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REVIEW

Valorization of Fish Processing by-Products: Biological and Functional Properties of Bioactive Peptides

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Abstract

Purpose of Review This review explores the potential of fish by-products as a source of bioactive peptides for the food, pharmaceutical, and cosmetic industries. Focusing on their diverse bioactive and functional properties, it offers insights into their industrial utilization, contributing to a better understanding of their applications.

Recent Findings Fish processing by-products, including wastewater and solid residues, serve as valuable sources of bioactive peptides exhibiting a remarkable range of biological activities, such as antioxidant, antimicrobial, and antihypertensive properties. These peptides exhibit a wide range of functional properties, such as solubility, water holding, fat binding, foaming, and emulsifying capacities. Moreover, they show significant potential for various industrial applications.

Summary Bioactive peptides derived from fish by-products are attracting interest in multiple industries due to their diverse biological activities and functional properties. These peptides have emerged as a valuable and largely untapped resource, as they can be extracted from underutilized, or waste materials generated during fish processing operations.

Keywords Fish processing by-products · Wastewater · Solid residues · Bioactive peptides · Bioactive properties · Functional properties

Introduction

Over the past two decades, there has been an increasing focus on marine food resources, driven by global population growth and the demand for high-quality protein [1]. Global fish production reached 179 million tonnes (MT) in 2018 and is projected to reach 194 MT by 2026 [2]. Processing procedures like canning, smoking, filleting, curing, and salting are applied to over 70% of captured fish, resulting in significant by-product generation. Fish by-products, such as heads, bones, skin, viscera, and other organs and tissues, account for 30 to 70% of the total fish weight, varying by species [2]. Moreover, during various processing operations such as washing, thawing, cooking, and fishmeal production, different wastewater is generated [3]. Proteins from marine sources provide essential nutrients for growth and development, and there is growing interest in exploring the bioactivities and functionalities of bioactive peptides embedded within these proteins [4]. Bioactive peptides, initially inactive within proteins, become active through processes like digestion, hydrolysis, and fermentation. These processes break down proteins into smaller peptide fragments, activating their functionality [5]. These peptides have diverse bioactivities, including anti-inflammatory [6], anticancer [7], antioxidant, antihypertensive [8], and antimicrobial effects [9]. They also have the potential to promote muscle regeneration, enhance cell growth [10], improve insulin sensitivity [11], and reduce the risks of cardiovascular diseases, metabolic syndrome [12], as well as type 2 diabetes [8]. Peptide bioactivities are influenced by structure, composition, and amino acid (AA)

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sequence [13]. Fish proteins possess functional properties like solubility, water holding capacity (WHC) [14], fat binding capacity (FBC), foaming, and emulsifying properties [15], making them intrigued as hydrocolloids. The immense promise of bioactive peptides derived from fish processing by-products has garnered significant attention across multiple industries, including food, pharmaceutical, cosmetic, and nutraceutical, as they offer a sustainable and innovative approach to developing novel, high-value products that can positively impact human health and well-being. This review brings together recent research on bioactive peptides derived from fish processing by-products, providing an exploration of their diverse bioactive and functional properties. It serves as an invaluable resource by summarizing data and providing insightful analysis of the latest advancements in the field of bioactive peptides derived from fish processing by-products. While previous reviews have covered the biological activities of fish-derived peptides, the unique aspect of this review is its comprehensive analysis of both the bioactive and functional properties of these peptides, providing a holistic understanding of their potential applications and value.

Exploring Fish Processing by-Products as an Alternative Source for Bioactive Peptides

Proteins serve a variety of essential roles, from providing structural and physiological support to promoting overall health and physical condition [16]. These proteins can be a rich source of bioactive peptides—peptide fragments derived from proteins that offer beneficial impacts on bodily functions and the well-being of living organisms. These functional peptide fragments typically contain 2 to 20 amino acid residues and are encrypted within the parent protein sequence. Through protein hydrolysis reactions, these peptide sequences are then released and become active [17]. However, the specific function of these bioactive peptides can vary depending on the source protein, the unique amino acid sequence that composes the peptide, and even the molecular weight of the peptide fragment. The amino acid composition of the parent protein is a critical factor in determining the properties and potential bioactivities of the derived bioactive peptides. The types, sequence, and proportions of amino acids within the protein can influence the physicochemical characteristics, structural features, and biological activities of the resulting peptides. For example, the presence of hydrophobic amino acids, such as leucine, isoleucine, and valine, can contribute to the peptide's ability to interact with cell membranes and receptors, potentially enhancing their absorption and bioavailability [18].

Recently, the volume of fish processing by-products has been rising rapidly, posing significant challenges in terms of proper management and disposal. These by-products, which account for approximately 45% of total fish production, often end up being improperly discarded, leading to economic losses and environmental degradation [19, 20]. Fishery effluents containing these by-products are typically rich in pollutants like salts, organic molecules, and high levels of biodegradable substances such as proteins and lipids, resulting in a high chemical oxygen demand (COD) [21]. However, this growing abundance of fish by-products also presents an opportunity to extract valuable bioactive proteins and peptides. The trend towards developing bio-functional ingredients from these underutilized resources has become a new focus, driven by the need to maximize the value of fish processing waste and mitigate the environmental challenges associated with their improper disposal. Implementing suitable wastewater treatment processes offers the potential to recover these valuable compounds, create value-added products, and provide both economic and environmental benefits [21, 22].

Solid by-Products from Fish Processing

Fish preparation involves multiple steps, including receiving, bleeding, gutting, de-heading, filleting, trimming, and slicing [23]. Additional steps may include descaling and skinning. Classification based on species and sizes is often necessary [24]. Bleeding fish minimizes the impact of blood on shelf life [25]. Chilling after bleeding reduces bacterial and enzymatic activity [26]. Gutting and further processing steps depend on the desired final product. Pre-processing steps are typically done at the catching site to enhance durability and facilitate transportation to processing units [27].

Fishery pre-processing yields various solid by-products, including the head (15–20%), trimming (2–5%), skin (1–5%), liver and gut (15–20%), bones (10–34%), and scales (2–4%) by weight [28–30]. During the filleting process, approximately 30–50% of the fish meat is typically left unused. This remaining part, accounting for over 45% of the whole fish body, consists of approximately 4–5% skin, 21–25% head, and 24–34% bones and is largely discarded [29].

Wastewater from Fish Processing

Fish processing wastewater is primarily generated during various operations in fish processing plants, including freezing, cleaning (slime removal, cutting heads, and washing), filleting, drying, fermenting, canning, and smoking [31]. On average, approximately 11 m^3 of water is consumed per tonne of prepared fish, with a significant part being directly discharged as wastewater [32]. In this context, fish processing wastewaters are briefly discussed as a rich source of potential protein-based value-added compounds. Figure 1A and B illustrate the operations involved in the fish processing industries, with a particular emphasis on the fish freezing and fish canning industries.

Blood Water

Blood is a notable by-product in fish processing, arising from various stages such as boat storage, unloading, butchering, scaling, filleting, skinning, and evisceration [33]. Pelagic processing, like acid-marinated and old-fashioned marinated herring production, generates blood-water with a high load. Valuable compounds have been successfully recovered from herring process waters, including proteins, peptides, AAs, vitamins, trace elements, and salt [34]. Slaughtering of farmed fish like salmon and trout in countries such as Norway, Iceland, and Russia also produce significant blood wastewater, accounting for around 3.5 to 4% of the live weight of these fish. For example, in Norway, around 2% of salmon blood, totaling approximately 26,000 metric tonnes (MT), is recovered. However, collecting, and drying fish blood present challenges for its utilization [35]. Fish blood separation is challenging due to its unique properties, which are different from those of warm-blooded animals. Contamination with substances like faeces, salt, and fish scales further complicates separation. Rapid coagulation can be prevented by adding anticoagulants like sodium citrate after slaughter. Blood-water composition varies based on factors like fish species, processing techniques, volume processed, temperature, and water volume used. High-fat fish like mackerel can result in elevated levels of fats, oils, and grease (FOG) in blood water [29]. In a study, significant amount of biopeptides with antioxidant and antimicrobial activities were recovered from codfish blood-water using ultrafiltration (UF) membranes, offering the potential for utilization in various sectors. [36].

Fish Canning Cooking Wastewater

The fish canning industry typically uses raw materials such as tuna or small pelagic fish, including sardines and anchovies. The canning process entails various steps, including washing, thawing, and cooking of the raw material, resulting in the generation of multiple wastewaters. Among these steps, cooking plays a crucial role and produces a substantial effluent known as cooking wastewater. This wastewater is characterized by high concentrations of organic materials and fats [21]. Studies have indicated that a fish canning plant can generate an estimated 15 to 27 MT per day of cooking wastewater. This wastewater is found to contain approximately 5% water-soluble proteins, including sarcoplasmic proteins and other proteins such as collagen, which consist of about 30% hydrophobic AAs [37, 38]. Similarly, in the processing of anchovies (*Engraulis japonicus*), approximately 1.5 MT of cooking wastewater is produced for each MT of processed species. During the cooking stage, elevated temperatures cause protein denaturation, releasing nitrogen into the wastewater. Amino groups, which form a significant part of the protein's molecular structure, contribute to this nitrogen release [39]. This wastewater is a valuable nutritional resource due to its crude protein content (5 g/L) and the presence of essential AAs [40].

Stickwater

Fishmeal is primarily produced from small marine fish with high bone and oil content, particularly from the *Clupeidae* family. The production process involves mincing, cooking, pressing, and drying whole fish or fish by-products [41]. The resulting liquid phase, called press liquor, is centrifuged to extract oil, leaving stickwater as wastewater. Stickwater constitutes around 60% of the processed fish weight, with proteins (5–9%) being significant components [42]. The fishmeal industry typically recovers dry matter from stickwater through evaporation. Concentrated solids obtained from evaporation are mixed with the press cake, increasing fishmeal production yield. However, some fishmeal plants may lack the required equipment for stickwater evaporation and concentration, leading to irregular operations. As a result, stickwater is often discharged directly into the sea or coastal areas, causing significant pollution [43]. The discharge of nutrient-rich wastewater contributes to algal bloom and eutrophication [44]. To address this concern and promote sustainable production, it is essential to investigate efficient approaches for recycling these by-products. Converting them into value-added products not only mitigates pollution but also promotes sustainable resource utilization.

In summary, the growing volume of fish processing by-products presents a significant challenge in terms of proper management and disposal, but also an opportunity to extract valuable bioactive proteins and peptides. Fish processing generates various solid and liquid by-products that are rich in proteins, peptides, and other valuable compounds. Implementing suitable treatment processes and recovery techniques offers the potential to extract these bioactive compounds and create value-added products. This not only mitigates the environmental challenges associated with improper disposal of fish processing by-products but also provides economic benefits. The trend towards developing bio-functional ingredients from these underutilized resources has become a new focus, driven by the need to maximize the value of fish processing waste and promote sustainable resource utilization.

Exploring the Bioactive Properties of Peptides Derived from Fish Processing by-Products

Natural ingredients without artificial preservatives are being explored to meet the demand for functional foods. Biologically active peptides and protein hydrolysates are being investigated as effective ingredients [45]. These

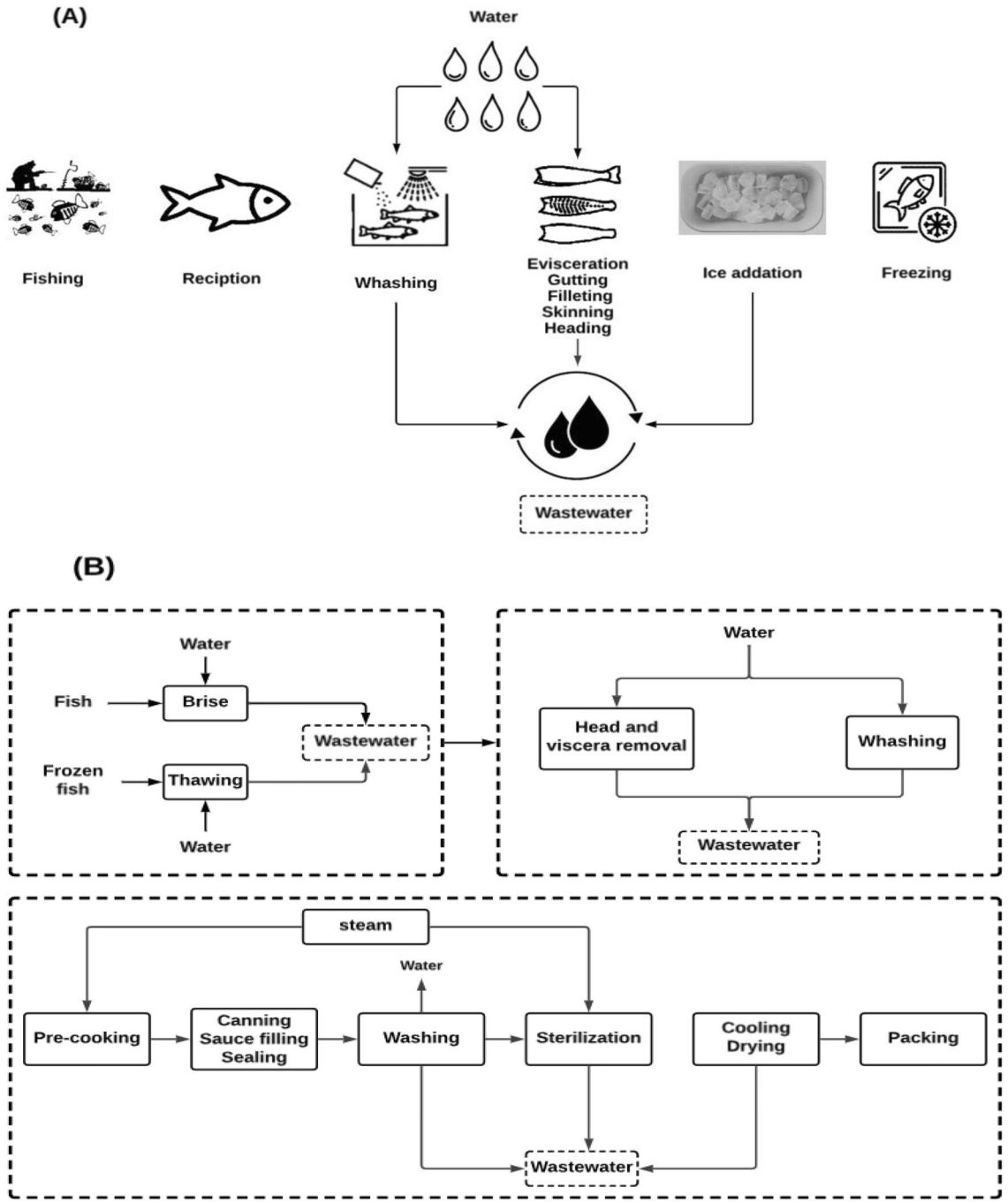

Fig. 1 Diagram describing the operations of fish processing industries, specifically focusing on (**A**) fish freezing and (**B**) fish canning industries [123]

peptides, released by proteolytic enzymes, regulate various physiological functions and exhibit antioxidant, antimicrobial, antihypertensive, antiviral, immunomodulating, antiproliferative, anticoagulant, and other activities [46]. Fish by-products are rich sources of diverse bioactive peptides, while bioactive peptides from aqueous raw materials offer additional properties such as anti-aging, anti-tuberculosis, anti-thrombotic, and anti-diabetic effects [47].

Antioxidant Activity

Oxidative damage occurs when the antioxidant defenses are diminished, resulting in the generation of detrimental free radicals and reactive oxygen species (ROS) [48]. This damage is associated with detrimental effects on lipids, enzymes, proteins, and DNA, contributing to conditions like cancer [48]. Antioxidant peptides, typically composed

of 3–16 AAs, play a crucial role in inhibiting free radical reactions and reducing oxidation processes. Common AAs found in these peptides include histidine, tyrosine, methionine, cysteine, tryptophan, and lysine. In the food industry, synthetic antioxidants are frequently employed to scavenge free radicals and preserve food quality [49]. Due to concerns about toxicity and carcinogenic effects, there is a growing demand for natural alternatives, and fish-derived bioactive peptides are a promising source of natural antioxidants with low toxicity [50]. Fish-derived bioactive peptides are effective antioxidants that scavenge free radicals, quench ROS, and chelate metal ions [51, 52]. They provide a safer alternative to synthetic antioxidants and have the potential to enhance food stability and address consumer concerns. These peptides can be used in functional food development to preserve and improve food quality.

Fish protein hydrolysates with antioxidant properties are gaining attention in the food industry [49]. Scientific studies have highlighted the presence of abundant biologically active antioxidant peptides in fish processing by-products, including solid residues and wastewater (Table 1). Incorporating these peptides into food formulations helps extend shelf life, prevent spoilage, and protect against foodborne pathogens. This meets the growing consumer demand for safer, healthier, and more sustainable food options.

Obtaining antioxidant bioactive peptides from fish processing by-products is complex and costly. Researchers are now focusing on optimizing production methods and practical applications of protein hydrolysates without isolating individual peptides to improve cost-effectiveness while preserving their natural bioactivity and antioxidant potential [53].

Antimicrobial Activity

Antimicrobial peptides (AMPs) are low-MW peptides with potent antimicrobial and immunomodulatory activities against various infectious agents. They exhibit diversity in structural characteristics, AA composition, and physicochemical properties, contributing to their broad antimicrobial effects. AMPs hold promise for novel therapeutic strategies against infections and diseases, including combating antibiotic resistance [54]. These peptides, typically composed of fewer than 50 AAs, have gained scientific interest for their antibacterial properties and potential in developing modern antibiotics and innovative food preservation approaches [55]. Certain fish families like, red sea bream, American plaice (*Pleuronectes americanus*), Atlantic halibut (*Hippoglossus hippoglossus*), and *Hippoglossoides platessoides*, possess body parts abundant in AMPs like pleurocidins [56, 57].

Fish proteins and by-products can be enzymatically hydrolyzed to generate AMPs. These diverse peptides exhibit antimicrobial activity against bacteria, fungi, viruses, and protozoa [58, 59], while also showing promise in suppressing cancer cells by disrupting intracellular signaling pathways [54]. Fish by-product-derived AMPs serve dual functionality as antimicrobial and anticancer agents, with fish skin mucus containing AMPs exhibiting cytotoxic effects against a wide range of cancer cells [60]. Pardaxin A, derived from various fish species, including *red sea bream*, *Salmo salar*, and *Pardachirus marmoratus*, has demonstrated inhibitory effects on multiple cancer cell lines, inducing apoptosis and promoting cell maturation and differentiation [61, 62].

Limited research has been conducted on antimicrobial activity in fish industry wastewater. AMPs were discovered in specific sources like codfish blood and sardine cooking wastewaters (*Gadus morhua L.* and *Sardina pilchardus*), respectively [10, 36]. Another study found antimicrobial activity in anchovy (*Engraulis japonicus*) cooking wastewater through enzymatic hydrolysis [40]. Fish industry by-products could serve as natural sources of antimicrobial agents, with AMPs found in wastewater holding promise for novel compounds. Table 1 summarizes studies purifying AMPs from different fish processing by-products.

Antihypertensive Activity

Angiotensin-converting enzyme (ACE) converts angiotensin I to angiotensin II, causing vasoconstriction and elevated blood pressure [46]. Peptide ACE inhibitors effectively block this conversion, preventing vasoconstriction and blood pressure elevation [46]. These inhibitors, found in proteins from diverse sources, including fish processing by-products, have shown promise in managing hypertension based on in vitro and in vivo studies [63]. Fish processing by-products yield ACE inhibitory peptides, highlighting their potential as natural ACE inhibitors (Table 1). These peptides, particularly those derived from processing by-products, hold promise for managing hypertension and related cardiovascular conditions. Peptides with lower molecular weights (MWs) and hydrophobic AAs tend to exhibit higher ACE inhibitory activity, while the presence of proline at the N or C terminals can influence ACE inhibition. Enzymatic hydrolysis of collagen using appropriate enzymes can produce ACE-inhibitory peptides [64]. Additionally, studies have identified ACE inhibitory peptides without proline at the N/C terminus [65]. Response surface methodology (RSM) aids in optimizing ACE inhibition for specific biopeptides by considering factors such as enzyme and protein substrate concentration, temperature, pH, and other parameters to optimize production processes [66]. Shortfin scad (*Decapterus macrosoma*) skin gelatin hydrolysates exhibited ACE inhibitory activity, and RSM was utilized to optimize inhibition levels ranging from 19.67% to 95.06% [66]. Fish by-products are a valuable

Table 1 Bioactive peptides derived *from fish processing by-products*

Fish species	Source of Peptide	Enzyme	Purification methods	Major findings of bioactive peptides	
Skipjack tuna (*Katsuwonus pelamis*) [105]	Skin Bones Head	Alcalase	SPE	Antioxidant	FPH-head showed the highest DPPH radical scavenging activity (~80%) and ABTS radical scavenging activity (~90%) at the highest tested concentration of 4 mg/mL
				Antimicrobial	The recovered FPHs demonstrated significant antibacterial activity against gram-positive bacteria, with inhibition zones of 7.00 mm for FPH-skin, 6.33 mm for FPH-skin, and 3.64 mm for FPH-bone against *L. monocytogenes*, *S. aureus*, and *B. cereus*, respectively, at a concentration of 10 mg/mL. Additionally, FPH-skin, FPH-head, and FPH-head showed the highest antibacterial activity against gram-negative bacteria, with inhibition zones of 5.83 mm for *E. coli*, 5.17 mm for *S. enterica*, and 5.00 mm for *S. typhimurium*, respectively, also at a concentration of 10 mg/mL
				ACE inhibitory	FPH-head exhibited the highest ACE inhibitory activity of ~25% at the highest tested concentration of 1 mg/mL
Codfish (*Gadus morhua L.*) [36]	Bloodwater	—	UF (10, 20, and 50 kDa)	Antioxidant	Bioactive peptides obtained from MW, GE Suez UF membrane (MWCO: 50 kDa and TMP: 0.1 bar) exhibited the highest ABTS radical scavenging activity of 17.34 mg AA/g of the sample
				Antimicrobial	A concentration of 40 mg/mL of bioactive peptides obtained from the Microdyn Nadir UP010 UF membrane (MWCO: 10 kDa and TMP: 0.1 bar) effectively reduced *E. coli* microbial growth. The inhibition halo sizes were not reported
Sardine (*Sardina pilchardus*) [36]	Cooking wastewater	—	UF (2.5 kDa)	Antioxidant	Bioactive peptides obtained from GH, GE Suez UF membrane (MWCO: 2.5 kDa and TMP: 1 bar) exhibited the highest ABTS radical scavenging activity of 4 mg AA/g of sample
				Antimicrobial	A concentration of 40 mg/mL of obtained bioactive peptides reduced the *E. coli* microbial growth, especially when using the GE Suez GH UF membrane (MWCO: 2.5 kDa and TMP: 1 bar). The inhibition halo size was not reported
Sardine (*Sardina pilchardus*) [10]	Cooking wastewater	*Cynara cardunculus*	UF (3 kDa) NF (120.37 g/mol) RO	Antioxidant	The highest ABTS radical scavenging activity was found in the unhydrolyzed UF protein/peptide retentate fraction (~115 µmol of TE/g), and the highest ORAC value was found in both unhydrolyzed and hydrolyzed UF retentate fractions (~150 µmol of TE/g)
				Antimicrobial	A concentration of 40 mg/mL reduced the *E. coli* microbial growth, specifically in the UF hydrolyzed protein/peptide retentate fraction. The inhibition halo size was not reported

Table 1 (continued)

Fish species	Source of Peptide	Enzyme	Purification methods	Major findings of bioactive peptides	
Bighead carp (*Hypophthalmichthys nobilis*) [59]	Head	Alcalase Alkaline Protease	UF (3, 10, and 30 kDa)	Antioxidant	The maximum DPPH radical inhibitory power of 82.76% was observed in the protein hydrolysate fraction (< 3 kDa) at a concentration of 2 mg/mL when using fish viscera alkaline protease. Additionally, the highest ABTS radical scavenging activity of 86.62% was reported in the same protein hydrolysate fraction at 2 mg/mL when using alcalase
				Antimicrobial	The highest inhibition zone of ~ 2 mm observed for *E. coli* in hydrolysate fraction (< 3 kDa) using alcalase and the highest inhibition zone of ~ 2.5 mm observed for *S. aureus* in hydrolysate fraction (< 3 kDa) using fish viscera alkaline protease
Yellowfin tuna (*Thunnus albacores*) [106]	Viscera	Protamex	UF (3, 10, and 30 kDa)	Antioxidant	The peptide fractions (< 3 kDa) exhibited the highest DPPH radical scavenging activity (~ 80%) at 3 mg/mL and the highest ABTS radical scavenging activity (66%) at 2 mg/mL
				Antimicrobial	The peptide fractions (< 3 kDa) exhibited the highest antimicrobial activity, with ~ 90% inhibition at 2 mg/mL against *E. coli* and *Pseudomonas*, and ~ 100% inhibition at 2 mg/mL against *Listeria* and *Staphylococcus*
Grenadier (*G, Macrourus sp.*) Megrim (*Me, Lepidorhombus boscii*) European hake (*Ha, Merluccius merluccius*) Boarfish (*Bo, Capros aper*) Atlantic horse mackerel (*HM, Trachurus trachurus*) [107]	Skins Bones Heads	Alcalase	Chemical process Bacteria (*Lactic acid*) fermentation	Antioxidant	The highest DPPH radical scavenging activity of 49.12% and the highest ABTS radical scavenging activity of 25.45 µg/mL were observed in boarfish (*Bo*, *Capros aper*) heads
				ACE inhibitory	Boarfish (*Bo*, *Capros aper*) heads exhibited the highest percentages of ACE inhibitory activity of 73.77%
kilka (*Clupeonella sp.*) [41]	Meat Fishmeal Stickwater	Alcalase 2018	Centrifugation	Antioxidant	The highest radical scavenging activities for DPPH and ABTS were demonstrated by kilka fishmeal, with IC_{50} values of 1.99 mg/mL and 2.00 mg/mL, respectively, at a concentration of 40 mg/mL
Tuna (*Thunnus tonggol*) [38]	Cooking juice	Orientase (*Bacillus subtilis*)	GFC		The DPPH radical-scavenging capacity of the protein hydrolysate reached its maximum at 80% after 60 min of hydrolysis
Tuna [37]	Cooking juice	Protease XXIII (*Aspergillus oryzae*)	Column chromatography		The hydrolyzed proteins obtained at a degree of hydrolysis of 25.68% (after 2.5 h of hydrolysis) exhibited the highest DPPH scavenging effect, achieving 82.19%
Redlip croaker (*Pseudosciaena polyactis*) [108]	Scales	Neutrase	UF (1, 3, and 5 kDa) Serial chromatography		The three identified peptides—GPEGPMGLE, EGPFGPEG, and GFIGPTE—exhibited the strongest scavenging activities against DPPH radicals (EC_{50} values of 0.59, 0.37, and 0.45 mg/mL, respectively), hydroxyl radicals (EC_{50} values of 0.45, 0.33, and 0.32 mg/mL), and superoxide anion radicals (EC_{50} values of 0.62, 0.47, and 0.74 mg/mL)

Table 1 (continued)

Fish species	Source of Peptide	Enzyme	Purification methods	Major findings of bioactive peptides	
Tuna (*Sarda Orientalis*) [109]	Black muscle by-product	Protamex	—		The FPH (< 14 kDa) exhibited a high DPPH scavenging activity of 70% at a concentration of 500 µg/mL
Fishes from bycatch (Micropogonias furnieri and *Paralonchurus brasiliensis)* [110]	Muscle Skin	Alcalase Protamex®	—		The protein hydrolysate from *Micropogonias furnieri* demonstrated the highest DPPH scavenging activity, achieving ~ 30%
Sharks Mullet Guitarfish Weakfish Snapper Ray Squid Seabass Pompano dolphinfish [87]	Skins Heads Skeletons	Alcalase	UF (1, 5, 10, and 30 kDa)		The peptide fraction (≤ 1 kDa) derived from hydrolyzed acid-soluble collagen obtained from a mixed by-product of various fish species exhibited the highest reducing power with an absorbance of 0.36 and demonstrated 91% hydroxyl radical scavenging activity at 15 mg/mL. Furthermore, this fraction showed the highest DPPH scavenging activity, achieving 81% at 5 mg/mL
Sturgeon (*Acipenser ruthenus*) [111]	Spermary	Papain	0.22 µm filter membrane chromatography system	Antimicrobial	Sturgeon spermary peptides exhibited the highest inhibition rate and the largest inhibition zone against *E. coli*, achieving an inhibitory rate of 76.46%
Anchovy (*Engraulis japonicus*) [40]	Cooking wastewater	Protamex	Liposome equilibrium dialysis combined with HPLC		The purified antimicrobial peptide demonstrated antibacterial activity against both gram-positive and gram-negative bacteria. *S. aureus* and *B. subtilis* were the most sensitive, exhibiting identical MICs of 16 µg/mL. The MICs for *S. pneumoniae*, *E. coli*, *S. dysenteriae*, *P. aeruginosa*, and *S. typhimurium* were 64, 32, 256, 64, and 32 µg/ml, respectively
Rainbow trout (*Oncorhynchus mykiss*) Sole (*Dover sole*) [112]	Skin Viscera Head	—	ASE PEF		Rainbow trout skin extracts using ASE and sole viscera extracts using PEF treatment demonstrated significant antibacterial activity against *S. aureus*, resulting in growth rates of 0.425 and 0.458 $\mu max \cdot h^{-1}$, respectively. Additionally, PEF extracts from rainbow trout head and skin enhanced the growth of *Lactobacillus casei*, with growth rates of 0.382 and 0.369 $\mu max \cdot h^{-1}$, while ASE extracts from rainbow trout head and skin promoted *Bifidobacterium lactis* growth at rates of 0.557 and 0.547 $\mu max \cdot h^{-1}$
Croaker (*Johnius sp.*) [113]	Skin	Protease from Rohu (*Labeo rohita*) Catla (*Catla catla*)	UF (1, 3, 5, and 10 kDa)	ACE inhibitory	Peptide fractions < 1 kDa from gelatin protein hydrolysate using catla protease and 1–3 kDa fractions from gelatin protein hydrolysate using Rohu protease exhibited the highest ACE activity, with IC_{50} values of 0.63 and 0.59 mg/mL, respectively

Table 1 (continued)

Fish species	Source of Peptide	Enzyme	Purification methods	Major findings of bioactive peptides
Lizardfish (*Synodus macrops*) [69]	Scale gelatin	Bromelain Alcalase Chymotrypsin Papain Acidic protease Trypsin	Nano-LC–MS/MS	ACE inhibitory peptide, identified as AGPPGSDGQPGAK, demonstrated an IC_{50} value of 420 µM
Sardine (*Sardina pilchardus*) [67]	Heads Cooked skins Wastewater of canning industry	Alcalase	UF (3 kDa)	Protein extract fractions (< 3 kDa) exhibited the strongest ACE activity, with an IC_{50} of 51 µg/mL

FPH, Fish protein hydrolysate; *DPPH*, 2,2-diphenyl-1-picrylhydrazyl; *ABTS* – 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); *MWCO*, Molecular weight cut-off; *TMP*, Transmembrane Pressure; *AA*, ascorbic acid; *TE*, Trolox equivalent; *ORAC*, oxygen radical absorbance capacity; *NF*, nanofiltration; *RO*, reverse osmosis; *UF*, ultrafiltration; *MIC*, minimum inhibitory concentration; *ASE*, accelerated solvent extraction; *PEF*, pulsed electric field; *SPE*, sulfated polysaccharide extraction; *GFC*, gel filtration chromatography; *HPLC*, high-performance liquid chromatography; *Nano-LC–MS/MS*, nanoscale liquid chromatography coupled to tandem mass spectrometry

source of collagen and ACE inhibitory peptides. Discarded seafood processing by-products contain proteins that can give rise to biopeptides with ACE inhibitory activity. ACE inhibitory activity has been demonstrated in low-MW fractions of protein extracted from sardine (*Sardina pilchardus*) heads and cooked skin wastewater [67]. Hydrolysates from tuna blood (*Katsuwonus pelamis*) have also been found to exhibit ACE inhibitory and antihypertensive properties [68].

Fish-derived biopeptides show promise as potent and safe ACE inhibitors and antihypertensive peptides, offering a potential alternative to synthetic drugs with minimal adverse effects. By exploring these biopeptides as alternative treatments for hypertension and cardiovascular conditions, abundant fish by-products can be effectively utilized [63]. This research contributes to the development of safer and more effective therapies for hypertension and related cardiovascular conditions.

Other Bioactive Properties

Fish by-product protein hydrolysates exhibit diverse bioactivities, including potential antitumor and antiproliferative effects. However, further investigation is needed to fully understand and harness their potential in these areas [69]. Tuna cooking juice hydrolysates, specifically the > 2.5 kDa ultrafiltration (UF) fraction, exhibit antiproliferative activity against MCF-7 cells by up to 25% while sparing normal breast epithelial cells. These findings highlight the potential of tuna cooking wastewater hydrolysates as a source of selective bioactive peptides with antiproliferative properties, suggesting their potential application in cancer research and therapeutics [70].

Fish by-product protein hydrolysates possess anti-inflammatory and immunomodulatory properties. For example, sardine by-product hydrolysate from the canning industry exhibited anti-inflammatory effects, particularly in the low MW fraction (< 10 kDa) [71]. Sturgeon peptides show remarkable anti-inflammatory effects [72]. Giant croaker (*Nibea japonica*) skin hydrolysate enhances the immune system by promoting cell-mediated and humoral immunity [73].

Food-derived bioactive peptides offer a safer alternative for diabetes treatment. They inhibit enzymes, suppress DPP-IV, and enhance insulin uptake, showing promising antidiabetic effects [74]. For instance, enzymatic hydrolysis of Atlantic salmon (*Salmo salar*) skin collagen produced DPP-IV-inhibitory peptides, including LDKVFR, with high activity [75]. Further research is needed to explore the potential of these peptides as DPP-IV inhibitors. Fish protein hydrolysates have potential as anticancer agents due to their essential biochemical properties and easy availability. They can serve as valuable components in the development of functional foods and pharmaceuticals. For example, a peptide derived from barred mackerel (*Scomberomorus*

commerson) by-products exhibited significant anticancer activity against the MCF-7 cell line [76]. Fish by-product protein hydrolysates possess strong metal-chelating abilities due to aromatic ring-containing AAs [77]. For example, salmon frame-derived protein hydrolysate exhibits copper-binding capacity, preventing copper deficiency-related issues [78]. Metal-chelating peptides help regulate metal imbalances, such as iron and calcium, by facilitating transport or absorbing excess ions [79]. Calcium-binding peptides have been extracted from Pacific cod (*Gadus macrocephalus*) bones [80], and iron-binding peptides have been identified in *Diplodus* proteins [81].

In conclusion, the exploration of the bioactive properties of peptides derived from fish processing by-products has unveiled significant potential for these natural ingredients as effective functional components. Fish processing by-products are rich sources of bioactive peptides that can effectively scavenge free radicals, quench reactive oxygen species, and chelate metal ions, providing a safer alternative to synthetic antioxidants. These peptides have demonstrated promise in enhancing food stability and addressing consumer demand for healthier, more sustainable food options. Additionally, fish-derived antimicrobial peptides exhibit inhibitory effects against a range of microorganisms, including bacteria, fungi, viruses, and protozoa, and some have also shown anticancer properties, suggesting their potential dual functionality. The antihypertensive activity of fish-derived biopeptides, particularly their ability to inhibit ACE, highlights their potential as natural alternatives to synthetic drugs for managing hypertension and related cardiovascular conditions. Beyond these well-studied bioactivities, fish by-product protein hydrolysates have also exhibited promising antiproliferative, anti-inflammatory, and immunomodulatory properties, warranting further investigation to fully harness their potential in these areas. Overall, the exploration of bioactive peptides from fish processing by-products represents a promising avenue for developing functional food ingredients and nutraceuticals that meet the growing demand for natural, sustainable, and health-promoting solutions.

Functional Properties of Peptides Derived from Fish Processing by-Products

Functional properties of proteins in food systems encompass attributes like solubility, water holding capacity (WHC), fat binding capacity (FBC), emulsification, and foaming properties. These properties, influenced by protein MW and structure, have applications in gelling, nutrition, and emulsification [82]. They play an important role in food product development, affecting sensory properties and consumer acceptability. Proteins possess varied functional properties that impact the texture and characteristics of processed foods [83].

Solubility

Fish protein hydrolysates possess important physicochemical properties, including solubility and AA composition, which contribute to the gelling, water retention, and texture of foods [84]. They are used as emulsifiers, binders, and nutritional supplements. Solubility is influenced by pH, with higher levels enhancing solubility due to negatively charged residues in proteins [85]. Solubility variation in peptides is influenced by net charge and surface hydrophobicity, promoting aggregation through hydrophobic interactions. pH plays a significant role in protein charge and solubility. Studies on ornate tilapia hydrolysate show solubility patterns at different pH levels, with low-MW peptides exhibiting high solubility [86]. This finding is consistent with other studies that link increased solubility to low-MW peptides [85, 87–89].

Hydrolysis converts hydrophobic protein groups into hydrophilic groups, increasing solubility [89]. This results in the release of small soluble peptides and the formation of new charged and polar groups from AAs, improving their solubility in water [15, 88]. The high solubility of nitrogen in protein hydrolysates enhances food product appearance and mouthfeel, providing an attractive visual presentation and a smooth sensation when consumed [87]. The study analyzed protein isolates from Atlantic salmon (*Salmo salar*), cod (*Gadus morhua*), and herring (*Clupea harengus*) by-products using the pH-shift method. Solubility resembled that of non-processed fish myofibrillar proteins. High solubility was observed at extreme pH levels, with lower solubility at pH 5.5. The pH-shift method preserved the original solubility characteristics [90]. In another study, fish protein hydrolysate (FPH) extraction from skipjack tuna head, bone, and skin showed a correlation between protein degradation, solubility, and MW reduction. Head-derived FPH had the highest solubility, indicating the potential for highly soluble hydrolysates [59]. Table 2 summarizes solubility data, enhancing our understanding of fish processing by-products' functional properties.

Water Holding and Fat Binding Capacities

Water holding capacity (WHC), which refers to a food product's ability to retain or bind water during processing, is highly valued in the food processing industry [91]. The solubility of fish protein has been linked to reduced WHC [86]. Studies on fish protein hydrolysates report WHC values ranging from 2.47 to 6.60 mL/g [85, 86, 88]. Table 2 summarizes WHC values for different fish processing by-products. Enzymatic hydrolysis increases polar groups like COOH and NH_2, which significantly affect water adsorption. For instance, rainbow trout hydrolysate with polar side chains like glutamic and aspartic acids demonstrates substantial WHC [88].

Fat binding capacity (FBC) refers to proteins' oil absorption and retention ability, impacting food taste [88, 92]. Factors

like enzyme specificity, emulsifying capacity, density, and hydrolysis affect FBC [93]. Fish protein hydrolysates show FBC values ranging from 1 to 10.8 mL/g [85–89]. Table 2 summarizes FBC values for fish processing by-products.

The protein's non-polar residue contributes to hydrophobic interactions at the oil–water interface, and protein-lipid interactions involve various bonding interactions [89]. Bluewing searobin fish protein hydrolysate shows decreased FBC with increasing peptide MW [85, 89]. In a study eel (*Monopterus sp.)* protein hydrolysate with different MW fractions (3, 5, and 10 kDa) was tested for water/oil adsorption capacity across pH 2 to 10. The 5 kDa fraction had the highest FBC at pH 2, while the 10 kDa fraction exhibited the highest WHC across all pH levels. Low-pH fish protein hydrolysate fractions show higher FBC, while neutral-pH fractions have better water retention ability [94]. Processing techniques can impact the functional properties of fish protein isolates. pH-shift treatment of Yellowfin Tuna Roe proteins improved WHC from 3.7 to 4 g/g of protein, enhancing overall functionality [95]. In a study on Argentine anchovy (*Engraulis anchoita*) processing by-products, the alkaline protein isolate displayed the highest WHC at pH 11, with increased FBC under alkaline pH conditions [96]. Whitemouth croaker (*Micropogonias furnieri*) protein by-products had good FBC but poor WHC due to their high lipid content. Alkaline-aided protein isolates (pH 11) showed higher solubility in FBC and WHC compared to acid-aided isolates (pH 3) [97].

Table 2 Functional properties of differ*ent fish processing by-products*

Fish Species	Source of protein	Functional properties							Reference
		Solubility (%)	WHC	FBC (g/g)	FC (%)	FS (%)	EAI (m^2/g)	ESI (min)	
Carp Fish	Heads Skins Skeletons	81.3–89.1	—	—	87.4	28.4 min	78.2–121.1	24.4–31.6	[114]
Milkfish (*Chanos chanos*)	Viscera Fins Bones	53.80–94.26	—	—	15–233	~50–200	28.18–53.51	31.98–43.83	[115]
Cod (*Gadus morhua*)	Frame	92.76–100	—	3.16–4.49	—	—	—	—	[116]
Perch (*Nemipterus japonicus*)	Head Viscera	89.8—99.7	—	—	10.7	81.2	—	—	[117]
Sole fish (*Cynoglossus arel*)	Skin	93.43	—	—	36	34	112.6	12.5	[118]
	Scale	90.33	—	—	54	42	98.7	16.5	
Oneknife Unicornfish (*Naso thynnoides*)	Skin	79.38–97.12	—	—	5.83–23.33	2.5–18.33	35.25–36.73	< 10%	[119]
Round sardinella (*Sardinella Baurita*)	By-products	~40–100	—	—	—	—	12.88–84.80	—	[120]
Atlantic salmon (*Salmo salar*) Atlantic Cod (*Gadus morhua*)	Backbone	—	—	—	—	—	9–13	4–33	[121]
Striped catfish (*Pangasianodon hypophthalmus*)	Viscera	14.78—> 100	0.71–0.89	1.03–1.39	87.50–137.50	25–126.67	13.96–61.70	16.23–87.98	[122]
Sharks, Mullet Guitarfish, Weakfish Snapper, Ray Squid, Seabass and pompano dolphinfish	Skins Heads Skeletons	≥ 95	—	—	78	60	130	42	[87]

WHC, Water holding capacity; *FBC*, fat binding capacity; *FC*, foaming capacity; *FS*, foaming stability; *EAI*, emulsifying activity index; *ESI*, emulsifying stability index

Foaming and Emulsifying Properties

Protein foaming is crucial for whipped toppings, ice cream mixes, and baked goods. Foaming capacity improves away from the isoelectric point (IP), particularly at alkaline pH [98]. Foam stability depends on the integrity of the protein film and its ability to absorb substances. Specialized foam stabilizers enhance protein–protein interactions, resulting in thicker and more effective protein films, improving foam stability [99]. Optimizing these factors ensures the desired texture and performance of foams in food applications. Protein's emulsifying ability contributes to desirable sensory attributes like smoothness and mouthfeel. Emulsifying properties are influenced by AA composition, sequence, and environmental factors like pH, temperature, and degree of hydrolysis [100]. Proteins perform multiple functions like emulsion stabilization, foam creation, surface adhesion, colloid protection, and film formation. These functions depend on the distribution of hydrophilic and hydrophobic AAs on the protein's surface, impacting their interaction with interfaces in food applications [101]. FPH from salmon, cod, and herring exhibits comparable emulsion stability and foaming capacity to soy protein. Cod protein outperforms salmon and herring proteins in emulsion and foaming capacity, with added viscoelastic properties. Understanding these properties is vital for developing textured and stable food products [90]. Tuna roe protein isolate shows excellent foaming capacity (128–142%) and high emulsification capacity (up to 10 g of oil per g of protein), with pH-shift treatment having no adverse effects on its properties. The resulting protein powder demonstrates comparable emulsifying capacity to casein [95]. The acid isolate from Big Eye Snapper (*Priacanthus tayenus*) had lower emulsifying activity index but superior emulsion stability compared to commercial whey protein and egg white. It also exhibited reduced foamability, indicating limited ability to generate and maintain foam structures [102]. Pollock viscera, liver, heads, trimmings, and frame powders (protein content: 65–79%) form viscoelastic emulsions with emulsion capacity and stability ranging from 29% to 34.5% [103]. Swordfish (*Xiphias gladius*) head muscle protein hydrolysis improves emulsifying and foaming properties across pH 2 to 10, with increased foaming abilities and decreased emulsifying activity at higher concentrations [104]. Table 2 provides an overview of the foaming and emulsifying properties of fish processing by-products.

In summary, the functional properties of peptides derived from fish processing by-products demonstrate their potential as functional ingredients in different industries. Peptides exhibit varied solubility, WHC, FBC, foaming, and emulsifying properties based on factors like MW, amino acid composition, and processing methods. Protein hydrolysis generally improves solubility by producing smaller peptides with more charged and polar groups. Lower-MW peptides exhibit higher solubility compared to larger peptides. The pH of the medium significantly impacts solubility, with extreme pH values typically showing higher solubility. Fish protein hydrolysates display a range of WHC and FBC values, influenced by the polarity of amino acid side chains and the degree of hydrolysis. Enzymatic hydrolysis increases polar groups, enhancing water adsorption and WHC. Hydrophobic interactions and protein-lipid bonding contribute to FBC, which can be affected by MW and pH. The foaming and emulsifying properties of fish protein hydrolysates are important for various food applications. Factors like amino acid composition, pH, and degree of hydrolysis influence these functional properties. Optimization of these properties is crucial for developing stable and textured food products. Overall, the functional properties of peptides derived from fish processing by-products highlight their potential as valuable ingredients in the development of nutritious and functional food products.

Conclusion

Extensive research has demonstrated the immense potential of fish by-products as a rich source of bioactive peptides. These peptides, abundantly present in fish processing by-products such as wastewater and solid residues, have garnered significant attention across the food, pharmaceutical, and cosmetic industries. They exhibit a wide range of biological properties, offering antioxidant, antihypertensive, antimicrobial, antiproliferative, anticancer, anti-diabetic, anti-inflammatory, immunomodulatory, and metal-chelating activities. Additionally, these bioactive peptides possess favorable functional characteristics, including water solubility, emulsification, and foaming abilities, making them highly desirable for diverse industrial applications.

This review highlights the potential of fish by-products and their bioactive and functional properties. It emphasizes the importance of utilizing these resources for product development and innovation across multiple sectors. This research sets the stage for future investigations and advancements in utilizing fish by-products to drive innovation. By leveraging the biodiversity of fish ecosystems and extracting valuable peptides, this approach promotes sustainable fish resource utilization and reduces waste. It also offers economic benefits by unlocking the untapped potential of fish by-products. The findings from this research contribute to the development of novel products and responsible fish resource management.

Abbreviations *MT*: Million tonnes; *AA*: Amino acid; *WHC*: Water holding capacity; *FBC*: Fat binding capacity; *COD*: Chemical oxygen demand; *MT*: Metric tonnes; *FOG*: Fats, oils, and grease; *UF*: Ultrafiltration; *NF*: Nanofiltration; *MWs*: Molecular weights; *ACE*: Angiotensin-converting enzyme; *ROS*: Reactive oxygen species; *AMPs*: Antimicrobial peptides; *RSM*: Response surface methodology; *ASE*: Accelerated solvent extraction; *PEF*: Pulsed electric field; *SPE*: Sulfated polysaccharide extraction; *LC–MS/MS*: Liquid chromatography-tandem mass spectrometry; *GFC*: Gel filtration chromatography; *UHPLC*: Ultra-high performance liquid chromatography; *HPLC*: High performance liquid chromatography; *FPH*: Fish protein hydrolysates; *FC*: Foaming capacity; *FS*: Foaming stability; *EAI*: Emulsifying activity index; *ESI*: Emulsifying stability index

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