The Annals of the University Dunarea de Jos of Galati
Fascicle VI – Food Technology (2022), 46(2), 188-206

REVIEW PAPER

ENZYMATIC HYDROLYSIS OF MARINE FISH GELATIN FOR PRODUCING ACE INHIBITOR PEPTIDES: META-ANALYSIS

AHMAD, SUKARNO*, SLAMET BUDIJANTO, AZIS BOING SITANGGANG

Department of Food Science and Technology, Faculty of Agricultural Engineering and Technology, IPB University, Bogor, Indonesia

*Corresponding author: dsukarno@apps.ipb.ac.id

Received on 8 June 2022
Revised on 23 September 2022

Abstract

Bioactive peptides showing Angiotensin-I-Converting Enzyme inhibitory (ACEi) activity can control blood pressure. They can be produced from various protein sources, including fish gelatin. This article provides a systematic review and meta-analysis on gelatin extraction, conditions of enzymatic hydrolysis, characteristics of ACEi peptides, and effect of hydrolysis procedure on ACEi peptides. Fish gelatin preparation uses various solutions such as acids, bases and distilled water. Enzymatic hydrolysis is carried out with an E/S ratio of 0.2 – 7% and temperature of 28.9 ºC (using bromelain) and 60.6 ºC (using Alcalase). Level of pH in hydrolysis also varied greatly, from 2 (using Pepsin) to 10 (using Purafect enzyme). The molecular weight of fish gelatin ACEi peptides ranged from 186-829 Da and the sequence of peptides was dominated by hydrophobic and aliphatic amino acids. Based on a meta-analysis, hydrolysis using enzyme combination resulted in more satisfying product than a single enzyme, represented with a combined effect size value of 0.593.

Keywords: angiotensin I converting enzyme, fish gelatin, hydrolysis, hydrolytic preparation, protein hydrolysis

Introduction

Hypertension is defined as having a systolic blood pressure greater than 140 mmHg and a diastolic blood pressure greater than 90 mmHg (WHO, 2020). Prihartono *et al*. (2022) reported that prevalence of hypertension has increased significantly in Indonesia over the last five years, with 46% of farmers in West Java experiencing hypertension. In Indonesia, 3322 people (56.2%) were found to have undiagnosed hypertension (Mahwati *et al.,* 2022). Synthetic drugs that act as ACEi have been used to treat hypertension. Angiotensin converting enzyme (ACE) is a

https://doi.org/10.35219/foodtechnology.2022.2.13

metallopeptidase that participates in the conversion of peptides such as angiotensin I to angiotensin II, which causes vasoconstriction and aldosterone action (Sturrock *et al*., 2004). ACE functions as a hydrolase in RAAS (Renin-Angiotensin Aldosterone System), which promotes the synthesis and release of aldosterone as well as the production of reactive oxygen, resulting in an increase in blood pressure or hypertension (Xu *et al*., 2021). Captopril, enalapril, zofenopril, ramipril, fosinopril, lisinopril, and SQ 29852 are commonly used ACEi (Ahmad *et al*., 2019). To enhance the efficacy, Man *et al*. (2020) studied a combined captopril, and this significantly reduced blood pressure.

The search for disease treatment has raised remarkably, including through functional food which provides health benefit beyond basic nutrition. Bioactive peptides are one of the most potential ingredients (Martirosyan and Singh, 2015), which meet functional food characteristics. Functionality of bioactive peptides (BPs) relates to various health-promoting properties such as anticancer, antihypertensive, antimicrobial, cholesterol-lowering, and antidiabetic activity (Barati *et al*., 2020). Technically, BPs are derived from the parent protein and then hydrolyzed into 3-20 peptide fragments with the aid of enzymes to form fish protein hydrolysates – FHPs (Wang, 2021). Body parts of fish are good source of collagen protein, including skin, bones, head, and scales.

Furthermore, collagen can be degraded into gelatin, which is an insoluble fraction (Gorgieva and Kokol, 2011). Collagen is the parent protein of fish gelatin which can be used as a source of bioactive peptides (Regenstein and Zhou, 2006). Fish by-products such as skin, bones and tendons, contain type 1 collagen (Ahmad *et al*., 2017). Several studies showed that gelatin could be employed as a protein source in the production of bioactive peptides. As argued by Mahmoodani *et al*. (2014), hydrolysis of catfish skin and bone gelatin produced antihypertensive peptides with IC50 activity of 3.2 g/ml and 1.3 g/ml, respectively. Hydrolysis of Theragra chalcogramma skin gelatin yielded antihypertensive peptide of 17.13 M (Byun and Kim, 2001). These findings revealed antihypertensive activity from peptide of fish gelatin, but the bioactivity of the BPs may differ remarkably, depending on how they are produced. Meanwhile, discussion on the impacts of hydrolysis method to BPs has remained inconclusive. To fill the gap, this systematic review and meta-analysis work discusses the effects of process conditions in manufacture of antihypertensive peptides from marine fish gelatin.

Materials and methods

Determination of research questions, inclusion and exclusion criteria

A laptop, Mendeley, Microsoft Word 2013, Microsoft Excel 2013, OpenMEE, and Colander were among the tools and materials used. The Sitanggang *et al*. (2021) method, with some PRISMA modifications (preferred reporting items for systematic review and meta-analysis). The literature search began with research questions as follows: How is gelatin substrate prepared, and what are the best conditions for enzymatic hydrolysis to produce ACEi peptides from fish gelatin protein?

PEO (population, exposure, and outcome) approach was applied as search strategy, i.e. population including "bone and skin gelatin from marine fish," exposure including "enzymatic hydrolysis", and outcome including "ace inhibitor." Then, for a more general Boolean search, alternative search of PEO synonyms was applied, i.e. ("enzymatic preparation" OR "enzymatic hydrolysis") AND ("fish gelatin" OR "marine fish" OR "collagen" OR "bone" OR "skin") AND ("ACE inhibitory" OR "bioactive peptide" OR "hypertension" OR "antihypertensive" OR "Angiotensin converting enzyme"). In several databases, such as Proquest, ScienceDirect, and Scopus, Boolean search was used as a keyword to find the journals required.

Criteria applied for article search focused on the relationship between activity of ACEi peptides and enzymatic hydrolysis preparations from marine fish gelatin (skin, scales, and bones). Subsequently, the collected articles were screened using the following steps: article type, duplication removal, as well as selection based on title and abstract by removal of articles unfitting to the inclusion criteria. The full-text articles were assessed for eligibility based on inclusion and exclusion criteria. Inclusion criteria included ACEi peptide activity, enzyme treatment, hydrolytic preparation, hydrolysis of gelatin, gelatin or fish collagen, English language article, no time limit for publication, non-reviewed and unduplicated research articles.

Article search

Article search was conducted on October 26, 2021, covering various databases fitting Boolean search, i.e. Scopus, Proquest, and Science Direct. There are minor technical differences in the use of colons, parentheses, and word count when searching each database. For the article screening process, PRISMA guidelines (figure 1) and a colander were used. The screening process resulted in 21 eligible articles based on inclusion criteria, and 3 of them were included in meta-analysis referring to Boolean search. The OpenMEE application was used to calculate individual and combined effect sizes in meta-analysis data using the dersimonian–laird random-effects model.

Results and discussion

Fish gelatin substrate preparation as a source of ACEi peptide

Fish protein can act as a promising source of protein for daily protein requirement, and it can be converted into gelatin. The fish by-products constitute a major raw material for gelatin production. However, some parts of the fish body are often used as parent proteins for production of ACEi bioactive peptides, including: head, skin, meat, scales, swim bladder, viscera and bones. Additionally, finished or semi-finished materials (have gone through a processing process) such as isolates and concentrates of fish are also can be parent proteins in the production of fish gelatin.

According to Shahidi (1994) and Alam (2007), fish protein is classified into myofibril, sarcoplasmic, stromal proteins; in this regard, collagen belongs to stromal protein, which is a main material for gelatin extraction. There are two major processes of gelatin manufacture: pretreatment and extraction (Ahmed *et al*., 2020).

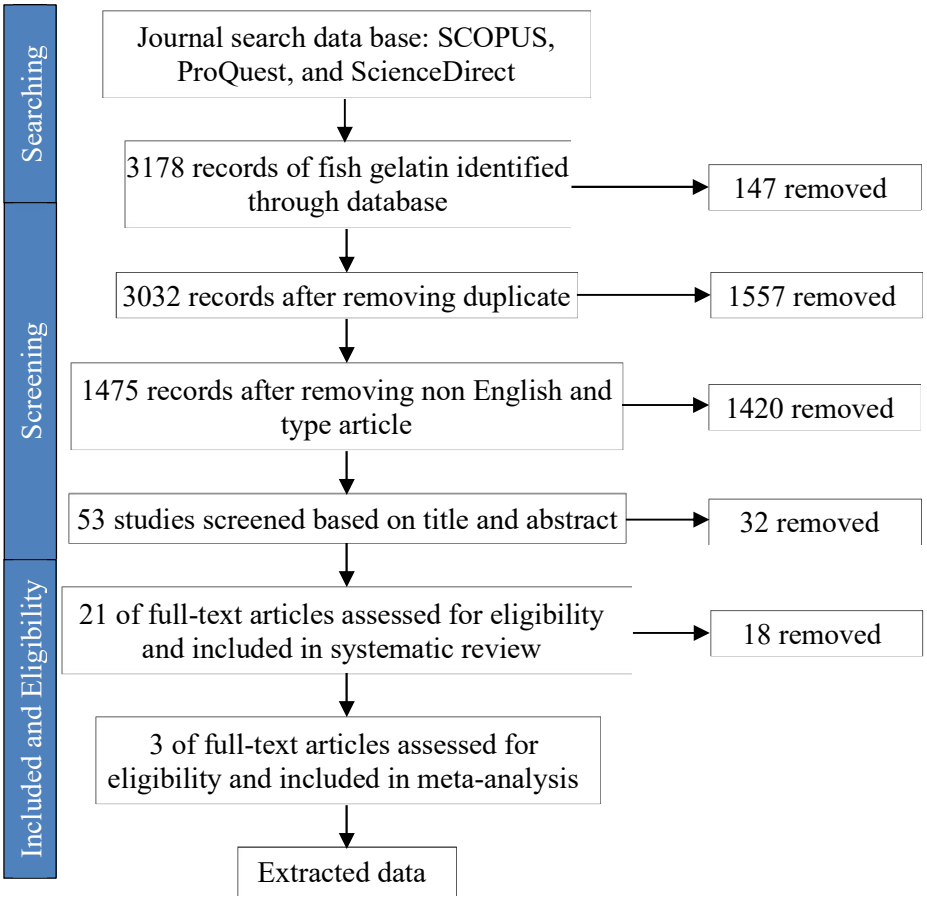

Figure 1. PRISMA guidelines for the inclusion of articles on the enzymatic preparation of ACEi peptides from fish gelatin proteins.

Based on the data in table 1, fish gelatin was often extracted from scales, skin, a mixture of skin and scales, as well as a mixture of bone and skin. Differences in materials affect the gelatin extraction process. Extraction of fish skin gelatin used 1-4 solvents, such as bases and acids, either strong or weak, and distilled water. Extraction of scale gelatin often used 1-5 solvents including such as salt solution (NaCl), strong base, alcohol, chelating (EDTA), strong acid, weak acid, enzymes and distilled water. Extraction of gelatin from a mixture of scales and skin using 3 solvents (NaOH, n-butanol, distilled water) was reported by Mirzapour-Kouhdasht *et al*. (2021a). Extraction of gelatin from a mixture of bone and skin using 2 alkaline solvents and distilled water (Neves *et al*., 2017), while another experiment worked with 4 solvents, including NaOH, H_2SO_4, $C_6H_8O_7$ and distilled water (Vázquez *et al*., 2019).

Table 1. Extraction of fish gelatin substrate.

Species	Fish parts	Extraction Condition				References
		Solution	t (h)	T(°C)	C	
Theragra chalcogramma	Skin	Alkaline	72	5	Ne	Byun and Kim (2001)
		Aquadest	Ne	Ne	Ne	
Sea bream	Scale	HCl	24	R	0.6 N	Fahmi *et al.* (2004)
Salmo salar L.	Skin	Alkaline	Ne	55	Ne	Gu *et al.* (2011)
Gadus macrocephalus	Skin	$Ca(OH)_2$	24	Ne	1%	Ngo *et al.* (2011)
		Aquadest	30	60	Ne	
Sparus aurata	Scale	NaCl	0.5	Ne	5%	Mosquera *et al.* (2014)
		NaOH	1	Ne	0.1 M	
		Isobutil alkohol	0.5	Ne	10%	
		EDTA	Ne	Ne	0.5 M	
		Aquadest	12	45	Ne	
Sparus aurata	Bone, scale	NaCl	0.5	Ne	5%	Akagündüz *et al.* (2014)
		NaOH	1	Ne	4 g/L	
		isobutil alcohol	0.5	Ne	10%	
		EDTA	12	Ne	146 g/L	
		CH_3CO_2H 99%	3	Ne	2.85 mL/L	
		Aquadest	12	60	Ne	
Okamejei kenojei	Skin	$Ca(OH)_2$	24	Ne	1%	Ngo *et al.* (2014)
		Aquadest	30	60	Ne	
Thunnus albacares	Skin	NaOH	69.6	10	1.9%	Han *et al.* (2015)
		Aquadest	4.7	56.6	Ne	
Ghodus macrocephalus	Skin	$Ca(OH)_2$	24	Ne	1%	Ngo *et al.* (2016)
		Aquadest	30	60	Ne	
Synodus fuscus	Scale	NaOH	6	4	0.1 M	Chen *et al.* (2017)
		CH_3CO_2H	24	Ne	0.5 M	
		NaCl	Ne	Ne	2 M	
		CH_3CO_2H	Ne	Ne	0.1 M	
		Aquadest	Ne	Ne	Ne	
Salmo salar	Bone, Skin	Alkaline	0.25	R	Ne	Neves *et al.* (2017)
		Aquadest	16	70	Ne	
Oncorhynchus mykiss	Skin	NaOH	0.5	4	0.2 M	Cheung and Li-Chan, 2017
		CH_3CO_2H	3	R	0.05 M	
		Aquadest	2	70	Ne	

Hemiramphus far	Skin	NaOH	2	4	0.05 M	Abdelhedi *et al.* (2017)
		CH_3CO_2H	48	4	0.1 M	
		Aquadest	18	40	Ne	
Synodus macrops	Scale	NaOH	2	Ne	0.05 M	Chen *et al.* (2018)
		Pepsin	5	50	1%	
Decapterus Macrosoma	Skin	NaOH	0.67	45	0.15%(b/v)	Rasli and Sarbon (2018)
		H_2SO_4	Ne	45	0.15%(b/v)	
		$C_6H_8O_7$	Ne	45	0.7% (b/v)	
		Aquadest	Ne	45	Ne	
Macrourus sp., Capros aper, Lepidorhombus boscii, Merluccius merluccius, Trachurus trachurus	Bone, skin	NaOH	0.5	22	0.2%	Vázquez *et al.* (2019)
		H_2SO_4	0.5	22	0.2%	
		$C_6H_8O_7$	0.5	22	1%	
		Aquadest	Ne	45	Ne	
Scomberomorus commerson	Scale, skin	NaOH	24	Ne	1 N	Mirzapour-Kouhdasht *et al.* (2021a)
		n-butanol	Ne	Ne	10%	
		Aquadest	5.8	70.7	Ne	
Gadus morhua	Skin	NaOH	4	25	0.1 M	Coscueta *et al.* (2021)
Salmo salar L.	Skin	Alkaline	Ne	55	Ne	Liu *et al.* (2021)
Salmo salar	Skin	NaOH	0.5	22	0.05 M	Vázquez *et al.* (2021)
		H_2SO_4	0.5	22	0.02 M	
		$C_6H_8O_7$	0.5	22	0.052M	

Gelatin extraction is performed using various types and quantities of solvents, depending on composition of raw material or parent protein. For example, fish bones and scales constitute a biocomposite material consisting of hydroxyapatite and type I collagen fibers, thus requiring additional demineralization before collagen isolation. The source of raw materials dramatically influences the extraction process, because each fish species possesses different structures, chemical composition, and complexity (Salvatore *et al*., 2020). In pre-treatment stage, solvent is applied differently in accordance with the target. Strong acid and strong base solvents in combination with temperature are usually applied to remove non-collagen compounds, but their presence may hydrolyze e collagen into gelatin. The soaking process using alkaline solutions such as NaOH and $Ca(OH)_2$ was applied for 0.5 to 72 hours at 4-55 ^oC, while soaking using acid solutions such as HCl and H_2SO_4 was performed for 0.5 to 24 hours at 22-45 ^oC. According to Benjakul *et al*. (2012), the effect of solution concentration was stronger than type of solution. For pre-treatment, salt solution (NaCl) and EDTA are also often applied. The soaking process in NaCl

enables to remove contaminants, but it can also facilitate extraction of targeted compound when added in further stages. The later condition is known as the salt solubilization extraction method. Chelating (EDTA) is employed for demineralization (Ahmed *et al*., 2020). Some acids such as HCl are intended to reduce minerals (Benjakul *et al*., 2012). Ahmed *et al*. (2020) argued that alcohol groups such as n-butanol were effective for removal of pigments and fats. Previous researches by Byun and Kim (2001); Ngo *et al*. (2011); Ngo *et al*. (2014); Ngo *et al.* (2016) applied skin pre-treatment that yielded a better ACEi peptide activity than others. The pre-treatment process was carried out by soaking in a solution of NaOH or $Ca(OH)_2$ for 1-3 days at 5 °C and room temperature. In addition, pre-treatment for bones/scales as reported by Fahmi *et al.* (2004) was applied using HCl solution for 24 hours at room temperature.

Hydrolysis conditions of fish gelatin for production of ACEi peptides

Hydrolysis conditions are influenced by the type of enzyme, pH, temperature, time and E/S ratio (Sitanggang *et al*., 2021). Enzyme showed a specific mode of action and differed each other. The origin of enzymes is a crucial factor that determines their activity, and as presented in Table 2, we recorded three groups of enzymes by isolation source as follows: microorganisms, plants and digestion. Microbial enzymes included Alcalase, Corolase, Neutrase, Esperase, Pronase, Protease, Purafect and Subtilisin A. Enzymes from plants included Bromelain, Papain and Actinidin, while digestive enzymes included Pepsin, Pancreas enzyme and Trypsin. *Bacillus* is predominant bacteria producing enzymes, such as Alcalase, Purafect and Subtilisin A enzymes produced by *Bacillus licheniformis* (Gu *et al*., 2011; Abdelhedi *et al*., 2017; Mirzapour-Kouhdasht *et al*., 2021a). Neutrase from *Bacillus amyloliquefaciens* (Eckert *et al*. 2019), Corolase from *Bacillus subtilis* (Cheung and Li-Chan, 2017), Esperase and Alkaline protease from *Bacillus sp* (Akagündüz *et al*., 2014; Fahmi *et al*., 2004). Pronase from *Streptomyces griseus* (Byun and Kim, 2001), Protease from microbial mold *Aspergillus satoi type XIII* (Ngo *et al*., 2014). As stated by Dos Santos Aguilar and Sato (2017), microbial enzymes are favorable since they can be produced in a short fermentation time and possibly engineered to modify the properties.

Regarding the mode of action, endopeptidase seems to be the most common. The enzyme activity depends on the site of action, commonly classified into two groups: exopeptidase and endopeptidase. Endopeptidase hydrolyzes middle chain of protein (Subin and Bhat, 2016). Each type of enzyme has a specific action and the work of the enzyme is influenced by the hydrolysis conditions. Based on the data obtained, the conditions of enzymatic hydrolysis of fish gelatin can be divided into 2 groups regarding the hydrolysis system, namely a single hydrolysis system (single enzyme hydrolysis) and a combination of enzyme hydrolysis. Both enzymatic actions predominantly occur in microbial enzymes, namely Alcalase, Corolase, Esperase and Neutrase. The combined hydrolysis of enzymes after Alcalase was dominated by Papain enzymes isolated from plants. According to Nasri (2017), Alcalase more often cleaves peptide bonds at C terminal containing hydrophobic amino acids.

Table 2. Enzymatic hydrolysis conditions and characteristics of fish gelatin ACEi peptides.

Hydrolysis Condition				Peptides		Activity of ACEi		References
T (ºC)	t (h)	pH	E/S	Residue	Da	%	µM	
Alcalase[en]--Pronase[en/ek]-- Kolagenase[en]								
50--50---37	2--2--2	8--8--7.5	2%[*]--3%[*]--1%[*]	GPM GPL	303 285	Ne Ne	17.13 2.6	Byun and Kim (2001)
Alkalin protease[en]								
60	1	8	5%[*]	GY VY GF VIY	238 280 222 393	Ne Ne Ne Ne	265 16 708 7.5	Fahmi *et al.* (2004)
Alcalase[en]--Papain[en]								
50--60	3--2	8.5--7	1%[*]--2%[*]	AP VR	186 273	Ne Ne	322 1216	Gu *et al.* (2011)
Papain[en]								
Ne	4	Ne	1%[*]	TCSP TGGGNV	406 503	81 68	Ne Ne	Ngo *et al.* (2011)
Esperase 8.0 L[en]								
60	3	8	7%[#]	Ne	Ne	Ne	Ne	Mosquera *et al.* (2014)
Alcalase[en]--Protease[en]								
50--37	6--6	7--7	1%[*]--1%[*]	MVGSAPGVL LGPLGHQ	829 720	Ne Ne	3.09 4.22	Ngo *et al.* (2014)
Esperase 8.0 L[en]								
60	3	8	7%[#]	Ne	Ne	Ne	Ne	Akagündüz *et al.* (2014)
Alcalase[en]								
60	6	Ne	1:1[*]	Ne	Ne	Ne	Ne	Han *et al.* (2015)
Pepsin[en]--Tripsin[en]--Pancreas[ek]								
Ne--55--37	Ne--Ne--1--2	Ne--8--6.3	Ne	Ne	Ne	Ne	Ne	Manikkam *et al.* (2016)
Pepsin[en]								
37	4	2	1%[Ne]	GASSGMPG LAYA	662 436		6.9 14.5	Ngo *et al.* (2016)
Neutrase[en]								
50	8	7	Ne	Ne	Ne	Ne	Ne	Chen *et al.* (2017)
Corolase PP[en]								
50	1	7	1%[*]	PP GF	212 222		1912.5 178.1	Neves *et al.* (2017)

				GPVA GGPAGPAV	343 626		445.61 673.16	
Corolase N[en]								
55	6	7.5	1%[Ne]	Ne	Ne	Ne	Ne	Cheung and Li-Chan (2017)
Purafect[en]								
50	3	10	30:1[U]	Ne	Ne	Ne	Ne	Abdelhedi *et al.* (2017)
Alcalase[en]								
60	2	9	2.92 %[Ne]	Ne	Ne	Ne	Ne	Rasli and Sarbon (2018)
Neutrase[en]								
47	2.83	7	3.2%[*]	AGPPGSDGQPGAK	827		420	Chen *et al.* (2018)
Alcalase[en]								
60.6	4	8.6	1%[#]	Ne	Ne	Ne	Ne	Vázquez *et al.* (2019)
Subtilisin A[en]--Actinidin[en]								
55--37	3--3	8.5--7.5	1%[*]--1%[*]	Ne	Ne	Ne	Ne	Mirzapour-Kouhdasht *et al.* (2021a)
Bromelain[en]								
28.9	0.5	4	0.5%[*]	Ne	Ne	Ne	Ne	Coscueta *et al.* (2021)
Alcalase[en]--Papain[en]								
50--60	3--2	8.5--7	1%[Ne]--2%[Ne]	GR RER GPR GPAG NVG LQ NK LN VL FTE PNH PH VDGK	231 459 328 300 288 259 260 245 230 395 366 252 416	61.1 52.2 47.7 20.9 15.5 13.7 12 11 9.08 8.12 7.90 1.71 1.07	3160 1938 3201	Liu *et al.* (2021)
Alcalase[en]								
60.3	4	8.8	0.2%[#]	Ne	Ne	Ne	Ne	Vázquez *et al.* (2021)

Ne - no data; * - w/w; # - v/w; en - endopeptidase; ek - exopeptidase; -- - sequential hydrolysis; U - (U/mg); Da - molecular weight; t - extraction time; T - temperature

The pH range was obtained from 2 to 10 based on data on the optimum hydrolysis conditions of fish gelatin in table 2. Enzymes from microorganisms were isolated at pH 7-10, while digestive enzymes such as Pepsin were isolated at pH 2 and Trypsin at pH 8. In addition, plant-sourced enzymes were obtained at pH 7-7.5. Dos Santos Aguilar and Sato (2017) states that proteases are classified into three types according to pH: acidic, neutral, and basic. Acid protease enzymes work properly at low pH and show a high affinity for amino acids with aromatic side chains. Neutral proteases show a high affinity for hydrophobic amino acids but they are prone to heat. Alkaline enzymes can hydrolyze tyrosine, phenylalanine, leucine (near the terminal carboxyl group), aspartate, histidine, and serine.

Based on the most types of enzymes used, the hydrolysis conditions using Alcalase ranged from pH 7-9 and temperature 50-60.6 ºC. The Corolase worked effectively at 50 ºC and pH 7 (Neves *et al*., 2017), which was slightly different from the research of Cheung and Li-Chan (2017), using a temperature of 55 ºC and pH 7.5. In terms of Esperase, two former studies applied similar conditions, i.e. pH 8 and temperature of 60 ºC (Akagündüz *et al*., 2014; Mosquera *et al*., 2014). Additionally, hydrolysis by Neutrase was carried out at temperature of 50 ºC and 47 ºC at pH 7 (Chen *et al*., 2017; Chen *et al*., 2018). The Papain enzyme is more commonly combined with other enzymes. In this regard, there are three studies involving the use of Papain, applied at 60 ºC and pH 7 (Gu *et al*., 2011; Ngo *et al*., 2011; Liu *et al*., 2021). Halim *et al*. (2016) mentioned that hydrolysis conditions using Alcalase on various fish substrates were found at pH 7.5-10 and 45-60 ºC. Neutrase worked properly at pH 8.5 and 55 ºC, while Papain performed appropriately at pH 6-7 and 50-60 ºC. Meanwhile, according to Villamil *et al*. (2017), Alcalase showed a satisfying hydrolytic activity on fish offal substrate at pH 8-8.5, temperature of 40-55 ºC, while proper activity for Neutrase was observed at pH 7 and 40-55 ºC. Sitanggang *et al*. (2021) mentioned that the dissimilar hydrolytic condition by enzymes results from physicochemical properties of each fish gelatin protein substrate.

Furthermore, the unit applied in E/S ratio (enzyme/substrate) differed between 21 articles assessed, including weight per weight (w/w), volume per weight (v/w), and enzyme activity (U/mg). The E/S ratio ranged from 0.2 to 7%, expressed in w/w and v/w. The hydrolysis time ranged from 30 minutes to 7 hours. It is known that the concentration of enzymes and substrates affects the hydrolysis time. According to O'Meara and Munro (1984), if the ratio is relatively similar, the enzyme hydrolyzes weakly bonded peptides with normal time, whereas if the enzyme concentration to the substrate is high, there will be a faster hydrolysis process on weak peptide bonds and the remaining hydrolysis time will continue on stronger peptide bonds. The concentration of the enzyme to the substrate is an important factor so that the addition of the enzyme to the substrate must be balanced. A lower substrate concentration makes the enzyme molecule in a free state, when the substrate increases the free enzyme molecule reacts to the substrate. As with the statement of Araujo *et al*. (2020), when the Alcalase enzyme concentration is constant and the substrate concentration is lower, the enzyme reaction rate is limited and when the substrate is increased the enzyme reaction increases. On the other hand, when substrate

concentration is higher, the substrate becomes a dead-end inhibitor, in which it presents at the active site of the enzyme but unable to involve in the hydrolysis reaction.

Bioactive characteristics of fish gelatin peptides as ACEi

In this work, bioactive peptides are obtained from fish gelatin. The gelatin is a hydrolyzed form of fish collagen classified as stromal protein (Nurdiani *et al*., 2020). Collagen contains low amount of methionine and tyrosine, but glycine accounts for about 33% of the collagen (Karayannakidis and Zotos, 2016). Collagen has three chains (alpha helix), and the three helical structure is bonded to each other (hydrogen bonds) between the chains. The chain is a double-stranded structure formed when two chains are covalently connected while the chain is a triple-stranded structure (Ahmad *et al*., 2017). According to Nurdiani *et al*. (2020), each collagen polypeptide contains the amino acid structure Gly-X-Y (X is usually proline and Y hydroxyproline). Gelatin has a combination of 52 amino acid residues with different molecular weights such as chain (80 – 125 kDa), chain (160 - 250 kDa), chain (240 - 375 kDa) and other low chains (Huang *et al*., 2019). Since bioactive peptides are available in nature at low level, production of bioactive peptides is required. Bioactive peptides generally consist of 2-20 amino acids and are generally rich in hydrophobic amino acids (Chakrabarti *et al*., 2018).

Based on Table 2, fish gelatin ACEi peptides were found to have molecular weights ranging from 186 to 829 Da and amino acid composition up to 13 residues. He *et al*. (2013), ACEi peptides from marine sources usually contain 2-20 amino acid residues and their molecular weight according to Halim *et al*. (2016), <3 or 4 kDa. Furthermore, according to Ishak and Sarbon (2018), the molecular weight of bioactive peptides from fish waste with ACEi activity and antioxidant properties were reported to be <1.2 kDa, <1 kDa and an average of 640–25,719 Da, respectively. The highest fish gelatin ACEi peptide activity was found in the study of Byun and Kim (2001), with GPL peptides (glycine, proline, leucine), while the lowest ACEi activity was in GPR peptides (glycine, proline, arginine) with 3201 M activity and a molecular weight of 328 (Liu *et al*. 2021). The molecular weight, composition and position of the amino acids formed determine the activity of ACEi (Sitanggang *et al*., 2021).

Based on the value of the termination coefficient in Figure 2, the correlation between molecular weight and ACEi IC50 shows a weak correlation value of 0.0439. This shows that the molecular weights ranging from 186 to 829 Da did not significantly affect the activity of the ACEi peptide produced. The molecular weight of ACEi peptides <1 kDa, the activity was more influenced by the composition and position of the amino acids formed. According to Sitanggang *et al*. (2021), peptides with molecular weight <1 kDa did not have a significant effect on the activity of ACEi produced, ACEi activity was more influenced by the composition and amino acid position of bioactive peptides. The composition and amino acid position of the ACEi peptide in the presence of proline at the C-terminus of the QRP peptide with a short distance led to an increase in ACEi. Meanwhile, according to He *et al*. (2013), tripeptides or dipeptides having a high ACEi activity usually contain the tryptophan,

phenylalanine, tyrosine, or proline at C-terminus, and branched aliphatic amino acids at the N-terminus. As reported by Wang (2021), in general, hydrophobic amino acids at C-terminus affected the activity of ACEi. In addition, there are amino acids Lys, Arg.

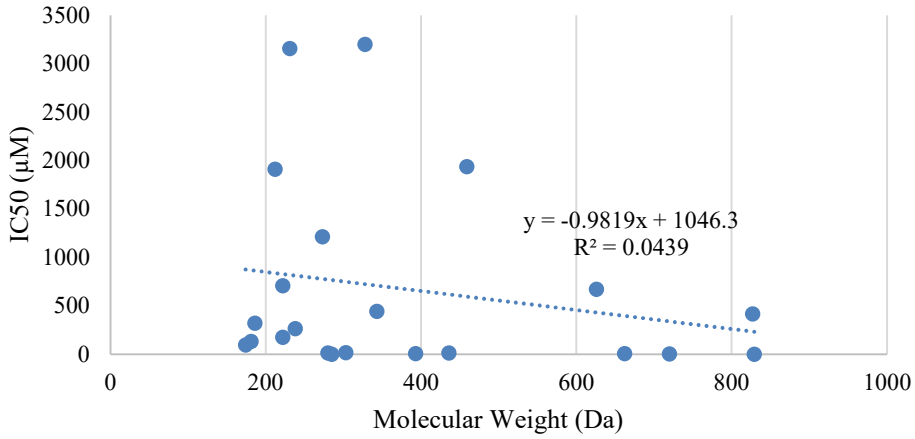

Figure 2. Correlation between ACEi activity and peptide's molecular weight. MWs and IC50 values are taken from Table 2.

The impacts of enzymatic hydrolysis systems on ACEi activity

As discussed previously, enzymes and hydrolysis conditions markedly affect production of ACEi peptides from fish gelatin. As explained by Rao *et al*. (2020), the production of bioactive peptides was influenced by protease specificity that was associated with the low molecular weight and length of the peptide structure. The specificity of the protease closely links to enzyme types that determine the position of cleavage peptide bond in the mother protein, hence the composition of peptide and position of amino acids that are formed are also influenced by the type of enzyme. According to Mirzapour-Kouhdasht *et al*. (2021b), ACEi activity positively correlated with the size of the low molecular weight peptide. The low molecular weight of the peptide to being influenced by the type of enzyme, it is further influenced by the enzyme concentration on the substrate and enzyme concentration included in hydrolysis conditions. Halim *et al*. (2016) mentioned key factors that affect final product, such as enzyme concentration, pH, time, and temperature. Protein substrates differ in their amino acid composition, and the specificity of the enzymes is unknown. According to Dos Santos Aguilar and Sato (2017), the active site of the enzyme determines substrate binding and how the substrate fits into the enzyme, and thus the active site determines the specificity of the protease on the substrate. The protease specificity determines the molecular weight, the number of peptides, amino acid composition, and an amino acid sequence, all of which affect the bioactive activity of the peptides produced. To improve the optimization process during the production of bioactive peptides, the appropriate enzymes and hydrolysis systems must be determined.

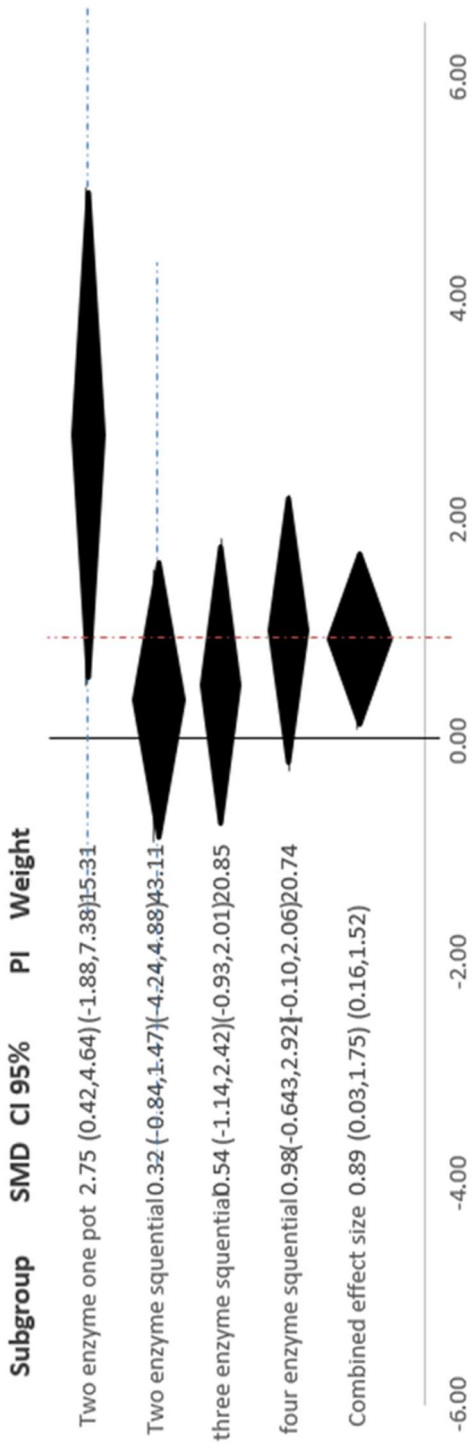

Figure 3. Subgroup analysis of the enzymatic hydrolysis system of fish gelatin. The notification subgroup is the name of the hydrolysis system group, SMD is the group effect size or the overall combined effect size, 95% CI is the confidence interval of the PI prediction interval and weight is the weighting.

Bioactive peptides can be produced through chemical hydrolysis, besides the enzymatic process, but as argued by Chakrabarti *et al*. (2018), Enzymatic hydrolysis is preferable to chemical, since chemically processed reaction requires a complicated controlling, and produces random peptides, as well as shows weak specificity (Zamora-Sillero *et al*., 2018). In the enzymatic process, there are a lot of methods for controlling the process, including the use of various enzyme sources (plant, microorganisms, gastrointestinal tract), fermentation with starter cultures, or hydrolysis of more than one enzyme to produce shorter peptides (system hydrolysis). Every method that was used needed optimization, in this regard discuss system hydrolysis more. There are 4 groups of hydrolysis systems obtained in this meta-analysis study: two enzymes one pot, two enzymes sequential, three enzymes sequential, and four enzymes sequential.

The results of the meta-analysis showed that the P value was 0.04. The magnitude of the effect is seen from the cumulative effect size value of 0.593. Based on the cumulative effect size, the treatment had a medium impact on producing ACEi activity or the hydrolysis of a combination of enzymes was 59.3% better than single hydrolysis in producing ACEi activity. Enzyme specificity is unique for each enzyme; however, when some enzymes are combined, the specificity may be broader, which enhances the hydrolysis of fish gelatin. The combination would make the formation of peptides small in size, and this potentially produces peptides with more optimal ACEi activity. The results of the meta-analysis study confirmed that the combination of enzymes produced a better ACEi activity value than the control. However, it should be noted that hydrolysis with a combination of enzymes is sometimes not better than a single enzyme because it is influenced by some aspects like the type of enzyme used, the protein substrate, and the hydrolysis conditions. According to Nasri (2017), certain types of enzymes can be combined and produce positive results (resulting in high activity) and some of them have no better results than hydrolysis with one type of enzyme. Hence, suitable of a combination system of hydrolysis and the type of enzyme that is used needs to be optimized to get the optimum hydrolysis conditions (E/S concentration, pH, temperature, hydrolysis time).

As seen in Figure 3, positive value indicates that the treatment (hydrolysis by combination of enzymes) is better than the control (hydrolysis by a single enzyme). The CI value shows a limiting effect size. Small CI value indicates a good level of precision, while large CI value indicates a less precise effect size. The CI value is also used to see the difference between control and treatment; if the CI value is tangent to the value 0 on the x-axis then the control and treatment are not significantly different. The CI value in the hydrolysis system group that is tangent to 0 on the x-axis means that the group is not significantly different from the control group. The PI value indicates the distribution of the effect size data that has not been observed (forecasting). Based on the analysis of subgroup hydrolysis in 2, 3, and 4 stages, there was no significant difference between control groups as seen from the p value. The p values for the 2, 3, and 4-stage hydrolysis systems were 0.363, 0.437, and 0.169, respectively. The one-step hydrolysis with the combination of two

enzymes was significantly different from the control but not significantly different from the effect size value of the overall subgroup combination with p value is 0.029.

The type of enzyme used in the meta-analysis study resulted in better activity than controls, followed with two enzymes one pot (bromelain+neutrase), two enzymes sequential (bromelain-corolase or alkalase-corolase or papain-corolase), three enzymes sequential (trypsin-pepsin-chymotrypsin), four enzymes sequential (trypsin-pepsin-chymotrypsin-trypsin). The recommended hydrolysis system for producing ACEi peptide from fish gelatin is two enzymes one pot and recommended types of enzymes are a combination of enzymes from microorganisms or enzymes from microorganisms combined with enzymes from plants.

Based on the results of the meta-analysis, the combined hydrolysis produced activities of ACEi better than single hydrolysis. This was due to the combination hydrolysis having a better possibility of producing low molecular weights. According to Rezvankhah *et al*. (2021), hydrolysis using more than one type of enzyme resulted in smaller molecular weight peptides. Daliri *et al*. (2017), showed that peptides with low molecular weight (<10 kDa) exerted more effective antihypertensive activity. Combination hydrolysis has a more variety of active sites so the enzyme and substrate in the hydrolysis process will be easier to match, even in some cases become more optimal. The use of various enzymes is the key to producing the desired amino acid composition or according to the active site of the ACE, so that it can be an option in engineering protein hydrolysis to produce ACEi peptides. According to Aluko (2018), protein hydrolysis occurs when the peptide bonds of the substrate are translocated into the active site of the enzyme, different active sites produce different peptides.

Conclusions

The review was conducted considering various articles discussing production of peptides with high ACEi activity from fish gelatin as parent protein. We found that optimum process of peptides ACEi production can be achieved by controlling two main stages: gelatin isolation and enzymatic hydrolysis. Gelatin extraction included 4 stages: pre-treatment, extraction, purification, and drying. The pre-treatment stage was recommended using a simple method such as soaking of raw materials in a strong acid such as HCl for 24 hours or using a strong base solution such as $NaOH/Ca(OH)_2$ for 24-72 hours without soaking in a salt solution or combination with other solvents. The extraction stage was carried out by soaking in distilled water for 30 hours at 60 ^oC. The final stage is separation by centrifugation and drying using a freeze dryer. Fish gelatin is further subjected to enzymatic hydrolysis. In the enzymatic hydrolysis process is recommended to use the Alcalase enzyme with the following hydrolysis conditions: pH 7-9, temperature 50-60.3 ^oC, E/S ratio 0.2-2%, hydrolysis time of 2-6 hours. Another type of enzymes recommended is isolated from microorganisms because releases peptides with high ACEi activity. The hydrolysis system can use single or different combinations of hydrolysis enzymes, which appeared to be more efficient.

References

Abdelhedi, O., Nasri, R., Mora, L., Toldrá, F., Nasri, M., Jridi, M. 2017. Collagenous proteins from black-barred halfbeak skin as a source of gelatin and bioactive peptides. *Food Hydrocolloids,* **70**, 123–133.

Ahmad, I., Ambarwati, N., Elya, B., Omar, H., Mulia, K., Yanuar, A., Negishi, O., Muńim, A. 2019. A new angiotensin-converting enzyme inhibitor from Peperomia pellucida (L.) Kunth. *Asian Pacific Journal of Tropical Biomedicine,* **9**(6),257–262.

Ahmad, T., Ismail, A., Ahmad, S.A., Khalil, K.A., Kumar, Y., Adeyemi, K.D., Sazili, A.Q. 2017. Recent advances on the role of process variables affecting gelatin yield and characteristics with special reference to enzymatic extraction: A review. *Food Hydrocolloids,* **63**, 85–96.

Ahmed, M., Verma, A.K., Patel, R. 2020. Collagen extraction and recent biological activities of collagen peptides derived from sea-food waste: A review. *Sustainable Chemistry Pharmacy,* **18**, 1–13.

Akagündüz, Y., Mosquera, M., Giménez, B., Alemán, A., Montero, P., Gómez-Guillén, M.C. 2014. Sea bream bones and scales as a source of gelatin and ACE inhibitory peptides. *LWT - Food Science and Technology*, **55**(2), 579–585.

Alam, N. 2007. Structure of fish muscles and composition of fish. *Participatory Training of Trainers. A New Approach Applied in Fish Processing,* **329**,38–48.

Aluko, R.E. 2018. Food protein-derived peptides: Production, isolation, and purification. Second Edition. *Proteins in Food Processing,* 394–412.

Araujo, J., Sica, P., Costa, C., Márquez M.C. 2020. Enzymatic hydrolysis of fish waste as an alternative to produce high value - added products. *Waste and Biomass Valorization,* 1-7.

Barati, M., Javanmardi, F., Mousavi, J.S.M.H., Jabbari, M., Rahmani, J., Barati, F., Nickho, H., Davoodi, S.H., Roshanravan, N., Mousavi, K.A. 2020. Techniques, perspectives, and challenges of bioactive peptide generation: A comprehensive systematic review. *Comprehensive Reviews in Food Science and Food Safety,* **19**(4), 1488–1520.

Benjakul, S., Kittiphattanabawon, P., & Regenstein, J. M. 2012. Fish Gelatin. In *Food Biochemistry and Food Processing*, 388–405. Wiley.

Byun, H.G., Kim, S.K. 2001. Purification and characterization of angiotensin I converting enzyme (ACE) inhibitory peptides from Alaska pollack (*Theragra chalcogramma*) skin. *Process Biochemistry,* **36**, 1155–1162.

Chakrabarti, S., Guha, S., Majumder, K. 2018. Food-derived bioactive peptides in human health: Challenges and opportunities. *Nutrients,* **10**(11):1–17.

Chen, J, Liu, Y., Yi, R., Li, L., Gao, R., Xu, N., Zheng, M. 2017. Characterization of collagen enzymatic hydrolysates derived from lizardfish (*Synodus fuscus*) scales. *Journal of Aquatic Food Product Technology,* **26**(1):86–94.

Chen, J., Liu, Y., Wang, G., Sun, S., Liu, R., Hong, B., Gao, R., Bai, K. 2018. Processing optimization and characterization of angiotensin-I-converting enzyme inhibitory peptides from lizardfish (*Synodus macrops*) scale gelatin. *Marin Drugs,* **16**(7), 1–17

Cheung, I.W.Y., Li-Chan, E.C.Y. 2017. Enzymatic production of protein hydrolysates from steelhead (Oncorhynchus mykiss) skin gelatin as inhibitors of dipeptidyl-peptidase IV and angiotensin-I converting enzyme. *Journal of Functional Foods,* **28**,254–264.

Coscueta, E.R., Brassesco, M.E., Pintado, M. 2021. Collagen-based bioactive bromelain hydrolysate from salt-cured cod skin. *Applied Sciences,* **11**(18), 1–12.

Daliri, E.B.M., Oh, D.H., Lee, B.H. 2017. Bioactive peptides. *Foods,* **6**(5):1–21.

Dos Santos Aguilar, G.J. and Sato, H.H. 2017. Microbial proteases: Production and application in obtaining protein hydrolysates. *Food Research International*, **103**, 1–54.

Eckert, E., Han, J., Swallow, K., Tian, Z., Jarpa-Parra, M., Chen, L. 2019. Effects of enzymatic hydrolysis and ultrafiltration on physicochemical and functional properties of faba bean protein. *Cereal Chemistry*, **96**(4), 725–741.

Fahmi, A., Morimura, S., Guo, H.C., Shigematsu, T., Kida, K., Uemura, Y. 2004. Production of angiotensin I converting enzyme inhibitory peptides from sea bream scales. *Process Biochemistry,* **39**(10), 1195–1200.

Gorgieva, S., Kokol, V. 2011. Collagen- vs. gelatine-based biomaterials and their biocompatibility: review and perspectives. In: Biomaterials Applications for Nanomedicine, Prof. Rosario Pignatello Ed, 1–19.

Gu, R.Z., Li, C.Y., Liu, W.Y., Yi, W.X., Cai, M.Y. 2011. Angiotensin I-converting enzyme inhibitory activity of low-molecular-weight peptides from Atlantic salmon (Salmo salar L.) skin. *Food Research International,* **44**(5), 1536–1540.

Halim, N.R.A., Yusof, H.M., Sarbon, N.M. 2016. Functional and bioactive properties of fi sh protein hydolysates and peptides : A comprehensive review. *Trends in Food Science and Technology,* **51**,24–33.

Han, Y., Byun, S.H., Park, J.H., Kim, S.B. 2015. Bioactive properties of enzymatic hydrolysates from abdominal skin gelatin of yellowfin tuna (Thunnus albacares). *International Journal of Food Science and Technology,* **50**(9), 1996–2003.

He, H.L., Liu, D., Ma, C.B. 2013. Review on the Angiotensin-I-Converting Enzyme (ACE) Inhibitor Peptides from Marine Proteins. *Applied Biochemistry and Biotechnology,* **169**(3), 738–749.

Huang, T., Tu, Z. cai, S.X., Sha, X., Wang, H., Zhang, L., Bansal, N. 2019. Fish gelatin modifications: A comprehensive review. *Trends in Food Science and Technology,* **86**, 260–269.

Ishak, N.H., Sarbon, N.M. 2018. A review of protein hydrolysates and bioactive peptides deriving from wastes generated by fish processing. *Food and Bioprocess Technology*, **11**(1), 2-16.

Karayannakidis, P.D. and Zotos, A. 2016. Fish processing by-products as a potential source of gelatin: a review. *Journal of Aquatic Food Product Technology,* **25**(1), 65–92.

Liu, W.Y., Feng, X.W., Cheng, Q.L., Zhao, X.H., Li, G.M., Gu, R.Z. 2021. Identification and action mechanism of low-molecular-weight peptides derived from Atlantic salmon (Salmo salar L.) skin inhibiting angiotensin I–converting enzyme. *Food Science and Technology*, **150**, 111911.

Mahmoodani, F., Ghassem, M., Babji, AS., Yusop, S.M., Khosrokhavar, R. 2014. ACE inhibitory activity of pangasius catfish (Pangasius sutchi) skin and bone gelatin hydrolysate. *Journal of Food Science and Technology,* **51**(9), 1847–1856.

Mahwati, Y., Nurrika, D., Latief, K. 2022. determinan hipertensi yang tidak terdiagnosis pada orang dewasa Indonesia: studi cross-sectional berdasarkan survei kehidupan keluarga indonesia 2014-2015. *Journal of Preventive Medicine and Public Health,* **55**, 60-67.

Man, S., Yang, L., Xiang, H., Lu, G., Wang, Y., Liu, C., Gao, W. 2020. Antihypertensive and renal protective effect of Shunaoxin pill combined with captopril on spontaneous hypertension rats. *Biomedicine & Pharmacotherapy*, **125**, 1–8

Manikkam, V., Mathai, M.L., Street, W.A., Donkor, O.N., Vasiljevic, T. 2016. Biofunctional and physicochemical properties of fish scales collagen-derived protein powders. *International Food Research Journal*, **23**(4), 1614–1622.

Martirosyan, D.M., Singh, J. 2015. A new definition of functional food by FFC : what makes a new definition unique ?. *Functional Foods in Health and Disease*, **5**(6), 209–223.

Mirzapour-Kouhdasht, A., Moosavi-Nasab, M., Lee, C.W., Yun, H., Eun, J.B. 2021a. Structure–function engineering of novel fish gelatin-derived multifunctional peptides

using high-resolution peptidomics and bioinformatics. *Scientific Reports,* **11**(7401), 1–15.

Mirzapour-Kouhdasht, A., Moosavi-Nasab, M., Yousefi, R., Eun, J.B. 2021b. Bio/multi-functional peptides derived from fish gelatin hydrolysates: Technological and functional properties. *Biocatalysis and Agricultural Biotechnology,* **36**(102152), 1–13.

Mosquera, M., Giménez, B., Da, S.I.M., Boelter, J.F., Montero, P., Gómez-Guillén, M.C., Brandelli, A. 2014. Nanoencapsulation of an active peptidic fraction from sea bream scales collagen. *Food Chemistry*, **156**, 144-150.

Nasri, M. 2017. Protein Hydrolysates and Biopeptides: Production, Biological Activities, and Applications in Foods and Health Benefits. A Review. *Adv Food Nutr Res,* **81**, 109–159.

Neves, A.C., Harnedy, P.A., O'Keeffe, M.B., FitzGerald, R.J. 2017. Bioactive peptides from Atlantic salmon (*Salmo salar*) with angiotensin converting enzyme and dipeptidyl peptidase IV inhibitory, and antioxidant activities. *Food Chemistry,* **218**, 396–405.

Ngo, D.H., Ryu, B.M., Kim, S.K. 2014. Active peptides from skate (okamejei kenojei) skin gelatin diminish angiotensin-i converting enzyme activity and intracellular free radical-mediated oxidation. *Food Chemistry,* **143**, 246–255.

Ngo, D.H., Ryu, B.M., Vo, T.S., Himaya, S.W.A., Wijesekara, I., Kim, S.K. 2011. Free radical scavenging and angiotensin-I converting enzyme inhibitory peptides from Pacific cod (*Gadus macrocephalus*) skin gelatin. *International Journal of Biological Macromolecules,* **49**(5), 1110–1116.

Ngo, D.H., Vo, T.S., Ryu, B.M., Kim, S.K. 2016. Angiotensin- I- converting enzyme (ACE) inhibitory peptides from Pacific cod skin gelatin using ultrafiltration membranes. *Process Biochemistry,* **51**(10), 1622–1628.

Nurdiani, R., Prihanto, A.A., Firdaus, M. 2020. Seafood as source of protein-based functional foods seafood as source of protein. in: encyclopedia of marine biotechnology: Five Volume Set, Se-Kwon Kim. Ed, . 2987-2997. John Wiley & Sons Ltd.

O'Meara, G.M., Munro, P.A. 1984. Effects of reaction variables on the hydrolysis of lean beef tissue by alcalase. *Meat Science* ,**11**(3), 227-238.

Prihartono, N.A., Fitria, L., Ramdhan, D.H., Fitriyani, F., Fauzia, S., Woskie, S. 2022. Determinants of hypertension amongst rice farmers in West Java, Indonesia. *International Journal of Environmental Research and Public Health,* **19**(3), 1152.

Rao, P.S., Bajaj, R., Mann, B. 2020. Impact of sequential enzymatic hydrolysis on antioxidant activity and peptide profile of casein hydrolysate. *Journal Food Science and Technology,* **57**(12), 4562–4575.

Rasli, H.I., Sarbon, N.M. 2018. Optimization of enzymatic hydrolysis conditions and characterization of Shortfin scad (*Decapterus Macrosoma*) skin gelatin hydrolysate using response surface methodology. *International Food Research Journal*, **25**(4), 1541-1549.

Regenstein, J.M., Zhou, P. 2006. Collagen and gelatin from marine by-products. *Woodhead Publishing Limited,* 279-303

Rezvankhah, A., Yarmand, M.S., Ghanbarzadeh, B., Mirzaee, H. 2021. Generation of bioactive peptides from lentil protein: degree of hydrolysis, antioxidant activity, phenol content, ACE-inhibitory activity, molecular weight, sensory, and functional roperties. *Journal of Food Measurements and Characterisation,* **15**(6), 5021–5035.

Salvatore, L., Gallo, N., Natali, M. L., Campa, L., Lunetti, P., Madaghiele, M., Blasi, F. S., Corallo, A., Capobianco, L., & Sannino, A. 2020. Marine collagen and its derivatives: Versatile and sustainable bio-resources for healthcare. *Materials Science and Engineering: C*, **113**, 110963.

Shahidi, F. 1994. Seafood proteins and preparation of protein concentrates. *Seafoods: Chemistry, Processing Technology and Quality,* 1-7.

Sitanggang, A.B., Putri, J.E., Palupi, N.S., Hatzakis, E., Syamsir, E., Budijanto, S. 2021. Enzymatic preparation of bioactive peptides exhibiting ace inhibitory activity from soybean and velvet bean: A systematic Review. *Molecules,* **26**(13), 1-14.

Sturrock, E.D., Natesh, R., Van, R.J.M., Acharya, K.R. 2004. Structure of angiotensin I-converting enzyme. *Cellular and Molecular Life Sciences,* **61**(21), 2677–2686.

Subin, S.R, Bhat, S.G. 2016. Enzymes: Concepts, Nomenclature, Mechanism of Action and Kinetics, Characteristics and Sources of Food-Grade Enzymes. In: *Enzymes in Food and Beverage Processing,* Muthusamy Chandrasekaran. Ed, 4–38. CRC Press Taylor & Francis Group, 6000 Broken Sound Parkway NW, Suite 300 Boca Raton, FL 33487-2742.

Vázquez, J.A., Hermida-Merino, C., Hermida-Merino, D., Piñeiro, M.M., Johansen, J., Sotelo, C.G., Pérez-Martín, R.I., Valcarcel, J. 2021. Characterization of gelatin and hydrolysates from valorization of farmed salmon skin by-products. *Polymers (Basel),* **13**(16), 2828.

Vázquez, J.A., Meduíña, A., Durán, A.I., Nogueira, M., Fernández-Compás, A., Pérez-Martín, R.I., Rodríguez-Amado, I. 2019. Production of valuable compounds and bioactive metabolites from by-products of fish discards using chemical processing, enzymatic hydrolysis, and bacterial fermentation. *Marine Drugs,* **17**(3), 139.

Villamil, O., Váquiro, H., Solanilla, J.F. 2017. Fish viscera protein hydrolysates : Production, potential applications and functional and bioactive properties. *Food Chemistry,* **224**, 160–171.

Wang, X. 2021. Natural bioactive compounds from fish. in: natural bioactive compounds. *Elsevier,* 393–408.

WHO, 2020. World Health Organization. Hypertension. (updated 2021 August 25; cited 2021 April 10). Available from: https://www.who.int/news-room/fact-sheets/detail/hypertension.

Xu, Z., Wu, C., Sun-Waterhouse, D., Zhao, T., Waterhouse, G.I.N., Zhao, M., Su, G. 2021. Identification of post-digestion angiotensin-I converting enzyme (ACE) inhibitory peptides from soybean protein Isolate: Their production conditions and in silico molecular docking with ACE. *Food Chemistry,* **345**, 128855.

Zamora-Sillero, J., Gharsallaoui, A., Prentice, C. 2018. Peptides from Fish By-product protein hydrolysates and its functional properties: an overview. *Marine Biotechnology,* **20**(2), 118–130.