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A review on fish peptides isolated from fish waste with their potent bioactivities

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ABSTRACT

Fish processing unit establishment is now seeing an increasing trend of interest and subsequent activities. Thus, as a collateral the accumulation of greater amount of fish waste (head, skin, frames, visceral parts, and scales) and underutilized (non-edible) fish presents a greater opportunity for further utilization if not accounted for at the right time. Fish waste obtained during fish catch and processing account for about 70% and therefore, if not treated and discarded properly becomes a threat to environment. Currently, efforts have been made for utilization of these waste in different ways that includes production of saleable products, like animal feed, biogas; isolation of valuable bioactive biomolecules and fractions; separation and extraction of free amino acids, peptides, enzymes, fats and oils; collagen, gelatin, chitin, chitosan, vitamins, minerals, and polysaccharides. Isolated bioactive compounds and fractions further may be used in formulation and synthesis of pharmaceuticals, cosmetics, and nutraceuticals. This review precisely outlines the present understanding on fish waste as a source of high-value fish protein hydrolysates which can further be used for formulating different nutraceutical and therapeutic products such as antimicrobial, anticarcinogenic, antioxidant, and antihypertensive peptides, fish collagen and gelatin; and enzymes.

1. INTRODUCTION

Fishery segment has grown manifold since last few decades. The capture fishery production has mounted up from 69 to 90 MN tons. Likewise, the aquaculture production all across the world has risen from 5 to 80 MN tons [1]. Food and Agricultural Organization [1] has reported that the demand for fish has increased from 67% in 1960s to about 87% at 2016. Consequently, this has led to increase in consumption, and hence generation of million tons of fish waste from fish processing and retailing units as a subsequent effect.

Fish waste in form of skin, head, frame, viscera, and scales etc., along with underutilized fish species have been accounting for about 70% of total fish catch [1]. Therefore, it has become a threat for the environment.

Numerous studies have been made to develop fish waste and underutilized fish to formulate, synthesize, and to produce commercially profitable products such as fish oil, enzymes, cosmetics, packing materials etc. [2]. Fish protein hydrolysates (FPH) can directly be used in nutraceuticals or can be processed and fractionated to be employed in pharmaceutical industries for therapeutic usage [3,4]. FPH is a concentrated and purified form of protein obtained by cleaving molecular bonds present in them by employing the processes of chemical or enzymatic hydrolysis; fermentation, solvent extraction methods, and various others [5].

This review summarizes the prevailing concepts and understanding on the usage of fish waste and underutilized fish as sources of free amino acids, bioactive peptides, enzymes, collagen, and gelatin and explores the isolation techniques involved in the procurement of such compounds.

2. COMPOSITION OF FISH WASTE

The quality and quantity as well the type of bioactive compounds present in fish waste depends upon various factors like type of species, sex, age, nutritional status, season, and health condition

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of the fish. The existence of relation between the quality and quantity of isolated products from fish waste and aforementioned factors have been established with comparative studies made in five fish species (Catfish, Cod, Flounder, Mackerel, and Salmon) [6]. Results of the study revealed that Mackerel contained 11.7% of fat, whereas Cod encompassed 0.1%. Likewise, protein content was found to highest in Salmon (23.5%) and lowest in Flounder (14%) [7]. On an average, fish waste contains about 58% proteins, 19% fat and minerals. In addition, fish waste also contain 22% of mono-saturated acids, palmitic acid and oleic acid [8].

3. BIOACTIVE PEPTIDES

Every living organism synthesize certain quantity of bioactive peptides within their body. Reports from various research articles reveals that these bioactive peptides can be used in various nutraceutical and pharmaceutical products. There are several ways to isolate such peptides from different sources. These include acid, alkali, and enzymatic hydrolysis, fermentation processes, solvent extraction techniques. In these processes, the complex proteins or polypeptides, present in the source sample, break down. Breaking of proteins or polypeptides takes place because of the breakage of peptide and other bonds present in the proteins which yield small peptides of 2–20 amino acids. Yield of small peptide, however, depends upon protein structure and folding of the whole protein, the type of cleavage treatment, degree of hydrolysis, enzymes specificity, and other additional conditions of hydrolysis and fermentation like concentration, temperature, and time [9,10]. Choice of solvent and their properties also imparts influence on the quantity and quality of the small peptides isolated from the living organisms.

The specific bioactivity of small peptides largely depends on the sequence and composition of amino acids. Currently, a series of research in all parts of the world is rendering momentum for isolation and characterization of small peptides from fish sources and further to explore their nutraceutical and therapeutic properties, as fish are considered to have higher amounts of proteins. Scientific investigations have also been taken up to isolate bioactive peptides from specific parts of fish that includes head, bone, frames, skin etc., and to confer specific bioactivities such as antimicrobial, antiviral, anticarcinogenic, anti-oxidative, antihypertensive, anticoagulative, analgesic, anxiolytic, immunomodulating, antidiabetic, and appetite- inhibiting properties [10–13] (Fig. 1).

4. GENERAL PROCESS OF BIOACTIVE PEPTIDE ISOLATION AND EXTRACTION

Isolation and extraction of bioactive peptides from fish waste can be obtained by various methods. Approaches included hydrolyzation of source sample with acid or alkali or digesting with one or more enzyme. Fermentation procedure may also be applied for cleaving the peptide and other bonds present in proteins. In

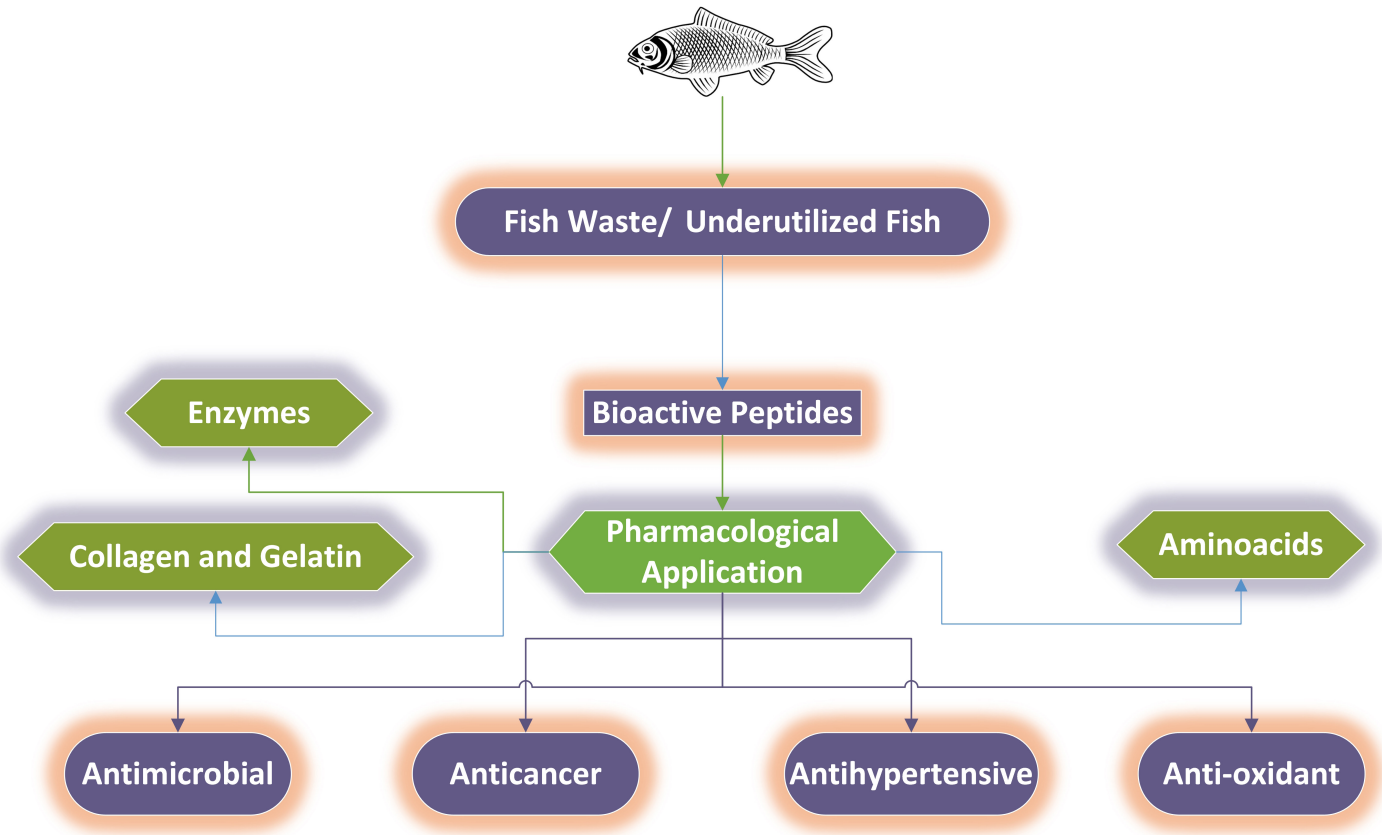

Figure 1: Different pharmacological application of bioactive peptides.

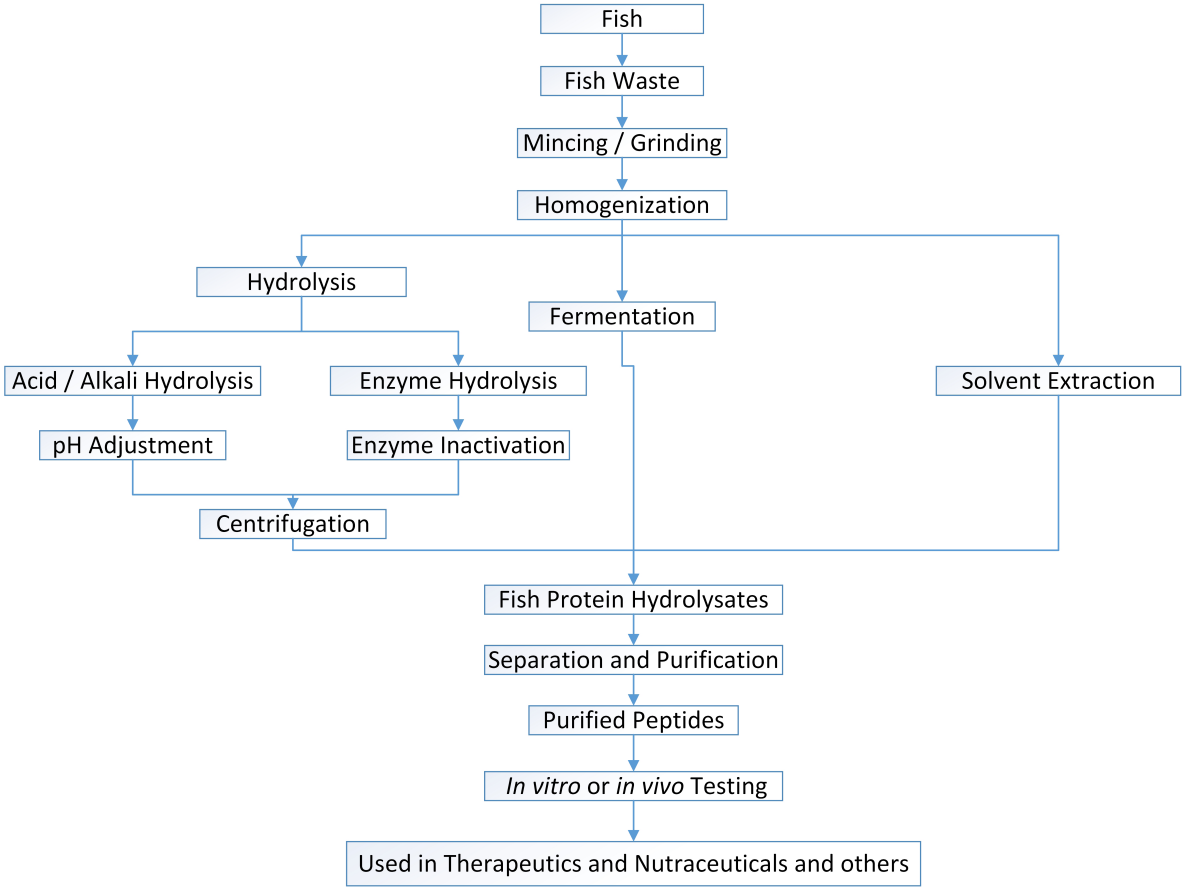

Figure 2: Flow chart shows different steps involved in the extraction and purification of bioactive peptides from fish waste.

fermentation technique, microorganisms replace enzymes for breaking down the proteins into peptides [14]. Besides, hydrolysis and fermentation, solvent extraction procedure can be offered for isolation of bioactive peptides from source sample. In this procedure different solvents are chosen and applied for separation of peptides of different sizes and properties from a mixture of peptides [14] (Fig. 2).

5. METHODS EMPLOYED FOR FISH PEPTIDE ISOLATION

Fish peptides can be isolated using chemical methods that include hydrolyzation of fish tissues with acids or alkali or digesting with proteolytic enzymes [15]. Apart from abovementioned methods, microbial fermentation of proteins; precipitation and process of salting out of high molecular weight compounds from decoctions or broth; solvent extraction processes can also be chosen as suitable methods for obtaining peptides from samples [10,16] (Fig. 2).

5.1. Acid and Alkali Hydrolysis

To pursue acid hydrolysis generally acids, such as Sulphuric and Hydrochloric acids, are used with different concentrations. Usually, the process of acid hydrolysis takes around 3–24 hours. Hydrolyzing the samples with acids ensures deeper proteolysis and rule out the likelihood of bacterial contamination of hydrolysates.

Alkaline hydrolysis of proteins can also be employed for isolation of bioactive peptides. However, alkaline hydrolysis generates residues of lanthionine and lysionoalanine which are toxic to organisms and hence restricts this technique for wider use.

5.2. Enzymatic Hydrolysis

Enzymatic hydrolysis is one the most adapted techniques for isolating bioactive peptides from tissue samples [17]. This technique uses proteolytic enzymes like proteases, α-chymotrypsin, papain, alcalase, thermolysin, pancreatin, trypsin, protamex, neutrase, chymotrypsin, pronase, and bromeline for digesting tissue samples [18]. During enzymatic hydrolysis, pH, temperature, enzyme concentration, and time duration are maintained to obtain desirable results. Enzymatic hydrolysis has added advantages over chemical treatments as the enzymes used for hydrolysis are specific in their catalytic activity and show substrate specificity. Desirable enzymes can be procured easily and are easy to handle and use [15]. In enzymatic hydrolysis, the final sample remains free of residual contaminants of chemical used during the process of isolation of bioactive peptides and so makes the peptides suitable for nutraceutical and pharmaceutical industries [13].

5.3. Fermentation

Fermentation is a time-tested process traditionally employed for protein hydrolysis and food preservation. This process enhances

flavor, taste, nutraceutical properties of food, and helps in the release of bioactive peptides by triggering the activities of both microbes and endogenous proteolytic enzymes. Bioactivity of several marine products like Thai fermented shrimp paste, shrimp by-products, squid miso, and other fermented fish products, have been reported to be prepared by this method [19]. Lopetcharat *et al.* [20] could able to trace the presence of soluble amino acids and peptides isolated from protein hydrolysates of muscle and visceral samples of fish. Likewise, Majumdar *et al.* [21] confirmed the presence of microbial and chemical properties in Shidal, a traditional fermented fish product of Northeast India. Shidal is rich in fatty acids like eicosapentaenoic, docosahexaenoic, arachidonic, linolenic, and linoleic acid [16].

5.4. Solvent Extraction

Peptide can also be isolated directly from fish tissues and from broth by precipitation of proteins of high molecular weight with organic solvents like ethanol, acetone, and other common organic solvents [16,22]. The process uses solvents to recover intrinsic bioactive peptides present in fish tissues, like antimicrobial peptides (AMPs), anticarcinogenic, and antihypertensive peptides. Since, this method generates very low amounts of peptides, hence, the use of solvent extraction method for fish protein isolation is limited only to only upstream research. However, after isolation and well-characterization, the peptide under study can be further synthesized in greater volumes for commercial purposes. The required volume of peptides can be synthesized at industrial scale with the use of modern biotechnological tools. Recently, Food and Drug Administration has approved a few bioactive peptides from marine sources which are now available in the market for consumer use and some peptides are in the process of acquiring validation through clinical trials [11].

6. PURIFICATION OF PEPTIDES

Peptides are very diverse groups of biomolecules, and this diversity has occurred due to the change in their amino acid sequences, molecular weight, charge, hydrophilicity, and hydrophobicity.

Multiplicity in the aforesaid properties of peptides bring difference in the pattern and type of bioactivities they exhibit. For purification of peptides, from tissue samples or from a mixture of proteins and peptides, it is necessary to know the physical and chemical properties of the peptide needed to be purified. Purification and isolation of peptides is generally done by the methods of ultrafiltration (UF), nanofiltration, and gel filtration (GF). However, all such methods need certain basic information about peptides to be isolated and purified. Information required take in molecular weight of the peptide to be isolated, functional groups of amino acids present in the peptides and charge of the peptides [23].

For instance, Ion Exchange Chromatography is employed to fractionate peptides based on their net charge. Some fractionations of peptides are carried out with Reversed-Phase High Performance Liquid Chromatography (RP-HPLC) which separates peptides on the basis of their hydrophobic and hydrophilic nature [24].

After purification of peptides, additional analysis of peptides are performed using Mass Spectroscopy (MS), Electrospray Ionization Mass (ESI), and Matrix-assisted Laser Deionization Time-of-Flight (MALDI–TOF). In an advance step, the purified and characterized fractions are sequenced for further use.

7. DIFFERENT FISH PARTS AS SOURCES OF BIOACTIVE PEPTIDES

Protein content in fish is high. Fish waste like frame, skin, viscera, head, bone, etc., thus, offer a rich source for biologically active peptides [25]. Fish fillets, cuttings, and backbones hold an enormous amount of protein with high nutritive value and a well-balanced amino acid composition [22]. Enzymatic hydrolysis of these materials often provides biologically active peptides with different physicochemical properties. The peptides have been reported to show antimicrobial, anti-oxidative, antihypertensive, anticoagulative, anticarcinogenic, immunomodulative, and other pharmacological and nutraceutical properties [10].

8. BIOLOGICAL ACTIVITIES

8.1. AMPs from Fish Waste

All living organism synthesize certain amounts of AMPs to defend themselves from foreign antigens. These defensive biomolecules participate in innate immune system of organisms. In unicellular animals, AMPs helps in destruction and killing of other organisms those share their biological niche in which they inhabit [26]. Majority AMPs are small cationic and amphipathic peptides. These peptides vary in length, structures, amino acid sequences, and molecular weights which generally remain in a range of <10 kDaltons (Da).

AMPs exhibit an extensive array of antimicrobial actively against both Gram-negative and Gram-positive bacteria. These molecules are also effective against fungi, viruses, and unicellular protozoans [27,28].

Presently, antibiotics have been used immensely for human usage and for cure and control of diseases of cattle, poultry, and aquatic animals [29]. However, overuse of antibiotics has led to the advent of antibiotics resistance against many pathogens and so when applied to cure and control a pathogen borne disease in human and animal, this thus becomes ineffective. Consequently, alternative molecules for the replacement of antibiotics in the treatment of human and animal diseases by other potent biomolecules is required. AMPs seems to be one of the most suitable and appropriate choice in replacement of antibiotics [30]. AMPs have been showing a broad range of antimicrobial activity along with immunomodulatory properties. However, it has an apparently low level of induced resistance [31].

Fish is an aquatic animal and hence lives in an environment which is overloaded with a number of saprophytic and pathogenic microbes. Skin is one of the prime barriers for entry of any pathogen into the body of an organism. It serves as the first line of defense system and is considered as one of the primary organs of immune system. Fish skin holds a large number of immunity factors like AMPs [32]. As mentioned above, AMPs are very

small peptides with a low molecular weight. These peptides are amphiphilic, in nature that provides innate immunity to Pisces.

AMPs found in fish belong to different families that include piscidin, defensin, hepcidin, cathelicidin, and histone-based peptide families [16]. These AMP families are species-specific, with piscidin being limited to Teleost. They show broad range antimicrobial activities against pathogenic bacteria, yeasts, molds, parasites, and viruses. Interestingly enough, AMPs can also demonstrate both pharmacological and anti-tumor activities [30].

The first natural AMP extracted from fish was paradaxin [33]. Later on, paradaxin was commercially synthesized. Since then, several AMPs have been extracted from various fish species under different environments. AMPs isolated from black-barred halfbeak gelatin and its hydrolysates showed antibacterial activities against three Gram-negative bacterial namely, *Klebsiella pneumonia*, *Salmonella enterica*, *and Salmonella typhi* and against three Gram-positive bacterial viz., *Micrococcus luteus*, *Staphylococcus aureus*, and *Bacillus cereus* [34].

Likewise, 2 glyceraldehyde- 3-phosphate dehydrogenase -related AMPs were isolated from the skin of Yellowfin Tuna and Skipjack Tuna. Yellow fin glyceraldehyde phosphate dehydrogenase (YFGAP) and Skipjack glyceraldehyde phosphate dehydrogenase (SJGAP), showed their antimicrobial properties against both Gram-positive and Gram-negative bacteria. YFGAP and SJGAP were also found effective against *Aeromona shydrophila*, *Streptococcus iniae*, and *Vibrio parahaemolyticus* [35,36].

Apart from exhibiting anti-bactericidal properties, these AMPs have also been found to be effective against viruses, fungi etc. For instance, TH15 peptide isolated from *Oreochromis mossambicus* (Tilapia), and epinecidin from Orange Spotted Grouper, exhibited, antiviral activity against nerve necrosis virus infection [16,37]. TH15 was also found effective against pancreatic necrosis when tested in CHSE214 embryonic cells of Salmon. Pancreatic necrosis infection is one of the most challenging infections caused in Salmon and accounts for significant financial losses during its cultivation [16,38].

Similarly, certain groups of AMPs isolated from fish species also display antifungal activities. For example, piscidin 2, secreted by Hybrid Striped Bass, found to rupture the plasma membranes of fungi [39]. In the same way, Epinecidin isolated from Rockfish found to be effective against *Candida albicans*, a human fungal pathogen [40].

Research findings of an *in vitro* experiment on HT1080 fibrosarcoma cells suggested potential anticarcinogenic and antitumorigenic properties of AMPs isolated from different fish species. These properties of fish AMPs were further confirmed by additional experimental findings. The application of Epinecidin and TH23 peptides isolated from fish helped in disintegration of cancer cells. These peptides also delayed the rate of cell proliferation along with a decline in cell motility [41,42]. In addition, at certain concentrations, TH23 protein did not produce any cytotoxic effects on normal human cells [41]. Correspondingly, it was observed that the protein hydrolysates of Atlantic Cod, *Gadus morhua*, marine Flounder *Pleuronectes platessa*, and Salmon, *Salmon salar* obtained from hydrolysis of respective fillets with alcalase enzyme exhibited significant *in vitro* antiproliferative activity in two human breast cancer cell lines [10,16].

8.2. Antioxidant Peptides from Fish Waste

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) gets generated from metabolic pathways involving oxygen and nitrogen. ROS and RNS fetch cellular damage and produce deleterious effect on organisms. ROS like superoxide anion radials (O_2^-), hydroxyl radical (OH^-), and non-free radical species like hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$) get generated during the process of cellular respiration and other endogenous metabolic pathway of a living organism. These ROS if present in bulk quantities create oxidative stress in cell and damage several other vital biomolecules like proteins, lipids, and DNA. These ROS start bond making and bond breaking processes with protein, lipid, DNA, RNA and other biomolecules through electron donation and acceptance mechanism and create breakage in the backbone of biomolecules especially of proteins and DNA molecules [43].

In human beings, neurodegenerative, hypertension, inflammation, diabetes, and cancer are caused due to interference of ROS and RNS [44,45]. ROS also cause harmful effects in food. Free radicals oxidize fats, oils, and proteins in food during their processing and storage. This creates detrimental effects on the nutritional quality of food. Notwithstanding, to counter the damage caused due to such reactive species (ROS and RNS) efficient antioxidant systems are present in every organism. Every organism bears a set of antioxidant enzymes to negate reactive species and free radicals. In many organisms' small peptides also display antioxidant properties. These peptides are regarded as more potent free radical scavengers than the free amino acids as at the end they generate a stable resultant peptide radical [46]. Although the exact relationship between structure and antioxidant properties of peptides are not known or yet to be established, still it is believed that amino acids which constitute these peptides play crucial role in determining the scavenging and biotransformation properties of such peptides. The type of amino acids, their placement in peptides, hydrophobicity nature of these amino acids and further of the whole peptide are the key factors in ascribing antioxidant properties of these peptides.

Generally, the most reactive amino acids are those that contain nucleophilic sulfur-containing side chains, like taurine, cysteine, and methionine or aromatic side chains like tryptophan, tyrosine, and phenylalanine [38]. Peptides those show antioxidant activities to offset reactive species also encompass metal chelating activities to work against excess free radicals. This mechanism involves ion chelation through pre-oxidative transition metals like Iron (Fe^{2+}), Copper (Cu^{2+}), and Lead (Pb^{2+}). These small peptides along with antioxidant activities, metal chelating activities also demonstrate anti-inflammatory, neuroprotective, and anti-allergy activities [47].

Multiple species of fishes have been reported to contain various kinds of antioxidant compounds. Compounds that are present in fish include amino acids, antioxidant enzymes and peptides, ascorbic acid, carotenoids, as well as phenolic compounds.

Antioxidant peptides purified from fish typically contain 2–16 amino acid residues [48]. The antioxidant efficacy of any protein is determined mainly by their amino acid composition, sequence, location of amino acids in the peptides, and their hydrophobicity. Studies reveal that peptides with higher antioxidant activities contain greater amounts of hydrophobic amino acids. These peptides are rich in histidine, proline, methionine, cysteine, tyrosine, tryptophan, and phenylalanine [49].

Recently, a peptide fraction with a molecular weight of approximately 10 kDa isolated from fish protein hydrolysate of Cod skeletons, confirmed to have (unlike fractions with molecular weights of 3, 5, and 30 kDa) high antioxidative activity [50]. Antioxidant activity was also found in the protein hydrolysates of Yellowfin sole, (*Limanda aspera)* extracted from its skeletons [51].

Antioxidative peptides have been isolated from different parts of fish (head, frames, bones, scales, viscera, etc.), and from different fish species employing enzymatic hydrolysis. In an experiment, the antioxidative properties of hydrolysates of Yellow stripe trevally, *Selaroides leptolepis*, were found to be dependent on the degree of hydrolysis of fractions and the type of the enzyme used [52]. A peptide fraction of 1.77 kDa molecular weight obtained employing enzymatic hydrolysis using Flavourzyme demonstrated an elevated amount of antioxidant effects. Similar antioxidant properties were exhibited by fish peptides fraction of 2.44 kDa, obtained using Alcalase enzyme.

Furthermore, antioxidative protein hydrolysates obtained from fish can be applied on foods, pharmaceuticals, cosmetics, and nutritional supplements. They may be used in place of synthetic antioxidants, such as butylated hydroxytoluene, butylated hydroxyanisole, tertbutyl hydroquinone, and propyl gallate in manufacture of different products [44].

Metals catalyzing lipid peroxidation reactions are among the major food spoilage agents [53]. Research studies explain that proteins and peptides help in prevention of food spoilage by obstructing the contact between lipids and metal ions in a steric manner or by forming a chelate with a metal ion.

FPH have shown exciting results in prevention of food spoilage caused due to lipid peroxidation. These peptides are found to be helpful in extending the shelf-life of foods, particularly in products containing haemoglobin or free iron that causes the rancidification of fats [54]. For instance, FPH from capelin helped in reduction of lipid oxidation by up to 60.4% when added to ground pork [55]. Similarly, Wu *et al.* [56], from their studies revealed that transitional peptides were able to enhance the lag time before oxidation as soon as they were added to a linoleic acid emulsion peroxidation system in comparison to small peptides with low molecular weight. In another study Jeon *et al.* [50], found that FPH fractions with different molecular weight showed diverse antioxidative properties. A study by Kristinsson *et al.* [57] on FPH isolated from muscle protein of catfish showed high-quality metal chelating ability. The metal chelating ability got increased with an increase of degree of hydrolysis. Similarly, FPH isolated from mackerel showed reduction in haemoglobin-mediated lipid oxidation in a linoleic acid system [58].

8.3. Antihypertensive Peptides from Fish Waste

Hypertension [or high blood pressure (BP)] is one the most prevalent risk factor of cardiovascular disease (CVD). Data on hypertension reveal that about 54% of strokes and 47% of heart diseases occurs due to hypertension. It has been estimated that every year approximately 17.9 million people all across the world perish due to CVD and this disease along with other cardiac diseases accounts for 45% of all deaths reported in European countries [59,60].

High BP can be regulated through synthetic angiotensin-converting enzyme (ACE) inhibitors molecules like captopril. It can also be treated with endothelial-dependent vasodilatation by up regulating the expression of nitric oxide (NO), phosphorylating endothelial nitric oxide synthase, and down regulating expression of endothelin expression. Relaxation of endothelial smooth muscle cell is carried out by the NO/cyclic guanosine monophosphate (cGMP)-mediated intracellular signaling pathway. In this pathway, there is an up regulation of cGMP-dependent protein kinase I. This then leads to decline in calcium levels through down regulation of the inositol-1, 4, 5-trisphosphate (IP-3) receptor.

Antihypertensive molecules are believed to act as inhibitors of ACEs. These antihypertensive molecules accomplish their action through Renin Angiotensin Aldosterone system. It has been revealed from structural studies of these peptides that antihypertensive peptide activity is determined by the C-terminal tripeptide residues. The tripeptide residues of antihypertensive molecule carry out competitive binding with the active site of ACE. It has been deduced that ACE prefers substrates that contain hydrophobic (aromatic or branched side chains) amino acid residues present at the three C-terminal positions [61].

Regular synthetic antihypertensive drugs bring about many undesirable side effects in human. Hence, the research for getting more natural sources of antihypertensive drugs is in progress.

Antihypertensive peptides from fish sources were first reported in Sardines. Antihypertensive effects of sardine fish peptides were confirmed through *in vitro* and *in vivo* experiments on spontaneously hypertensive rat (SHR) rat cell line [16]. In another study, antihypertensive peptides purified from protein hydrolysates of skeletal discard of Walleye Pollock, showed significant results in lowering down ACEs [16].

ACE inhibitory peptides have also been identified and isolated from several fish species that include Tuna, Bonito, Salmon fish and different Shell-fishes. In an experiment, it was found that purified catfish protein on digestion with the commercial enzymes Protamex, ACE inhibitory peptides got released [16]. Garcia-Mora *et al.* [58] reported the release of ACE inhibitory peptides from Mediterranean fish discard using enzymes subtilisin. The peptide Valine-Alanine-Methionine-Proline-Phenylalanine, purified from small-spotted cat shark hydrolysates, is one of the most promising peptides because of its low IC_{50} value which is found to be 0.44 μM (micromolar) analyzed through quantitative structure–activity relationship models model. In an animal study, Fujita and Yoshikawa [62] showed that thermolysin digest of Bonito released a Leu-Lys-Pro-N-Met peptide. Additionally, this peptide showed an ACE inhibition and reduced BP in a dose-dependent manner.

In another experiment, it had been observed that following an oral administration of thermolysin hydrolysates isolated from Chum Salmon muscle, the systolic BP (SBP) of SHRs reduced significantly with respect to control rats. The highest reduction in SBP took place soon after 4 hours of peptide ingestion implying that short chain peptides were responsible for lowering down of BP. Reduction in BP after a time period of 6–8 hours of oral administration is often considered to be the effect of peptide with large chain length as a large peptides chains get converted from the substrate into true ACE inhibitors and this process in *in vivo* consume time for conversion of long chains into short chain and to work further [63]. Additional observation was made that the SBP of rats of all test groups came down to their initial level after 24 hours of peptide intake which suggest that the hydrolysates of Chum Salmon are capable of lowering the BP temporarily which makes them temporary hypotensors.

Likewise, fish hydrolysates obtained through enzyme hydrolysis of fillet of Pacific hake, *Merluccius* showed an inhibitory activity against ACE in *in vitro* experiment at an IC_{50} of 165 µg/ml. Correspondingly, protein hydrolysates from the skeleton and head discard of Cod and *Limanda aspera* demonstrated a reduction in ACE activity [64]. Protein hydrolysates from fillets of catfish with alkaline hydrolysis showed various fractions of hydrolysates with different degree of hydrolysis [65]. All fractions confirmed of holding considerable amount of antioxidant properties, as well as ACE inhibiting activity. Experiments with FPH also showed existence of both anticoagulative and antithrombotic effects in laboratory conditions suggesting that the fish peptides has a role in activating blood coagulation pathway [66].

In a study by Cheung and Li-Chan [67], steelhead rainbow fish hydrolysates were obtained using enzymatic hydrolysis. A peptide of <3 kDa was purified by UF from crude FPH. This fraction when examined for the presence of ACE inhibitory activity, showed more ACE inhibitory activity in comparison to the crude FPH. Similar results were obtained with fish skin peptide. Bioactivity shown by the fractions obtained from crude FPH were found to be small peptides with very low molecular weights suggesting that small peptides have more bioactive potency than the whole and crude hydrolysates [68].

In another study, Thuanthong *et al.* [69], found five ACE inhibitory peptides fractions namely, GIV, GAP*GF, GFP*GPA, SGNIGFP*GPK, GIPGPIGPP*GPR from Nile tilapia skin gelatin with an IC_{50} concentration value of the bioactive peptides molecules ranging between 760 and 1,490 µM. Likewise, Ngo *et al.* [70] obtained ACE inhibitory peptides from Pacific cod skin gelatin after an enzymatic digestion with pepsin. The peptides identified were with a sequence of amino acid (GASSGMPG and LAYA) and with IC_{50} values of 6.9 and 14.5 µM respectively. The molecular weight of both the peptides were found to be <1 kDa which envisage that these peptides could able to cross the intestinal barrier and exhibit bioactivities.

8.4. Anticancer Peptides from Fish Waste

Cancer is a leading global disease. Estimate suggest that the cancer cases will upsurge from 14 (2012) to 22 million cases in next 20 years [71,72]. In cancer, the cells grow and proliferate abnormally and spread to neighboring cells and tissues. Hence, drugs are designed to inhibit the abnormal growth of cells and further to deregulate cell proliferation [73]. Presently, radiotherapy and chemotherapy are two most promising therapy for cancer treatment [74]. Arguably, these two processes are painful, costly and exhibit much side effects. Therefore, cancer biologists and physicians all across the globe are in search of alternate processes and drugs for cancer treatment.

In recent developments in cancer therapy, scientists are using protein hydrolysates and peptides from different food sources of both animal and plant origin. Research findings show that bioactive compounds in form of soy isoflavones, lycopene, resveratrol, omega-3-fatty acids, pomegranate, curcumin, vitamin E, selenium from different sources such as milk, egg, fish, crabs, shrimp, sea cucumber, oyster, mussel, chlorella (algae), spirulina, rice, corn, common bean, chickpea, and rape seed are effective against cancer [75–86]. Generally, enzymatic hydrolysis, fermentations with bacterial strains are in use for isolation of anticancer protein hydrolysates or peptides from aforementioned sources [87–89]. For enzymatic hydrolysis, a lot of commercial proteases like pepsin, alcalase, trypsin, pancreatin, protamex, neutrase, chymotrypsin, pronase, papain have been employed [90]. However, among all enzymes, pepsin is the enzyme of choice for enzymatic hydrolysis because pepsin has the ability to generate peptides with anticancer activities. This is because when a tissue sample is hydrolyzed with pepsin, it hydrolyses peptide bonds containing hydrophobic amino acids specifically aromatic amino acids in the substrate molecules. These hydrophobic peptides usually remain hidden in the core of the parent proteins, but hold the capacity to inhibit the growth of cancer cells. Thus, when these hydrophobic peptides are exposed after getting hydrolyzed with pepsin, they activate the apoptotic pathway by activating the molecules of apoptosis process and also sometimes by activating the cell cycle inhibitors and checkpoints molecules [91].

Although isolation of anticancer peptides from different parts of fish is a recent research idea still a lot of work has been carried out from past one decade. Hsu *et al.* [92] isolated two peptides of 12.06 and 11.24 kDa by using papain and protease enzymes from the muscle tissue of Tuna fish (*Thunnus tonggol*) and effectively showed their anticancer activities in Human breast cancer (MCE-7) cell line. Both the peptides showed dose dependent inhibition on MCF-7 cells. Similarly, Kannan *et al.* [77] reported the presence of anticancer activities in the peptides isolated from Shrimp shell hydrolysates. They used cryotin and pepsin enzymes for digesting shrimp shells. The purified shrimp peptides showed antiproliferative activity (one of the anticancer properties) against both human colon cancer (caco-2) and human liver cancer (HepG2) cell lines. In the same year, You *et al.* [93] purified peptides ranging between <3 and >10 kDa from muscle tissues of *Misgurnus anguillicaudatus* (Loach) and tested them against HepG2 (liver), MCF-7 (breast), and Caco-2 (Colon) cancer cell lines. They reported that these peptides significantly lowered down the rate of proliferation of cancer cells. Antiproliferative activities were also found in the peptides isolated from Tuna fish on MCF-7 [94]. The investigators assumed that lowering of proliferative rate

in cancer cells caused due to the activation of S-phase cell cycle inhibitor molecules which helped the cells to remain arrested in the S-Phase of cell cycle.

Likewise, Song *et al.* [95], Chalamaiah *et al.* [87], and Yang *et al.* [96] isolated peptides from muscle tissue of Half-fin anchovy (*Setipinna taty*) of 670.35 kDa, roe of Rohu (*Labeo rohita*) of <10 kDa and eggs of Giant Grouper (*Epinephelus lanceolatus*) of <5 kDa respectively. The extracted and purified peptides from the aforementioned fishes showed anticancer properties against human prostate cancer cells (PC-3), human colon cancer cells (Caco-2) and two oral cancer cell lines (Ca9-22 and CAL-27). The peptides (isolated from eggs of Giant Grouper) activated programmed cell death in Ca9-22 oral cancer cell line.

Later in the year 2016, Sae-leaw *et al.* [97] and Pan *et al.* [89] peptides from skin of Barramundi and cartilage of Skate fish digesting with Alcalase enzyme. Peptides isolated from skin of Barramundi showed antiproliferative activities against human colon and liver cancer cell lines. Similarly, peptides isolated from cartilage of Skate fish exhibited antiproliferative activities against HeLa cell lines.

Recently, Ting and Chen [98], Sruthy *et al.* [99], Chee *et al.* [100], and Lugo *et al.* [101] reported the presence of anticancer activities of peptides, purified from, Nile Tilapia, Indian white shrimp, Orange spotted grouper, North African catfish on different cancer cells respectively (Table 1). Reports on isolation of peptides with anticancer properties from several other fish is continuing.

8.5. Amino Acids

Amino acids are one of the most chief biomolecules present in every living organism. They are nutritionally important, and hence are critical in nutraceutical and pharmaceutical industries. In nutraceutical industries they are used as food additives for human and animal feed. They are employed in pharmaceutical industries as antioxidant supplements.

Fish hydrolysate is biochemically a mixture of an array of amino acid, oligopeptides, nucleosides with their respective bases, short chain organic acid molecules, aldehydes, esters vitamins, and minerals [48]. FPH contain nearly 75%–80% protein constituents. Out of this, 60% are peptides whereas 40% constitute amino acids. The major amino acids found in FPH are glutamine ranging between 14% and 16% of total hydrolysates, asparagine and lysine at about 10% each. The combined input of branched chain amino acids (Lysine, Leucine, and Isoleucine) is about 23%.

Presently, head and frame discard of fish from fishery industries are treated with various enzymes to obtain FPH. These FPH are further used in various nutraceutical and pharmaceutical industries for manufacturing food supplements for human consumption and animal feed and for antioxidant supplements and for inhibition of tumor necrosis factor - α (TNFα) [107–109].

Monosodium glutamate, alanine, aspartate, and arginine are some of the important amino acids employed in food industry to give aroma and taste to food items. Likewise, lysine, methionine, threonine, and tryptophan have their use in animal feed industry to enrich aquaculture feed and livestock fodder. Amino acids like arginine, glycine, glutamate, and histidine, are used as filler in protein drugs [7,108]. Taurine, a natural amino acid, is also found in higher concentration in fish and has proved to inhibit synthesis of TNFα, the pro-inflammatory interleukin-6, interleukin 1β, and NO synthase inducible nitric oxide synthase [48,109].

8.6. Collagen and Gelatin

Fish skin and bones are extraordinary sources of collagen and gelatin. Collagen and gelatin isolated from fish can serve as an alternative to mammalian collagen protein in food and pharmaceutical industries [110].

Collagen extracted from fish skin, scales, and bones are extensively exploited to be utilized as scaffolds and transporters. These proteins (collagen and gelatin) show biocompatibility and are by far recyclable and exhibit low antigenicity, as a result of which cells easily grow on them [111].

Similarly, gelatin has an extensive usage in food and pharmaceutical industry. It plays a key role in the improvement of the structure and durability of sweets. In food industries it is generally used for making toppings, soups, dairy products and fruit juices whereas in pharmaceutical industries, gelatin is used in making of capsules, ointment, emulsion, creams, cosmetics and pill coating [10,112]. It is also utilized in making of vitamins and nutritional supplements [10].

Fish gelatin indorse excellent film-forming properties, and hence often used to produce transparent, water-soluble, and tough films [10,112]. Gelatin isolated from cold-water fish has a low gel melting temperature and therefore can be used in iced up food stuffs or for cold storage items that are to be consumed as soon as they are brought out from cold conditions. Additionally, this low melting gelatin can be used in products which need to get dissolved at a faster rate for instance, in microencapsulated nutritional supplements like vitamins, enzymes, and cosmetic product and other pharmaceutical stuff like colorants, drug additives, etc. [110].

Gelatin also plays an inhibitory role in lipid peroxidation. It is believed that the lipid peroxidation is brought by hydrophobic amino acids like glycine, valine, alanine, proline and hydroxyproline found in gelatin [25,38].

Fish gelatin also shows antioxidant properties. In a study in Nile tilapia (*Oreochromis. niloticus*), it was found that gelatin obtained from fish scale protein by enzymatic hydrolysis (using alcalase, pronase E, trypsin, and pepsin) proved to be effective in providing protection to DNA from getting damaged due to oxidative stress. The DNA damage was reported to be blocked by about 70% [45].

8.7. Enzyme

Fish visceral parts are rich source of different enzymes which include pepsin, trypsin, chymotrypsin, and collagenase. These enzymes show excellent catalytic properties, strong efficiency at lower temperatures, reduced sensitivity to substrate concentration and higher stability to a wide range of pH [25,113]. A lot of endogenous enzymes have also been isolated, purified and characterized and are further used to carry out protein hydrolysis [63].

Table 1: Table shows some of anticancer peptides isolated from different fish, with their process of isolation, method of characterization and type of anticancer activity shown against different types of cancers cells.

SI no.	Source/scientific name	Common name	Fish part used for isolation of peptides	Enzyme used for enzymatic hydrolysis	Purification method	Characterization	Molecular mass	Activity	References
1.	*Thunnus tonggol*	Tuna	Dark muscle	Papain and protease XXIII	UF	MALDITOF-MS	12.06 kDa and 11.24 kDa	Anti-cancer activity against MCF-7 (human breast cancer) cell line	[92]
2.	*Pleuroncoides planipes, Peneaus setiferus*	Shrimp	Shrimp shell	Cryotin and pepsin	UF	NM	<10 and 10–30 kDa	Antiproliferative Activity against Caco -2 and HepG2 (human colon cancer and liver cancer) cell lines	[102]
3.	*Misgurnus anguillicaudatus*	Loach	Muscle	Papain	UF	NM	>10 kDa, 5–10 kDa, 3–5 kDa, <3 kDa	Antiproliferative activity against MCF-7 and Caco-2 (human breast cancer and colon cancer) cell lines	[93]
4.	*Nemipterus japonicus*	Japanese threadfin bream	Backbone	Trypsin	FPLC and GFC	NM	NM	Cytotoxic activity against HepG2 (human liver cancer) cell line	[103]
5.	*Thunnus tonggol*	Tuna	Cooking juice	Protease XXIII	GFC	Q-TOF	24.49292 KDa and 25.62405 KDa	Antiproliferative activity against MCF-7 (Human Breast Cancer) cell line and induction of S-phase cell cycle inhibitors to arrest cell cycle in S-phase	[94]
6.	*Setipinna taty*	Half-fin anchovy	Sauce	Pepsin	SGC, TLC, RP-HPLC	MALDI-MS	NM	Induction of apoptosis against U937 cells	[95]
7.	*Setipinna taty*	Half-fin anchovy	Muscle	Pepsin	GFC RP-HPLC	NM	670.35 Da	Antiproliferative activity against PC-3 (human prostate cancer) cell line	[95]
8.	*Setipinna taty*	Half-fin anchovy	Muscle	Pepsin	GPC	NM	5,000 Da	Antiproliferative activity against DU145 (human prostate cancer) cell line and human esophagus cell line (109)	[95]
9.	*Labeo rohita*	Rohu	Roe (egg)	Pepsin	NM	NM	<10 kDa	Antiproliferative activity against Caco-2 (human colon cancer)	[87]

Continued

SI no.	Source/scientific name	Common name	Fish part used for isolation of peptides	Enzyme used for enzymatic hydrolysis	Purification method	Characterization	Molecular mass	Activity	References
10.	*Epinephelus lanceolatus*	Giant Grouper	Roe	Protease N	UF	Pico-Tag MTS	<5 kDa	Reduction in oral cancer cell viability Ca 9-22 and CAL 27 cells (oral cancer) cell lines Induction of programmed cell death in oral cancer cell (Ca9-22)	[96]
11.	*Lates calcarifer*	*Barramundi*	Skin	Alcalase	RP-HPLC	NM	Up to 7 kDa	Antiproliferative activity in Caco-2 and HepG2 (human colon cancer and liver cancer) cell lines	[97]
12.	*Okamejei kenojei*	Skate	Cartilage	Alcalase and trypsin	UF, RPHPLC	Q-TOF MS, ESI	726.9 Da	Antiproliferative activity against HeLa cell lines	[89]
13.	*Epinephelus coioides*	Orange-spotted grouper	NM	NM	NM	NM	2,985.63 Da	Antiproliferative activity against U937 (human leukeamia) cell line	[100]
14.	*Clarias gariepinus*	North African catfish	NM	NM	NM	NM	NM	Antiproliferative activity against H460 (human lung cancer) cell line	[101]
15.	Epinecidin-1 from *Epinephelus coioides*	Grouper	Gills and intestine	NM	NM	SPM using Fmoc Chemistry	2.336 kDa	Growth inhibition of human epithelial carcinoma cell line, HeLa cell line and fibro sarcoma cancer cell line (HT-1080)	[104]
16.	Epinecidin-8 from *Epinephelus coioides*	Grouper	NM	NM	NM	SPM using Fmoc Chemistry	1.872 kDa	Growth inhibition of human epithelial carcinoma cell line, HeLa cell line and fibro sarcoma cancer cell line (HT-1080)	[104]
17.	Pardaxin-1 from *Pardachirus marmoratus*	Garden sole	NM	NM	NM	SPM using Fmoc Chemistry	3.324 kDa	Growth inhibition of human epithelial carcinoma cell line, HeLa cell line and fibro sarcoma cancer cell line (HT-1080)	[104]

Continued

SI no.	Source/scientific name	Common name	Fish part used for isolation of peptides	Enzyme used for enzymatic hydrolysis	Purification method	Characterization	Molecular mass	Activity	References
18.	Pardaxin-6 from *Pardachirus marmoratus*	Garden sole	NM	NM	NM	SPM using Fmoc Chemistry	2.778 kDa	Growth inhibition of human epithelial carcinoma cell line, HeLa cell line and fibro sarcoma cancer cell line (HT-1080)	[104]
19.	Hepcidin TH2-3*Oreochromis mossambicus*-	Tilapia	NM	NM	NM	NM	2.302 kDa	Anti-tumour activity against human fibrosarcoma cell line	[105,106]
20.	*Oreochromis niloticus*	Nile tilapia	NM	NM	RP-HPLC	MS	2.982 kDa	Cell death in NSCLC cell line through necrosis in combination with Epidermal growth factor receptor kinase inhibitors against lung cancer cells	[98]
21.	*Penaeus indicus*	Indian White shrimp	NM	NM	NM	NM	2.435 kDa	Anticancer activity against H460 and HEP-2 (human lung and liver cancer) cell lines	[99]

NM, not mentioned; UF, ultra filtration; MS, mass spectroscopy; MALDITOF-MS, matrix-assisted laser desorption/ionization-time of flight mass spectrometry; FPLC, fast protein liquid chromatography; GFC, gel filtration chromatography; SGC, sensor gas chromatography; TLC, thin layer chromatography; SPM, solid phase methods; Fmoc, 9-fluorenylmethoxycarbony; MTS, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium; Q-TOF MS, quadrupole time-of-flight mass spectrometry; ESI, electrospray ionization.

In several industries, especially food, substrate catalysis is carried out with enzymatic degradation as enzymes are very specific, robust, and helpful in preservation of raw materials, energy, chemicals, water in comparison to other traditional methods [114]. Recently, isolation and characterization of fish proteolytic enzymes and their employment in food industries are garnering high attention. Proteases found in the alimentary canal of fish are same as found in mammals. Among them pepsin, trypsin, chymotrypsin, collagenase, and elastase are of prime importance.

Fish belonging to cold habitat contain enzymes which get activated even at low temperatures. However, their thermal stability is comparatively low [115]. Since food industries require enzyme that can remain active even at low temperature and retention of thermal stability is desirable during food processing; enzymes isolated from fish residing in cold habitat play a great role in such process as these enzymes can be inactivated even at low temperature [116].

Additionally, enzymes isolated from fish can be employed in other fields. Instances include utilization in removal of fish bones and scales, Roe cleaning, traditional fish sauce production, and in manufacturing of several biosensors for quality control [64].

Extraction, isolation, and characterization of different enzymes from different fish species has been done by various investigators. Enzymes like trypsin, pepsin, and other proteases have been successfully obtained from fish tissues and employed for other research works [10,65,66]. However, the processes adapted for isolation and characterization of enzymes are not common. For instance, Gildberg [117] isolated pepsin from Cod stomach through autolysis and Reece [118] extracted enzyme protease from Mackerel and Cod waste by applying UF and ion—exchanged chromatography. Pepsin was obtained @ 9 g/l and protease were isolated @ 1 g/kg of fish waste respectively. Nonetheless, application of enzymes from fish waste is restricted to seasonal availability of fish waste and on nutritional and health status of fish [10,66]. Also to add, the extraction of enzymes from any natural sources is very costly and hence restricts its employment in industries as industries need a large amount of enzymes [10]. Conversely, more cost effective methods of enzyme extraction from fish waste must be tried out on a large scale to utilize fish waste in an effective way [65].

9. CONCLUSION

In the present review, we have discussed widely about different types of bioactive peptides isolated successfully from fish waste and underutilized fish. The bioactive peptides isolated and characterized from different fish sources have been successfully employed as antimicrobial, antioxidant, antihypertensive, and anticarcinogenic agents. Besides this, discussion was carried out on the scientific procurement of important amino acids, and enzymes from different fish parts with their commercial and research utilization. Furthermore, discussions have been made on utilization of collagen and gelatin isolated from fish bone and skin waste. Again, thoughts were also made on the isolation and employment of enzymes from fish waste for different purposes. Nevertheless, more research is needed for determination several other important bioactive properties like neuroprotective, anti-inflammatory, immunomodularoy etc., and to find out effective methods to scale up the production process, amicability with food nutrient molecules, to remain stable in alimentary canal, all time availability and safety all along with time.

10. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

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