

A Review of Preparation Techniques and Functional-biological Properties of Fish Protein Hydrolysate

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ABSTRACT

Fish protein hydrolysates (FPH), which are made from low-value fish and by-products of fish processing, have drawn a lot of interest because of their remarkable nutritional, functional, and bioactive qualities. The preparation techniques, functional characteristics, and biological activities of FPH are all covered in detail in this review. After evaluating the three main hydrolysis methods—enzymatic, alkaline, and acidic—enzymatic hydrolysis is shown to be the most advantageous method due to its gentle processing conditions, specificity, and preservation of nutritional quality. The review highlights enzymes including alcalase, papain, and pepsin and gathers the best enzymatic conditions for the generation of FPH from different fish species and waste materials. The adaptability of FPH in the food, pharmaceutical, and nutraceutical industries is demonstrated by the exploration of functional qualities such as solubility, emulsification, foaming, and the capacity to hold water and oil across several species. Furthermore, due to certain peptide sequences, FPH has a variety of bioactivities, such as ACE-inhibitory, anti-inflammatory, antihypertensive, antioxidant, and antibacterial properties. The potential of FPH as a sustainable and valuable element is highlighted in this review, which also calls for more study into improving its industrial uses and manufacturing circumstances.

Key words: *Fish protein hydrolysate (FPH), Enzymatic hydrolysis, Fish processing by-products, Bioactive peptides*

Introduction

Globally, people eat fish and fish products. It offers the best source of high-quality protein in the world, making up to 14–16% of all animal protein consumed globally, along with other seafood (Boyd, McNevin and Davis, 2022). More than 196 million tonnes of fish are expected to be processed in 2025, indicating the growth of the fish producing business. A significant percentage of the by-products produced by this industry—such as heads, skin, trimmings, fins, viscera, frames, and occasionally muscle—are currently thrown away, underutilised,

or used to make low-value goods like fish meal and fish silage (Gao *et al.*, 2021).

Proteins, lipids, and water make up the majority of fish, with minor amounts of other components such as carbohydrates, minerals, vitamins, and nitrogenous non-protein compounds. The Food and Agriculture Organisation (FAO) has classified fish proteins into three primary categories: Approximately 70–80% of muscle protein is composed of myofibrillar (structural) proteins, which include important contractile proteins including actin, myosin, troponin, and actomyosin. 2. Sarcoplasmic proteins: These water-soluble proteins, which make up

about 25–30% of the total, include globulins, myoalbumin, and other enzymatic proteins.

3. Connective tissue proteins: Mostly collagen, these proteins are found in trace levels and make up as much as 10% of the total protein in cartilaginous fishes (elasmobranchs) and roughly 3% in teleost fishes.

Proteins are vital biopolymers that are found in both plant and animal sources. They are widely acknowledged as a plentiful supply of nutrients required for the growth and development of the organism (Khan *et al.*, 2022). Proteins and amino acids derived from freshwater, brackish, and marine fish are vital for physiological functions and offer significant health benefits, particularly in preventing cardiovascular and related diseases (Tørris, Småstuen and Molin, 2018). The use of protein from fisheries and aquaculture products falls into three categories:

(i) optimising yield by using proteins from conventional fish processing; (ii) turning industrial fish and by-products into materials resembling surimi; and (iii) creating targeted protein or peptide products from these sources (Thorkelsson and Kristinsson, 2009).

Fish and shellfish processing wastes have generated interest in the past few years in recovering digestive enzymes for biotechnological uses in a variety of industries, such as cheese production, herring fermentation, fish skinning, roe processing, specialty kit manufacturing, and medical applications (Shahidi and Kamil, 2001; Fernandes, 2016; Jemli *et al.*, 2016). Fish viscera are an excellent source of protein and polyunsaturated fats, making up around 20% of the biomass of fresh fish. If these wastes or low-quality raw materials are not used, they might lead to issues with the environment, human health, and the economy (Vidotti *et al.*, 2003).

Fish product such as Fish Protein Hydrolysate (FPH) and Fish Protein Concentrate (FPC) can be obtained from the fish and fish waste. Functional proteins/peptides (FPPs) found in FPH and FPC can be used in a range of industries and products (Ramakrishnan *et al.*, 2023).

Bioactive peptides, present in FPH are protein fragments with precursor sequences. After being released by protein hydrolases, they can interact with the relevant receptors and affect physiological processes demonstrating antioxidant, antibacterial, antifungal, antiviral, immunomodulating, antiproliferative, antithrombotic, anticoagulant and antihypertensive properties. They also act as angio-

tensin-converting enzyme inhibitors (ACE-inhibitors) and have a role in haemolytic, opioid and calcium binding (García-Moreno *et al.*, 2014; Kang, Ishak and Sarbon, 2018; Tkaczewska, 2020). This review focuses on the optimal condition for enzymatic production of fish protein hydrolysates from different fishes. Review also focuses on FPH functional properties and bioactive properties

Fish protein hydrolysate (FPH)

Fish protein hydrolysate (FPH) is a value-added product derived from fish processing waste or low-value fish, consisting of a complex mixture of free amino acids and peptides of varied molecular weight. It is usually accessible in liquid form or as a hygroscopic, amorphous powder. FPH's nutritional composition typically includes 81-93% protein, 3-8% ash, less than 5% fat content, and moisture levels ranging from 1-8% (Das, Nayak and Dash, 2021). Fish and fish left-overs may be raw materials for the processing of FPH. Fish meat is further treated with Trypsin, Alcalase, chymotrypsin, Pepsin, and other enzymes under controlled conditions of pH and temperatures (Toldra and Kim, 2016). FPH find its application in food, cosmetics, pharmaceutical, nutraceutical and functional food industry. The liquid FPH is a fluid mixture of hydrolyzed proteins that contains almost 90% moisture, making it difficult to transport and especially unstable for long-term storage. Therefore, dry fish protein hydrolysates (FPH) are preferable due to their longer shelf life, greater stability, and convenient portability compared to liquid FPH. However, the main obstacle and expensive part of producing dry FPH is having to remove over 90% of the water from liquid FPH (Silva *et al.*, 2014).

To achieve optimal protein fraction recovery, the raw materials at the processing site must be thoroughly assessed (Das *et al.*, 2021). The manufacturing of fish protein hydrolysate (FPH) begins with the acquisition of raw fish materials, which often comprise byproducts such as viscera, fins, scales, and corpses. These leftover components are initially cleaned by rinsing them with water to remove surface pollutants such as dirt and debris. After cleaning, the materials are mechanically chopped into smaller bits to improve the efficacy of enzymatic treatment and protein extraction.

Widely used fish species

The main marine species in worldwide capture over

years were the families of Carangidae, Clupeidae, Engraulidae, Gadidae, Nemipteridae, Ommastrephidae, Portunidae, Scomberesocidae, Scombridae, Trichiuridae (Petrova *et al.*, 2018). The most important fish families farmed in aquaculture are Cyprinidae (Carp), Salmonidae (Salmon, Trout), Serranidae (Seabass), Acipenseridae (Sturgeon), Scophthalmidae (Turbot), Sparidae (Sea Bream), Mytilidae (Mussels), Ostreidae (Oysters) and some families of clams. Thus, due to the abundance of aforesaid species in worldwide fishing production, an attention must be paid on better utilization of a big amount of by-products rested after their processing. Both fish and fish leftovers can be used as raw materials to produce FPH. Given that using solely fish byproducts or entire fish may have an impact on processing (Petrova *et al.*, 2018).

Methods of preparation of FPH

In order to produce products with high added value and economic significance that form peptides of various sizes, the creation of protein hydrolysates from fish viscera aims to solubilise the protein source using chemical or enzymatic processes to increase its biological and nutritional value (Das *et al.* 2021). Chemical and biological processes, which entail acid and alkaline hydrolysis, are the most often employed ways for producing protein hydrolysates in industrial activities. In contrast, enzymatic hydrolysis and autolysis are examples of biochemical techniques. Endopeptidases and exopeptidases are two examples of the natural proteolytic enzymes that break down animal-derived proteins during the autolysis process. By enabling the breakdown of longer peptide chains into smaller peptides and free amino acids, the use of commercially available enzymes speeds up this degradation process, also known as enzymatic hydrolysis. As interest in refining this bioconversion method has grown, many scientific studies in recent years have concentrated on new and enhanced methods for hydrolysing fish proteins (Ortiz *et al.*, 2023).

Acid hydrolysis

Proteins can be chemically hydrolysed by cleaving peptide bonds with an acid. Several methods have been proposed for hydrolysing fish proteins in acidic solutions (Kristinsson and Rasco, 2000). Due to its simplicity, ease of use, and low cost, this method is significant on an industrial scale. High heat and high pressures have been used to hydro-

lyse fish proteins completely using hydrochloric acid or, occasionally, sulphuric acid. Following hydrolysis, the resulting mixture is typically neutralized to a pH range of 6.0 to 7.0, subsequently concentrated, and then subjected to drying processes. Complete hydrolysis of fish protein substrates can be achieved within 18 hours under conditions of 118 °C using 6 M hydrochloric acid. This method is commonly employed to convert low-value or underutilized fish-derived raw materials into products such as fertilizers, owing to its cost-effectiveness and capacity for extensive protein breakdown (Petrova *et al.*, 2018; Das *et al.*, 2021). However, during acid hydrolysis, essential amino acids including tryptophan, methionine, and cysteine are often lost. Additionally, asparagine and glutamine are transformed to aspartic acid and glutamic acid. Acid hydrolysis has downsides, including high NaCl concentration, making the resulting FPH unsuitable for food and biological uses (Petrova *et al.*, 2018).

Alkali hydrolysis

Large water-soluble polypeptides are quickly broken down during the alkaline hydrolysis of fish protein, and they then break down more slowly into simpler molecules (Elavarasan, 2019). In the presence of alkaline agents like calcium, sodium, or potassium hydroxide, alkali hydrolysis is maintained at lower temperatures (usually 27°–54 °C) for several hours until the required level of hydrolysis is achieved (Pasupuleti and Braun, 2008). In contrast to acid hydrolysis, tryptophan is unaffected by alkaline hydrolysis, which destroys other amino acids including serine and threonine (Pasupuleti and Braun, 2008). Alkaline treatment of proteins can adversely affect protein integrity by causing amino acid racemization, cysteine degradation, formation of abnormal amino acids, and inhibition of enzymatic hydrolysis. (Elavarasan, 2019). Protein hydrolysis using alkali, mainly sodium hydroxide, frequently leads to poor functioning and, more significantly, can have a negative impact on the hydrolysate's nutritional content. Nevertheless, the food sector uses limited alkali treatment to recover and solubilise a variety of proteins (Kristinsson and Rasco, 2000).

Enzymatic hydrolysis

For simple acceptance in the food business, protein is extracted enzymatically under regulated pH settings without sacrificing its nutritious value. En-

zymes are used to break down the proteins in fish flesh or processing waste to create fish protein hydrolysate (FPH). Enzymatic hydrolysis is more popular as it offers greater control over both the process and the end product's quality. The process typically lasts from a few hours under controlled and mild conditions. These conditions generally include moderately elevated temperatures, ranging from 35°C to 65°C, and a pH level optimized according to the specific enzymatic system employed. It is preferable to grind the raw material i.e fish and its by-products (waste) and mix with water (2:1 w/w) before being moved to the reactor vessel and heated to the proper temperature in the enzymatic pre-treatment step. FPH should have a well-controlled fat concentration (<0.5% w/w) since a larger fat level might cause lipid oxidation leading to darkening of the finished goods, resulting in brown colours (Siddik *et al.*, 2021). The enzymes, originating from animal, plant, or microbial sources, are utilized for the production of fish protein hydrolysates (FPH). The kinetics of an enzyme-catalyzed reaction are greatly influenced by temperature and pH, with each enzyme responding uniquely to these conditions. Enzymes typically exhibit optimal activity within a specific range of temperature and pH. Deviations from these optimal conditions can lead to enzyme denaturation, resulting in a loss of activity (Vázquez *et al.*, 2004). Hydrolysis is performed at low pH using animal-derived enzymes such as pepsins, while neutral conditions of hydrolysis involves plant-based enzymes like papain and bromelain, or microbial enzymes such as alkalase, neutrase, and flavorzyme. Hydrolysis in alkaline condition is primarily conducted using microbial enzymes like alkalase. Microbially derived enzymes are thought to have better stability in terms of pH and temperature (He, Franco and Zhang, 2013). Enzymatic hydrolysis of peptide bonds occurs under moderate conditions, offering greater precision and easier regulation. This method does not compromise nutritional value or produce adverse effects, while also facilitating efficient protein recovery and peptide purification. (Tavano, 2013). However, enzymatic hydrolysis of proteins is a complicated process because of the large number of peptide bonds and their unique susceptibility to enzymatic reactions. The peptide profile of the finished product is influenced by a number of parameters, including pH and temperature, in addition to enzyme specificity.

The following chart (Table 1) provides a compre-

hensive summary of various studies on the enzymatic production of fish protein hydrolysates (FPH), highlighting the different fish parts, enzymes, and optimized conditions used by researchers. By compiling data from multiple authors, this summary serves as a valuable reference for understanding the key factors affecting FPH production and optimizing hydrolysis conditions for improved efficiency.

Characterization of FPH: The FPH produced is further characterized for their physical and biological properties. The Table 2 provides comprehensive summary of different functional properties of the fish hydrolysate presented by different researchers. These properties mainly include solubility, water holding capacity, oil holding/binding capacity, emulsifying properties foaming capacity etc. Protein structure, including amino acid sequence, molecular weight, shape, and charge distribution, affects its functional capabilities (Casarin, *et al.*, 2008). FPH showed notable distinctions in its physicochemical and other functional features, outperforming FPC in terms of functionality. The FPH exhibited favourable bulk density, emulsion, protein digestibility, solubility, colour, and microstructure.

Solubility

The solubility of peptide fractions was observed to be influenced by the pH of the solvent, with minimal solubility recorded under acidic conditions. However, as the pH increased, no significant variation in solubility was noted (González-Serrano *et al.* 2022). Influence of pH was also seen in Protein Hydrolysate from Ribbon Fish (*Lepturacanthus Savala*). There was a minimal solubility at pH 4 and a maximum at pH 6–8 (Yathisha *et al.*, 2022). It is possible to attribute variations in the solubility of the peptide fractions to both surface hydrophobicity, which promotes aggregation by hydrophobic contact, and peptide net charge, which rises as pH moves away from the isoelectric point (Noman *et al.*, 2018; González-Serrano *et al.*, 2022). The increased solubility of hydrolysed protein's is also caused by its reduced molecular size as compared to the intact protein as well as by additional carboxylic and amine groups from amino acids that make the hydrolysate more hydrophilic (Dos Santos *et al.*, 2011).

Foaming properties

The foam capacity (FC) and foam stability (FS) of the isolated peptide fractions are the two major parameters for the foaming characteristic of proteins and

Table 1. Summary of enzymatic fish protein hydrolysate preparation

Fish species	Raw materials	Enzyme used for FPH Production	Optimal conditions used	References
	Filletts	Alcalase	50 °C, pH 6 and 90 min	(Dos Santos <i>et al.</i> , 2011).
Bluewing searobin (<i>Prionotus punctatus</i>)		Flavourzym and 90 min	50 °C, pH 5	
Pink perch (<i>Nemipterus japonicus</i>)	Muscle	Trypsin	37 °C, pH 8.8 and 240 min	Naqash and Nazeer, 2013
		Pepsin	37 °C, pH 2 and 240 min	
		Papain	37 °C, pH 6 and 240 min	
Indian mackerel (<i>Rastrelliger kanagurta</i>)	Fish backbone	Papain	37 °C, pH 6,360 min	(Sheriff <i>et al.</i> , 2014)
Fresh water carp (<i>catla catla</i>)	Muscle	Pepsin	37 °C,	pH 2, 360 min
		Alcalase	60 °C, pH 9, 30min	(Elavarasan, Naveen Kumar and Shamasundar 2014)
		Bromelain	50 °C, pH 6.5, 60 min	
		Flavorzyme	50 °C, pH 6.5, 75 min	
		Protamex	50°C, pH 6.5, 60 min	
Sardine (<i>Sardina pilchardus</i>)	Whole fish	Subtilisin	50°C, pH 8, 120 min	(García-Moreno <i>et al.</i> , 2014)
		Trypsin		
Horse mackerel (<i>Trachurus mediterraneus</i>)	Whole fish	Subtilisin	50°C, pH 8, 120 min	(García- Moreno <i>et al.</i> 2014)
Seabream (<i>Pagellus acarne</i>)	Whole fish	Trypsin	50°C, pH 8, 120 min	
		Subtilisin		
Bogue (<i>Boops boops</i>)	Whole fish	Trypsin	50°C, pH 8, 120 min	
		Subtilisin		
Catshark (<i>Scyliorhinuscanicula</i>)	Whole fish	Trypsin	50°C, pH 8, 120 min	
		Subtilisin		
Rainbow trout (<i>Oncorhynchus mykiss</i>)	Viscera	Pepsin	37°C, pH 3, 90 min	(Wald <i>et al.</i> , 2016)
	Muscle	Crude enzyme preparation from the fungus <i>P. digitatum</i>	55°C, pH 7, 180 min.	(Da Rocha <i>et al.</i> , 2018)
Red scorpionfish (<i>Scorpaena notata</i>)				
<i>Secutor insidiator</i> (Pugnose ponyfish)	Whole fish	Papain	50 °C, pH 7.0, 90 min	(Dinakarkumar <i>et al.</i> , 2022)
Asian swampeel (<i>Monopterus albus</i>)	Muscle	Proteinase, Alcalase enzyme	37 °C, pH 7.0, 120 min 40°C, pH 8.5, 120 min 60°C, pH 9.5, 180 min 60°C, pH 10.5, 300 min	(Baharuddin, Halim and Sarbon, 2015)
Chinese sturgeon (<i>Acipenser sinensis</i>)	Whole fish	Papain	50 °C pH 5.5, 6, 6.5, and 7, temp 35, 40, 45, time in min 15, 30, 60, and 120	Noman <i>et al.</i> , 2018
Argentine croaker (<i>Umbrina canosai</i>)	Protein mycrofybrills	Alcalase	50°C, pH 8,90 min	(Da Rocha <i>et al.</i> 2018)
Rohu (<i>Labeo rohita</i>)	Viscera	Protamex Alcalase	50 °C, pH 7.0,90 min pH 8 (35 °C, 45 °C and 55 °C), time (30 min, 60 min and 90 min	Mohanty <i>et al.</i> , 2021
Yellowfin tunatrimmings (<i>Thunnus albacares</i>)	Whole fish	Alcalase enzyme	50 °C, pH, 8.0300 min	(Cai <i>et al.</i> , 2022)
Ribbon Fish (<i>Lepturacanthus Savala</i>)	Viscera	Alcalase enzyme	50 °C, pH, 8.590 min	Yathisha <i>et al.</i> , 2022

Table 2. Functional properties of fish protein hydrolysate

Fish species	Functional properties	Range of activities	References
Bluewing sea robin (<i>Prionotus punctatus</i>)	Solubility	10-40 %	(Dos Santos <i>et al.</i> , 2011)
	Water holding capacity	2.37 g water g _{protein} ⁻¹	
	Oil holding capacity	3.22-5.22 mL oil g _{protein} ⁻¹	
Pink perch (<i>Nemipterus japonicus</i>)	Emulsifying activity index	55-124 m ² g solid ⁻¹	(Naqash and Nazeer, 2013)
	Solubility	25-70 %	
Fresh water carp (<i>catla catla</i>)	Emulsifying	25-75%	(Elavarasan <i>et al.</i> , 2014)
	Foaming	75-130%	
Asian swamp eel (<i>Monopterus albus</i>)	water holding capacity	6-8 ml/g	(Baharuddin <i>et al.</i> , 2015)
	Solubility	80-90 %	
Skipjack Tuna (<i>Katsuwonus pelamis</i>)	foaming properties	10-50%	(Klomkiao and Benjakul, 2018)
	Solubility	91-100%	
	Emulsifying	20-38 m ² /g	
Chinese sturgeon (<i>Acipenser sinensis</i>)	Foaming	100-140%	(Noman <i>et al.</i> , 2018)
	Solubility	85-95%	
	Emulsifying	90-95%	
Rohu (<i>Labeo rohita</i>)	Oil binding	2.59%	Mohanty <i>et al.</i> , 2021
	Foaming	76%	
Ribbon Fish (<i>Lepturacanthussavala</i>)	Emulsifying	11-29 %	(Yathisha, Vaidya and Sheshappa, 2022)
	Foaming	72-106 %	
Secutor insidiator (<i>Pugnose ponyfish</i>)	Solubility	20-80 %	(Dinakarkumar <i>et al.</i> , 2022)
	Oil holding capacity	2-6 g/g	
	Emulsifying	15-85 m ² /g	
Common carp (<i>Cyprinus carpio</i>)	Oil binding	0.2-0.6 ml/g	(González-Serrano <i>et al.</i> 2022)
	Foaming	10-95 %	
	Solubility	80-100%	
	Emulsifying	55-160 m ² /g	
	Foaming	60-150%	

peptides. Three aspects drive foam formation: the transportation, penetration, and restructuring of molecules at the water–air interface. A protein with a good foam forming characteristic is capable of moving swiftly through the water–air contact, unfolding, and rearranging there (González-Serrano *et al.*, 2022). Fish protein hydrolysates (FPHs) possess excellent foaming and emulsifying properties, making them suitable for use as emulsifying and stabilizing agents in various products. They can aid in the formation and stabilization of foam-based formulations across a range of applications (Nalinanon *et al.*, 2011). The literature shows foaming properties ranging from 10-150% varying depending on the fish, the extraction conditions and mainly the molecular size of the hydrolysates. Smaller size peptides give better solubility and foaming, however, too small frac-

tions result in reduced foaming (Kristinsson and Rasco, 2000; Panyam and Kilara 1996; Damodaran and Parkin 2017).

Emulsifying

The emulsifying capacity of protein hydrolysates is closely associated with the ability of peptides to lower interfacial tension between hydrophobic and aqueous phases within food systems (Dos Santos *et al.*, 2011). Solubility, Degree of Hydrolysis (DH), and enzyme specificity are the main determinants of emulsifying qualities because they affect the molecular size and hydrophobicity of peptides produced by hydrolysis (Baharuddin *et al.*, 2015). The literature shows that the emulsifying property is highest in common carps and sturgeons. The study shows that the modification of *Acipenser surgeon* pro-

tein with metal-phenolic networks enhances its antioxidant and emulsifying properties (Chen *et al.* 2025). Similarly, common carp has high emulsifying activity index i.e the ability to form and stabilize an emulsion, which makes them suitable candidate in food processing (Ullah *et al.*, 2014; González-Serrano *et al.*, 2022).

Biological Activity

The broad and powerful biological activities of fish protein hydrolysates (FPH) have garnered significant scientific attention, positioning them as useful tools for disease prevention and health promotion. Many researchers have efficiently proven the antioxidant qualities of FPH. Peptides with substantial free radical scavenging activity, metal-chelating ability, and lipid peroxidation inhibition are among the processes that help reduce oxidative stress and the diseases that are linked to it (Samaranayaka and Li-Chan, 2011). Apart from its antioxidative properties, FPH has notable antihypertensive effects, mainly by inhibiting the angiotensin-I converting enzyme (ACE), which is essential for controlling blood pressure (Mendis *et al.*, 2005). Numerous peptides produced from FPH exhibit multifunctionality, frequently combining anti-inflammatory, antihypertensive, and antioxidant properties in a single preparation (Kemp and Kwon, 2021a; Barrios-Rodríguez *et al.*, 2022). Their ability to manage chronic illnesses and advance general health is facilitated by these synergistic activities (Chalamaiah, Yu and Wu, 2018). FPH are therefore becoming more widely acknowledged as beneficial ingredients in pharmaceutical formulations, nutraceuticals, and functional foods (Zhang and Zhao, 2017). The table 3 summarizes the different biological activities proposed by various authors based on the fish parts of different fish species used.

Antioxidant property

Among the various categories of bioactive peptides, antioxidant peptides have been the subject of the most extensive scientific investigation. These peptides contribute to oxidative stability by disrupting free radical chain reactions, thereby inhibiting both enzymatic and non-enzymatic oxidation pathways. Typically consisting of short chains containing 3 to 16 amino acid residues, antioxidant peptides often feature amino acids such as histidine, tyrosine, methionine, cysteine, tryptophan, and lysine. Notably, these residues exhibit inherent antioxidant proper-

ties even in their unbound, free forms (Sánchez and Vázquez, 2017). Changes in microbial growth and lipid oxidation are the most significant markers of food deterioration. Food oxidation has an impact on carbs, proteins, and fats. But the primary factor degrading food quality is lipid oxidation, which causes rancidity and shortens shelf life (Di Bernardini *et al.* 2011). Using antioxidants is the most often used therapy to slow down the processes that cause food to get rancid. Their impact on the direction of food oxidation might vary greatly depending on the structure. According to reports, the antioxidant peptides possess metal-chelating or hydrogen/electron-donating properties that can stop a chain reaction or stop free radicals from forming (Nwachukwu and Aluko 2019). Among the literature cited, different parts of the fishes including their scales, wastes, bones have exhibited the antioxidant properties. The activity differs depending on various factors such as the sours, hydrolysis conditions, peptide size, amino acid composition, method of extraction and the type of assay (Chalamaiah *et al.*, 2015).

Angiotensin-I converting enzyme (ACE) inhibitory activity

The activity of the angiotensin I converting enzyme (ACE), which cleaves the C-terminal of the histidyl-leucine of the decapeptide angiotensin I to form the octapeptide angiotensin II, is intimately linked to hypertension as strongly acting vasoconstrictor. ACE inhibition is therefore a successful therapeutic strategy for the treatment of hypertension. For a peptide to have ACE-inhibition action, it must have a hydrophobic amino acid at either of its terminals, namely phenylalanine, proline at the C-terminal and isoleucine, valine at the N-terminal (Abachi, Bazinet and Beaulieu, 2019). The peptides' molecular weight, chain length, and molecular interaction are closely correlated with their display of antihypertensive activity. In line with previous research, the smaller, lower molecular weight peptides had a greater ACE inhibitory capacity than the bigger, higher molecular weight peptides (Ghassem *et al.* 2011; Lin, Lv and Li, 2012). There are few clinical studies showing the effect of fish protein hydrolysates as antihypertensive ((Vázquez *et al.*, 2020) 2019).

Antimicrobial activity

The ability of peptides to interact with bacterial membranes is influenced by several factors. While

Table 3. Bioactive peptides and bioactivity of Fish protein hydrolysate

Fish species	Source	Bioactivity	References
Pink perch (<i>Nemipterus japonicus</i>)	Muscle	Antioxidant	Naqash and Nazeer, 2013)
Indian mackerel (<i>Rastrelliger kanagurta</i>)	Fish backbone	Antioxidant	(Sheriff <i>et al.</i> , 2014)
Fresh water carp (<i>catla catla</i>)	Muscle	Antioxidant	(Elavarasan <i>et al.</i> , 2014)
Sardine (<i>Sardina pilchardus</i>)	Whole fish	Antioxidant	(García-Moreno <i>et al.</i> , 2014)
Horse mackerel (<i>Trachurus mediterraneus</i>)	Whole fish	Antioxidant	(García-Moreno <i>et al.</i> , 2014)
Seabream (<i>Pagellus acarne</i>)	Whole fish	Antioxidant	(García-Moreno <i>et al.</i> , 2014)
Bogue (<i>Boops boops</i>)	Whole fish	Antioxidant	(García-Moreno <i>et al.</i> , 2014)
Catshark (<i>Scyliorhinus canicula</i>)	Whole fish	Antioxidant	(García-Moreno <i>et al.</i> , 2014)
Turbot (<i>Scophthalmus maximus</i>)	Fish fillet	Antioxidant	(Vázquez <i>et al.</i> , 2020)
Rohu (<i>Labeo rohita</i>)	Roes	Antihypertensive Antiproliferative ACE-inhibitory	(Chalamaiah <i>et al.</i> , 2015)
Sardinelle (<i>Sardinella aurita</i>)	Head and viscera	(ACE) inhibitory	(Bougatef <i>et al.</i> 2008)
Tuna fish (<i>Thunnus tonggol</i>)	Whole	Antiproliferative	(Hsu, Li-Chan and Jao, 2011)
Rainbow trout (<i>Oncorhynchus mykiss</i>)	Viscera	Antibacterial	(Wald <i>et al.</i> 2016)
Asian swampeel (<i>Monopterus albus</i>)	Muscle	Angiotensin-I converting enzyme (ACE) inhibitory activity	(Baharuddin <i>et al.</i> , 2015)
Red scorpionfish (<i>Scorpaena notata</i>)	Muscle	Antioxidant angiotensin-I converting enzyme (ACE) inhibitory activity	(Aissaoui <i>et al.</i> 2017)
Argentine croaker (<i>Umbrina canosai</i>)	Protein myofibrils	Antimicrobial, Anti-inflammatory Antioxidant	(Da Rocha <i>et al.</i> , 2018)
Milkfish (<i>Chanos chanos</i>) scales		Anti-inflammatory Antioxidant	(Chen <i>et al.</i> , 2018)
(<i>Engraulis encrasicolus</i>)	Waste	Anchovy Anti-inflammatory, Antioxidant	(Giannetto <i>et al.</i> , 2020)
Common carp (<i>Cyprinus carpio</i>)	Whole fish	Antioxidant activity	(González-Serrano <i>et al.</i> 2022)
Yellowfin tuna trimmings (<i>Thunnus albacares</i>)	Whole fish	Antioxidant activity	(Cai <i>et al.</i> 2022).
Ribbon Fish (<i>Lepturacanthus savala</i>)	Viscera	Antioxidant angiotensin-I converting enzyme (ACE) inhibitory activity	(Yathisha <i>et al.</i> 2022)

molecular weight may affect solubility and the tendency to form aggregates, the primary determinants of effective membrane contact are the exposure of specific amino acids, particularly those with positive charges, and the acquisition of appropriate secondary structure. This enables peptides to bind to and disrupt the bacterial membrane, with molecular weight playing a secondary role compared to sequence, charge, and structure (Najafian and Babji, 2012). Antimicrobial peptides are short chains typically composed of fewer than 50 amino acids, with a significant proportion—approximately 50%—be-

ing hydrophobic. These peptides usually have a molecular weight below 10 kDa. Their net positive charge promotes initial binding to the negatively charged bacterial cell membranes, thereby facilitating membrane penetration by peptides such as hydrolysate-derived antimicrobial agents (Da Rocha *et al.*, 2018). The extent of protein hydrolysis (DH%) has been shown to have a substantial impact on antibacterial performance, with notable enhancements in inhibitory activity against food-related contaminants and bacteria relevant to aquaculture observed in the initial phases of the hydrolysis process (Wald *et al.* 2016).

Anti-inflammatory activity

Fish protein hydrolysate has strong anti-inflammatory properties, suggesting that it could be used as a bioactive substance to control inflammatory reactions. Its importance as a functional element in the management of inflammation-related disorders is supported by the existence of bioactive peptides that can influence major inflammatory pathways, which may be responsible for the reported effects (Kemp and Kwon, 2021b). The immune system's reaction to external stimuli is inflammation, which restores normal functionality. ROS can either directly cause inflammation or indirectly do so by influencing inflammatory activity through cytoplasmic proteins (Chen *et al.*, 2018). The inflammatory pathway's protein expression analysis revealed that Anchovy (*Engraulis encrasicolus*) hydrolysate treatment significantly reduced the expression of pro-inflammatory mediators like COX-2, stopped I κ B- α from degrading, and inhibited NF- κ B's nuclear translocation in response to LPS-induced inflammation (Giannetto *et al.*, 2020). The salmon byproduct peptide PAY, which reduced NO generation in macrophages activated by LPS. At a dosage of 0.75 mM, the authors also observed that PAY inhibited pro-inflammatory cytokines such TNF- α , IL-6, and IL-1 β (Ahn, Cho and Je, 2015).

Conclusion

In recent years, enzymatic hydrolysis has emerged as the preferred method for producing fish protein hydrolysates (FPH). As highlighted in this review, the quality and functional characteristics of FPH are significantly influenced by the specific preparation techniques employed. FPH exhibits broad applicability due to its rich content of essential amino acids and bioactive peptides. It is utilized in various forms—ranging from nutritional supplements and food additives with emulsifying and foaming control properties to antioxidant agents and nutraceutical products. One of the most promising and practical applications of FPH is its use as a sustainable feed additive in fishmeal production. Since conventional fishmeal often relies on fresh fish that could otherwise be used for direct human consumption, utilizing fish processing by-products for hydrolysate production offers a more environmentally and economically sustainable alternative. Given their multifunctional benefits, FPHs hold consider-

able potential for enhancing the quality and innovation of food products across multiple sectors. Continued research focused on refining processing parameters may further expand their role in meeting modern consumer demands for nutritious, flavorful, and sustainable food solutions.

Conflict of Interest - None

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