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REVIEW

# Bioactive angiotensin converting enzyme inhibitory activity and antihypertensive activity derived from fish protein hydrolysate: a systematic review

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**Abstract**

In the fish processing sector, fish have been processed in large quantities, are generated and discarded into the sea or dumped into the oceans. However, by employing effective strategies, this fishery waste can be utilized and converted into fish protein hydrolysate (FPH). FPH is a rich source of amino acids and peptides that have biological properties such as angiotensin-converting enzyme (ACE) inhibition, antimicrobial, antioxidant, and anticancer activity. The FPH has been used as a functional food perspective related to improving human health, primarily by maintaining blood pressure and normal heart function as measured by antihypertensive activity. FPH can be produced by various fish species, parts, and hydrolysis methods. Several studies have been published on the acceptability of FPH in obtaining bioactive properties from various fish, each using a different method to obtain bioactive properties. FPH is commonly produced by fish species such as Atlantic Cod, Lizard Fish, Atlantic Chub Mackerel, Atlantic Horse Mackerel, and Kawakawa (Mackerel Tuna). Furthermore, we summarized the various methodologies used by various researchers based on raw material data collection, method of production, ACE-inhibitory assay, ACE-inhibitory activity, degree of hydrolysis, molecular weight, purification method, and ACE-I peptide structure in this review.

## 1. Introduction

Fish production is a promising sector in the industry, with more than 196 million tons of fish expected to be processed in 2025 (Gao *et al.,* 2021). Many countries worldwide produce fish, and approximately about 50-60% of fish by-products are generated as waste (Gao *et al.,* 2021; Idowu *et al.,* 2021). Malaysia's annual waste disposal is estimated to be around 20 million tonnes, accounting for roughly 25% of total output (Baco et al., 2022). As a consequence, effective strategies for using fish byproducts are required. To solve the problem of waste from fish industries, they have converted the waste into fertilizer, fish meal or animal feed. Nowadays, numerous studies have explored essential nutrients such as can improve human health by boosting the immune system against disease, and consequently can control pollution problems (Idowu *et al.,* 2021). Furthermore, fish are a rich source of proteins, vitamins, omega-3 fatty acids and minerals that are fundamental to human health (Slizyte *et al.,* 2016). However, fish by-products have been content crude protein varying from 8 to 35% and are potentially, utilized as sources of essential amino acids, collagen, gelatin, polyunsaturated lipids, and enzymes (Gao *et al.,* 2021). Fish protein hydrolysate (FPH) has have been reported consisting bioactive properties such as antihypertensive, antioxidative, anticancer and immunomodulatory activities (Slizyte *et al.,* 2016; Gao *et al.,* 2021) and also exhibit relatively small bioactive peptides (3–20 amino acids) (Idowu *et al.,* 2021). Currently, peptide products can be obtained in the market from bonito, which have been shown able to reduce blood pressure (Gao *et al.,* 2021). For instance, peptides have been identified and isolated from animal and vegetable sources such as chicken skin, chicken legs, broccoli, wheat and soy (López-García et al., 2022). In addition, the antihypertensive actions of protein hydrolysates are mainly recognized for their inhibition of Angiotensin converting enzyme (ACE) (Slizyte *et al.,* 2016). Therefore, the FPH has been explored as a therapeutic approach to treat hypertension through the inhibition of ACE. In this review paper, we have described on the acceptability of FPH in obtaining

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bioactive properties from different fish and parts were used, with a different method of synthesis to obtain bioactive properties. Furthermore, through this review, we summarized the different methodologies conducted by different researchers based on data collection of raw materials, method of production, ACE-inhibitory assay, ACE-inhibitory activity, degree of hydrolyzation, molecular weight, purification method, and ACE-I peptides structure were also discussed. Lastly, the limitations and future directions are discussed.

## 2. Materials and methods

### *2.1 Search strategy*

This study consists of a systematic review of the existing scientific literature on antihypertensive activity of FPH. A literature search was performed using the following databases (from inception 2000 to 2020): Google Scholar, PubMed, Semantic Scholar, and Scopus. The following keywords were used, "Antihypertensive Activity" or "ACE Inhibitor", and "Fish Protein Hydrolysate". The search was slightly revised for each database as appropriate according to these criteria 1) all studies published without restriction to the year of publication; 2) all articles in scientific journals; 3) articles published in the English language. Hence, additional citations were identified by manually searching the references of articles retrieved from the computerized databases and relevant reviews. Initially, 951 scientific articles were grouped by online search based on year of publication and type of publication. Following a read the publication indicated that 727 articles from Google Scholar, 119 articles from Semantic Scholar, 41 articles from PubMed, and 64 articles from Scopus. According to Google Scholar, only 430 articles were chosen manually due to the inclusion of criteria, while 654 articles were selected. As a consequence, 72 articles were chosen after the manuscript was revised. The flowchart shown in Figure 1 summarizes the study selection.

### *2.2 Analysis of data*

Numerous review articles were published on the acceptability of FPH in obtaining bioactive properties from different fish, with different methods to obtain bioactive properties. Furthermore, through this review, we summarized the different methodologies conducted by different researchers based on data collection of raw materials, method of production, ACE-inhibitory assay, ACE-inhibitory activity, degree of hydrolysis, molecular weight, purification method, and ACE-I peptide structure.

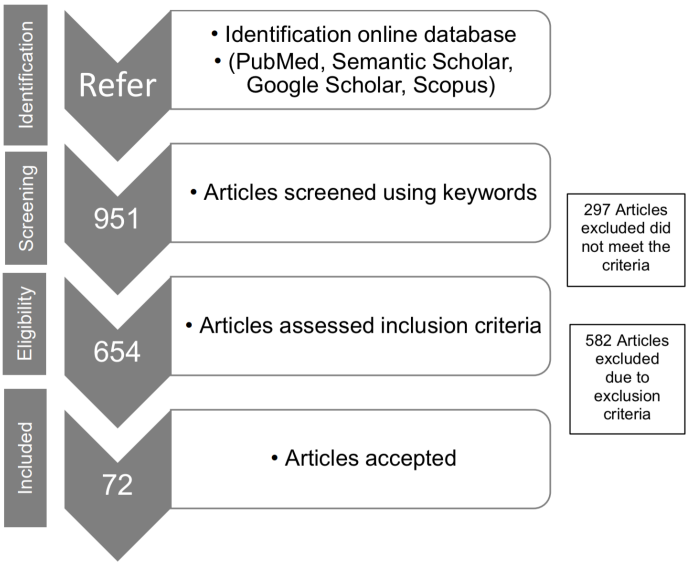


Figure 1. Flowchart of phases of the systematic review.

## 3. Results and discussion

### *3.1 Fish species and parts used in producing fish protein hydrolysate*

The fish species used in producing FPH are often main captures of fisheries, by-catches and aquaculture farmed fishes such as Atlantic Cod, Haddock, Lizard Fish, Atlantic Chub Mackerel, Atlantic Horse Mackerel, and Kawakawa (Mackerel Tuna) (Petrova *et al.,* 2018). Recently, researchers from Universiti Malaysia Sabah, Malaysia has been successfully produced new species of hybrid grouper, which is a cross-bed species from *Epinephelus lanceolatus* × *Epinephelus*, which is able to produce high-quality FPH (Chan *et al.,* 2020). Based on the Department of Fisheries Malaysia (2019) (Kementerian Pertanian dan Industri Makanan dan Jabatan Perikanan Malaysia, 2019), Malaysia, is one of the main contributors to the country's Gross Domestic Product (GDP) fishery sector. Furthermore, the fish waste generated from fishery remains of processed fishes like bones or frames, head, skin, trimmings, scale and viscera are the potential raw material for producing FPH (Intarasirisawat *et al.,* 2013; Mongkonkamthorn *et al.,* 2020). In addition, the parts of skipjack tuna blood and roe can also be utilized (Intarasirisawat *et al.,* 2013; Mongkonkamthorn *et al.,* 2020) and could be influenced by the antihypertensive activity of the FPH produced due to differences in chemical composition (Petrova *et al.,* 2018).

### *3.2 Method of hydrolysis*

According to Petrova *et al.* (2018), there are two methods that can be used to produce FPH: (A) Chemical Hydrolysis and (B) Biochemical Hydrolysis. Chemical hydrolysis involves acid and alkali treatment while biochemical hydrolysis is carried out by proteolytic enzymes or protease (Gao *et al.,* 2021). In addition,

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enzymatic hydrolysis is the current biotreatment that offers a safe, fast, and easily controlled method in order to produce protein hydrolysates through the hydrolytic breakdown of peptide bonds (Gao *et al.,* 2020). In this review, the production of FPH is highlighted through enzymatic hydrolysis by different enzymes. This might be due to the biotreatment can produce a better quality of FPH and have a shorter reaction time at optimal conditions while also does not form toxic chemicals (Siddik *et al.,* 2021). Recently, Gao *et al.* (2020) have suggested that changes in enzyme type, pH, time, temperature, enzyme-to-substrate ratio, solid-liquid ratio, and enzyme amount resulted in the different peptides produced. There are several types of proteases used in producing FPH. Protease can be categorized into three types i.e. acidic protease (a), neutral protease (b), and alkaline protease (c) (Ao *et al.,* 2018). The enzymes used to produce FPH are extracted from crude enzymes derived from bacteria, fungi, fish viscera, mammals, purified enzymes, and plant enzymes.

### *3.3 Angiotensin-converting enzyme inhibitory assay*

To further understand the principle of the assay methods, the regulation of blood pressure and the role of angiotensin converting enzyme (ACE) must be understood. Blood pressure is regulated by Renin-Angiotensin-Aldosterone System (RAAS) (Fountain and Lappin, 2020). ACE is found primarily in the vascular endothelium of the lung and kidneys (Fountain and Lappin, 2020; Herman *et al.,* 2020). The production of ACE can cause blood pressure to rise through two types of reactions. The first reaction is when ACE convert Angiotensin-I to Angiotensin-II. Angiotensin-I is inactive and produced by the cleavage of Angiotensinogen by renin. Upon conversion by ACE, angiotensin-II formed will bind on angiotensin II type I and type II receptors. Also, angiotensin-II also stimulates the production of aldosterone. This causes vasoconstriction and salt retention and thus the rising of blood pressure. The second reaction is when ACE convert bradykinin, a vasodilator into inactive fragments. As a result, the blood pressure rises. Therefore, antihypertensive drugs aim to inactivate ACE by acting as an ACE-inhibitor, preventing the formation of Angiotensin-II. The mechanism of ACE-inhibitors is not fully known. However, it can be linked to their interference with the RAAS (Herman *et al.,* 2020). The ACE-inhibitory activity of antihypertensive drugs or natural antihypertensive can be measured using substrates that can be hydrolysed ACE. This is due to ACE-Inhibitor preventing the formation of ACE, thus reducing the hydrolysis of substrate by ACE, therefore the product will be lesser. Absorbance is then measured using spectrophotometer or microplate reader (Ahmad *et al.,* 2017). Basically, the purpose of ACE-inhibitor assay is to provide a comparison of the effectiveness of antihypertensive in lowering blood pressure. The activity of ACE in RAAS is shown in the chart in Figure 2.

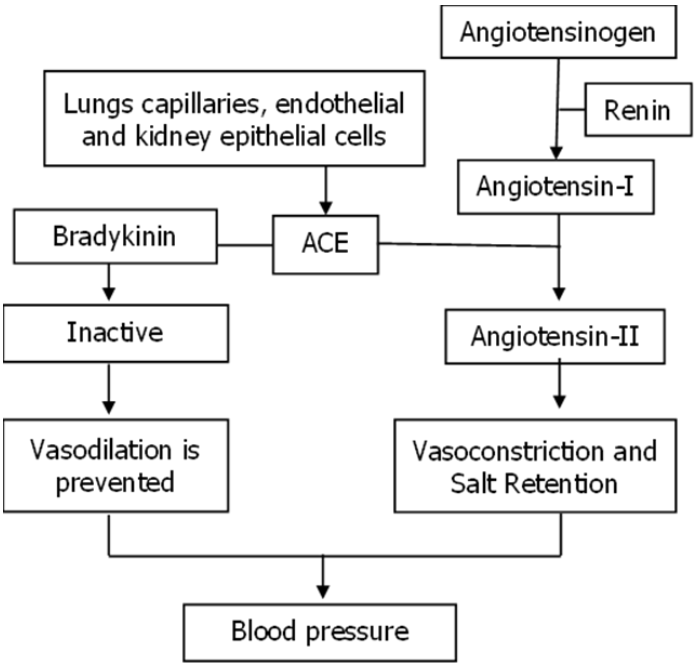


Figure 2. Effect of Angiotensin-Converting Enzyme (ACE) in Renin-Angiotensin-Aldosterone System (RAAS).

Indeed, there are several Angiotensin-converting Enzyme **(**ACE) inhibitory assays used in measuring the antihypertensive activity of FPH. The general principle of the assay is to measure the concentration of the hydrolysed product of substrate by ACE. In the presence of ACE-inhibitors, the concentration of the hydrolysed products will be lesser (Ahmad *et al.,* 2017). Table 1 shows the *in vitro* ACE-inhibitory assay method, substrate and measurement method used in determining antihypertensive activity of FPH. There are six *in vitro* assay methods that can be used to measure ACE inhibitory activity based on the substrate used: (1) Cushman and Cheung method, (2) Holmquist method, (3) Elbl and Wagner method, (4) Baudin method, (5) Carmel and Yaron method, and (6) Lam method (Ahmad *et al.,* 2017). The substrates used are HHL, FAPGG, o-amino benzoyl glycyl-p-nitrophenilalanilproline and 3HB-GGG. Modifications usually involve the addition of colour reagents, buffers, indicators, method of absorbance measurement and wavelength measured. The assay of Cushman and Cheung (1971) and Holmquist *et al.* (1979) are the most used in determining antihypertensive activity of FPH, followed by Carmel and Yaron (1978) and Lam *et al.* (2009). Shalaby *et al.* (2006) performed a study on the comparison of the performance of assay using HHL (Cushman and Cheung method) and FAPGG (Holmquist method) as substrate. Both assays show good performance and repeatability when used for studies of comparisons of inhibitory activity, like the antihypertensive activity of FPH from

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Table 1. ACE-Inhibitory assay methods to measure ACE inhibitory activity based on the different substrates used.

| Method | Substrate | Measurement |
|---|---|---|
| Cushman and Cheung (1971) | Hippuryl-histidyl-leucine (HHL) | HHL has been hydrolysed into HA. The concentration of HA was measured at 228 nm using Ultraviolet-Visible (UV-Vis) Spectrophotometer or High Performance Liquid Chromatography (HPLC). |
| Modified Cushman and Cheung by Hayakari *et al.* (1978) | Hippuryl-histidyl-leucine (HHL) | Addition of 2,4,6-trichloro-s-triazine (TT) in the mixture with HA for reaction to occur. Absorbance was measured at 382 nm. |
| Modified Cushman and Cheung by Jimsheena and Gowda (2009) | Hippuryl-histidyl-leucine (HHL) | Benzene-sulfonyl-chloride (BSC) is a colour agent in the presence of quinoline. The yellow colour developed is measured at 410 nm using microtiter plate reader or spectrophotometer. |
| Modified Cushman and Cheung by Salampessy *et al.* (2015) | Hippuryl-histidyl-leucine (HHL) | 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer has been used. Absorbance was measured at 228 nm. |
| Holmquist *et al.* (1979) | Furanacryloyl-L-phenylalanylglycylglycine (FAPGG) | FAPPG has been hydrolysed into dipeptide and furanacryloyl-phenylalanine, which was measured at 238 nm and 352 nm using High Performance Liquid Chromatography-Ultraviolet (HPLC- |
| Modified Holmquist by Udenigwe *et al.* (2009) | Furanacryloyl-L-phenylalanylglycylglycine (FAPGG) | FAPGG was mixed with ACE and measured at an absorbance of 345 m with a microplate reader. |
| Modified Holmquist by Shalaby *et al.* (2006), Vermeirssen *et al.* (2002), | Furanacryloyl-L-phenylalanylglycylglycine (FAPGG) | The assay has been measured at an absorbance of 340 nm using a microplate scanning spectrophotometer or UV-Vis double beam spectrophotometer. |
| Modified Carmel and Yaron by Sentandreu and Toldrá (2006) | o-amino benzoyl glycyl-p-nitrophenilalanilproline | The substrate was hydrolysed into *o*-amino benzoyl glycyl which has fluorescence which can be measured at absorbance 355 nm (excitation) and 405 nm (emission) using a multi-scanning microplate fluorometer. |
| ACE-Inhibition Screening Kit (WST-1) or Lam *et al.* (2009) | 3-Hydroxybutyryl-Gly-Gly-Gly (3HB-GGG) | Detection of 3-Hydroxybutyric acid (3HB) by enzymatic method. The indicator was used to form colour. The measurement at absorbance 450 nm using a microplate reader. |

various species and parts of fishes. Hence, the Holmquist method is shown to have advantages such as being faster, simpler, and able to perform up to 30 samples a day compared to Cushman and Cheung method only can perform six to 12 methods a day while requiring HPLC separation step which additional time and equipment are needed. However, the method by Cushman and Cheung is better for samples that can affect the absorbance such as turbidity or coloured (Shalaby *et al.,* 2006). ACE-Inhibition Screening Kit modified from the Lam method (Lam *et al.,* 2009) is also used in the study on boarfish protein hydrolysate (Hayes *et al.,* 2016). Compared to the other method, this method is rapid, simple, convenient and can be used to test many samples. Based on Lam *et al.* (2009), this method when compared to Cushman and Cheung method which is the most common method, had no significant difference in precision. In fact, Lam's method – Flow Injection Analysis shows a correlation with WST-1 method ($R^2$ = 0.984) and with Cushman and Cheung method ($R^2$ = 0.994) (Lam *et al.,* 2009).

### *3.4 Angiotensin-converting enzyme-Inhibitory activity of fish protein hydrolysate*

ACE-inhibitory activity is expressed in percentage (%) and in $IC_{50}$. $IC_{50}$ is an expression which indicates the concentration of ACE-inhibitory peptides that can inhibit 50% of ACE activity. In general, a lower $IC_{50}$ value indicates higher antihypertensive activity. This means that a lower concentration of ACE-inhibitory peptides is required to achieve the inhibition of 50% of ACE activity. Table 2 shows the ACE-inhibitory activity of FPH produced from different species and parts of fish, hydrolysis by different enzymes with their degree of hydrolysis (DH), ACE-I peptides amino acid sequence, and the molecular weight. Based on Table 2, the FPH produced from different parts of fish show different antihypertensive activity. Valcarcel *et al.* (2020) reported different antihypertensive activity of FPH obtained from European seabass and seabream hydrolysates. For seabass, the $IC_{50}$ value for the head is 989.2 µg/mL, frames and trimming hydrolysate was 801.3 µg/mL, and for viscera hydrolysate was 1398.3 µg/mL. For seabream, the $IC_{50}$ value for head hydrolysate was 1034.5 µg/mL, frames and trimming is 793.2 µg/mL, and for viscera hydrolysate was 1245.8 µg/mL. For both

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Table 2. ACE-Inhibitory activity of fish protein hydrolysates

| Fish Species | Fish Part | Enzymes | DH % | Peptide Sequence | Molecular Weight | Antihypertensive Activity | References |
|---|---|---|---|---|---|---|---|
| Zebra Blenny (*Salaria basilisca*) | Muscle | Zebra Blenny Protease | - | - | - | 93.6 µg/mL | Ktari *et al.* (2014) |
| | | Smooth Hound Proteases | - | - | - | 130.0 µg/ml | |
| | | Sardinelle Proteases | - | - | - | 182.0 µg/mL | |
| Boarfish (*Capros aper*) | Head | Alcalase | 17.55 | - | - | 178.3 µg/mL | Vázquez, Meduíña, Durán *et al.* (2019) |
| | Whole | Alkaline Protease | 5.4 | - | - | 72.08% | Hayes *et al.* (2016) |
| Catfish (*Pangasius sutchi*) | Muscle | Protamex | 5 | - | <20 kDa | 90.60% | Theodore and Kristinsson (2007) |
| | | | 15 | - | <20 kDa | 70.00% | |
| | | | 30 | - | < 5 kDa | 73.90% | |
| | Skin | Alcalase | 64.87 | - | - | 3.2 µg/mL | Mahmoodani *et al.* (2014) |
| | Bone | Alcalase | 68.48 | - | - | 1.3 µg/mL | |
| | Skin | Alcalase | 64.87 | - | - | 0.77 µg/mL | Mahmoodani *et al.* (2012) |
| Mekong Giant Catfish (*Pangasianodon gigas)* | Skin Gelatin | Visceral Peptidase | 43.51 | - | - | 67.75% | Ketnawa *et al.* (2017) |
| | | Bovine Trypsin | 39.67 | - | - | 41.00% | |
| Cobia (*Rachycentron canadum*) | Skin | Protamex | - | | 630-450 Da | 0.221 mg/mL | Lin *et al.* (2019) |
| | | Protease N | - | - | 630-450 Da | 0.291 mg/mL | |
| Atlantic Cod (*Gadus morhua*) | Muscle | Pepsin and Trypsin-Chymotrypsin mixture | - | - | - | 1.4 µg/mU | Jensen *et al.* (2014) |
| | | Trypsin-Chymotrypsin mixture | - | - | - | 0.130 mg/mL | Girgih *et al.* (2015) |
| Eel (*Monopterus* sp.) | Muscle | Alcalase | - | - | 3 kDa | 71.90% | Azemi *et al.* (2017) |
| | | | | | 5 kDa | 49.05% | |
| | | | | | 10 kDa | 17.52% | |
| Goby (*Zosterisessor ophiocephalus*) | Muscle | Alcalase | 7.82 | - | - | 3.19 mg/mL | Nasri *et al.* (2013) |
| | | Crude enzyme from *B. licheniformis* NH1 | 9.63 | - | - | 3.33 mg/mL | |
| | | Crude enzyme from *B. mojavensis* A21 | 13.21 | ARS | 332 | 1.36 mg/mL | |
| | | | | VVAPFAHGT | 897 | | |
| | | | | RSTA | 433 | | |
| | | | | FYPP | 522 | | |
| | | | | RCSAGV | 591 | | |
| | | Crude enzyme from *B. pumilus* A1 | 10.41 | - | - | 2.07 mg/mL | |
| | | Crude enzyme from *B.subtilis* A26 | 10.24 | - | - | 1.74 mg/mL | |
| Grenadier (*Macrourus* sp.) | Skin, Bones | Alcalase | 15.36 | - | - | 361.1 µg/mL | Vázquez *et al.* (2019) |
| | Head | Alcalase | 18.45 | - | - | 195.6 µg/ mL | |

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Table 2 (Cont.). ACE-Inhibitory activity of fish protein hydrolysates

| Fish Species | Fish Part | Enzymes | DH % | Peptide Sequence | Molecular Weight | Antihypertensive Activity | References |
|---|---|---|---|---|---|---|---|
| Hybrid grouper (*Epinephelus lanceolatus* × *Epinephelus fuscoguttatus)* | Head and Bones | Alcalase | 17.7 | - | - | 59.12% | Chan *et al.* (2020) |
| | | Trypsin | - | - | - | 48.64% | |
| | | Proteinase K | 41.3 | - | - | 55.92% | |
| | | Pepsin | - | - | - | 53.38% | |
| Haddock (*Melanogrammus aeglefinus*) | Muscle | Pepsin and Trypsin-Chymotrypsin mixture | - | - | - | 1.1 µg/mU | Jensen *et al.* (2014) |
| Cape Hake (*Merluccius capensis*) | Sawdust and Cut-offs | Protamex | 19 | - | - | 1.00 mg/mL | Pires *et al.* (2015) |
| | | | 12.8 | - | - | 1.00 mg/mL | |
| | | | 12.6 | - | - | 1.00 mg/mL | |
| European Hake (*Merluccius merluccius)* | Head | Savinase | 5.3 | - | 3492 Da | 1.4 mg/mL | Karoud (2019) |
| | | | 6.5 | - | 2271 Da | 0.94 mg/mL | |
| | | | 7.7 | - | 1859 Da | 0.63 mg/mL | |
| | | | 8.6 | - | 1216 Da | 0.26 mg/mL | |
| Pacific Hake (*Merluccius productus*) | Whole | Endogenous protease (pepsin, trypsin, chymotrypsin) | - | - | - | 161 µg/mL | Samaranayaka *et al.* (2010) |
| | Muscle | Alcalase | - | - | - | 165 µg/mL | Cinq-Mars and Li-Chan (2007) |
| Atlantic Halibut (*Hippoglossus hippoglossus)* | Muscle | Pepsin and Trypsin-Chymotrypsin mixture | - | - | - | 1.77 µg/mU | Jensen *et al.* (2014) |
| Greenland Halibut (*Reinhardtius hippoglossoides*) | Muscle | Pepsin and Trypsin-Chymotrypsin mixture | - | - | - | 2.14 µg/mU | Jensen *et al.* (2014) |
| Lizard Fish (*Saurida elongata*) | Muscle | Neutral Protease | 24 | SPRCR | 617 Da | 41 µM | Wu *et al.* (2012) |
| | | | 24 | RVCLP | - | 175 µM | Wu *et al.* (2015) |
| | | | - | GMKCAF | 655.2 Da | 47.3 µM | Lan *et al.* (2015) |
| | | | - | RYRP | 592.0 Da | 52 µM | Sun *et al.* (2017) |
| | Scale | Neutral Protease | 9.5 | AGPPGSDGQP GAK | <2 kDa | 420 µM | Chen *et al.* (2018) |
| Pond Loach (*Misgurnus anguillicaudatus*) | Muscle | Bromelain | - | AHLL | 452 Da | 40.3 µM | Li *et al.* (2012) |
| Atlantic Chub Mackerel (*Scomber colias*) | Whole | Subtilisin and Trypsin | 14 | - | - | 360 µg/mL | García-Moreno *et al.* (2013) |
| | | Subtilisin and Trypsin Simultaneously | 14 | - | - | 345 µg/mL | |
| Atlantic Horse Mackerel (*Trachurus trachurus*) | Whole | Subtilisin and Trypsin | 16 | - | - | 395 µg/mL | |
| | | Subtilisin and Trypsin Simultaneously | 16 | - | - | 364 µg/mL | |

Table 2 (Cont.). ACE-Inhibitory activity of fish protein hydrolysates

| Fish Species | Fish Part | Enzymes | DH % | Peptide Sequence | Molecular Weight | Antihypertensive Activity | References |
|---|---|---|---|---|---|---|---|
| Horse mackerel (*Trachurus mediterraneus*) | Whole | Subtilisin and Trypsin | 15.94 | - | - | 330.2 µg/mL | Pérez-Gálvez *et al.* (2016) |
| | | Trypsin (47.5 °C) | - | - | - | 272.1 µg/mL | |
| | | Trypsin (55 °C) | - | - | - | 253.0 µg/mL | |
| | | Subtilisin and Trypsin Simultaneously | 21 | HLALT | 553.32 Da | 5.11 µM | García-Moreno *et al.* (2015) |
| | | | - | RQLAGP | 640.32 Da | 6.24 µM | |
| | | | - | ELSAP | 515.31 Da | 7.08 µM | |
| Milkfish (*Chanos chanos*) | Non-extruded scale | Flavourzyme | - | - | <3 kDa | 762 µg/mL | Huang *et al.* (2018) |
| | | Alcalase and Flavourzyme mixture | - | - | <3 kDa | 472 µg/mL | |
| | Extrusion-pretreated scale | Flavourzyme | - | - | <3 kDa | 592 µg/mL | |
| | | Alcalase and Flavourzyme mixture | - | - | <3 kDa | 547 µg/mL | |
| Monkfish (*Lophius piscatorius*) | Head | Alcalase | - | - | 1576 Da | 931.3 µg/mL | Vázquez, Menduíña, Nogueira *et al.* (2020) |
| | Viscera | | - | - | 947 Da | 1142.5 µg/mL | |
| European Pilchard (*Sardina pilchardus*) | Whole | Subtilisin and Trypsin | - | - | - | 400 µg/mL | García-Moreno *et al.* (2013) |
| | | Subtilisin and Trypsin Simultaneously | - | - | - | 430 µg/mL | |
| | Muscle | Brewer's spent yeast (BSY) (*Saccharomyces pastorianus*) | - | - | - | 164 µg/mL | Vieira and Ferreira (2017) |
| European Plaice (*Pleuronectes platessa*) | Muscle | Pepsin and Trypsin-Chymotrypsin mixture | - | - | - | 1.7 µg/mU | Jensen *et al.* (2014) |
| Saithe (*Pollachius virens*) | Muscle | Pepsin and Trypsin-Chymotrypsin mixture | - | - | - | 1.69 µg/mU | Jensen *et al.* (2014) |
| Alaska Pollack (*Theragra chalcogramma*) | Skin | Alcalase, Pronase E, and Collagenase | - | GPM | <2 kDa | 17.3 µM | Byun and Kim (2001) |
| | | | - | GPL | <2 kDa | 2.65 µM | |
| Thornback Ray (*Raja clavata*) | Skin | *Bacillus subtilis* A26 proteases | - | APGAP | - | 170.28 µM | Lassoued *et al.* (2015) |
| | | *Bacillus amyloliquefaciens* Neutrase | - | GIPGAP | - | 27.9 µM | |
| | Muscle | Alcalase | - | - | - | 84.00% | |
| | | Neutrase | - | - | - | 87.00% | |
| | | *B. subtilis* A26 crude enzyme | - | - | - | 87.00% | |
| | | Crude alkaline protease from *R. clavata* | - | - | - | 51.00% | |

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Table 2 (Cont.). ACE-Inhibitory activity of fish protein hydrolysates

| Fish Species | Fish Part | Enzymes | DH % | Peptide Sequence | Molecular Weight | Antihypertensive Activity | References |
|---|---|---|---|---|---|---|---|
| Ribbonfish (*Trichiurus haumela*) | Backbone | Acidic Protease | - | LW | 317.25 Da | 5.6 µM | Zou *et al.* (2014) |
| Atlantic Salmon (*Salmo salar)* | Skin, Bone and Residual Meat | Carolase PP | - | - | - | 0.13 mg/mL | Neves *et al.* (2017) |
| | Muscle | Pepsin and Trypsin-Chymotrypsin mixture | - | - | - | 2.7 µg/mU | Jensen *et al.* (2014) |
| | Backbone | Trypsin | - | - | - | 0.2 – 0.9 mg/mL | Slizyte *et al.* (2016) |
| | | Carolase PP, Carolase 7089, Protamex, Papain, Bromelain, Protex, | - | - | - | 2.8 – 4.6 mg/mL | |
| | | Seabzyme | - | - | - | 9.5 mg/mL | |
| | Head, Backbone and Frames | Acid Fungal Protease by *Aspergillus niger* | 43.86 | - | - | 95.50% | Nurdiani *et al.* (2016) |
| | Head | Alcalase | - | - | <2 kDa | 478.5 µg/mL | Vázquez, Sotelo, Sanz *et al.* (2019) |
| | Trimming and Frames | | - | - | <2 kDa | 653.7 µg/mL | |
| Chum Salmon (*Oncorhynchus keta*) | Skin | Trypsin | 70.05 | GLPLNLP | 722 Da | 18.7 µM | Lee *et al.* (2014) |
| Round Sardinella (*Sardinella aurita*) | Head and Viscera | *Bacillus licheniformis* NH1 Protease | 11 | - | - | 2.1 mg/mL | Bougatef *et al.* (2008) |
| | | Alcalase | 8 | - | - | 2.3 mg/mL | |
| | | Sardine viscera protease | 8 | - | - | 1.2 mg/mL | |
| | | Chymotrypsin | 7.5 | - | - | 1.8 mg/mL | |
| | | *Aspergillus clavatus* ES1 Protease | 8 | - | - | 7.4 mg/mL | |
| | Muscle | *Bacillus subtilis* A26 Protease | 4 | - | - | 1.16 mg/mL | Jemil *et al.* (2017) |
| Red Scorpionfish (*Scorpaena notata*) | Muscle | *Penicillium digitatum* Protease | 15.12 | LVTGDDKTNLK | 1204.67 Da | 6.72 µM | Aissaoui *et al.* (2017) |
| | | | - | DTGSDKKQL | 992.51 Da | 5.44 µM | |
| | Head | | 10.78 | - | - | 0.489 mg/mL | |
| European Seabass (*Dicentrarchus labrax*) | Head | Alcalase | - | - | 1787 Da | 989.2 µg/mL | Valcarcel *et al.* (2020) |
| | Frames and Trimming | | - | - | 1381 Da | 801.3 µg/mL | |
| | Viscera | | - | - | 2023 Da | 1398.3 µg/mL | |

Table 2 (Cont.). ACE-Inhibitory activity of fish protein hydrolysates

| Fish Species | Fish Part | Enzymes | DH % | Peptide Sequence | Molecular Weight | Antihypertensive Activity | References |
|---|---|---|---|---|---|---|---|
| Seabream (*Sparus aurata*) | Head | Alcalase | - | - | 1894 Da | 1034.5 µg/mL | Valcarcel *et al.* (2020) |
| | Frames and Trimming | | - | - | 1494 Da | 793.2 µg/mL | |
| | Viscera | | - | - | 1936 Da | 1245.8 µg/mL | |
| | Scale | Esperase 8.0L | 16.1 | - | <3 kDa | 59.9 µg/mL | Akagündüz *et al.* (2014) |
| Stone fish Sea Cucumber (*Actinopyga lecanora*) | Whole with removed viscera | Bromelain | - | ALGPQFW | 794.44 Da | 0.012 mM | Auwal *et al.* (2019) |
| | | | - | KVPPKA | 638.88 Da | 0.980 mM | |
| | | | - | LAPPTM | 628.85 Da | 1.310 mM | |
| | | | - | EVLIQ | 600.77 Da | 1.440 mM | |
| | | | - | EHPVL | 593.74 Da | 1.680 mM | |
| | | | 44.59 | - | - | 84.26% | |
| Kilka Fish (*Clupeonella cultriventris*) | Whole | Alcalase | 2.98 | - | - | 26.00% | Qara and Habibi Najafi (2018) |
| | | Neutrase | 2.78 | - | - | 53.00% | |
| Skate (*Okamejei kanojei*) | Skin | Alcalase and then Neutral Protease | - | LGPLGHQ | 720 Da | 4.22 µM | Ngo *et al.* (2015) |
| | | | - | MVGSAPGVL | 829 Da | 3.09 µM | |
| Small-spotted catshark (*Scyliorhinus canicula*) | Muscle | Alcalase | - | - | - | 114.5 µg/mL | Vázquez *et al.* (2017) |
| | | Esperase | - | - | - | 83.6 µg/mL | Vázquez *et al.* (2017) |
| | | Subtilisin and Trypsin Simulataneously | 17.3 | YLGW | 547.26 Da | 0.09 µM | García-Moreno *et al.* (2015) |
| | | | | VAMPF | 563.27 Da | 0.44 µM | |
| | | | | PGCF | 422.19 Da | 1.33 µM | |
| | | | | HLF | 415.22 Da | 9.56 µM | |
| Smooth Hound (*Mustelus mustelus*) | Head and viscera | Intestinal crude enzyme from *M.mustelus* | 18.2 | - | - | 1.21 mg/mL | Sayari *et al.* (2016) |
| | | Gastric Proteases | 16.5 | - | - | 1.25 mg/mL | |
| | | Porcine Pancreatin | 11 | - | - | 1.28 mg/mL | |
| | Viscera | Esperase | - | - | 3-1 kDa | 101.61 µg/mL | Abdelhedi *et al.* (2017) |
| | | | - | - | <1 kDa | 92.75 µg/mL | |
| | | Neutrase | - | - | 3-1 kDa | 75.05 µg/mL | Abdelhedi *et al.* (2018) |
| | | | - | - | <1 kDa | 117.76 µg/mL | |
| | | Purafect | - | - | 3-1 kDa | 85.86 µg/mL | |
| | | | - | - | <1 kDa | 53.31 µg/mL | |

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Table 2 (Cont.). ACE-Inhibitory activity of fish protein hydrolysates

| Fish Species | Fish Part | Enzymes | DH % | Peptide Sequence | Molecular Weight | Antihypertensive Activity | References |
|---|---|---|---|---|---|---|---|
| Yellowfin Sole (*Limanda aspera*) | Frame | α-chymotrypsin | - | MIFPGAGGPEL | 1.3 kDa | 28.7 μg/mL | Jung *et al.* (2006) |
| Nile Tilapia (*Oreochromis niloticus*) | Gelatin | Alcalase | - | DPALATEPDPMPF | 1382 Da | 62.2 μM | Vo *et al.* (2011) |
| | Skeleton | Alcalase | 67 | - | 5-3 kDa | 0.634 mg/mL | Borges-Contreras *et al.* (2019) |
| | | Flavourzyme | 52 | - | 5-3 kDa | 0.470 mg/mL | |
| | Skin | Pepsin | 6.48 | - | - | <39.00% | Lin and Alashi (2017) |
| | | Papain | 7.44 | - | - | <39.00% | |
| | | Bromelain | 10.33 | - | - | 39.00% | |
| | | | 50.59 | - | - | 90.54% | Choonpicharn *et al.* (2015) |
| | | Flavourzyme | 56.61 | - | - | 91.44% | |
| | | Trypsin | 44.55 | - | - | 92.55% | |
| | | Alcalase | 16.05 | - | - | 92.53% | |
| | | Papain | 59.55 | - | - | 89.41% | |
| | | Neutrase | 15.71 | - | - | 89.02% | |
| | | Pepsin and α-chymotrypsin | - | LSGYGP | - | 2.577 μmol/L | Chen *et al.* (2020) |
| | Frame | Pepsin | 23.46 | - | <1 kDa | 0.41 mg/mL | Lin and Alashi (2017) |
| | | | | - | 1-3 kDa | 0.55 mg/mL | |
| | | | | - | 3-5 kDa | 0.79 mg/mL | |
| | | | | - | 5-10 kDa | 0.83 mg/mL | |
| | | Bromelain | 15.38 | - | - | - | |
| | | Papain | 20.01 | - | - | - | |
| | Head, Tail and Frames | Alcalase | - | - | 10 kDa | 64.32% | Roslan *et al.* (2017) |
| | | | - | - | 5 kDa | 71.83% | |
| | | | - | - | 10/5 kDa | 75.59% | |
| | | | - | - | 5/5 kDa | 84.04% | |
| | | | - | - | <5 kDa | 88.26% | |
| | Muscle | Proteinases of *Virgibacillus halodenitrificans* SK1-3-7 | 48 | MCS | 340.39 Da | 0.29 μM | Toopcham *et al.* (2017) |
| | Scale | Alcalase | 12.88 | - | 200-2000 Da | 0.73 mg/mL | Zhang *et al.* (2019) |
| Trevally (*Pseudocaranx* sp.) | Whole | Bromelain | - | AR | 245.28 Da | 448.47 μM | Salampessy *et al.* (2015) |
| | | | - | AV | 188.23 Da | 321.89 μM | |
| | | | - | APER | 471.51 Da | 190.88 μM | |

Table 2 (Cont.). ACE-Inhibitory activity of fish protein hydrolysates

| Fish Species | Fish Part | Enzymes | DH % | Peptide Sequence | Molecular Weight | Antihypertensive Activity | References |
|---|---|---|---|---|---|---|---|
| Rainbow Trout (*Oncorhynchus mykiss)* | Frames | Alcalase | - | - | 300-500 Da | (Microwave): 0.015 mg/mL (EFUF): 0.017 mg/mL (Cationic peptides): 0.0036 mg/mL | Ketnawa *et al.* (2019) |
| | | | - | - | | | |
| | | | - | - | | | |
| | | | - | - | | | |
| | | | - | - | | | |
| | Head | Alcalase 2.4L | - | - | <2 kDa | 508.9 µg/mL | Vázquez, Sotelo, Sanz *et al.* (2019) |
| | Trimming and Frames | | - | - | <2 kDa | 975.4 µg/mL | |
| Kawakawa (*Euthynnus affinis*) | Muscle | Pepsin from skipjack tuna stomach | 21.7 | - | <1 kDa | 0.44 mg/mL | Taheri and Bakhshizadeh (2020) |
| | | | | - | 1-3 kDa | 0.52 mg/mL | |
| | | | | - | 3-10 kDa | 1.86 mg/mL | |
| | | | | - | >10 kDa | 1.94 mg/mL | |
| Skipjack Tuna (*Katsuwonus pelamis*) | Roe | Alcalase | - | MLVFAV | 678 Da | 3.07 µM | Intarasirisawat *et al. (2013)* |
| | | | - | FVSACSVAG | 839 Da | 3.49 µM | |
| | | | - | MCYPAST | 771 Da | 58.73 µM | |
| | | | - | DLDLRKDLYAN | 1334 Da | 67.43 µM | |
| | | | - | YVNDAATLLPR | 1231 Da | 105.85 µM | |
| | | | - | LADGVAAPA | 854 Da | 317.75 µM | |
| | | | - | DWMKGQ | 763 Da | 422.92 µM | |
| | Blood | Alcalase | 40.4 | - | - | 0.26 mg/mL | Mongkonkamthorn *et al.* (2020) |
| | | Neutrase | 43.7 | - | - | 0.28 mg/mL | |
| | | Flavourzyme | 46 | - | - | 0.19 mg/mL | |
| | Frame | Pepsin | 74.5 | GDLGKTTVSNWPPKYKDTP | 2482 Da | 11.28 µM | Lee *et al.* (2010) |
| Bullet Tuna (*Auxis rochei*) | Viscera | Subtilisin | 3 | - | - | 0.53 mg/mL | Derouiche Ben Maiz *et al.* (2019) |
| | | | 6 | - | - | 0.40 mg/mL | |
| | | | 9 | - | - | 0.43 mg/mL | |

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Table 2 (Cont.). ACE-Inhibitory activity of fish protein hydrolysates

| Fish Species | Fish Part | Enzymes | DH % | Peptide Sequence | Molecular Weight | Antihypertensive Activity | References |
|---|---|---|---|---|---|---|---|
| Bullet Tuna (*Auxis rochei*) | Viscera | Endogenous Enzyme | 3 | - | - | 0.31 mg/mL | Derouiche Ben Maiz *et al.* (2019) |
| | | | 6 | - | - | 1.10 mg/mL | |
| | | | 9 | - | - | 1.16 mg/mL | |
| Turbot (*Scophthalmus maximus*) | Head | Alcalase | - | - | 1622 Da | 1273.6 µg/mL | Vázquez, Rodríguez-Amado, Sotelo *et al.* (2020) |
| | Trimming and Frames | | - | - | 1200 Da | 1063.4 µg/mL | |
| | Viscera | | - | - | 2146 Da | 212.7 µg/mL | |
| Oneknife Unicornfish (*Naso* | Skin | Protease from *Bacillus* sp. | - | - | <10 kDa | 10.18 µg/mL | Alolod *et al.* (2019) |
| *Merlangus merlangius* | Muscle | Pepsin and Trypsin-Chymotrypsin mixture | - | - | - | 1.4 µg/mU | Jensen *et al.* (2014) |
| *Micromesistius poutassou* | Whole | Subtilisin | 12 | - | - | 172 µg/mL | García-Moreno *et al.* (2017) |

species, the frame and trimming hydrolysate have the lowest $IC_{50}$ value. Other than this, some articles show fish bones having better antihypertensive activity. Mahmoodani *et al*. (2014) reported that catfish bone hydrolysate (3.2 µg/mL) exhibits better antihypertensive activity compared to its skin hydrolysate (1.3 µg/mL). FPH produced from fish head hydrolysate was reported to give the best antihypertensive activities (Vázquez, Meduíña, Durán *et al.,* 2019; Vázquez, Sotelo, Sanz *et al.,* 2019; Vázquez *et al.,* 2020). Grenadier head protein hydrolysate (195.6 µg/mL) has a lower $IC_{50}$ value than skin and bone hydrolysate (361.1 µg/mL) (Vázquez, Meduíña, Durán *et al.,* 2019). FPH produced from rainbow trout head hydrolysate (508.9 µg/mL) has a lower $IC_{50}$ value than trimming and frames hydrolysate (975.4 µg/mL). This is the same for Atlantic salmon head hydrolysate (478.5 µg/mL) having a lower $IC_{50}$ value than trimming and frame hydrolysate (653.7 µg/mL) (Vázquez, Sotelo, Sanz *et al.,* 2019). FPH produced by monkfish head shows a lower $IC_{50}$ value (931.3 µg/mL) than its viscera (1142.5 µg/mL) (Vázquez *et al.,* 2020). The difference in findings between protein sources of head and viscera might be caused by crude material of viscera containing protein of smaller size than head substrates, in which the highest $IC_{50}$ value was produced in trimming and frames hydrolysate.

Several studies have reported on the effect of enzymes on the antihypertensive activity of FPH (Nasri *et al.,* 2013; Chan *et al.,* 2020). According to Nasri *et al.* (2013), the goby muscle protein hydrolysate produced from different enzymes shows different ACE-inhibitory activities. The $IC_{50}$ of goby FPH produced with Alcalase (3.19 mg/mL), crude enzyme from *B. licheniformis* NH1 (3.33 mg/mL), *B. mojavensis* A21 (1.36 mg/mL), *B. pumilus* A1 (2.07 mg/mL), and *B. subtilis* A26 (1.74 mg/mL). The factor causing a difference in ACE-I activity can be caused by different enzyme specificity. Some of the peptides might be bio-inactive or bioactive. Therefore, the specificity of the enzyme used is crucial (Nasri *et al.,* 2013). The FPH produced from hybrid grouper head and bones also shows different $IC_{50}$ when hydrolysed with different enzymes: Alcalase (59.12%), Trypsin (48.54%), Proteinase K (55.92%), and pepsin (50.76%) (Chan *et al.,* 2020). The author concludes that Alcalase might be able to produce a mixture of peptides with more bioactive peptides. Other than commercial enzymes, FPH can be produced using crude enzymes extracted from the fish itself. The crude enzymes used in the reviewed journals are crude zebra blenny protease (Ktari *et al.,* 2014), crude Mekong giant catfish visceral peptidase (Ketnawa *et al.,* 2017), sardine viscera protease (Bougatef *et al.,* 2008), and smooth hound intestinal crude enzyme (Sayari *et al.,* 2016). These FPH obtained with crude protease from fish itself show a higher antihypertensive activity when compared with hydrolysis using other enzymes. This higher ACE-I activity might be due to better suitability (Bougatef *et al.,* 2008), substrate specificity, and the characteristics of the peptides produced (Ktari *et al.,* 2014; Sayari *et al.,* 2016). According to Bougatef *et al.* (2008), other types of enzymes used might result in lower ACE-I activity due to the cleaving of bioactive peptides at a position that would affect their bioactivity. García-Moreno *et al.* (2013) make a comparison on the effects of using trypsin after subtilisin enzyme and using both enzymes simultaneously on ACE-I activity of Atlantic chub mackerel, Atlantic horse mackerel, and European pilchard. The finding reported simultaneous usage of both enzymes having lower $IC_{50}$ values which are 345 µg/mL, 364 µg/mL, and 430 µg/mL respectively. The $IC_{50}$ value for hydrolysis using trypsin after subtilisin are 360 µg/mL, 395 µg/mL, and 400 µg/mL respectively. Furthermore, Mongkonkamthorn *et al*. (2020) investigated the interaction effect of hydrolysis parameters such as hydrolysis time and enzyme concentration on the $IC_{50}$ of DPPH and ABTS scavenging activity as well as the FRAP value of Tuna blood hydrolysate (TBH). Furthermore, Ghanbari *et al*. (2015) found that increasing the hydrolysis time improved the DPPH scavenging activity of sea cucumber hydrolysates using Alcalase and Flavourzyme.

The degree of hydrolysis (DH) is known to influence the characteristics and bioactivity of the peptide formed (Nasri *et al.,* 2013). Derouiche Ben Maiz *et al.* (2019) reported that the DH impacted the antihypertensive activity of bullet tuna viscera hydrolysate. For FPH produced with subtilisin, the $IC_{50}$ is the lowest at DH = 6% (0.40 mg/mL), whereas the others were DH = 3% (0.53 mg/mL) and DH = 9% (0.43 mg/mL). For FPH produced with endogenous enzyme, DH = 3% produced the lowest $IC_{50}$ (0.31 mg/mL), followed by DH = 6% (1.10 mg/mL) and DH = 9% (1.16 mg/mL). According to Chan *et al.* (2020), the result obtained shows that a higher degree of hydrolysis will produce FPH with higher ACE-inhibitory activities. However, it is until around DH = 6%, that the ACE-inhibitory activities would not increase despite increasing DH. Another finding by Bougatef *et al.,* (2008) shows that FPH with higher DH did not have higher ACE-inhibitory activities. However, Ghanbari *et al*. (2015) reported that cleavage to small peptides was caused by increased DH and ACE inhibition activity. Small peptides containing hydrophobic amino acids such as methionine, histidine, and tyrosine are suggested to improve the efficiency of antioxidant and ACE inhibitory activities by increasing antioxidant activity (Li *et al*., 2014). Kasiwut *et al*. (2019) discovered that a high DH value enzyme Alcalase contained low MW peptides and had a high ACE

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inhibitory activity when tested with a tuna cooking juice hydrolysate.

Molecular weight (MW) is also observed to have an influence on the antihypertensive activity of the peptide formed. Based on Karou's (2019) study, the $IC_{50}$ value of European hake protein hydrolysate decreased with increasing DH and decreasing MW. The lowest $IC_{50}$ was 0.26 mg/mL (DH = 8.6%, MW = 1216 Da), followed by 0.63 mg/mL (DH = 7.7%, MW = 1859 Da), 0.94 mg/mL (DH = 6.5%, MW = 2271 Da), and 1.4 mg/mL (DH = 5.3%, MW = 3292 Da). This is similar to another finding (Azemi *et al.,* 2017), which reported that fractioned eel muscle hydrolysate showed higher ACE-I activity at lower molecular weight, 71.90% (3 kDa). While higher molecular weight shows lower ACE-I activity: 49.05% (5 kDa) and 17.52% (10 kDa). Lin and Alashi (2017) reported that lower MW shows higher antihypertensive activity. The $IC_{50}$ of the Nile tilapia protein hydrolysates were 0.41 mg/mL (<1 kDa), 0.55 mg/mL (1-3 kDa), 0.79 mg/mL (3-5 kDa), and 0.83 mg/mL (5-10 kDa). Kawakawa muscle hydrolysate also showed a similar pattern on $IC_{50}$ (Taheri and Bakhshizadeh G, 2020). The lowest $IC_{50}$ is obtained from MW of <1 kDa (0.44 mg/mL), followed by 1-3 kDa (0.52 mg/mL), 3-10 kDa (1.86 mg/mL), and >10 kDa (1.94 mg/mL). There is however a slight difference in another finding (Abdelhedi *et al.,* 2018). The $IC_{50}$ of smooth hound viscera hydrolysate produced by Purafect showed a similar pattern, which is 53.31 µg/mL (<1 kDa) and 85.86 µg/mL (3-1 kDa). However, the hydrolysate produced by Neutrase showed a different pattern, where the lowest $IC_{50}$ is 75.05 µg/mL (3-1 kDa), followed by 117.76 µg/mL (<1 kDa). However according to Abdelhedi *et al.* (2018), a MW <300 Da will exhibit high ACE-inhibitory activity and that unfractionated FPH and peptides with >4 kDa MW will have lower bioactivity. In short, there is a similarity in the findings, where fractions with lower molecular weight have better antihypertensive activity. ACE-Inhibitory peptides are isolated or purified from FPH to obtain peptides with vary structure properties such as molecular weight, peptide length, peptide sequence and amino acid composition, which these characteristics will affect the ACE-I activity of the peptides (Abachi *et al.,* 2019). Bioactive peptides can be isolated through different purification methods that are often used in combination. Most of the FPH is fractionated first using ultrafiltration membranes to obtain fractions with a lower molecular weight FPH (Byun and Kim, 2001). After that, fractions with the best ACE-inhibitory properties will have their peptides fractionated again by purification methods such as chromatographic methods and membrane filtration methods (Abachi *et al.,* 2019). The isolation of peptides from FPH is necessary to characterize peptides with interesting properties such as antihypertensive properties. This is because certain properties of a protein might be contributed by only a fraction of the protein itself (Berg *et al.,* 2002). Also, isolated ACE-I peptides have stronger ACE-Inhibitory activity than the crude FPH. This is because peptides of lower molecular weight and chain length have a higher ACE-I activity (Yathisha *et al.,* 2019). Table 2 shows the peptide sequence, molecular weight, purification method and ACE-I activity of bioactive peptide purified from FPH from various fish species and parts. Furthermore, smaller peptides may be absorbed more easily and quickly in the intestine by the digesting system, as evidenced by the MW <1 kDa fraction with the highest antioxidant and ACE inhibition activity (Mongkonkamthorn *et al*., 2020).

The ACE-I activity of the peptides varies. This is due to the difference in peptide structure which involves amino acid component and sequence, and the chain length (Daskaya-Dikmen *et al.,* 2017). In general, ACE-I peptides inhibit ACE activity by binding into the active sites of ACE. In Daskaya-Dikmen *et al.* (2017) review, large peptides with long chain lengths cannot bind effectively into the active sites (Murray and FitzGerald, 2007). The binding of the ACE-I peptides to the active sites also depends on the amino acid at C-terminal. Peptides with (tyrosine, phenylalanine, tryptophan, proline, and lysine) at the position of the C-terminal exhibit better ACE-Inhibitory properties (Murray and FitzGerald, 2007). This structure and activity relationship is similar to another finding (Lin *et al.,* 2019) where peptides with Trp on the C-terminal exhibit a better ACE-inhibitory activity or lower IC50 than the other two with Trp on N-terminal. According to Jung *et al.* (2006), peptides cleaved by α-chymotrypsin will contain amino acids (proline, alanine, valine, leucine, and histidine) on the C-terminal which exhibit ACE-inhibitory activities. This is similar to Intarasirisawat *et al.* (2013) findings, where the peptide with valine on the C-terminal exhibit the highest ACE-inhibitory properties. For peptides obtained from lizardfish protein hydrolysate, they show different peptide characteristics. According to Wu *et al.* (2012), peptides with hydrophobic amino acid (Arg) at the position of the C-terminal, in addition to its positive side chain group will show good ACE-inhibitory properties. Compared to Wu *et al.* (2015), the peptide obtained contain a hydrophilic amino acid group (Pro) on its C-terminal, therefore, it shows a lower ACE-inhibitory activity than the previous. This is supported by Lan *et al.* (2015) findings, where smaller and hydrophobic amino acid at the C-1 position is better at binding with the active sites of ACE.

FPH can be purified in different ways such as according to their size, charge, and binding affinity and

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Table 3. Characteristics of antihypertensive peptides isolated from fish protein hydrolysates

| Peptide Sequence | Purification Method | Molecular Weight | $IC_{50}$ | References |
|---|---|---|---|---|
| Trp-Ala-Ala | UF, SEC, RP-HPLC | 630 – 450 Da | 118.50 µM | Lin *et al.* (2019) |
| Ala-Trp-Trp | | 630 – 450 Da | 9.40 µM | |
| Ile-Trp-Trp | | 630 – 450 Da | 0.51 µM | |
| Trp-Leu | | 630 – 450 Da | 26.80 µM | |
| Ala-Arg-Ser | SEC, RP-HPLC | 332 Da | - | Nasri *et al.* (2013) |
| Val-Val-Ala-Pro-Phe-Ala-His-Gly-Thr | | 897 Da | - | |
| Arg-Ser-Thr-Ala | | 433 Da | - | |
| Phe-Tye-Pro-Pro | | 522 Da | - | |
| Arg-Cys-Ser-Ala-Gly-Val | | 591 Da | - | |
| Ser-Pro-Arg-Cys-Arg | UF, SEC, HPLC | 617 Da | 41 µM | Wu *et al.* (2012) |
| Arg-Val-Cys-Leu-Pro | UF, SEC, IEC, HPLC | - | 175 µM | Wu *et al.* (2015) |
| Gly-Met-Lys-Cys-Ala-Phe | MAM-ACE, RP-HPLC | 655.2 Da | 47.3 µM | Lan *et al.* (2015) |
| Arg-Tyr-Arg-Pro | UF, RP-HPLC, IMAC | 592.0 Da | 52 µM | Sun *et al.* (2017) |
| Ala-Gly-Pro-Pro-Gly-Ser-Asp-Gly-Gln-Pro-Gly-Ala-Lys | IEC, SEC | <2 KDa | 420 µM | Chen *et al.* (2018) |
| Ala-His-Leu-Leu | UF, SEC, RP-HPLC | 452 Da | 40.3 µM | Li *et al.* (2012) |
| Gly-Pro-Met | UF, SEC, IEC, RP-HPLC | <2 Kda | 17.13 µM | Byun amd Kim (2001) |
| Gly-Pro-Leu | | <2 Kda | 2.65 µM | |
| Ala-Pro-Gly-Ala-Pro | SEC, RP-HPLC | - | 170.28 µM | Lassoued *et al.* (2015) |
| Gly-Ile-Pro-Gly-Ala-Pro | | - | 27.9 µM | |
| Leu-Trp | SEC, RP-HPLC | 317.25 Da | 5.6 µM | Zou *et al.* (2014) |
| Pro-Pro | SEC, RP-HPLC | - | 1912.46 µM | Neves *et al.* (2017) |
| Gly-Phe | | - | 178.14 µM | |
| Gly-Pro-Val-Ala | | - | 445.61 µM | |
| Gly-Gly-Pro-Ala-Gly-Pro-Ala-Val | | - | 673.16 µM | |
| Arg | | - | 98.04 µM | |
| Tyr | | - | 132.84 µM | |
| Gly-Leu-Pro-Leu-Asn-Leu-Pro | SEC, RP-HPLC | 722 Da | 18.7 µM | Lee *et al.* (2014) |
| Leu-Val-Thr-Gly-Asp-Asp-Lys-Thr-Asn-Leu-Lys | UF, SEC, RP-HPLC | 1204.67 Da | 6.72 µM | Aissaoui *et al.* (2017) |
| Asp-Thr-Gly-Ser-Asp-Lys-Lys-Gln-Leu | | 922.51 Da | 5.44 µM | |
| Ala-Leu-Gly-Pro-Gln-Phe-Tyr | RP-HPLC, IEF | 794.44 Da | 0.012 mM | Auwal *et al.* (2019) |
| Lys-Val-Pro-Pro-Lys-Ala | | 638.88 Da | 0.980 mM | |
| Leu-Ala-Pro-Pro-Thr-Met | | 628.85 Da | 1.310 mM | |
| Glu-Val-Leu-Ile-Gln | | 600.77 Da | 1.440 mM | |
| Glu-His-Pro-Val-Leu | | 583.4 Da | 1.680 mM | |
| Leu-Gly-Pro-Leu-Gly-His-Gln | UF, FPLC, SEC | 720 Da | 4.22 µM | Ngo *et al.* (2015) |
| Met-Val-Gly-Ser-Ala-Pro-Gly-Val-Leu | | 829 Da | 3.09 µM | |
| Arg-Gln-Leu-Ala-Gly-Pro | SEC in FPLC system | 640.32 Da | 6.24 µM | García-Moreno *et al.* (2015) |
| Glu-Leu-Ser-Ala-Pro | | 515.31 Da | 7.08 µM | |
| His-Leu-Ala-Leu-Thr | | 553.32 Da | 5.11 µM | |
| Tyr-Leu-Gly-Trp | | 537.26 Da | 0.09 µM | |
| Val-Ala-Met-Pro-Phe | | 563.27 Da | 0.44 µM | |
| Pro-Gly-Cys-Phe | | 422.19 Da | 1.33 µM | |
| His-Leu-Phe | | 415.22 Da | 9.56 µM | |
| Met-Ile-Phe-Pro-Gly-Ala-Gly-Gly-Pro-Glu-Leu | UF, IEC, HPLC, RP-HPLC | 1.3 kDa | 28.7 µg/mL | Jung *et al.* (2006) |
| Asp-Pro-Ala-Leu-Ala-Thr-Glu-Pro-Asp-Pro-Met-Pro-Phe | IEF, HPLC | 1382 Da | 62.6 µM | Vo *et al.* (2011) |
| Leu-Ser-Gly-Tye-Gly-Pro | - | - | 2.577 µmol/L | Lin *et al.* (2019) |
| Met-Cys-Ser | SEC | 340.39 Da | 0.29 µM | Toopcham *et al.* (2017) |

https://doi.org/10.26656/fr.2017.7(4).044

Table 3 (Cont.). Characteristics of antihypertensive peptides isolated from fish protein hydrolysates

| Peptide Sequence | Purification Method | Molecular Weight | $IC_{50}$ | References |
|---|---|---|---|---|
| Ala-Arg | UF, RP-HPLC | 245.28 Da | 448.47 μM | Salampessy *et al.* (2015) |
| Ala-Val | UF, RP-HPLC | 188.23 Da | 371.89 μM | |
| Ala-Pro-Glu-Arg | UF, RP-HPLC | 471.51 Da | 190.88 μM | |
| Met-Leu-Val-Phe-Ala-Val | UF, IEC, RP-HPLC | 678 Da | 3.07 μM | Intarasirisawat *et al.* (2013) |
| Phe-Val-Ser-Ala-Cys-Ser-Val-Ala-Gly | UF, IEC, RP-HPLC | 839 Da | 3.49 μM | |
| Met-Cys-Tye-Pro-Ala-Ser-Thr | UF, IEC, RP-HPLC | 771 Da | 58.73 μM | |
| Asp-Leu-Asp-Leu-Arg-Lys-Asp-Leu-Tyr-Ala-Asn | UF, IEC, RP-HPLC | 1334 Da | 67.43 μM | |
| Tyr-Val-Asn-Asp-Ala-Ala-Thr-Leu-Leu-Pro-Arg | UF, IEC, RP-HPLC | 1231 Da | 105.85 μM | |
| Leu-Ala-Asp-Gly-Val-Ala-Ala-Pro-Ala | UF, IEC, RP-HPLC | 854 Da | 317.75 μM | |
| Asp-Trp-Met-Lys-Gly-Gln | UF, IEC, RP-HPLC | 763 Da | 422.92 μM | |
| Gly-Asp-Leu-Gly-Lys-Thr-Thr-Thr-Val-Ser-Asn-Trp-Ser-Pro-Pro-Try-Lys-Asp-Thr-Pro | IEC, RP-HPLC | 2482 Da | 11.28 μM | Lee *et al.* (2010) |

the efficiency of purification can be accessed by electrophoresis (Berg *et al.,* 2002). Based on Table 3, most of the peptides are isolated using a combination of different methods. There are two articles that focus on presenting new purification methods to improve the process, which is the MAM-ACE followed by RP-HPLC method (Lan *et al.,* 2015), and UF followed by IMAC and RP-HPLC method (Sun *et al.,* 2017). These two methods separate peptides based on their binding affinity. The first method by Lan *et al.* (2015) proposed a rapid purification method using magnetic affinity separation. The method involves using a magnet to recover the magnetic agarose microsphere immobilized ACE (MAM-ACE) extract, followed by purification using RP-HPLC. This method is studied to give faster and more effective results. The second method by Sun *et al.* (2017) uses Immobilized Metal Affinity Chromatography (IMAC) with nickel (II) ion ($Ni^{2+}$). The general mechanism is to obtain fractions that have a binding affinity with $Ni^{2+}$. Fractions that bind with $Ni^{2+}$ will then undergo further purification through RP-HPLC. The author concludes that this method requires fewer steps, produces peptides with higher purity and is more efficient, faster, and cheaper.

## 4. Conclusion

In this review article, the various species of fish and proteolysis methodologies offer different FPH and bioactive peptides, which can reduce its potentially negative environmental impact and could be a potential bioactive ingredient for food, or dietary supplement industries. Based on data collection and results obtained, the enzymatic hydrolysis method is the most commonly method used for fish waste treatment. The fish hydrolysate product from this method has shown their potential industrial applications as functional food ingredients, by producing favourable amino acid profiles, and beneficial biological activities. Furthermore, many researchers discovered the strongest correlation between molecular weight and antihypertensive activity, with lower molecular weight peptides having a lower $IC_{50}$ value and higher antihypertensive activity. However, several limitations need to be overcome before they can be used in large-scale consumption. The production of peptide FPH is still in small-scale production since it is expensive and time-consuming for commercial manufacture. The identification and understanding of protein peptide composition and peptide structure-function activities, are still required and need more study.

## Conflict of interest

The authors declare no conflict of interest.

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