Antibacterial activity of functional bioactive peptides derived from fish protein hydrolysate

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Abstract. By-product removal in fish processing is estimated to be between 25 and 70% due to improper fish production handling and significant problems in the fish industry today. Therefore, one of the ways to manage the raw material of by-product is through protein hydrolysis. However, one of the most effective methods for managing this raw material, which includes skin, bones, heads, and viscera, is to convert their protein into peptides via hydrolysis methods, resulting in fish protein hydrolysate (FPH). FPH has been shown to have bioactive properties such as antibacterial, antihypertensive, antioxidative, anticancer, and anticoagulant properties. Bioactivity could be fully utilised in the future in both the nutraceutical and food industries. Numerous studies have been published on the acceptability of FPH in obtaining bioactive properties from various fish, particularly antibacterial activity. For example, the antibacterial peptide was identified as FPIGMGHGSRPA, consisting of 12 amino acids. Its antibacterial activity was tested against B. subtilis using 800 g/mL ampicillin. The inhibition zone increased with peptide concentration. This review discusses functional bioactive peptides derived from fish protein hydrolysate that can be used as antibacterial agents by inhibit Gram-positive and Gram-negative bacterial growth. It also covers fish species, parts, and hydrolysis methods to maximise yields.

Keywords: antibacterial activity; bioactive peptides; fish protein hydrolysate; food industry; different fish species

1. Introduction

In 2025, many countries worldwide the world's fish production is expected to be around with a total of 196 million tonnes of fish processed [1]. As a result, fish processing from by-products ranges from 25 to 70% of heads, skins, viscera, backbone, trimmings, and blood [2]. Malaysia's annual waste disposal is estimated to be around 20 million tonnes, accounting for approximately 25% of total production [3]. The majority of fish waste is dumped, with no effort made to reduce the harmful effects on the environment, which will also cause disposal issues. To solve the problem, effective methods for converting fish by-product wastes into essential nutrients that can improve human health and nutritional value, such as producing Fish Protein Hydrolysate (FPH), are required [4,5]. Furthermore, FPH has been reported to have a wide range of bioactive properties such as antibacterial activity, antihypertensive, antioxidant, anticancer, and anticoagulant, which could be fully utilised in the nutraceutical and food industries [5].
Furthermore, the crude protein content of fish by-products ranges from 8 to 35% and can be used as a source of collagen, gelatin, polyunsaturated lipids, enzymes, and essential amino acids [1]. According to Petrova et al. [6], FPH is a product made from fish by-products using a protein hydrolysis method, which involves the breakdown of protein into a minor part of peptide and then into amino acid. Compared to raw protein, the protein hydrolysate produced contained peptides and amino acids that were easily absorbed [7]. Several methods have been used to extract proteins and peptides from fish by-products, including acidic or alkaline hydrolysis, bacterial fermentation, and enzymatic hydrolysis. There are various advantages; enzymatic and chemical hydrolysis is the most commonly used methods [8]. Ennaas et al. [9] reported using protamex protein hydrolysate, which successfully hydrolyses four antibacterial peptides (SIFIQRFTT, RKSGDPLGR, AKPGDGA, and GLPGPLPAGPK) derived from marine organisms. Furthermore, Bi et al. [10] discovered an antibacterial peptide Sm-A1 (GITDLRGM-KRLKKMK) from turbot (Scophthalmus maximus) that has excellent antibacterial activity against both Gram-positive and Gram-negative bacteria by compromising cell membrane integrity. The bioactive antibacterial activity obtained from FPH inhibits the growth of microorganisms, which may increase the life expectancy of a product. This review discusses the acceptability of FPH in obtaining bioactive properties from various fish and parts, using a different method of hydrolysis to obtain bioactive properties. This review also discussed the potential of bioactive antibacterial peptides that function as antibacterial agents by inhibiting the growth of Gram-positive and Gram-negative bacteria.

2. Source of fish protein hydrolysate (FPH)

2.1. Fish species and parts of fish

Numerous studies have successfully confirmed the antibacterial activity of FPH, such as by using tilapia (Oreochromis niloticus), half-fin anchovies (Setipinna taty), and sardin (Sardinella aurita) as the main fish that has been used as antibacterial activity [11-14]. While Barbel (Barbus callensis), Japanese eel fish (Anguilla japonica) silver grouper fish (Hypophthalmichthys molitrix), grouper fish (Ctenopharyngodon idella), Atlantic inflatable fish (Scomber scombrus), and Atlantic cod fish (Morhua girl) also main selection in producing of antibacterial activity through antibacterial peptides. The second preferred fish in the study of antibacterial activity is the large-headed fish (Hypophthalmichthys nobilis) and shark hound (Mustellus mustellus) [9, 15, 16]. Thus, other fish species used in antibacterial activity include tuna (Thunnini) [17], barb fish (Barbonymus schwenfeldii) [18], anchovies (Engraulis japonicus) [19] and rainbow trout (Oncorhynchus mykiss) [20]. Bioactive peptides can be obtained from various fish parts, including the muscle, skin, viscera, and bone. However, the mucous or fluid layer is the primary source of antibacterial peptides. For effective use, the target part of fish sections must be considered [21]. Antibacterial peptides are found primarily in the mucous layer and prevent pathogenic bacteria from entering the skin layer, such as the hagfish (Eptatretus burgeri) [22]. Furthermore, antibacterial peptides also were successfully isolated pleurocidsins from the mucus of the winter flounder (Pleuronectes americanus), American plaice fish, (Hippoglossoides platessoides), and Atlantic halibut fish (Hippoglossus hippoglossus), have been discovered in several studies [23].

2.2. Hydrolysis methods efficiency

FPH can be produced using various techniques, including chemical hydrolysis (acid and alkaline hydrolysis), bacterial fermentation, and enzymatic hydrolysis. A specified chemical agent breaks the connection between different groups of peptides in the order of protein during the chemical hydrolysis process. This procedure is quick and produces a high level of protein recovery [6]. However, because this procedure is carried out under rather intense work conditions (high acid or alkaline concentration and high temperature), the hydrolysis process is nearly uncontrollable. Furthermore, there is minimal control over the uniformity of the hydrolyzed models, with significant fluctuations in the free amino acid profile as a result of non-specific peptide bond breaking. [8]. The hydrolysis of fish proteins produced via chemical hydrolysis has a relatively limited range of applications. Hence, for bacterial fermentation, hydrolysis is a procedure the growth of lactic acid bacteria produce acid and antimicrobial
substances that interrupt competing bacteria; however, this method does not allow for the removal of lipids. [24]. Enzymatic hydrolysis is a widely used technology that uses a shorter reaction time to produce precise hydrolysates while keeping the nutritional content of the source protein by concentrating on specific peptide bonds and amino acids that exhibit optimal activity under particular conditions [4]. Each step of the process involves substrate preparation, enzyme selection, determining the level of enzymatic hydrolysis, uniformity and thermal to inactivate endogenous enzymes, hydrolysis, and termination [21]. Numerous application enzymes are routinely used in enzymatic hydrolysis, including alcalase, neutrase, papain, pepsin, and trypsin [6]. During hydrolysis, conditions such as enzyme concentration, pH, time, and temperature must be tightly controlled and maintained and vary according to the type of enzyme used. Enzyme concentrations ranging from 0.01 to 5.00 % (w/w) and pH values ranging from 1.5 to 11 have been determined [21]. The final enzymatic hydrolysis products do not include any leftover organic solvents or hazardous compounds [25]. According to table 1, many fish species and parts contain protein hydrolysate. They discovered several hydrolysis methods and antibacterial activity by correlating Gram-positive and Gram-negative bacteria.

3. Potential bioactive antibacterial peptides
Antibacterial peptides are amino acid chains of less than 10 kDa and 50 amino acids, nearly half of which are hydrophobic. The charge or hydrophobic ratio of this antibacterial cationic peptide can also change its activity [10]. These peptides can form pores or block membrane ion gradients, causing bacterial cell death. Furthermore, some peptides can cause bacterial depletion without membrane lysis by altering cell metabolism [20]. Furthermore, it can be used as an antibacterial peptide that is possible against Gram-negative and Gram-positive bacteria and subsequently used to make modern antibiotics and antibacterial agents for the food industry [44].

Antibacterial peptides from FPH were analysed using the ‘Agar well diffusion method using Gram-negative or positive strain [26]. Recently, Bi et al. [10] have investigated Sm-A1, Sm-A2 and Sm-A3 are positively charged peptides from the turbot fish (Scopthalmus maximus) that inhibit Gram E. coli, S. aureus, and B. subtilis, and formation of biofilm very well through assessment of salmon fish preservation. For instance, an antibacterial peptide from fish waste using membrane ultrafiltration resulted that the lowermost molecular weight fraction (< 3 kDa) had the highest (P < 0.05) percentage of bacteria inhibition against pathogenic Gram-positive (Listeria and Staphylococcus) and Gram-negative (E. coli and Pseudomonas) [44] significantly. According to Aissaoui et al., [38], the peptide with the highest antibacterial activity was identified, resulting in the sequence FPIGMHGSRPA, consisting of 12 amino acids. Its antibacterial activity was tested against the B. subtilis strain using ampicillin at an 800 μg/mL concentration. It was discovered that as the amount of peptide increased, so did the inhibition zone increase. The structure of the activity has been studied, and it appears that the positively charged amino acids will bind to the negatively charged molecule on the pathogen’s membrane, resulting in the formation of a pore that will decompose or damage the pathogen membrane. Furthermore, the antibacterial activity of hound shark (Mustellus mustellus) from peptides SHVH-E9, SHVH-EE, and SHVH-P were successfully against M. luteus [45]. In addition, the mucous crust of the large-headed grouper (Hypophthalmichthys nobilis) has antibacterial activity against S. epidermidis and E. coli [16]. The Argentine croaker protein antibacterial peptide (Umbrina canosa) showed inhibition of Gram-positive such as L. innocua, L. monocytogenes and S. aureus, and Gram-negative, followed by Gram-negative A. hydrophilia and Y. enterocolitica. Interestingly, this hydrolysate does not show inhibition of some microorganisms such as probiotics, namely B. bifidum, L. acidophilus and L. helviticus. Thus, this hydrolysate can be used in food formulations containing this microorganism [26]. Hence, the peptide sequences SIFIQRFTT, RKSHPDLGR, AKPGAGSGPR, and GLPGPLPGAPPK inhibited E. coli and L. innocua [9]. In addition, collengin also is an antifungal peptide of inflatable fish protein hydrolysis [46].
Table 1. Summarises study of various fish species producing antibacterial activity by inhibiting the growth of gram positive and gram negative bacteria.

| Fish species               | Type of ecosystem | Part     | Hydrolysis                  | Tested Bacterial Strain                          | Country  | References |
|----------------------------|-------------------|----------|-----------------------------|--------------------------------------------------|----------|------------|
| Tuna                       | Saltwater         | Viscera  | Papain                      | Gram (-) E. coli, S. Tipymirium, V. parahaimoliticus | Indonesia| [17]       |
| Turbot \((Scopthalmus maximus)\) | Saltwater         | Viscera  | Trypsin and pepsin          | Gram (+) B. subtilis, Listeria monocytogens, S. aureus, Gram (-) E. coli, Salmonella Typhimurium | China    | [10]       |
| Pacific chub mackerel \((Scomber japonicus)\) | Saltwater         | Fin      | Papain, pepsin, trypsin and Protease Alcalase and protamex | Gram (-) E. Coli, Salmonella                      | Sri Lanka| [26]       |
| Argentine croaker \((Umbrina canosai)\) | Saltwater         | Muscle   | Alcalase, flavourzyme and trypsin | Gram (+) B. thermosphacta, L. innocua, L. monocytogenes, S. aureus, Gram (-) A. hydrophila, Y. enterocolitica | Brazil   | [27]       |
| Yellowfin tuna \((Thunnus albacares)\) | Saltwater         | Muscle   | Pepsin                      | Gram (+) S. aureus, Gram (-) E. coli             | Italy    | [28]       |
| Silver carp \((Hypophthalmichthys molitrix)\) | Freshwater        | Muscle   | Papain, alacase, flavourzyme and trypsin | Gram (+) S. aureus, Gram (-) E. coli              | China    | [29]       |
| Tuna fish \((Sardinella aurita)\) | Saltwater         | Muscle   | Protease from Bacillus subtilis A26 | Gram (+) S. aureus B. cereus, Micrococcus luteus, Enterococcus faecalis | Tunisia  | [30]       |
| Fish species                           | Type of ecosystem | Part                  | Hydrolysis                      | Tested Bacterial Strain                                                                 | Country | References |
|---------------------------------------|-------------------|-----------------------|---------------------------------|----------------------------------------------------------------------------------------|---------|------------|
| Short nosed tripod fish (Triacanthus biaculeatus) | Saltwater         | Muscle                | Methanol and acetone            | Gram (-) *E. coli*, *Pseudomonas aeruginosa*, *K. pneumoniae*, *S. enterica*, *S. typhi* | India   | [31]       |
| Carp (Hypophthalmichthys nobilis, Ctenopharyngodon idella, Cyprinus carpio) | Saltwater         | Skin mucus            | Alkaline-Sodium chloride        | Gram (+) *Micrococcus Leteus*, Gram (-) *Edwardsiella tarda*, *Aeromonas sp.*, *A. hydrophila* | India   | [32]       |
| Giant mudskipper (Periophthalmodon srxlosseri) | Brackish water    | Skin mucus            | Ethanol                         | Gram (-) *Proteus mirabilis*, *P. aeruginosa*, *E. coli*, *S. aureus*, *S. typhi*, *Vibrio cholerae*, *B. anthracis*, *K. pneumoniae* | India   | [33]       |
| Anchovy (Engraulis japonicus)         | Saltwater         | Whole part            | Enzymatic with protamex        | Gram (+) *S. aureus*, *B. subtilis*, *S. pneumoniae*                                 | China   | [19]       |
| Nile Tilapia (Oreochromis niloticus)  | Freshwater        | Head, frame, fin, belly flap meat, Alkaline followed by | Gram (+) *Listeria monocytogene*, *S. aureus*                                         |          | Thailand [12] |
| Fish species | Type of ecosystem | Part | Hydrolysis | Tested Bacterial Strain | Country | References |
|--------------|------------------|------|------------|-------------------------|---------|------------|
| Indian major carp *(Cirrhinus mrigala)* | Saltwater | Skin mucus | Acetic acid | Gram (-) *S. Typhimurium, E. coli*, Gram (+) *S. aureus* | India | [34] |
| Half-fin anchovy *(Setipinna taty)* | Saltwater | Whole part | Pepsin | Gram (-) *E. coli* | China | [14] |
| Oblong Blowfish *(Takifugu oblongus)* | Saltwater | Skin, Muscle, Liver and Gonads | Acetic acid | Gram (-) *E. coli, Pseudomonas aeruginosa, S. aureus, K. pneumoniae, B. subtilis* | India | [35] |
| Atlantic mackerel *(Scomber scombrus)* | Saltwater | Viscera, digestive gland, stomach gonads, heart, intestines, liver and spleen | Protamex | Gram (+) *Listeria innocua* | Canada | [9] |
| Indian major carps *(Labeo rohita, Catla catla)* Chinese carps *(Hypophthalmichthys molitrix, Ctenopharyngodon idella)* | Saltwater | Mucus | Trypsin | Gram (-) *Aeromonas hydrophila, Aeromonas sobria, Pseudomonas fluorescens, Vibrio anguillarum* | Bangladesh | [15] |
| African catfish *(Clarias gariepinus)* | Saltwater | Gill, suprabranchial organ and viscera | Protease K, pepsin and trypsin | Gram (+) *S. aureus* | United State | [36] |
| Fish species                       | Type of ecosystem | Part                                                                 | Hydrolysis                        | Tested Bacterial Strain                                      | Country   | References |
|-----------------------------------|-------------------|----------------------------------------------------------------------|-----------------------------------|---------------------------------------------------------------|-----------|------------|
| Japanese eel (Anguilla japonica)  | Saltwater         | Skin mucus, gill, kidney, liver and spleen                          | Acid-acetic acid; and acetone     | Gram (-) Edwardsiella tarda, Aeromonas sp., A. hydrophila, Micrococcus Leteus | China     | [37]       |
| Small red scorpionfish (Scorpaena notata) | Saltwater         | Viscera                                                              | Protease from *Trichoderma harzianum* | Gram (+) B. cereus, B. subtilis, Staphylococcus aureus | Tunisia   | [38]       |
| Big Head Carp (Hypophthalmichthys nobilis) | Fresh water       | Skin mucus                                                           | Sodium chloride                   | Gram (+) S. aureus, S. epidermidis and B. Cereus             | India     | [16]       |
| Clown barb fish (Barbodes everetti) | Fresh water       | Skin mucus                                                           | Alkaline-Sodium chloride          | Gram (-) B. cereus, E. coli, *Listeria monocytogenes*, P. aeruginosa, A. hydrophilla | Malaysia  | [39]       |
| Channel catfish (Ictalurus punctatus) | Saltwater         | Bone                                                                 | Alcalase, neutrase, papain, pepsin and trypsin | Gram (-) E. coli                                   | China     | [40]       |
| Tilapia (Oreochromis niloticus)    | Freshwater        | Head, frames and viscera                                            | Protamex                          | Gram (+) *Bacillus megaterium*                              | France    | [41]       |
| Fish species                  | Type of ecosystem | Part            | Hydrolysis | Tested Bacterial Strain                              | Country   | References |
|------------------------------|-------------------|-----------------|------------|------------------------------------------------------|-----------|------------|
| **Barbe** *(Barbus callensis)* | Freshwater        | Muscle          | Alcalase   |Gram (+) *B. cereus, L. monocytogenes, S. aureus, Micrococcus Luteus* Gram (-) *E. coli, Enterobacter sp.* | Tunisia   | [42]       |
| **Tilapia** *(Oreochromis niloticus)* | Freshwater        | Frame and head  | Papain     |Gram (+) *S. aureus, B. subtilis* Gram (-) *E. coli* | India     | [13]       |
| **Tinfoil barb fish** *(Barbonymus schwanenfeldii)* | Freshwater        | Skin mucus      | Ethanol    |(+)*Staphylococcus sp., B. cereu* Gram (-) *Shigellaboydii, E.coli* | India     | [18]       |
| **Rainbow trout** *(Oncorhynchus mykiss)* | Saltwater         | Viscera         | Pepsin     |Gram (+) *(R. salmoninarum, W. minor, W. paramesentoides, Micrococcus luteus, B. cereus, Ent. Faecalis)* Gram (-) *(A. media, A. salmonicida, F. auraucaanum, F. psychrophilum, C. freundii, E. Coli, Pro. Mirabilis, P. flureszens)* | Jerman    | [20]       |
| **Japanese eel** *(Anguilla japonica)* | Saltwater         | Liver           | Chymotrypsin |Gram (-) *E. tarda,* | China     | [43]       |
| Fish species | Type of ecosystem | Part | Hydrolysis | Tested Bacterial Strain | Country | References |
|--------------|------------------|------|------------|-------------------------|---------|------------|
| Aeromonas sp., A. hydrophila | | | | | | |

Note: Gram (+): Gram-positive bacteria; Gram (-): Gram-negative bacteria

4. Conclusions
In conclusion, FPH from fish by-products including skin, mucus, bones, heads, and viscera demonstrated significant antibacterial action against Gram-positive and Gram-negative bacteria by disrupting the cell membrane integrity. Short-chain peptides with lower molecular weight have diverse, active chemicals that contribute to their antibacterial activity by forming an inhibitory zone. Furthermore, enzymatic hydrolysis is a promising, safe process and does not contain any organic solvents or hazardous substances in the end products. However, by chemical hydrolysis, the varying nutritional composition may provide issues in producing a consistent end-product and treated at high temperatures. This high temperature may cause amino acid degradation and racemisation. This knowledge can be used to optimise production conditions, increase the yield of selected peptides, and gain a better understanding of the beneficial bioactive peptides involved in the development of antibacterial activity on a diverse selection of fish species and parts.

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