Journal of Food Science and Technology (Iran)

Homepage:www.fsct.modares.ir

Scientific Research

Investigation of Antidiabetic, Antihypertensive, and Antioxidant Properties of Bioactive Peptides Extracted from the Waste of Green Tiger Shrimp (Penaeus semisulcatus)

Mahya damali amiri[1*], Reza hasan sajedi[2]

1- master of biochemistry, Tarbiat modares university, Jalal al ahmad, Kargar shomali.
2- phd of biochemistry, professor Tarbiat modares university, Jalal al ahmad, Kargar shomali.

ARTICLE INFO

Article History:

Received: 2024/11/03

Review: 2025/10/12

Accepted: 2025/10/16

Keywords:

marine peptide,

enzymatic hydrolysis,

bioactive peptide,

Green tiger shrimp,

antidiabetic properties

DOI: **10.48311/fsct.2026.83919.0**

*Corresponding Author E-

mahya.amirii76@gmail.com

ABSTRACT

In shrimp processing industries, approximately 40-45% of the raw material is discarded as waste, including heads, viscera and, shells,. The aim of this study was to extract and evaluate the biological activity of peptides derived from the processing waste of the green tiger shrimp (Penaeus semisulcatus). The shrimp processing waste was prepared, and target tissues were used for enzymatic treatment. Results related to the approximate composition of the raw material based on dry weight with 74.5% moisture showed that the highest proportion was shrimp body tissue, followed by protein at 44% and ash at 2.2% in the green tiger shrimp tissue content. Subsequently, three types of enzymes—alkaline protease, Alcalase, and Ovataz—were used for hydrolysis and peptide production from shrimp waste. The endogenous shrimp enzymes were inactivated by heating at 85°C, and the pH was adjusted to 8/0. Hydrolysis was carried out for 8 and 16 hours, and the total protein content of the supernatant was measured using the Kjeldahl technique for analysis and selection of the best sample. The 16-hour hydrolysate treated with Alcalase, containing 35.5% protein, was selected as the optimal sample. The proteins extracted and hydrolyzed using Alcalase were separated by ultrafiltration into three molecular weight fractions: less than 3, 10, and 30 kDa. The results indicated that the peptide fraction with a molecular weight below 3 kDa exhibited the highest biological activity; specifically, inhibition of the DPP-IV enzyme up to 73.35%, ACE inhibition up to 55%, and free radical scavenging activity in the DPPH assay of 69.61%.

1- Introduction

Nowadays, health problems arising from hypertension, diabetes, cancer, and other lifestyle-related metabolic disorders have become major global health concerns [1]. The growing population, aging, poor dietary habits, obesity, and sedentary lifestyles have contributed to the increased prevalence of these diseases. In diabetes, nutritional therapy plays a key role in the metabolic management of the disease, as it can help reduce blood glucose levels and improve insulin sensitivity. One of the pharmacological strategies for controlling metabolic disorders such as diabetes is the inhibition of the dipeptidyl peptidase enzyme; however, continuous use of these enzyme inhibitors is often associated with side effects, including gastrointestinal and renal complications, which highlights the need for natural alternatives [1]. Bioactive peptides derived from food sources—particularly aquatic proteins—have recently attracted significant attention as natural compounds with multiple biological functions. These peptides are typically obtained through enzymatic hydrolysis and, depending on their molecular weight, amino acid sequence, and spatial structure, may exhibit various biological activities such as inhibition of dipeptidyl peptidase and angiotensin-converting enzyme, as well as antioxidant, antihypertensive, anti-inflammatory, and even anticancer properties [2, 3].

Numerous studies have investigated the biological activities of these peptides derived from various sources. Nongonierma and FitzGerald (2017) reported that bioactive proteins extracted from food sources have a high potential for regulating the glycemic index [3]. Wang et al. examined the optimal conditions for enzymatic hydrolysis of *Chlorella pyrenoidosa* alga proteins and successfully isolated a peptide with angiotensin-converting enzyme (ACE) inhibitory activity [4]. Chalamaiah et al. (2012), in a comprehensive review of fish protein hydrolysates, emphasized that these compounds possess a wide range of bioactive peptides with strong antioxidant properties and therefore have great potential for application in the production of functional foods [5]. Ramazani (2018) demonstrated that peptides derived from *ponyfish* waste exhibit significant antioxidant and antihypertensive activities [6]. Similarly, Ko et al. evaluated the ACE inhibitory activity in protein hydrolysates obtained from various algal species [7]. Ishak et al. found that peptides derived from blackfin fish waste possess notable antioxidant and antihypertensive properties [8]. In another study, Shahidi et al., through a structured review, highlighted that peptides derived from a tropical fish species can play a key role in scavenging free radicals [9].

In Iran, Nahvi et al. reported the extraction of bioactive peptides from *kilka* fish, which exhibited free radical scavenging activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radicals [10]. Despite the growing number of studies on various fish species, limited research has been conducted on the by-products of the green tiger shrimp (*Penaeus semisulcatus*). This species is one of the economically valuable and native aquatic organisms of southern Iran, and its processing by-products — including the shell, head, and tail — are rich in usable proteins.

2- Materials and Methods

2.1. Preparation of Raw Material

The enzymes Alcalase® 2.4L FG (Novozymes, Denmark), alkaline protease (Cat. No. P4860; Sigma-Aldrich, USA), and Ovozyme® (DSM Food Specialties, Netherlands) were obtained for the study. The DPP-IV activity assay kit (Cat. No.

MAK203), along with other chemical reagents including DPPH, ABTS, and the ACE activity assay kit (Cat. No. CS0002), were purchased from Sigma-Aldrich (USA). Additional chemicals and solvents were supplied by Merck (Germany). The by-products of green tiger shrimp (*Penaeus semisulcatus*), including the shell, head, and tail, were collected from the waters of the Persian Gulf in Hormozgan Province. The samples, with an average body length of approximately 5 cm, were transported to the laboratory of the Faculty of Marine Sciences, Tarbiat Modares University, in ice boxes with an ice-to-shrimp ratio of 1:2. Then 100 g portion of the frozen sample was subjected to freeze-drying and then ground into powder. The powdered material was homogenized, and impurities were removed. The sample was sieved and stored in a dry place, protected from direct sunlight, until further use.

2.2. Proximate Composition Analysis of the Raw Material

The contents of moisture, total protein, dry matter, and ash were determined according to the standard procedures recommended by the Association of Official Analytical Chemists (AOAC). For moisture determination, samples were dried in an oven at 105 °C for 24 hours until a constant weight was achieved. For ash content, 0.5 g of each sample (previously dried at 65 °C for 48 hours) was placed in a porcelain crucible and incinerated in a muffle furnace at 550 °C for 5 hours. The total protein content was determined using the Kjeldahl method. Specifically, 1 g of the powdered sample (before hydrolysis), 0.553 g of the freeze-dried supernatant powder from shrimp hydrolyzed with Ovozyme for 16 hours, 0.253 g of the corresponding sample hydrolyzed with Alcalase for 16 hours, and a 16-hour control sample were subjected to Kjeldahl analysis [11, 12]. The weight of the sample before and after oven drying was measured for the determination of dry matter percentage [13]. In this step, 1 mL of the sample was centrifuged at 12,000 rpm for 15–25 minutes, and the weights of the supernatant and precipitate were measured separately.

2.3. Production of Protein Hydrolysates

To obtain the highest yield, optimization of hydrolysis conditions was carried out. For this purpose, enzyme concentration (2%), hydrolysis time (ranging from 1 to 8 hours and 16 hours), and different types of commercial alkaline proteases of microbial origin—including Alcalase, Ovozyme, and Robuzyme alkaline protease—were selected as independent variables.

A total of 20 g of shrimp powder was mixed with distilled water at a ratio of 1:5 (sample:water). The pH of the mixture was adjusted to 8.0, which represents the optimal activity for Alcalase, using NaOH and HCl. The samples were then incubated at 55 °C. Enzymes were added to the mixture at a concentration of 2% (20 mL Alcalase, 20 mg Ovozyme, and 20 mg Robuzyme alkaline protease), and the samples were stirred continuously for the designated time intervals. To terminate enzymatic activity, the samples were heated at 85 °C for 15 minutes at each time point. After cooling, the samples were centrifuged, and the supernatant was collected and stored at −80 °C until further analysis [9, 14].

2.4. Peptide Fractionation

Based on the obtained data, the hydrolysate produced using Alcalase showed the most favorable results. Therefore, peptide fractionation was performed using ultrafiltration with Amicon membrane filters of three different molecular weight cutoffs (3, 10, and 30 kDa). Several peptide fractions were obtained from the Alcalase hydrolysate. The peptides were separated according to their molecular weight using the Amicon ultrafiltration system [15, 16].

2.5. Peptide Analysis

The molecular weight distribution profile of the Alcalase hydrolysate and its peptide components was determined using sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). Since the Coomassie Brilliant Blue staining produced faint bands, silver nitrate staining was employed for clearer visualization of the peptide bands [15].

2.6. Determination of Peptide Absorbance and Concentration

To ensure uniform experimental conditions and enable comparative evaluation of different samples, the protein concentration of the supernatant fractions and their optical density (OD) were measured at 280 nm using an ELISA reader. These measurements were conducted to confirm consistency across all samples prior to further analyses.

2.7. Determination of Antioxidant Activity

The antioxidant activity of the peptide samples was evaluated using two assays based on free radical scavenging capacity: the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay.

2.7.1. DPPH Free Radical Scavenging Activity

The DPPH radical scavenging capacity of the samples was determined according to the following equation [16, 17]:

Free radical scavenging(%) = (A blank – A sample) × 100/ A blank

A sample = absorbance of the sample mixed with the DPPH solution,
A blank = absorbance of the DPPH solution mixed with ethanol.

2.7.2. ABTS Radical Scavenging Capacity

The ABTS free radical scavenging activity of the synthesized peptide samples was determined using the following equation [16, 17]:

Free radical scavenging (%) = (A blank – A sample) ×100/ A blank

where:
Asample = absorbance of the sample mixed with the ABTS solution,
Ablank = absorbance of the control solution without any active compound.

2.8. Determination of Dipeptidyl Peptidase-IV (DPP-IV) Inhibitory Activity

The inhibitory activity of DPP-IV was measured using a DPP-IV inhibition assay kit, which operates based on the cleavage of a non-fluorescent substrate. The assay was conducted by mixing 25 µL of the sample at different concentrations with 50 µL of the enzyme solution in each well of a 96-well microplate. The plate was then incubated at 37 °C for 10 minutes. Subsequently, 25 µL of the DPP-IV substrate solution was added to each reaction well, including test samples, enzyme controls, and blanks.

The assay was performed in a black 96-well microplate, and absorbance readings were taken using a microplate reader. The buffer solution was used instead of the enzyme solution as a control. After allowing 15–30 minutes for complete mixing of all reagents, fluorescence (FLU; λ_ex = 360 nm, λ_em = 460 nm) was recorded using a microplate reader operating in kinetic mode (one reading per minute) at 37 °C. The slope between two time points (T_1 and T_2) within the linear range of the DFLU/min curve was calculated. The slope values of all tested samples were subtracted from those of the corresponding blank samples to obtain the accurate DPP-IV inhibitory activity [3].

2.9. Determination of Angiotensin-I Converting Enzyme (ACE) Inhibitory Activity

The antihypertensive activity was evaluated based on the inhibition of angiotensin-I converting enzyme (ACE). The assay was performed using a commercial ACE inhibition kit (Sigma-Aldrich, USA). Briefly, 10 µL of each sample was mixed with 40 µL of the enzyme solution (diluted in the assay buffer), followed by incubation at 37 °C for 5 minutes. Subsequently, 50 µL of the diluted substrate solution was immediately added to the mixture.The fluorescence intensity was then measured at an excitation wavelength of 320 nm and an emission wavelength of 405 nm for five cycles over a total duration of 5 minutes using a microplate reader [18].

2.10. Statistical Analysis

All experiments were carried out in triplicate, and the results were expressed as mean ± standard deviation (SD). Microsoft Excel software was used for data processing and graphical presentation.

3-Results and Discussion

3.1. Proximate Analysis of Raw Material

The results of the proximate composition analysis of the raw shrimp material, based on the dry weight of the sample, are presented in Table 1. Moisture content, at 74.5%, represented the major component of *Penaeus semisulcatus* (green tiger shrimp) body composition. Overall, the shrimp body contained 74.5% moisture, 44% protein, and 2.2% ash. Determining the proximate composition of this species provides valuable information regarding the protein content of the raw material used in this study.

Table 1. Approximate ingredients of oven-dried green tiger shrimp

(٪) Moisture	(٪) Dry matter	(٪) Ash	(٪) Sample	Protein
Oven dried sample	44	2/2	99/6	74/5

3.2. Determination of Total Protein Content

The total protein content of the supernatant was measured using the Kjeldahl method to analyze and compare the produced samples and to select the most suitable one, as shown in Table 2. In addition, the obtained results were further verified through measurements of dry matter percentage and supernatant yield after 16 hours of enzymatic hydrolysis with Alcalase, which served as a complementary confirmation of the previous data (Table 2). The 16-hour Alcalase hydrolysate was selected as the main sample for subsequent experiments.

Table 2. Supernatant protein percentage of hydrolyzed samples with Alcalase and Evatase enzymes in the production process

Sample Protein(٪)	The percentage of dry matter	The percentage of the supernatant
Treated with	8	35/5
69 Alkalase	4	15/5
Control		
44/15		

3.3. Determination of Soluble Protein Concentration by the Biuret Method

The results of the protein concentration analysis for the 16-hour hydrolyzed samples are presented in Figure 1. As expected, the Alcalase enzyme exhibited the highest soluble protein content compared to the other hydrolyzed samples and the control group. In contrast, the alkaline protease from Robozym consistently showed the lowest protein concentration in most experiments and was therefore excluded from further analyses.

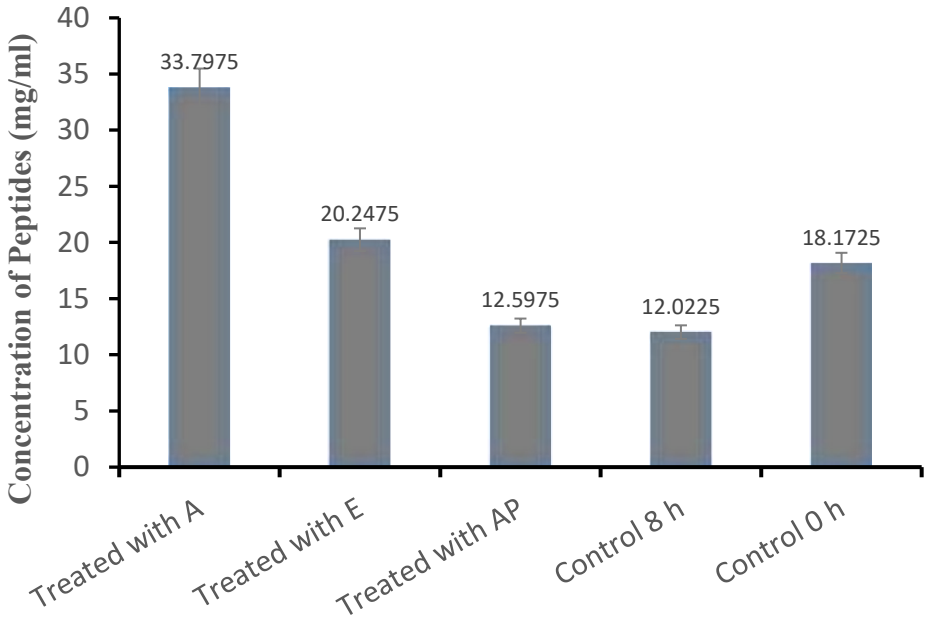


Fig 1. Comparison of the concentration of peptides in the control samples, protein hydrolyzed with A, E and AP by the Biuret method

3.4. Peptide Analysis

As shown in Figure 2, the molecular weight distribution profile of the protein hydrolysate obtained using Alcalase, along with its peptide

components, was determined by SDS–PAGE electrophoresis**.** A standard molecular weight marker was used to identify peptides with different molecular weights. Since the gel stained with Coomassie Brilliant Blue was faint, silver nitrate staining was performed to obtain clearer visualization of the bands. In the samples with molecular weights below 30 kDa, no prominent peptide bands were observed, and the detected bands appeared faint—consistent with the findings of Bordbar et al. [15]**.**

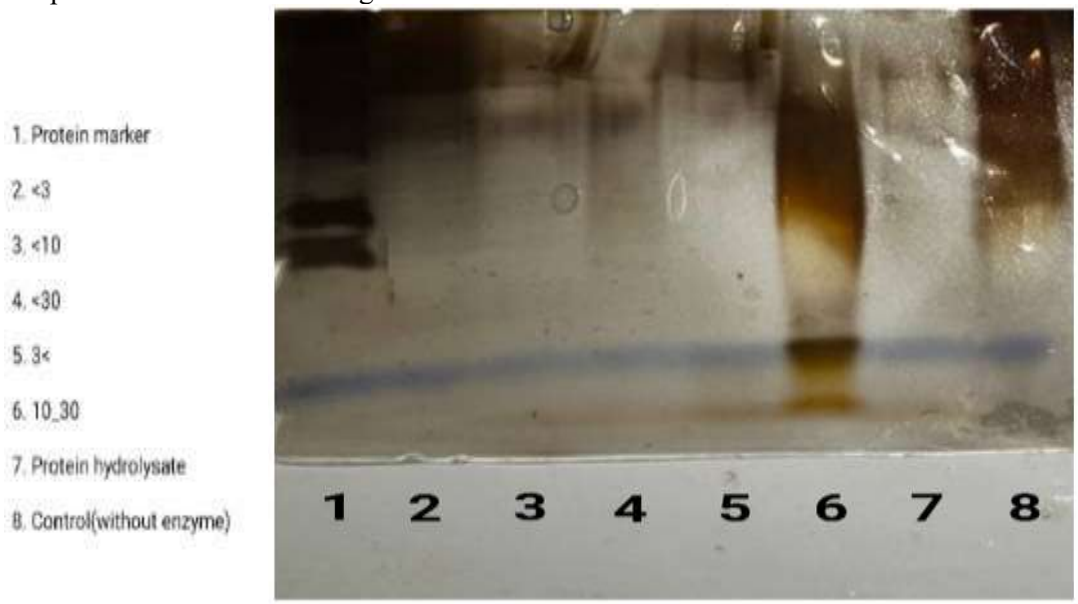


Fig 2. SDS-PAGE patterns of unfractionated and ultrafiltrated fractions

3.5. Analysis of the Concentration of Ultrafiltrated Peptides

The results of peptide concentration measurements for the ultrafiltrated samples are shown in Figure 3. Numerous studies have investigated the enzymatic hydrolysis of various fish parts using commercial proteases. Many of these studies have examined the effects of different enzymes, including papain (plant origin**),** trypsin and chymotrypsin (animal origin), and Alcalase**,** Protamex**,** Flavourzyme, and Neutrase (microbial origin) [8,19].

The protein recovery yield reflects the efficiency of an enzyme in releasing proteins from a substrate and depends on the characteristics of the raw material, the hydrolysis conditions, and the enzyme's catalytic activity [20]. The high efficiency of Alcalase in cleaving proteins and producing small peptides is consistent with the findings of Bordbar et al. [15]**.**

The generation of low-molecular-weight peptides by Alcalase suggests that the green tiger shrimp protein may contain segments particularly susceptible to enzymatic cleavage by Alcalase. According to Ishak et al. [8], Alcalase hydrolyzes proteins into smaller peptide fragments; therefore, the presence of low-molecular-weight peptide bands observed in this study may indicate the formation of peptides with strong bioactive properties**.**

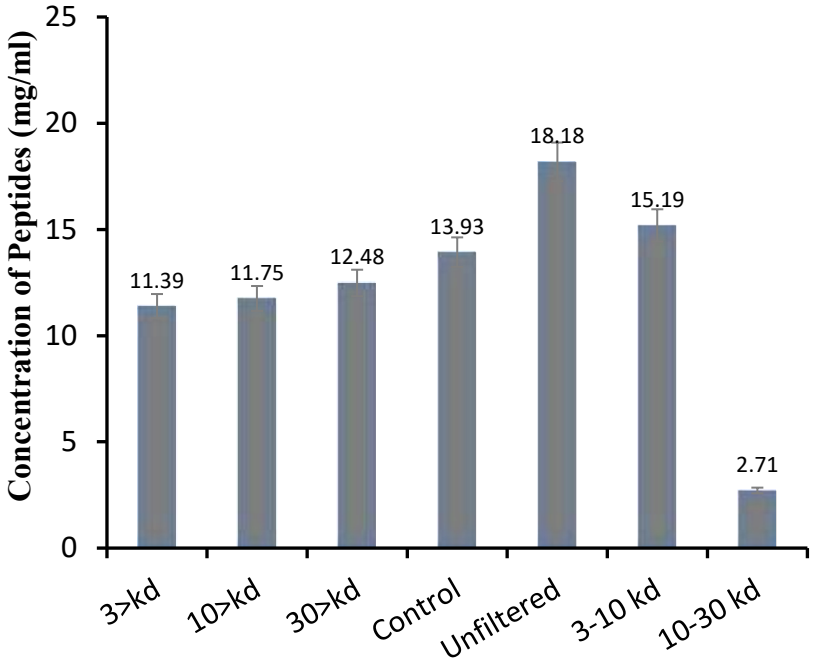


Fig 3. Comparison of ultrafiltrated peptides concentration in different components by biuret

3.6. Evaluation of Anti-Oxidant Activity

3.6.1. DPPH Free Radical Scavenging Activity

One of the key indicators used to assess the antioxidant activity of proteins is their ability to scavenge DPPH free radicals**.** DPPH is a stable, ethanol-soluble free radical that exhibits maximum absorbance at 517 nm**.** This radical can be neutralized by electron-donating compounds such as antioxidants, resulting in a decrease in absorbance. The results of the antioxidant activity assessment of the hydrolyzed compounds demonstrated that the hydrolysate exhibited strong antioxidant capacity at all tested concentrations up to 3 dilution after 10 mg/mL. The highest radical scavenging activity was observed in the <30 kDa fraction**,** with a value of 69.61 ± 0.15% at a concentration of 10 mg/mL, as shown in Figure 4**.** Compared to the non-hydrolyzed control sample, all hydrolyzed samples displayed significantly higher DPPH radical scavenging activity ($p \leq 0.05$).

The enzyme used in this study was Alcalase, an alkaline protease known for its strong ability to hydrolyze peptide bonds and produce low-molecular-weight peptides. The results of this research showed that protein hydrolysis with Alcalase led to a significant increase ($p \leq 0.05$) in the antioxidant activity of the samples compared to the non-hydrolyzed control. This enhancement is likely attributed to the release of bioactive peptides with DPPH radical scavenging capacity. Previous studies have also reported that hydrolysis with Alcalase can generate peptides with high antioxidant potential, depending on the nature and amino acid sequence of the released fragments [21]. In particular, a study conducted on ponyfish protein [17] demonstrated that Alcalase markedly improved antioxidant activity, with

the DPPH radical scavenging rate reaching 75.59 ± 1.6% in the hydrolyzed samples [21].

The results of the comparison of DPPH radical scavenging activity between the hydrolyzed protein and the peptide fractions with different molecular weights are presented in Figure 4. The findings indicated that as the concentration decreased, the scavenging activity increased, and all peptide fractions (<3, 10–30, and >30 kDa) exhibited the ability to scavenge DPPH radicals.

Ascorbic acid (vitamin C) was used as a positive control at concentrations ranging from 0.001 to 2 mg/mL, and its antioxidant activity at a concentration of 1 mg/mL was calculated to be 70.5%**.** This compound was selected due to its well-known properties in neutralizing free radicals and its strong antioxidant potential. Vitamin C, as a natural antioxidant with a high radical scavenging capacity, is widely employed in laboratory studies for comparison with other antioxidant compounds [22]. In the present study, vitamin C exhibited 70.5% antioxidant activity at a concentration of 1 mg/mL, which served as the reference standard for comparing the radical scavenging potential of the Alcalase-hydrolyzed samples. Moreover, the highest DPPH radical scavenging activity (69.61 ± 0.15%) was observed at a concentration of 10 mg/mL.

Similar results have been reported by other researchers. Ramazanzadeh et al. [16] stated that the highest DPPH radical scavenging activity and reducing power of hydrolyzed rainbow trout protein using Alcalase was 39.8% at a concentration of 11 mg/mL. Furthermore, according to Ramazanzadeh et al. [17**]**, the orange-spotted grouper exhibited more than 70% DPPH radical scavenging activity during 1–4 hours of hydrolysis. Therefore, fish protein-derived peptides demonstrate a strong potential to exert antioxidant activity across various oxidative systems [22].

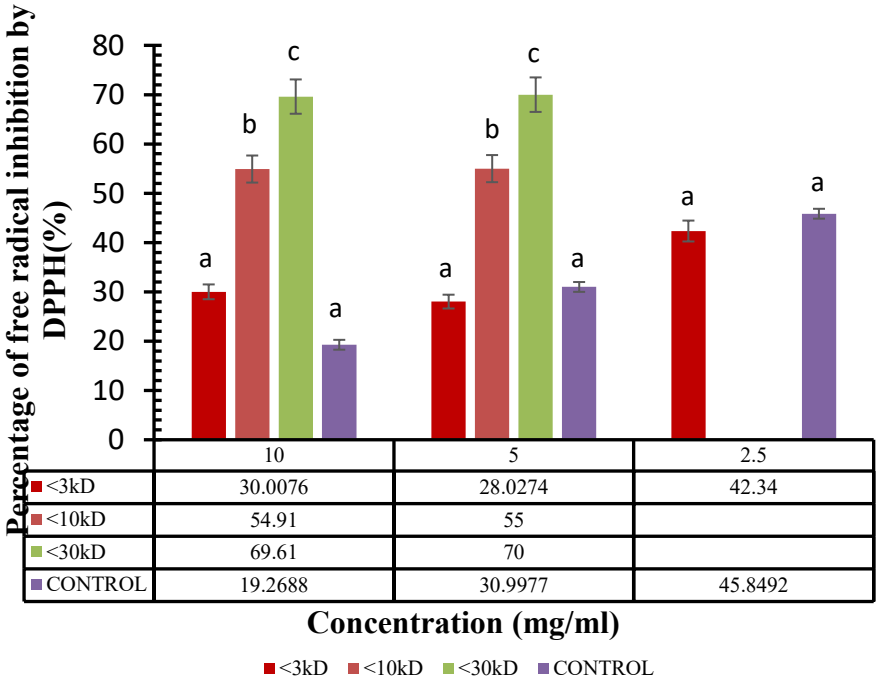
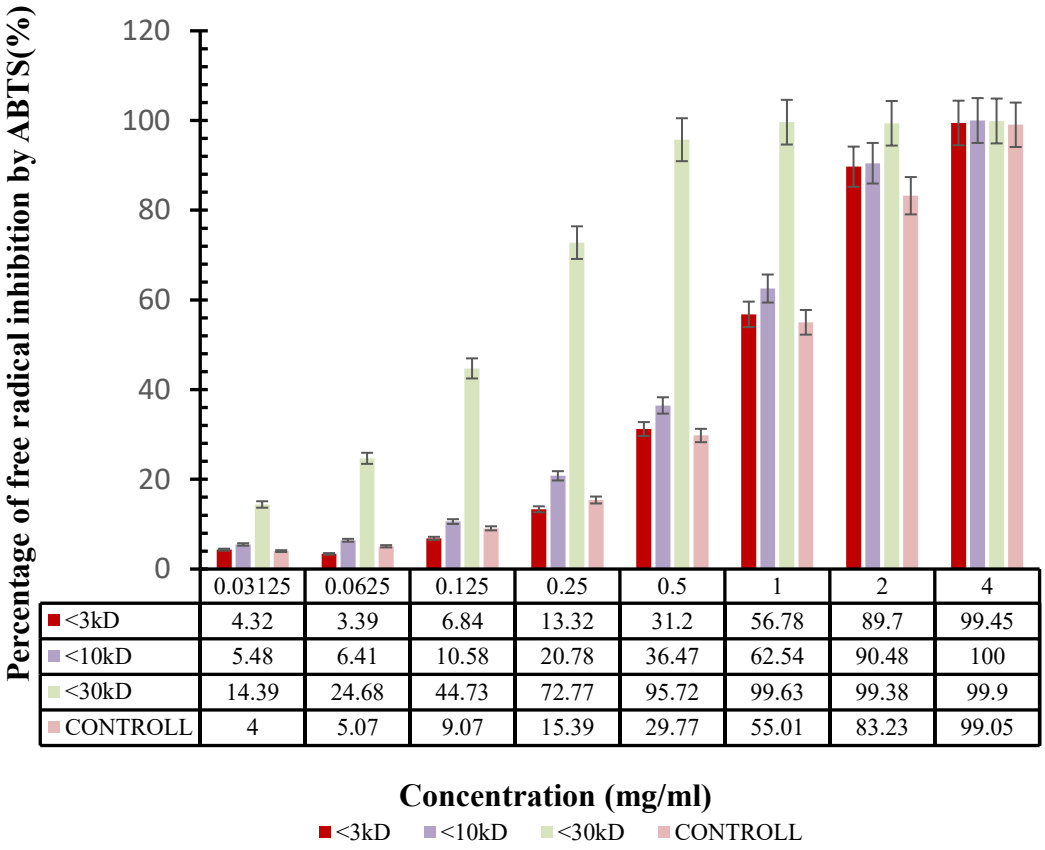


Fig 4. Comparison chart of DPPH free radical inhibition percentage of different samples

3.6.2. ABTS Radical Scavenging Capacity

The ABTS radical is a relatively stable free radical that can be easily scavenged by antioxidants [23]. The higher scavenging capacity observed against the ABTS radical compared to the DPPH radical in the tested samples is consistent with the findings of Shahidi et al. [9]. Free radicals represent one of the main mechanisms underlying the antioxidant activity of protein hydrolysate-derived peptides, which is mainly attributed to the presence of amino acids such as valine, leucine, proline, and alanine in their peptide sequences [24, 25]. High levels of amino acids such as Asp, Pro, Ala, Trp, Tyr, Met, Cys, Leu, Arg, and His in the protein structure, as reported by Zhong et al. [25], likely contribute to this activity. The findings of Zhong et al. [26] further indicate that the presence of these hydrophobic and aromatic amino acids can enhance the antioxidant activity of peptides.

According to the results presented in Figure 5, both the peptide fractions and the protein hydrolysate demonstrated the ability to scavenge ABTS radicals. Although the hydrolyzed samples at different molecular weights exhibited higher radical scavenging activity than the unhydrolyzed control, the differences among the samples at various concentrations were not statistically significant ($p > 0.05$).



	0.03125	0.0625	0.125	0.25	0.5	1	2	4
<3kD	4.32	3.39	6.84	13.32	31.2	56.78	89.7	99.45
<10kD	5.48	6.41	10.58	20.78	36.47	62.54	90.48	100
<30kD	14.39	24.68	44.73	72.77	95.72	99.63	99.38	99.9
CONTROLL	4	5.07	9.07	15.39	29.77	55.01	83.23	99.05

Fig 5. Comparison chart of ABTS free radical inhibition percentage of different samples

3.7. Determination of Angiotensin I-Converting Enzyme (ACE) Inhibitory Activity

The ACE inhibitory activity of the protein hydrolysate and peptide fractions obtained through ultrafiltration, at a concentration of ug/mL, is presented in Figure 6**.** The results showed that all samples exhibited the ability to inhibit ACE activity, with inhibition rates ranging from 33% to 55%**.** The highest inhibitory activity was observed in the peptide fraction with a molecular weight below 3 kDa (54.88%), which showed a statistically significant difference compared to the <10 kDa and <30 kDa fractions ($p < 0.05$, ANOVA). Moreover, the peptide fractions with molecular weights below 30 kDa displayed the lowest bioactive properties. Various factors can influence the bioactivity of peptides, including the type of protease enzyme used, the degree of hydrolysis, molecular weight, amino acid composition, and hydrolysis conditions such as temperature, time, and pH [27]. However, among these, the molecular weight is considered the most critical factor.

This can be attributed to the influence of molecular weight on the composition and diversity of amino acids within peptides, which significantly affects their ability to inhibit ACE activity. It has been demonstrated that peptides with molecular weights below 3 kDa possess an optimal amino acid composition for effective inhibition of ACE enzyme activity.

Forghani *et al.* [18] investigated the ACE inhibitory capacity of hydrolyzed peptides produced using various proteases, including Alcalase, Flavourzyme, Trypsin, Papain, Bromelain, and Protamex. Their results were consistent with those of the present study, showing that peptides hydrolyzed by Alcalase exhibited the highest antihypertensive activity overall. According to Ishak *et al.* [8], peptide fractions obtained from the ultrafiltration of hydrolyzed proteins are strongly influenced by the peptide sequence and the presence of specific amino acids at the C-terminal end of the peptides—particularly Trp, Tyr, Phe, Pro, and other hydrophobic residues that tend to interact with the active site of ACE.

The inhibitory property may also be partially attributed to the protein source, the peptide extraction process, and the type of ultrafiltration membrane used. In general, fractionation of peptides based on molecular size enhances the bioactivity of the protein hydrolysate [19]. As observed in the present results, changes in peptide molecular weight caused a significant variation in ACE inhibitory capacity. The highest inhibitory activity was found in the <3 kDa fraction, whereas the lowest activity was observed in the >30 kDa fraction. These findings confirm that low-molecular-weight peptides are more effective ACE inhibitors than high-molecular-weight ones [19].

These results are consistent with the findings of Wu and colleagues [26], who observed the highest ACE inhibitory activity in low–molecular-weight fractions. Overall, in our study, the inhibitory activity ranged between 33% and 55%, which is in agreement with the results reported by Borges-Contreras et al. [27] regarding the antihypertensive effect of peptides obtained from shrimp shell hydrolysis. Previous studies on ACE-inhibitory peptides have shown that peptides with lower molecular weights exhibit stronger inhibitory activity compared to those with higher molecular weights [18,19], which is consistent with the results obtained in the present study. According to the study by Ngo and colleagues [28], peptides with molecular weights of 5–10 kDa derived from Atlantic cod fish skin gelatin exhibit approximately 60% inhibitory activity. Since the primary activity of ACE is the cleavage of C-terminal dipeptides from oligopeptide substrates with broad specificity, strong ACE inhibitors are,according to the findings of Ngo and

colleagues [28], strongly influenced by the C-terminal tripeptide sequence.

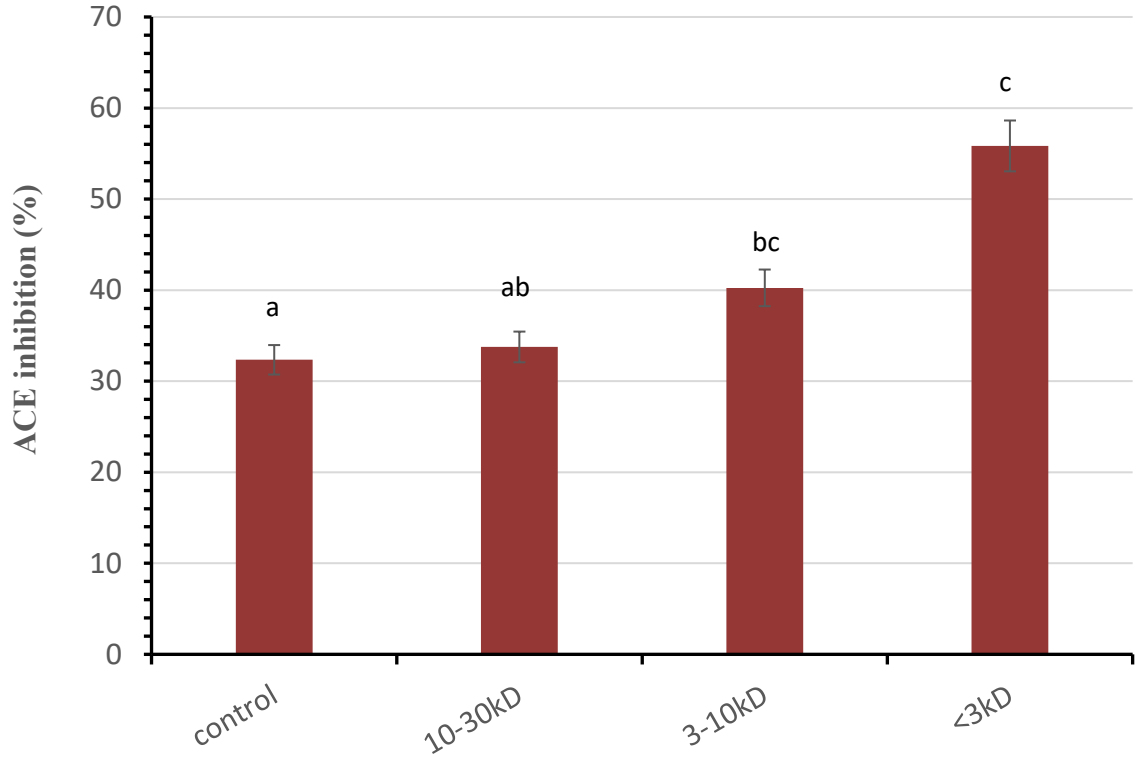


Fig 6. Comparison chart of ACE enzyme inhibition percentage at 12.5 (µg/ml) concentration of different hydrolyzed samples

3.8. Evaluation of Antidiabetic Activity through Dipeptidyl Peptidase-IV (DPP-IV) Inhibitory Assay

The DPP-IV inhibitory activity of the protein hydrolysate and its ultrafiltration-derived peptide fractions at a concentration of *mg/mL* is shown in Figure 7. According to the results, all samples exhibited DPP-IV inhibitory activity, with inhibition levels ranging from 73.35% (the highest activity observed in the peptide fraction <3 kDa) to 28.12% (the lowest activity in the 3–10 kDa fraction). These results were generally consistent with those obtained for the ACE inhibitory assay. Nongonierma and FitzGerald [3] reported that structural parameters of peptides may be associated with their DPP-IV inhibitory properties, and that peptide chain length does not necessarily have a direct effect on inhibitory performance. The differences in DPP-IV inhibitory activity among the various hydrolysates may therefore be attributed to variations in peptide structure, as Huang *et al.* [29] indicated that the DPP-IV inhibitory potential of a protein hydrolysate is determined primarily by the amino acid composition and sequence of its peptides.

Several researchers have reported that the optimal molecular weight for DPP-IV inhibitory peptides ranges between 200 and 1000 Da [30]. In a study conducted by Hsu *et al.* [30], it was

found that the peptide fraction with a molecular weight below 1.5 kDa, derived from porcine skin gelatin hydrolysate, exhibited the highest DPP-IV inhibitory activity compared to higher-molecular-weight fractions.

As expected, in the present study, the <3 kDa fraction also demonstrated the greatest inhibitory activity compared to the total hydrolysate and other peptide fractions. However, higher-molecular-weight peptides (such as 10 and 30 kDa fractions) also showed notable antidiabetic activity, which may be attributed to their specific three-dimensional structures or different mechanisms of action. Statistical analysis (ANOVA) revealed that the differences among the groups were significant ($p < 0.05$). These findings suggest that molecular weight alone cannot fully determine the antidiabetic potential of peptides, and that other factors such as peptide structure and mechanism of interaction also play crucial roles.

Wang *et al.* [4] utilized the skin of various warm-water and cold-water fish species to produce gelatin hydrolysates and compared their DPP-IV inhibitory activities**.** Their results indicated that the >1.5 kDa fraction of tilapia (Oreochromis niloticus) skin gelatin exhibited 51.9% DPP-IV inhibition at a concentration of 1 mg/mL, which is comparable to the activity of the <3 kDa fraction observed in the present study. Similarly, in the study conducted by Ramezanzadeh [17]**,** at a concentration of 2 mg/mL, the 3–10 kDa peptide fraction derived from ponyfish showed the highest DPP-IV inhibitory activity, with approximately 55% inhibition**,** which is consistent with our findings regarding the strong inhibitory effect of <3 kDa peptides on DPP-IV enzyme activity in diabetic assay kit.

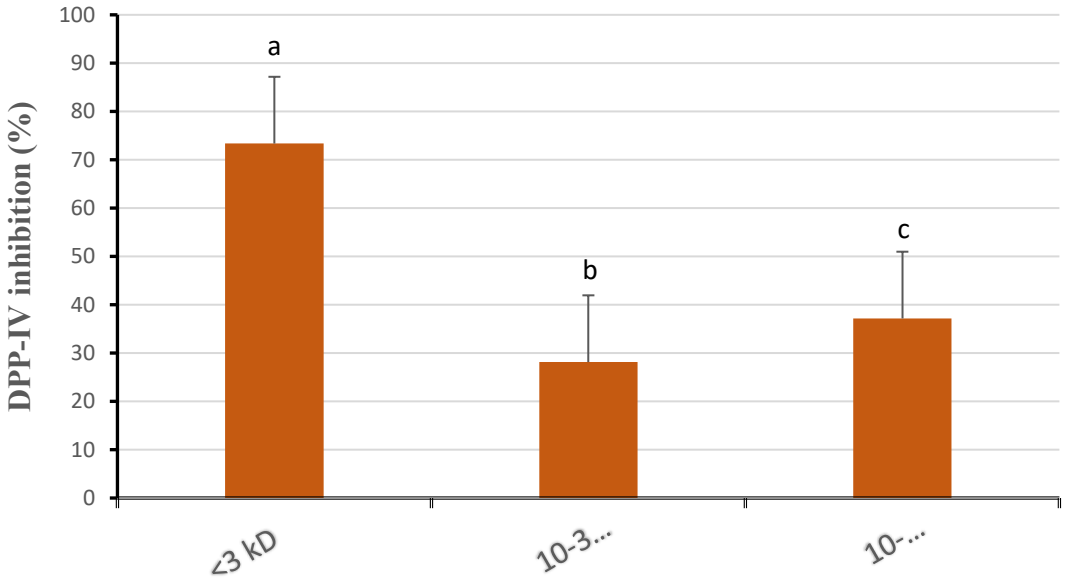


Fig 7. Comparison of DPP-IV inhibitory activity of hydrolyzed protein and peptide components with different molecular weights at a concentration of 2 (mg/ml)

4-Conclusion

With the rapid growth of the global population, the demand for new and sustainable protein sources is greater than ever. To meet this need, efficient and intelligent utilization of marine resources, particularly the by-products of seafood processing industries, is of great importance. Despite significant efforts in extracting bioactive compounds from aquatic by-products, this field still offers vast research and industrial potential.

In shrimp processing industries, by-products such as the head, thorax, shell (carapace), and tail, which account for approximately 40–45% of the total shrimp weight, are often used as aquaculture feed or animal feed supplements. However, the head and thorax are rich in proteins and amino acids that can be hydrolyzed into bioactive peptides with various food and pharmaceutical applications. These peptides, due to their antioxidant and antihypertensive properties, can be utilized as natural high-value additives in the food and nutraceutical industries.

Moreover, shrimp head and thorax are valuable sources of calcium and other minerals, which may serve as dietary supplements. The shell also contains bioactive peptides, chitin, and chitosan, which can be converted into chitosan for applications in biomedicine and biodegradable material production. Overall, the valorization of these by-products can contribute to sustainable development in the seafood industry and significantly reduce environmental pollution.

For instance, chitosan is utilized in the production of edible films, antibacterial drugs, and dietary supplements. The shrimp tail contains proteins and bioactive peptides that can be hydrolyzed into peptides exhibiting antihypertensive and antibacterial properties. Certain shrimp by-products also contain nutritionally valuable oils in their tails, which can serve as a source of omega-3 fatty acids and other beneficial compounds. Bioactive peptides with diverse biological activities are promising candidates for the development of such value-added products.

In this study, a 16-hour enzymatic hydrolysis using Alcalase was performed to extract peptides from the by-products of *Penaeus semisulcatus* (green tiger shrimp). The results demonstrated that all peptide fractions possessed inhibitory activity against DPPH and ABTS free radicals, as well as DPP-IV and ACE enzymes. Among them, peptides with a molecular weight lower than 3 kDa exhibited the highest levels of bioactivity.

Data Availability

The data used to support the finding of this study are available from the corresponding author upon request.

Conflict Of Interest

The authors have no conflicts interest to report.

Funding Statemant

The researchers did not receive any specific grant from funding agencies the public, commercial or not-for-profit sectors.

5-References

[1] Ma, W., Li, N., Lin, L., Wen, J., Zhao, C., & Wang, F. (2023). Research progress in lipid metabolic regulation of bioactive peptides. *Food Production, Processing and Nutrition*, *5*(1), 10.

[2] Hu, S., Li-Chan, E. C. Y., Wan, Y., Zhang, Y., & Pan, S. (2019). Identification of anti-diabetes peptides from *Spirulina platensis*. *Journal of Functional Foods, 56*, 333–341.

[3] Nongonierma, A. B., & FitzGerald, R. J. (2019). Features of dipeptidyl peptidase IV (DPP-IV) inhibitory peptides from dietary proteins. *Journal of Food Biochemistry, 43*(1), e12451.

[4] Li, Y., He, F., Liu, S., Wang, J., & Zhu, H. (2021). Investigation of *Chlorella pyrenoidosa* protein as a source of novel

angiotensin I-converting enzyme (ACE) and dipeptidyl peptidase-IV (DPP-IV) inhibitory peptides. *Nutrients, 13*(5), 1624.

[5] Chalamaiah, M., Hemalatha, R., & Jyothirmayi, T. (2012). Fish protein hydrolysates: Proximate composition, amino acid composition, antioxidant activities, and applications: A review. *Food Chemistry, 135*(4), 3020–3038.

[6] Ramazani, M. (2018). Effect of hydrolysis intensity on functional properties of hydrolyzed protein from the ponyfish (*Leiognathus bindus*). *Journal of Food Processing and Preservation, 10*(2), 137–149.

[7] Ko, S.-C., Kim, E.-A., & Jung, W.-K. (2012). A novel angiotensin I-converting enzyme (ACE) inhibitory peptide from a marine *Chlorella ellipsoidea* and its antihypertensive effect in spontaneously hypertensive rats. *Process Biochemistry, 47*(12), 2005–2011.

[8] Ishak, N., & Sarbon, N. (2018). Physicochemical characterization of enzymatically prepared fish protein hydrolysate from waste of shortfin scad (*Decapterus macrosoma*). *International Food Research Journal, 25*(6), [pages].

[9] Klompong, V., Benjakul, S., Kantachote, D., & Shahidi, F. (2008). Comparative study on antioxidative activity of yellow stripe trevally protein hydrolysate produced from Alcalase and Flavourzyme. *International Journal of Food Science & Technology, 43*(6), 1019–1026.

[10] Nahvi, A. N., Ali, M., & Masoud, R. (2017). Production of hydrolyzed protein from Kilka fish by enzymatic hydrolysis and evaluation of its bioactive properties. *Physiology and Biotechnology of Aquatics, 5*(3), 39–58.

[11] Zhang, Y., Duan, X., & Zhuang, Y. (2012). Purification and characterization of novel antioxidant peptides from enzymatic hydrolysates of tilapia (*Oreochromis niloticus*) skin gelatin. *Peptides, 38*(1), 13–21.

[12] Ng, K., & Mohd Khan, A. (2012). Enzymatic preparation of palm kernel expeller protein hydrolysate (PKEPH). *International Food Research Journal, 19*(2), [pages].

[13] Owisi, H. R. (2009). Inhibitory properties of hydrolyzed aquatic proteins on angiotensin I-converting enzyme activity and its effect in reducing the risk of heart attack. In *Proceedings of the First National Congress on Veterinary Laboratory Sciences* (pp. [pages]).

[14] Nejad, K. (2019). Comparison of separate and combined levels of commercial multi-enzymes on the feeding efficiency and chemical composition of common carp (*Cyprinus carpio*) carcasses. *Journal of Veterinary Research, 74*(1), 35–43.

[15] Bordbar, S., Ebrahimpour, A., Zarei, M., Abdul Hamid, A., & Saari, N. (2018). Alcalase-generated proteolysates of stone fish (*Actinopyga lecanora*) flesh as a new source of antioxidant peptides. *International Journal of Food Properties, 21*(1), 1541–1559.

[16] Ramezanzade, L., et al. (2018). Recovery of bioactive peptide fractions from rainbow trout (*Oncorhynchus mykiss*) processing waste hydrolysate. *Ecopersia, 6*(1), 31–40.

[17] Ramezanzade, L., et al. (2022). Evaluation of bioactive properties of peptide fractions from enzymatic hydrolysis of orangefin ponyfish (*Leiognathus bindus*). *Journal of Fisheries Science and Technology, 11*(2), 106–122.

[18] Forghani, B., et al. (2012). Enzyme hydrolysates from *Stichopus horrens* as a new source for angiotensin-converting enzyme inhibitory peptides. *Evidence-Based Complementary and Alternative Medicine, 2012*, Article ID 236384.

[19] Auwal, S. M., et al. (2018). Enhanced physicochemical stability and efficacy of angiotensin I-converting enzyme (ACE)-inhibitory biopeptides by chitosan nanoparticles optimized using Box-Behnken design. *Scientific Reports, 8*(1), 10411.

[20] Kristinsson, H. G., & Rasco, B. A. (2000). Fish protein hydrolysates: Production, biochemical, and functional properties. *Critical Reviews in Food Science and Nutrition, 40*(1), 43–81.

[21] Herawati, E., Akhsanitaqwim, Y., Agnesia, P., Listyawati, S., Pangastuti, A., & Ratriyanto, A. (2022). In Vitro Antioxidant and Antiaging Activities of Collagen and Its Hydrolysate from Mackerel Scad Skin (*Decapterus macarellus*). *Marine Drugs*, *20*(8), 516. https://doi.org/10.3390/md20080516

[22] Bashir, K. M. I., Chakniramol, S., Mansoor, S., Jahn, A., Cho, M.-G., & Choi, J.-S. (2024). Antioxidant activity of protein hydrolysates from redlip mullet (*Chelon haematocheilus*) muscle and byproducts. *Foods, 13*(18), 3009.

[23] Umayaparvathi, S., Meenakshi, S., Vimalraj, V., Arumugam, M., Sivagami, G., & Balasubramanian, T. (2014). Antioxidant

activity and anticancer effect of bioactive peptide from enzymatic hydrolysate of oyster (Saccostrea cucullata). *Biomedicine & Preventive Nutrition*, *4*(3), 343-353.

[24] Oh, Y., Ahn, C. B., Nam, K. H., Kim, Y. K., Yoon, N. Y., & Je, J. Y. (2019). Amino acid composition, antioxidant, and cytoprotective effect of blue mussel (*Mytilus edulis*) hydrolysate through the inhibition of caspase-3 activation in oxidative stress-mediated endothelial cell injury. *Marine Drugs, 17*(2), 135. https://doi.org/10.3390/md17020135

[25] Zhong, S., et al. (2011). Antioxidant properties of peptide fractions from silver carp (*Hypophthalmichthys molitrix*) processing by-product protein hydrolysates evaluated by electron spin resonance spectrometry. *Food Chemistry, 126*(4), 1636–1642.

[26] Wu, S., et al. (2012). Optimization of hydrolysis conditions for the production of angiotensin-I converting enzyme-inhibitory peptides and isolation of a novel peptide from lizard fish (*Saurida elongata*) muscle protein hydrolysate. *Marine Drugs, 10*(5), 1066–1080.

[27] Borges-Contreras, B., et al. (2019). Angiotensin-converting enzyme inhibition in vitro by protein hydrolysates and peptide fractions from mojarra of Nile tilapia (*Oreochromis niloticus*) skeleton. *Journal of Medicinal Food, 22*(3), 286–293.

[28] Ngo, D.-H., et al. (2016). Angiotensin-I-converting enzyme (ACE) inhibitory peptides from Pacific cod skin gelatin using ultrafiltration membranes. *Process Biochemistry, 51*(10), 1622–1628.

[29] Huang, S.-L., et al. (2012). Dipeptidyl-peptidase IV inhibitory activity of peptides derived from tuna cooking juice hydrolysates. *Peptides, 35*(1), 114–121.

[30] Hsu, K. C., Tung, Y. S., Huang, S. L., & Jao, C. L. (2013). Dipeptidyl peptidase-IV inhibitory activity of peptides in porcine skin gelatin hydrolysates. *Bioactive food peptides in health and disease*, (2), 205-218.

مجله علوم و صنایع غذایی ایران

سایت مجله: www.fsct.modares.ac.ir

مقاله علمی-پژوهشی

بررسی خواص ضددیابتی، ضدفشارخون و ضداکسیدانی پپتیدهای زیست فعال استخراج شده از ضایعات میگوی ببری سبز (*Penaeus semisulcatus*)

محیا دمالی امیری[۱]*، رضا حسن ساجدی[۲]

۱- کارشناسی ارشد بیوشیمی، دانشگاه تربیت مدرس، جلال آل احمد، کارگر شمالی

۲- دکتری بیوشیمی، استاد، دانشگاه تربیت مدرس، جلال آل احمد، کارگر شمالی

اطلاعات مقاله

تاریخ های مقاله :

تاریخ دریافت: ۱۴۰۳/۰۸/۱۳

تاریخ داوری: ۱۴۰۴/۰۷/۲۰

تاریخ پذیرش: ۱۴۰۴/۰۷/۲۴

کلمات کلیدی:

پپتید دریایی،

هیدرولیز آنزیمی،

پپتید زیست‌فعال،

میگوی ببری سبز،

خاصیت ضد دیابت

DOI: 10.48311/fsct.2026.83919.0

* مسئول مکاتبات:

mahya.amirii76@gmail.com

چکیده

در صنایع عمل‌آوری میگو حدود ۴۵-۴۰٪ از ماده اولیه به صورت مواد زائد شامل سر، امعا و احشا و پوسته دور ریز می‌شوند. هدف از این مطالعه، استخراج و ارزیابی فعالیت زیستی پپتیدهای حاصل از ضایعات فرآوری میگوی ببری سبز (Penaeus semisulcatus) بود. ضایعات حاصل از عمل‌آوری میگو تهیه و بافت های هدف جهت تیمار آنزیمی مورد استفاده قرار گرفت. نتایج مربوط به تعیین ترکیب تقریبی ماده خام اولیه بر اساس وزن خشک نمونه با رطوبت ۷۴/۵٪، بیش ترین ترکیب بدن میگوی ببری سبز و سپس پروتئین ۴۴٪ و خاکستر ۲/۲٪ محتوای بافت میگوی سبز را تشکیل می‌دادند. در ادامه، از سه نوع آنزیم آلکالین پروتئاز، آلکالاز و اواتاز جهت هیدرولیز و تولید پپتیدها از ضایعات میگو استفاده شد. سپس آنزیم‌های درونی میگو بوسیله دمای ۸۵ درجه غیرفعال شده و در محدوده pH ۸/۰ تنظیم گردید. هیدرولیز در ۸ و ۱۶ ساعت انجام شد و میزان پروتئین کلی بخش رویی توسط تکنیک کجدال، جهت آنالیز و بررسی نمونه‌های تولیدشده و انتخاب بهترین نمونه، سنجیده شد و ساعت ۱۶ هیدرولیز شده با آنزیم آلکالاز با ۳۵،۵٪ پروتئین به عنوان نمونه‌ی بهینه انتخاب شد. پروتئین‌های استخراج‌شده با استفاده از آنزیم آلکالاز هیدرولیز شده و در سه بازه وزنی کمتر از ۳، ۱۰ و ۳۰ کیلو دالتون با روش اولترافیلتراسیون جداسازی شدند. نتایج نشان دادند که فراکسیون پپتیدی با وزن مولکولی کمتر از ۳ kDa بیشترین فعالیت زیستی را داراست؛ به‌طوری که مهار آنزیم DPP-IV تا ۷۳.۳۵٪، مهار ACE تا ۵۵٪ و فعالیت مهار رادیکال آزاد در آزمون DPPH برابر با ۶۹.۶۱٪ بود.