro* digestion and stability of bioactive peptides. Several studies indicated that the difference in ACE inhibitory activity can be attributed to the differences in degree of hydrolysis, peptide chain length and sequences and, their hydrophobic nature (Pangestuti & Kim, 2014; Benjakul et al., 2014; Suetsuna & Osajima, 1986). The interaction between the active site and substrate determines the mode of ACE inhibition. Almost all the ACE inhibitors bind to the catalytic site at the C-terminal, except for the hydrophobic binding and blocking of the N-terminal catalytic site. This is the reason for ACE inhibitory peptides having hydrophobic amino acids at their ends. In contrary, these hydrophobic amino acids tend to have strong binding effect by hydrophobic interactions between the enzyme and the ligand (Elavarasan & Shamasundar, 2014). The structure–activity relationship studies of bioactive peptides forecast that hydrophobic amino acids residues lead to a better ACE-inhibitory activity (Dara et al., 2020c). Several studies on bioactive peptides from marine sources reported that the hydrophobic amino acids valine, isoleucine, alanine, methionine, leucine at N-terminal end, and tryptophan, tyrosine, phenylalanine proline at C-terminal end may contribute to ACE activity (Dara et al., 2020b). ACE inhibitory peptides derived from fish waste and the ACE inhibitory activity or IC_{50} values are given in the Table 3 & 4.

Antioxidative properties

Oxidation is one of the main causes of diseases and pathogenesis in humans. Free radicals released in the body during oxidative stress implicates large number of disorders includes cardiovascular diseases, cancer and auto immune diseases like diabetes and rheumatism (Patras et al., 2013). Many research studies have implicated a direct link between reactive free radicals and oxidative damage. The reactive free radicals include reactive oxygen species (ROS), superoxide radicals (SOR), and reactive nitrogen species (RNS). ROS and RNS are both produced to help maintain the homeostasis

Table 3. Type of Hydrolysis and ACE inhibitory activity of protein hydrolysates obtained using different proteases*

Source	Enzyme	ACE inhibitory activity or IC 50	Reference
Chum salmon cartilage and skin	Thermal hydrolysis	62.6 mg ml^{-1}	Nagai et al., 2006
Sea cucumber	Bromelain and alcalase	0.0142 mg ml^{-1}	Zhao et al., 2007
Chicken collagen	*Aspergillus oryzae*	0.26 mg ml^{-1}	Saiga et al., 2008
Alaska Pollock	Pronase and Flavourzyme	0.49 mg mL^{-1}	Park et al., 2009
Atlantic salmon skin	Alcalase and papain	1.165 mg ml^{-1})	Gu et al., 2011
Squid skin	Pepsin	0.33-105 g ml^{-1}	Lin et al., 2012
Blacktip shark skin	Papaya crude enzyme	0.94-1.77 gm L^{-1}	Kittiphattanabawon et al., 2013
Skate skin	*Alcalase, flavourzyme, Neutrase and protamex	*72.8%	Ngo et al., 2014
Thornback ray skin	*Bacillus subtilis* A26 *Bacillus amyloliquefaciens*	0.94 mg ml^{-1} 2.07 mg ml^{-1}	Lassoued et al., 2015
Pacific cod skin	*Pepsin, papain, α-chymotrypsin, trypsin, neutrase, and alcalase	*14.5 μM	Ngo et al., 2016
Giant catfish skin	Visceral peptidase	67.75 %	Ketnawa et al., 2017
Tilapia skin gelatin	Gastrointestinal proteases	80.90 and 68.35 μM	Ling et al., 2018
Camel skin	Alcalase and Protease	< 1 mg ml^{-1}	Mudgil et al., 2019
Croaker Skin gelatin	Gastrointestinal proteases	0.64 and 0.63 mg ml^{-1}	Dara et al., 2020

*Source: Pavan Kumar Dara, 2018

Table 4. Sequences of bioactive peptide exhibiting ACE inhibitory and antioxidant activities derived from fish processing waste*

Source	Peptide sequence	Bioactive property	Reference
Atlantic salmon (*Salmo salar L.*) skin collagen	AP (186.2 Da) VR (273.3 Da)	ACE inhibitory activity	Gu et al., 2011
Salmon byproduct	VWDPPKFD (1003.1Da) FEDYVPLSCF (1219.4 Da) FNVPLYE (881 Da)	ACE inhibitory	Ahn et al., 2012
Thornback ray skin gelatin	AVGAT (417 Da) GGVGR (444Da)	ACE inhibitory and antioxidant activities	Lassoued et al., 2015
Thornback ray skin gelatin	LESRT (605 Da) SPGPMGPR (798 Da) WIGYSF (772 Da) VPGQPGSP (795 Da) PGPTGEKGSKGEPGL (1410 Da)	ACE inhibitory and antioxidant activities	Lassoued et al., 2015
Bluefin leatherjacket heads	WEGPK (615.69 Da) GPP (269.33 Da) GVPLT (485.59 Da),	Antioxidant activity	Chi et al., 2015
Half-fin anchovy (*Setipinna taty*)	AGDDARPA (771.35 Da) ESAGLHE (741.33 Da) NKVKGELD (901.45 Da) TLWSGAGQTVNT (1233.63 Da) EMSAGLHE (872.37 Da) WRKKDPLND (1171.60 Da),	Antioxidant activity	Song et al., 2015
Pacific cod skin gelatin	GASSGMPG (662 Da) LAYA (436 Da)	ACE inhibitory	Ngo et al., 2016
Nile tilapia skin	GPEGPAGAR (810.87 Da) GETGPAGPAGAAGPAGPR (1490.61 Da)	ACE inhibitory activity	Choonpicharn et al., 2016
Salmon gelatin	Gly-Gly-Pro-Ala-Gly-Pro-Ala-Val, Gly-Pro-Val-Ala, Pro-Pro and Gly-Phe)	ACE inhibitory activity	Neves et al., 2017
Tilapia skin gelatin	VGLPNSR (741.4133 Da) and QAGLSPVR (826.4661 Da)	ACE inhibitory activity	Ling et al., 2018
Cyprinus carpio	Ala-Tyr	ACE inhibitory activity	Tkaczewska et al., 2019
Croaker Skin gelatin	GLTGRPGDAGPQGK (1310.67 Da) GFPGER (662.32 Da)	ACE inhibitory and antioxidant activities	Dara et al., 2020

*Source: Pavan Kumar Dara, 2018

of live cells in normal healthy tissues and both play an important role as cell-signalling molecules. The reactive free radical with unpaired electron, are extremely unstable and have tendency to react quickly with other molecules to achieve a stable configuration. These excessive amounts of ROS and RNS create imbalance between antioxidants and oxidants which is technically termed as 'oxidative stress/damage'. This oxidative damage vulnerably targets the unsaturated fatty acids, thiol groups in proteins and nucleic acid bases in DNA (Yeum et al., 2010).

Many synthetic antioxidants butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate (PG) have been used in the food industry to retard the oxidation process. However, the use of synthetic antioxidants is under strict

regulation due to potential health hazards. The search for natural antioxidants as alternatives to synthetic ones is therefore of great interest among researchers. Nevertheless, natural commodities such as vegetables, fruits, herbs, spices, teas, oilseeds, nuts, cereals, and legumes are rich sources of natural antioxidants. These dietary antioxidants include vitamins (vitamin E, C, and β-carotene), plant phenols (flavonoids, other phenolic compounds), and essential minerals (selenium, zinc, manganese, and copper) that form important antioxidant enzymes. Natural antioxidants inhibit lipid oxidation reactions by radical scavenging, protein complexing, and synergistic effects by reducing oxidized antioxidants and metal chelation (Msagati, 2012). The methods generally used to determine antioxidant activity are the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay (Blois, 1958), ferric ion reducing antioxidant parameter (FRAP) assay (Benzie & Strain, 1999), 2,22 -azino-bis-(3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) assay (Miller et al., 1993), β-carotene-linoleic acid bleaching activity (Amarowicz et al., 1993).

Over the past decades, the antioxidant activity of bioactive peptides derived from fish sources has been determined by various methods such as DPPH, ABTS, peroxide, carbon-centred, hydroxyl, and superoxide radical scavenging activities, reducing power, chelating ability, and inhibition of the peroxidation of linoleic acid (Dara et al., 2020b). The precise mechanism underlying their antioxidant effects of peptides have not been fully elucidated and appears to be far more complex and subtle than previously realized (Msagati, 2012). It is well known that the degree of hydrolysis is inverse to the peptide size, which means that the smaller peptides can arise only if higher DH is achieved. And this smaller peptides or lower molecular weight peptides can easily react with free radicals and exhibits more antioxidant activity (Dara et al., 2020c). Further, the tryptophan, tyrosine and methionine amino acids followed by cysteine, histidine, and phenylalanine exhibited the highest antioxidant activity (Guaadaoui et al., 2014). Moreover, the amino acids with aromatic residues donate protons to free radicals which enhance the radical scavenging property of bioactive peptides.

Many studies have proposed that the peptides with amino acid histidine, showed hydrogen donating, radical trapping and ion chelating ability, whereas the peptides with amino acid cysteine, plays antioxidant role independently by direct interaction with free radicals (De Castro et al., 2015). There are several factors which determine the potential of peptides to exhibit bioactive properties. Though certain amino acids are present in the peptide sequence, their position plays an important role in exhibiting antioxidant activity of nutraceuticals, functional foods and bioactive peptide-based food supplements (Noomhorm et al., 2014). Amino acid composition and peptide sequence, as well as chain length, are the crucial factors that mainly govern the antioxidant activity of peptides. Enzymatic hydrolysis of proteins is influenced by several factors like source of protein, pH, temperature, duration of hydrolysis and type of enzyme used for hydrolysis. The nature of enzymes, substrate and hydrolysis conditions will determine the properties of protein hydrolysate. The physicochemical conditions such as temperature, pH, and time of hydrolysis need to be optimized for the activity of an enzyme for that specific substrate (Benjakul et al., 2014). Apparently, the *in vivo* studies on bioactive peptides from aquatic commodities were demonstrated for the enhancement of antioxidant enzymes such as superoxide dismutase (SOD), catalase and glutathione peroxidase (GSH-Px) and reduction of malondialdehyde (MDA) enzyme activity (Agyei et al., 2015). Specific peptide linkages and structural features influence the three-dimensional (3D) conformation of bioactive proteins and therefore affects the biological activity. It has been claimed that the peptide defines the synergistic and antagonistic effects of antioxidant capacity of bioactive peptides (Pangestuti & Kim, 2014). The bioactive peptides derived from skin gelatin is found to have higher antioxidant activity than the bioactive peptides from meat protein probably due to higher percentage of Glycine and Proline amino acid (De Castro et al., 2015). Antioxidant peptides derived from fish processing waste are given in the Table 4.

Conclusion

In conclusion, many researchers have investigated the biological activities of bioactive peptides derived from aquatic food processing waste proteins including myosin, collagen etc. Numerous bioactive peptides with antihypertensive and antioxidant activities have been characterized from sea/marine food sources. These derived bioactive peptides could be utilized as novel bio-functional ingradients and molecules to develop food supplements, nutraceuticals, food formulations with antihyper-

tensive and/ antioxidant properties. The bioactive peptides have been extensively demonstrated predominantly *in vitro* or *in vivo* using animal experimental models. Thus, more technologies and cost-effective methods need to be developed for the production of pure and stable bioactive peptides. Simultaneously, clinical studies need to be evolved to understand the underlying mechanisms of gastrointestinal digestive stability, bioavailability and safety of these bioactive peptides before exploitation for human nutrition as functional foods.

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