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IDENTIFICATION AND CHARACTERISATION OF A NOVEL ANGIOTENSIN-I-CONVERTING ENZYME (ACE) INHIBITORY PEPTIDE FROM RED BIGEYE (*Priacanthus macracanthus*) MUSCLE HYDROLYSATE

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ABSTRACT

Bioactive peptides from marine organisms hold strong potential as angiotensin-converting enzyme (ACE) inhibitors. This study aimed to identify and characterise ACE inhibitory peptides from the muscle hydrolysate of small red bigeye fish produced using Alcalase®. Peptide isolation was carried out through ultrafiltration, fast protein liquid chromatography (FPLC), and reversed-phase high-performance liquid chromatography (RP-HPLC). The purified ACE inhibitory peptide was further characterised by determining its amino acid sequence, inhibition mechanism, and stability under different pH levels, temperatures, and simulated gastrointestinal (GI) digestion conditions. A novel ACE inhibitory peptide identified as Asp-Lys-Gln-Thr-Pro-Ser-Gly-Tyr was successfully purified, achieving a 2.45-fold increase in purity, 93% ACE inhibitory activity, and an IC_{50} value of 0.163 mg/mL. This peptide exhibited a competitive inhibition mode towards ACE. Stability tests indicated that the pure peptide remained stable at a pH between 2 and 8, with decreased stability above pH 8. At temperatures of 30°C and 90°C, the peptide's stability was measured at 70.1% and 37.9%, respectively. Additionally, the pure peptide showed resilience against *in vitro* GI enzymes. This study highlights a novel antihypertensive peptide from red bigeye hydrolysate that exhibits good stability under varying pH conditions, temperature fluctuations, and *in vitro* GI digestion.

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Introduction

Hypertension has emerged as a major global health issue, affecting populations in both developed and developing nations, driven largely by factors such as economic growth and an aging population (Zhou *et al*., 2021). This chronic disease poses a significant health risk, impacting approximately 42% of adults aged 30 and above, and remains one of the leading causes of mortality worldwide (WHO, 2023). Current management strategies primarily utilise angiotensin-converting enzyme (ACE) inhibitors, which are effective in reducing the risks of coronary artery disease, cardiovascular mortality, and heart failure (Shih *et al*., 2019). Nevertheless, despite the wide availability of ACE inhibitors such as captopril and enalapril, cardiovascular-related mortality continues to increase. These drugs function by blocking the conversion of angiotensin I to angiotensin II, a crucial process in blood pressure regulation (Sarbon *et al*., 2019). However, prolonged use of ACE inhibitors is often associated with side effects, including inflammation, persistent dry cough, altered taste perception, kidney impairment, angioneurotic oedema, and skin rashes (Daskaya-Dikmen *et al*., 2017; Yilmaz

et al., 2018). Consequently, there is a pressing need to develop new therapeutic options that are safer, more cost-effective, and equally or more efficacious in managing hypertension.

Bioactive peptides obtained from food sources that inhibit ACE represent a promising strategy for managing hypertension with fewer side effects (Sukarno *et al*., 2022; Ishak *et al*., 2021). This has led to growing interest in isolating ACE-inhibitory compounds from natural foods as safer alternatives to synthetic drugs (Ishak *et al*., 2021). In particular, the discovery and characterisation of ACE-inhibitory peptides from marine organisms have attracted considerable attention for their potential role in hypertension management. Various marine organisms, including fish gelatine (Sukarno *et al*., 2022), mud crab (Zaliha, 2021), blood cockle (Aishah *et al*., 2020), Channa striata (Chasanah *et al*., 2022), and ribbonfish (Yathisha *et al*., 2021), have been investigated for their bioactive peptides. These peptides exhibit strong ACE-inhibitory activity, often supported by molecular docking studies and *in vitro* assays that clarify their binding mechanisms and inhibitory capabilities. Fish ACE inhibitory peptides are emerging as a noteworthy natural option for managing high blood pressure (Suo *et al*., 2022; Hu *et al*., 2023).

Recent systematic reviews have underscored the growing scientific and therapeutic relevance of marine-derived ACE-inhibitory peptides, highlighting their excellent bioavailability and potential as natural agents for blood pressure regulation (Jo *et al*., 2024). These bioactive compounds are increasingly viewed as safer and more sustainable alternatives to conventional synthetic ACE inhibitors, offering promising prospects for their incorporation into functional foods and novel antihypertensive therapies.

Research has revealed the presence of ACE-inhibitory peptides in a wide range of marine species. For example, collagen-derived peptides SEGPK, FDGPY, and SPGPW extracted from the swim bladders of monkfish (*Lophius litulon*) demonstrated strong inhibitory activity against ACE, with IC_{50} values of 0.63, 0.94, and 0.71 mg/mL, respectively (Hu *et al*., 2023). Similarly, peptides obtained from the swim bladders of mi-iuy croaker (*Miichthys miiuy*), specifically SHGEY and SPYGF, showed notable ACE-inhibitory effects, recording IC_{50} values of 0.86 mg/mL and 0.37 mg/mL, respectively (Zhu *et al*., 2022).

In addition, several peptides identified from blue mussel (*Mytilus edulis*) including IK, YEGDP, WF, and SWISS, displayed strong ACE-inhibitory properties, with IC_{50} values of 0.77, 0.19, 0.40, and 0.32 mg/mL, respectively, indicating high affinity towards ACE binding sites (Suo *et al*., 2022a). Likewise, studies on skipjack tuna (*Katsuwonus pelamis*) milt identified ACE-inhibitory peptides ICY, LSFR, and IYSP, which exhibited potent activity with IC_{50} values of 0.48, 0.59, and 0.76 mg/mL, respectively (Suo *et al*., 2022b). Molecular interaction analyses revealed that these peptides bind to ACE through hydrogen bonds, electrostatic interactions, and hydrophobic forces, emphasising their strong potential as natural therapeutic agents for hypertension management.

The expanding research on marine-derived bioactive peptides highlights their impressive diversity and potential health-promoting properties, while also pointing to the need for a deeper understanding of their mechanisms and biological effects. Future studies should focus on conducting clinical trials to confirm the antihypertensive efficacy of these peptides in humans and explore efficient methods for their large-scale production and integration into functional foods and nutraceuticals. Advancing this line of research could play an important role in combating global health challenges such as hypertension and promote the wider use of marine-sourced bioactive compounds in preventive and therapeutic healthcare.

In Malaysia, a total catch of 26,135 tonnes of red bigeye fish was recorded, with an average market price of RM5.38 per kilogramme (Department of Fisheries Malaysia, 2024). This species is mainly caught in coastal waters located 20–30 nautical miles offshore, at depths

of 30–40 metres. In Malaysian waters, red bigeye fish typically range from 5 to 34 cm in length, with an average size of 20–25 cm, and generally weigh between 0.5 and 1 kg, though some individuals can reach up to 2 kg. For surimi production, fish measuring between 20 and 36 cm are preferred. Those smaller than 15 cm are categorised as low-value species in the Asian market; these are often less desirable for direct consumption and are instead utilised for animal feed or, in the case of the smallest ones, as fertiliser. To date, there have been no reported studies on angiotensin-converting enzyme (ACE) inhibitory peptides derived from red bigeye muscle. Therefore, this study seeks to identify and characterise ACE-inhibitory peptides from red bigeye muscle hydrolysates.

Methodology

Materials and Reagents

Fresh whole red bigeye fish (*Priacanthus macracanthus*) were sourced from the Fisheries Development Authority of Malaysia (LKIM) at Pulau Kambing, Terengganu. Approximately 50 kilograms of fish, each measuring under 15 cm in length, were transported to the laboratory on ice to maintain freshness. Upon arrival, the samples were thoroughly rinsed with tap water, eviscerated, and washed several times with chilled water before being stored at temperatures below 5°C. The cleaned fish were then filleted, homogenised using a Panasonic food processor, and kept frozen at −20°C until further use. Prior to experimental analysis, the frozen samples were thawed and finely minced.

Angiotensin-converting enzyme (ACE) from rabbit lung (≥ 2.0 units/mg protein, modified Warburg–Christian) (0.1 UN), Alcalase® (≥ 0.75 Anson units/mL), pepsin from porcine gastric mucosa (≥ 2,500 units/mg protein), Kjeldahl tablets, Hippuryl-L-Histidyl-L-Leucine (HHL), and pancreatin from porcine pancreas (8 × USP) were obtained from Sigma-Aldrich (Saint Louis, USA). All other reagents and chemicals used in this study were of analytical grade.

Determination of Crude Protein

Before preparing the red bigeye muscle hydrolysate, the crude protein content of the homogenised muscle was determined using the Kjeldahl method, as described by AOAC (2002). This value was then used to calculate the required amounts of fish muscle, water, and enzyme for the enzymatic hydrolysis process (Kristinsson & Rasco, 2002). The crude protein content of the lyophilised red bigeye muscle hydrolysate was also analysed using the same method.

Preparation of Lyophilised Red Bigeye Muscle Hydrolysate (RH)

The enzymatic hydrolysis of red bigeye (*Priacanthus macracanthus*) muscle was performed based on the procedure described by Ahn *et al*. (2012), with minor adjustments. Briefly, 110 g of homogenised fish muscle was mixed with 66 g of distilled water and preheated at 90°C for 10 minutes to deactivate native enzymes. Once cooled to room temperature, the pH of the mixture was adjusted to 8.5 using 1 N NaOH. Hydrolysis was then initiated by adding 40 g of an Alcalase® solution, prepared by dissolving 0.17 g of enzyme in 39.83 g of distilled water to achieve a 2% enzyme-to-substrate ratio. The reaction mixture was incubated at 50°C for 6 hours in a water bath. Enzyme activity was terminated by reheating the sample at 90°C for 10 minutes, followed by cooling to room temperature. The hydrolysed sample was then transferred into 500 mL centrifuge tubes and centrifuged at 6000×g for 15 minutes at 4°C. The obtained supernatant was filtered through Whatman No. 1 paper to remove residual lipids, frozen at -80°C overnight, and subsequently freeze-dried using a Labconco FreeZone 4.5 L benchtop lyophiliser (USA). The resulting dried hydrolysate powder was stored at -20°C for further experimental analysis.

Determination of Degree of Hydrolysis (DH) of Red Bigeye Muscle Hydrolysate

The degree of hydrolysis (DH) of the red bigeye (*Priacanthus macracanthus*) muscle hydrolysate was assessed using a modified version of the trichloroacetic acid (TCA) method proposed by Klompong *et al*. (2007). In this procedure, 20 mL of the protein hydrolysate was mixed with an equal volume of 20% (w/v) TCA solution to obtain the TCA-soluble fraction. The mixture was allowed to stand for 30 minutes to promote protein precipitation, after which it was centrifuged at 7800 g for 15 minutes. The nitrogen content of both the original hydrolysate and the TCA-soluble supernatant was measured using the Kjeldahl method.

The degree of hydrolysis (DH) was then determined using the following equation:

$$\text{DH (\%)} = \frac{\text{Soluble N in TCA 10\% (w/v) x 100}}{\text{Total N in the sample}}$$

Determination of ACE Inhibitory Activity

The angiotensin-converting enzyme (ACE) inhibitory activity of the red bigeye muscle hydrolysate was evaluated using a modified version of the method described by Ahn *et al*. (2012). The hydrolysate was first diluted to a concentration of 1 mg/mL using a 50 mM sodium borate buffer containing 0.3 M NaCl, with the pH adjusted to 8.3. For the assay, 50 µL of the diluted sample was combined with 50 µL of ACE solution (25 mU/mL) in a microcentrifuge tube and pre-incubated at 37°C for 10 minutes in a shaking water bath (Memmert GmbH, Germany). The reaction was initiated by adding 100 µL of a 25 mM HHL substrate, followed by incubation at 37°C for 30 minutes. The reaction was terminated by introducing 250 µL of 1 M HCl. Hippuric acid (HA), the reaction product, was extracted with 500 µL of ethyl acetate. After centrifugation at 800×g for 15 minutes, 200 µL of the upper organic phase was transferred into a clean tube and evaporated at 80°C for one hour using an oven (Memmert GmbH, Germany). The dried residue was then dissolved in 1 mL of distilled water, and absorbance was measured at 228 nm using a UV–vis spectrophotometer (Shimadzu Corporation, Japan) equipped with a quartz cuvette. The inhibitory concentration (IC_{50}), defined as the concentration of the sample needed to inhibit 50% of ACE activity, was determined from the resulting absorbance data.

Fractionation of Red Bigeye Hydrolysate using Ultrafiltration

The red bigeye fish hydrolysate was fractionated according to molecular weight using ultrafiltration (Vivaspin 20 sample concentrators, Sartorius, Japan) equipped with membranes of 10, 5, and 3 kDa molecular weight cut-offs (MWCO). Initially, 3 g of the hydrolysate was dissolved in 30 mL of distilled water, resulting in a concentration of 100 mg/mL. Each ultrafiltration membrane was pre-rinsed with 20 mL of distilled water to ensure proper conditioning. Approximately 20 mL of the prepared hydrolysate solution was loaded into the 10 kDa MWCO concentrator and centrifuged at 6,000×g for 30 minutes at 4°C using a refrigerated centrifuge (Kubota Tabletop 5500, Japan). The filtrate obtained was collected and subjected to subsequent filtration through the 5 kDa and then the 3 kDa MWCO concentrators under identical conditions.

The final filtrates and retentates from each ultrafiltration step were freeze-dried and weighed to determine their relative yields within the hydrolysate. Each fraction was then tested for angiotensin-converting enzyme (ACE) inhibitory activity. The fraction demonstrating the lowest IC_{50} value, indicating the highest ACE-inhibitory potential, was selected for further purification and lyophilised for detailed characterisation.

Purification of ACE Inhibitory Peptides using Fast Protein Liquid Chromatography (FPLC)

The peptide fraction with a molecular weight below 3 kDa, obtained from the ultrafiltration process, was further purified using a HiPrep™

26/60 Sephacryl™ S-100 HR gel filtration column (Cytiva, Sweden) connected to an ÄKTA Avant FPLC system (Cytiva, Sweden). Prior to sample loading, the column was equilibrated with phosphate-buffered saline (PBS). The peptide solution, adjusted to a concentration of 5 mg/mL, was injected into the column and eluted with a linear gradient of the same buffer at a constant flow rate of 1.5 mL/min. An injection volume of 3.2 mL was used, corresponding to two column volumes (CV). The elution profile was monitored at a wavelength of 214 nm.

Each fraction collected during elution was subsequently freeze-dried and tested for angiotensin-converting enzyme (ACE) inhibitory activity. The fraction exhibiting the lowest IC_{50} value, indicating the most potent ACE-inhibitory effect, was selected for further purification and detailed analysis.

Purification of ACE Inhibitory Peptides using Semi-Preparative High-Performance Liquid Chromatography (HPLC)

The peptide fraction exhibiting the strongest ACE-inhibitory activity (designated as F3) underwent further purification using semi-preparative high-performance liquid chromatography (HPLC), with modifications based on the method of Ahn *et al*. (2012). The F3 fraction, identified as the most active ACE inhibitor from the previous FPLC purification, was applied to a reversed-phase C18 column (5 µm, 4.6 × 150 mm; Shimadzu Corporation, Japan). Before injection, the sample was diluted to a concentration of 5 mg/mL with distilled water and filtered through a 0.45 µm cellulose acetate syringe filter (Sartorius, Germany) to remove any particulates.

The column was pre-equilibrated with 25% acetonitrile for 30 minutes. A 100 µL aliquot of the prepared sample was then injected, and separation was performed using a gradient elution system consisting of solvent A (water with 0.1% trifluoroacetic acid, TFA) and solvent B (acetonitrile with 0.1% TFA). The gradient was programmed to increase from 10% to 70% acetonitrile over a 25-minute run at a flow rate of 1 mL/min. Elution was monitored at 215 nm. The fraction corresponding to a single, well-defined peak was collected, freeze-dried, and stored for subsequent characterisation and analysis.

Determination of Amino Acid Sequence of the Purified ACE Inhibitory Peptides

Liquid Chromatography–Quadrupole–Time-of-Flight Mass Spectrometry (LC-Q-TOF-MS/MS) analysis was conducted following the general approach outlined by Kinter and Sherman (2005), with some modifications. The purified peptide samples were dissolved in 50 µL of 0.1% formic acid prepared in deionised water and transferred into 250 µL polypropylene LC vials (Agilent, USA) fitted with 0.2 µm regenerated cellulose membrane filters (Sartorius, Germany) to remove any impurities. A 25 µL portion of each sample was then injected automatically into the LC-Q-TOF-MS/MS system. Chromatographic separation was performed using a Hypersil Gold C18 column (2.1 × 150 mm, 3 µm; Thermo Scientific, USA) with a binary solvent system consisting of mobile phase A (90% deionised water with 0.1% formic acid and 10% acetonitrile) and mobile phase B (acetonitrile with 0.1% formic acid). The analysis was carried out at a constant flow rate of 15 µL/min. The gradient programme began with an initial 5-minute equilibration, followed by a linear increase from 5% to 95% mobile phase B over 120 minutes, before re-equilibrating back to 10% B for the final 15 minutes. Mass spectrometric data for MS/MS scans were collected across an m/z range of 100–2000. The four most abundant precursor ions, excluding those with single or undefined charge states, were selected for fragmentation based on the predicted peptide ion list. Ionisation was achieved using an electrospray ion source at 3.5 kV, with nitrogen gas maintained at 350°C and a flow rate of 10 L/min to facilitate collision-induced dissociation (CID).

Determination of ACE Inhibitory Mode

The inhibition mechanism of the purified peptide against angiotensin-converting enzyme (ACE) was determined using a modified version of the procedure described by Lau *et al*. (2013). The assay was performed spectrophotometrically, employing hippuryl-L-histidyl-L-leucine (HHL) as the substrate. In brief, 50 µL of ACE solution (25 mU/mL) was combined with 50 µL of the peptide solution at concentrations of 0.25, 0.5, and 1.0 mg/mL. The mixtures were incubated at 37°C for 30 minutes. Subsequently, 100 µL of HHL at different concentrations (0.63, 1.25, 2.5, 5.0, and 10.0 mM) was added, and the reactions were allowed to proceed for 60 minutes in a shaking water bath maintained at 37°C.

The reactions were terminated by adding 250 µL of 1.0 M HCl. Hippuric acid, the reaction product, was extracted with 500 µL of ethyl acetate. From the resulting mixture, 200 µL of the upper layer was collected and evaporated at 80°C in a drying oven (Memmert GmbH, Germany). The residue was dissolved in 1.0 mL of distilled water, and the absorbance was measured at 228 nm using a UV-Vis spectrophotometer (Shimadzu Corporation, Japan). Enzyme activity was compared between reactions conducted in the presence and absence of the peptide, with the latter serving as the control. To determine the type of inhibitions (competitive, non-competitive, or uncompetitive) Lineweaver-Burk plots were generated and analysed based on the kinetic data obtained.

Stability of Purified Peptide at Different pH

The effect of pH on the stability of the ACE-inhibitory peptide was evaluated following a modified version of the procedure described by Zuo *et al*. (2017). The purified peptide was dissolved in distilled water to prepare a stock solution at a concentration of 10 mg/mL. The pH of the peptide solutions was then adjusted to 2, 4, 6, 8, 10, and 12 using 1 M HCl or 1 M NaOH. All pH adjustments were carried out at ambient temperature (approximately 25°C), and the samples were incubated for one hour under these conditions. After incubation, the pH of each sample was readjusted to a neutral level (pH 7.0), and the residual ACE-inhibitory activity of each solution was measured to determine the peptide's stability across varying pH environments.

Stability of Purified Peptide at Different Temperatures

To evaluate thermal stability, the purified peptide solutions were incubated at four temperature conditions: 30°C, 50°C, 70°C, and 90°C, in a water bath (Memmert GmbH, Germany) for 10 minutes, as adapted from the method of Zuo *et al*. (2017). Following the heat treatment, all samples were immediately cooled in an ice bath (0°C) to halt any further thermal effects before assessing their remaining ACE-inhibitory activity.

In Vitro Gastrointestinal Digestion on ACE Inhibitory of Peptides Against Gastrointestinal Enzymes

The *in vitro* gastrointestinal digestion of the purified peptide was conducted using a modified version of the procedure described by Zaliha (2021). Two digestion treatments were performed: (1) Pepsin digestion only and (2) sequential digestion with pepsin followed by pancreatin. To begin, the purified peptide solution (10 mg/mL) was adjusted to pH 2.0 with 1 M HCl, after which pepsin was added at an enzyme-to-substrate ratio of 1:25 (w/w). The mixture was incubated at 37°C in a shaking water bath for 2 hours. After incubation, the sample was divided into two equal portions. For the pepsin-only treatment, one portion was heated in boiling water for 10 minutes to deactivate the enzyme, immediately cooled on ice, and centrifuged at 4,000×g for 15 minutes. The resulting supernatant was collected and stored at -20°C for subsequent analysis of ACE-inhibitory activity.

For the sequential digestion treatment, the second portion was adjusted to pH 7.5 using 1

M NaOH before adding pancreatin at the same enzyme-to-substrate ratio (1:25, w/w). This mixture was then incubated for another 2 hours at 37℃ in a shaking water bath. Enzymatic activity was terminated by boiling for 10 minutes, followed by rapid cooling on ice and centrifugation at 4,000×g for 15 minutes. The supernatants obtained from both digestion processes were stored at -20°C until further evaluation of ACE-inhibitory activity and IC_{50} values.

Results and Discussions

Crude Protein Content and Degree of Hydrolysis (DH)

Table 1 shows the crude protein content of raw red bigeye muscle and its lyophilised hydrolysate. The crude protein content of the raw red bigeye muscle was 17.21 ± 0.03%, which increased significantly to 84.62 ± 0.50% in the lyophilised hydrolysate.

These results are consistent with findings from other fish species. For example, black tilapia (*Oreochromis placidus*) has been reported to contain 20.28% protein in raw muscle and 92.53% in its lyophilised hydrolysate (Kasran *et al*., 2023). Similarly, the protein content of the lyophilised red bigeye hydrolysate in this study aligns with values reported for lyophilised hydrolysates from tilapia (*Oreochromis niloticus*) muscle (85.4%) and red snapper (*Lutjanus vitta*) muscle (87.36%) (Foh *et al*., 2011; Khantaphant *et al*., 2011).

The substantial increase in crude protein content observed in the lyophilised red bigeye hydrolysate aligns with the concentration effects reported in previous studies. This increase can be attributed to the freeze-drying process, enhanced protein solubilisation during hydrolysis, and the removal of non-protein insoluble materials through centrifugation (Halim & Sarbon, 2017). Eliminating these non-protein components results in higher protein purity. The elevated protein content of fish hydrolysates makes them valuable as nutritional supplements for human consumption and as promising sources of bioactive peptides.

The DH and crude protein content are fundamental indicators of the efficiency and quality of protein hydrolysates. In this study, the red bigeye hydrolysate achieved a DH of 73.75 ± 0.83% after 6 hours of enzymatic hydrolysis. This is comparable to DH values reported in previous studies, such as 69.0% for eel flesh hydrolysate, 77.03% for salmon skin hydrolysate, and 78.72% for bigeye tuna dark muscle hydrolysate (Qian *et al*., 2007; See *et al*., 2011; Baharuddin *et al*., 2016). These high DH values reflect substantial protein degradation into smaller peptides and free amino acids, leading to improved solubility, bioavailability, and overall functional properties of the hydrolysates.

High DH values, like those observed in the red bigeye hydrolysate, are linked to the generation of low molecular weight peptides. These peptides tend to be more bioavailable and frequently demonstrate improved functional properties. DH is influenced by several hydrolysis parameters, including enzyme type, temperature, and pH. For instance, bigeye tuna muscle hydrolysed with Protamex™ under optimised conditions achieved a DH of 39.04% and a crude protein content of 76.37% (Abd El-Rady *et al*., 2023). In this study, the red bigeye hydrolysate exhibited a high DH along with a notable increase in crude protein content, demonstrating the efficiency of the enzymatic hydrolysis process. These results are consistent with findings from other fish protein hydrolysate studies and further underscore the potential of such hydrolysates as valuable sources of bioactive peptides and functional food ingredients.

Table 1: The crude protein content of raw red bigeye muscle and its lyophilised hydrolysate

Component	Raw Red Bigeye Muscle	Lyophilised Red Bigeye Hydrolysate
Crude protein content (%)	17.21 ± 0.03^{b}	84.62 ± 0.50^{a}

Note: Means with different letters (a to b) were significantly different ($p < 0.05$).

Fractionation of Red Bigeye Hydrolysate Using Ultrafiltration

In this research, ACE-inhibitory peptides from red bigeye muscle hydrolysate were successfully isolated through a multi-step purification process involving ultrafiltration, gel filtration chromatography (FPLC), and reverse-phase high-performance liquid chromatography (RP-HPLC). The ultrafiltration process utilised membranes with molecular weight cut-offs (MWCO) of 10, 5, and 3 kDa. As presented in Table 2, a clear negative correlation was observed between peptide size and ACE-inhibitory activity. The fraction containing peptides smaller than 3 kDa demonstrated the highest inhibitory effect against ACE (91.65 ± 0.02%) and exhibited the lowest IC_{50} value (0.26 mg/mL), suggesting that lower molecular weight peptides possess greater bioactivity. Conversely, the fraction containing peptides larger than 10 kDa displayed the weakest inhibition (56.33 ± 0.06%) and the highest IC_{50} value (0.86 mg/mL), indicating reduced ACE-inhibitory potential.

These results align well with previous studies reporting that smaller peptides tend to exhibit greater ACE-inhibitory activity. For example, research on the deep-sea mussel *Gigantidas vrijenhoeki* revealed that peptides smaller than 1 kDa displayed the highest ACE inhibition, with the peptide KLLWNGKM showing exceptional potency (IC_{50} = 0.007 μM) [(Heo *et al*., 2023)]. Similarly, Ishak *et al*. (2021) found that peptide fractions under 3 kDa from shortfin scad (*Decapterus macrosoma*) hydrolysates exhibited stronger ACE inhibition (81.50%) and lower IC_{50} values (2.20 mg/mL) compared to larger peptide fractions. In another study, Sarbon *et al*. (2019) demonstrated that chicken skin peptides smaller than 2 kDa had the most pronounced inhibitory effect on ACE (92%) with IC_{50} = 0.04 mg/mL (Gly-Pro-Ile-Gly-Pro-Pro-Ser-Gly-Gly-Phe-Asp). Likewise, Lin *et al*. (2017) reported similar observations in tilapia frame hydrolysates, where peptides below 1 kDa exhibited 81% inhibition (IC_{50}= 0.41 mg/mL), outperforming the 5–10 kDa fractions.

Taken together, these studies reinforce the notion that peptides with lower molecular weights generally display superior bioactive potential, particularly in terms of ACE inhibition and antioxidant capacity.

In agreement with previous findings, peptides smaller than 3 kDa derived from shortfin scad (*Decapterus macrosoma*) hydrolysates have been shown to possess moderate ACE-inhibitory activity (77.4%) (Nasir & Sarbon, 2019). This observation is consistent with the results of the present study, where the <3 kDa fraction exhibited a comparable inhibitory effect, reflected by an IC_{50} value of 0.26 mg/mL. In contrast, larger peptides with molecular weights exceeding 10 kDa tend to show weaker ACE-inhibitory effects, primarily due to their limited bioavailability and reduced capacity to interact with the enzyme's active sites (Su *et al*., 2021).

The enhanced activity of smaller peptides can be attributed to their structural advantage, which allows for more efficient interaction with ACE. For instance, the low molecular weight dipeptide Ser-Pro (SP) isolated from skipjack tuna (*Katsuwonus pelamis*) demonstrated strong

Table 2: The ACE inhibitory activity of fractionate red bigeye hydrolysate

Fraction	ACE Inhibitory Activity (%)	IC_{50} (mg/mL)
MW>10 kDa	56.33 ± 0.06^{d}	0.86
MW5-10 kDa	59.19 ± 0.04^{c}	0.81
MW3-5 kDa	72.06 ± 0.02^{b}	0.50
MW < 3 kDa	91.65 ± 0.02^{a}	0.26

Note: Means with different letters (a to d) were significantly different ($p < 0.05$).

ACE inhibition, with an IC_{50} value of 0.06 mg/mL, owing to its effective binding to the enzyme's catalytic region (Zheng *et al*., 2022).

Overall, the data presented in Table 2 are consistent with earlier reports, reinforcing that peptides with molecular weights below 3 kDa are generally more potent ACE inhibitors due to their superior bioavailability and stronger affinity for ACE binding sites. These findings highlight the potential of such peptides for incorporation into functional food formulations aimed at hypertension control. Moreover, they support the broader consensus that ACE-inhibitory peptides, typically composed of 3–20 amino acids, exhibit greater physiological efficacy when shorter in length, as they can more readily pass through the intestinal barrier and exert biological effects (Ma *et al*., 2023).

Purification of ACE Inhibitory Peptides using Fast Protein Liquid Chromatography (FPLC)

To achieve further purification, the peptide fraction from ultrafiltration with the strongest ACE-inhibitory activity (IC_{50} < 3 kDa) was processed using gel filtration chromatography on a HiPrep™ 26/60 Sephacryl™ S-100 HR column (Cytiva, Sweden), connected to a fast protein liquid chromatography (FPLC) system. The sample was fractionated into eight separate components across 1.5 column volumes at a constant flow rate of 1.5 mL/min. Each eluted fraction was subsequently freeze-dried and analysed for its ACE-inhibitory potential. The chromatographic profile of the eight collected fractions, detected at 214 nm, is shown in Figure 1.

The data in Figure 2 and Table 3 indicate that purified sample F3 exhibited the highest ACE inhibitory activity (92.67 ± 0.05%) and the lowest IC50 value (0.18 mg/mL), suggesting superior efficacy among the fractions tested.

The findings revealed that fraction F3 displayed the most potent ACE-inhibitory activity, characterised by a notably low IC_{50} value, suggesting its strong potential as a bioactive peptide for managing hypertension through ACE suppression. This indicates that F3 could be a promising candidate for incorporation into functional foods or nutraceutical products designed to promote cardiovascular health. The variation in activity observed among different fractions also underscores the importance of precise purification techniques in obtaining

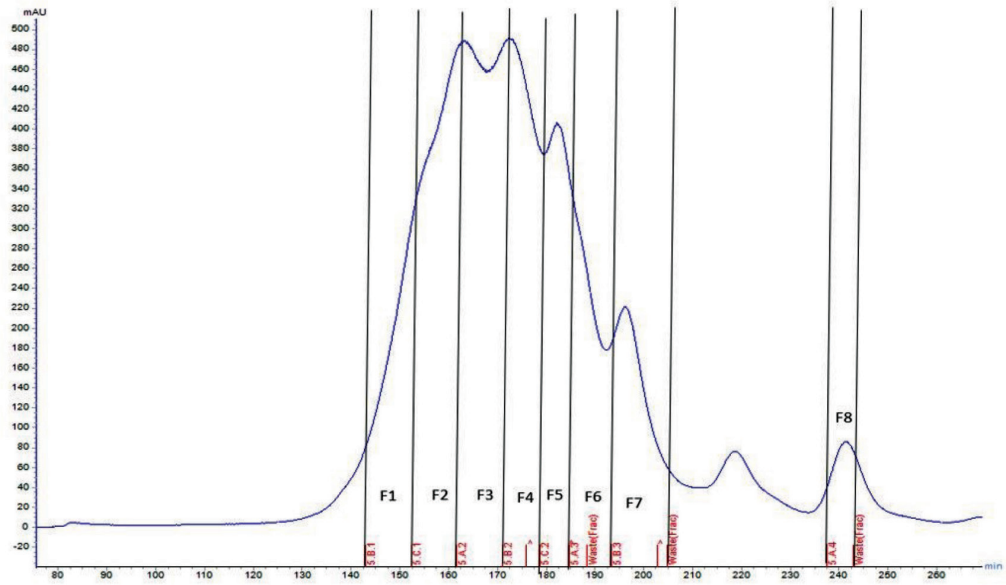

Figure 1: The chromatogram of red big eye hydrolysate < 3 kda using gel chromatography fraction in FPLC eluted at 214 nm

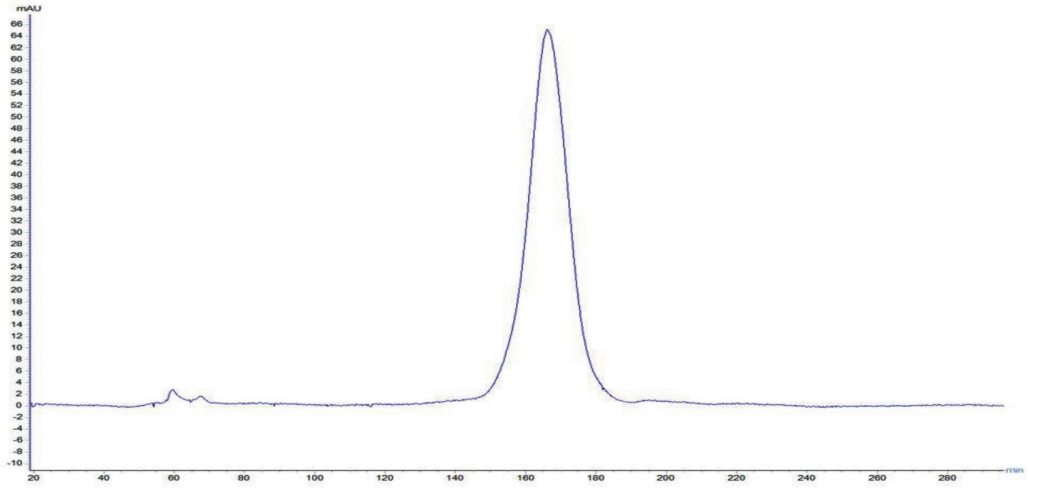

Figure 2: The chromatogram of active fraction F3 red bigeye bioactive peptide in FPLC eluted at 214 nm

Table 3: The ACE inhibitory activity (%) and IC_{50} of purified sample from FPLC

Purified Sample	ACE Inhibitory Activity (%)	IC_{50} (mg/mL)
F1	58.65 ± 0.04^{e}	0.66
F2	77.43 ± 0.08^{b}	0.42
F3	92.67 ± 0.05^{a}	0.18
F4	75.96 ± 0.09^{c}	0.41
F5	66.21 ± 0.02^{d}	0.54
F6	54.65 ± 0.03^{f}	0.86
F7	49.32 ± 0.03^{g}	1.02
F8	40.53 ± 0.03^{h}	1.36

Note: Means with the different letters (a to h) were significantly different ($p < 0.05$).

peptides with optimal bioactivity. Overall, these results contribute to the expanding research interest in naturally derived ACE inhibitors as safer, cost-effective alternatives to conventional synthetic drugs, with fewer associated side effects.

Purification of ACE Inhibitory Peptides using Semi-Preparative Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC)

Figure 3 illustrates the RP-HPLC chromatogram of the most active peptide obtained from the F3 fraction, analysed using a Shimadzu 5 µm C18 column. A comparison of ACE-inhibitory performance between the crude hydrolysate and the purified F3 fraction, isolated through FPLC and RP-HPLC, is summarised in Table 4. The results demonstrate a marked improvement in ACE inhibition, reaching 92.67% and 93.20%, respectively, along with reduced IC_{50} values of 0.18 mg/mL and 0.16 mg/mL, indicating that purification significantly enhanced the peptide's inhibitory potency.

In a related study, Ahn *et al*. (2012) reported that an ACE-inhibitory peptide isolated from salmon and purified through sequential steps involving FPLC with a HiPrep 16/10 DEAE FF ion-exchange column, followed by Sephadex G-25 gel filtration, exhibited IC_{50} values of 0.17

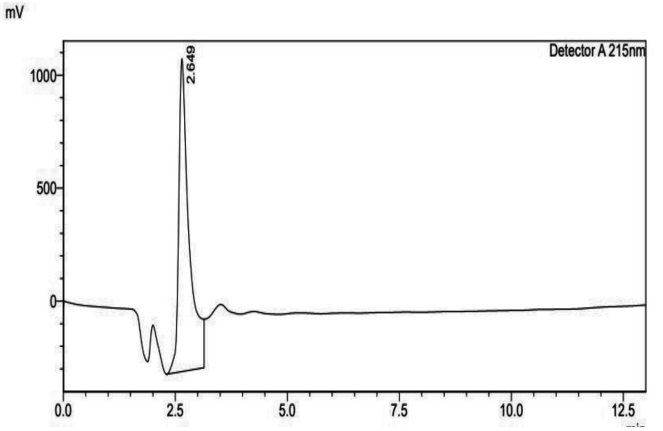

Figure 3: The RP-HPLC chromatogram of potent peptide loaded on Shimadzu 5 µm C18 column

mg/mL and 0.14 mg/mL at different purification stages. They identified three highly active peptides: Val-Trp-Asp-Pro-Pro-Lys-Phe-Asp (P1), Phe-Glu-Asp-Tyr-Val-Pro-Leu-Ser-Cys-Phe (P2), and Phe-Asn-Val-Pro-Leu-Tyr-Glu (P3), which showed strong ACE inhibition with IC_{50} values of 9.10 µM, 10.77 µM, and 7.72 µM, respectively. Likewise, Ghassem *et al.* (2014) found that peptides derived from snakehead fish, purified using gel chromatography followed by RP-HPLC with a C18 column, demonstrated ACE inhibitory activities of 75.23% and 78%. The two most active peptides identified, LYPPP and YSMYPP, exhibited IC_{50} values of 1.3 µM and 2.8 µM, respectively.

Consistent findings were also reported by Ishak *et al*. (2021), who isolated ACE-inhibitory peptides from shortfin scad (*Decapterus macrosoma*) using FPLC with gel filtration followed by RP-HPLC on a C15 Sephadex column. The purified peptides showed ACE inhibition levels of 81.47% and 87.99%, corresponding to IC_{50} values of 1.59 mg/mL and 0.20 mg/mL, respectively. In another study, Wu *et al*. (2015) identified the peptide Arg-Val-Cys-Leu-Pro (RVCLP) from lizard fish muscle, which demonstrated an IC_{50} value of 175 µM after purification through ultrafiltration, size-exclusion chromatography, ion-exchange, and HPLC techniques.

As shown in Table 4, the sequential purification process, including ultrafiltration, FPLC, and RP-HPLC, significantly enhanced the ACE-inhibitory activity of the red bigeye hydrolysate compared to the crude extract. A progressive increase in both ACE inhibition and purification efficiency was observed as the sample underwent each purification step. The crude hydrolysate initially showed an inhibitory activity of 89.17% with an IC_{50} value of 0.40 mg/mL. Following purification through RP-HPLC, the inhibition level rose to 93.20%, while the IC_{50} decreased to 0.16 mg/mL, corresponding to a 2.45-fold improvement in purification.

Table 4: Purification table of ACE inhibitory peptide from red bigeye hydrolysate

Purified Sample	ACE Inhibitory Activity (%)	IC_{50} (mg/mL)	Purification Fold
Red big eye hydrolysate	89.17 ± 0.15^{d}	0.40	1
Ultrafiltration (< 3kda)	91.65 ± 0.02^{c}	0.26	1.54
FPLC (Gel filtration)	92.68 ± 0.05^{b}	0.18	2.18
RP-HPLC	93.20 ± 0.04^{a}	0.16	2.45

Note: Means with the different letter (a to d) were significantly different ($p < 0.05$).

A similar pattern was reported by Ahn et al. (2012), who found that salmon-derived peptides exhibited purification folds of 2.56 and 5.48 after gel filtration and high-performance liquid chromatography, respectively.

This consistent enhancement highlights the effectiveness of combining ultrafiltration, gel filtration, and RP-HPLC techniques to isolate peptides with high ACE-inhibitory potency. Comparable findings have been reported in recent studies. For instance, Sun *et al*. (2019) successfully purified ACE-inhibitory peptides from the marine green alga Ulva intestinalis, identifying Phe-Gly-Met-Pro-Leu-Asp-Arg (FGMPLDR; MW 834.41 Da) and Met-Glu-Leu-Val-Leu-Arg (MELVLR; MW 759.43 Da), with IC_{50} values of 0.183 mg/mL and 0.179 mg/mL, respectively. Furthermore, Jo *et al*. (2024) reviewed advances in marine-derived ACE-inhibitory peptides, emphasising their therapeutic potential for cardiovascular disease prevention. Overall, these studies corroborate the current findings and demonstrate that stepwise purification is a highly effective strategy for obtaining potent, bioactive ACE-inhibitory peptides from marine sources.

The implications of this work are substantial for the development of natural antihypertensive agents. The improved ACE inhibitory activity achieved through purification suggests that these peptides may be suitable for incorporation into functional foods or nutraceuticals designed to help manage hypertension. Moreover, utilising marine resources such as red bigeye hydrolysate offers a sustainable and potentially cost-effective approach to obtaining valuable bioactive compounds.

The Amino Acid Sequence of Pure ACE Inhibitory Peptide from Red Bigeye Muscle Hydrolysate

The newly identified ACE-inhibitory peptide from red bigeye, designated as DKQTPSGY (Asp–Lys–Gln–Thr–Pro–Ser–Gly–Tyr), is an octapeptide exhibiting strong inhibitory activity, achieving 93.2% ACE inhibition with an IC_{50} value of 0.16 mg/mL, as illustrated in Figure 4. This peptide sequence is not listed in the BIOPEP-UWM database, suggesting it is a novel compound. Its distinct amino acid arrangement likely contributes to its potent inhibitory properties by promoting effective binding interactions with the ACE active site. The coexistence of both hydrophilic and hydrophobic residues within the sequence may enable multiple stabilising interactions, such as hydrogen bonding, electrostatic attraction, and hydrophobic forces, enhancing the peptide's affinity for ACE. Moreover, its relatively small molecular size likely supports higher absorption

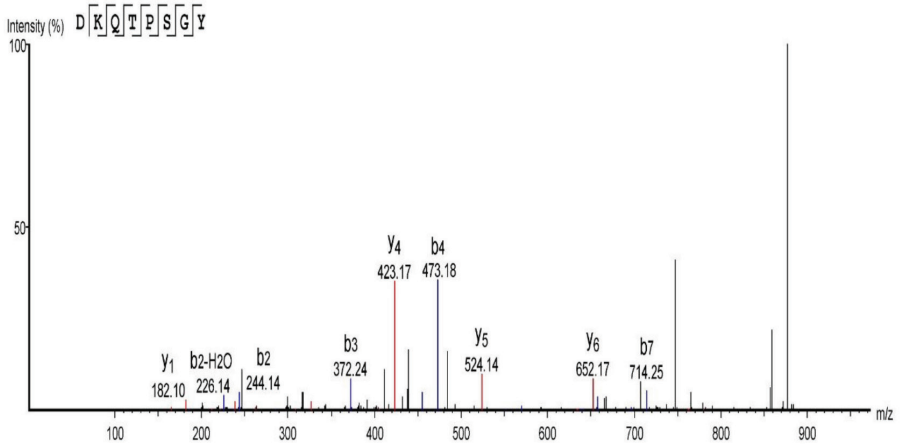

Figure 4: The amino acid sequence of the purified peptide from red bigeye was acquired over the m/z range of 100 to 900

and bioavailability, making it a strong candidate for development as a natural antihypertensive agent.

These findings align with previous research by Wang *et al*. (2024) who also identified highly active ACE-inhibitory peptides from marine organisms using similar purification strategies. Wang *et al*. (2024) isolated three potent non-competitive ACE inhibitors from sea cucumber gonads: DDQIHIF, HDWWKER, and THDWWKER, with IC_{50} values of 333.5, 583.6, and 1291.8 μmol/L^{-1}, respectively. Molecular docking analyses revealed that these peptides fit snugly into the ACE catalytic pocket, forming stable enzyme–peptide complexes through strong binding interactions. Collectively, these findings emphasise the promising potential of marine-derived peptides, including the newly discovered DKQTPSGY, as natural therapeutic agents for hypertension management.

The discovery of novel ACE-inhibitory peptides offers considerable potential for advancing the development of functional foods and nutraceuticals designed to help control hypertension, a key contributor to cardiovascular disease. Newly identified peptides such as DKQTPSGY serve as promising models for creating bioactive compounds with improved activity, selectivity, and safety, providing natural alternatives to synthetic antihypertensive drugs. Future studies are expected to increasingly employ computational tools such as molecular docking and molecular dynamics simulations to predict, refine, and enhance peptide ACE interactions. Furthermore, sourcing bioactive peptides sustainably from underutilised marine species presents a valuable opportunity for innovation while promoting environmental conservation in marine resource utilisation.

Several studies have reported comparable findings. For example, Ishak *et al*. (2021) identified a bioactive peptide from shortfin scad (*Decapterus macrosoma*), Arg-Gly-Val-Gly-Pro-Val-Pro-Ala-Ala (RGVGPVPAA), which displayed potent ACE inhibition with an IC_{50} value of 0.2 mg/mL. Similarly, Ngo *et al*. (2016) discovered two low-molecular-weight ACE-inhibitory peptides from Pacific cod skin gelatin hydrolysates: GASSGMPG (eight amino acids, 662 Da) and LAYA (four amino acids, 436 Da) which demonstrated 96% ACE inhibition with IC_{50} values of 6.9 μM and 14.5 μM, respectively. Liu *et al*. (2021) also identified three active peptides, Gly-Arg, Arg-Glu-Arg, and Gly-Pro-Arg, from Atlantic salmon (*Salmo salar L*.) skin collagen hydrolysates, with IC_{50} values of 0.73, 0.89, and 1.05 mg/mL. Their strong inhibitory activities were attributed to stable hydrogen bonding and hydrophobic interactions within ACE binding sites. In another study, Chen *et al*. (2020) isolated Leu-Ser-Gly-Tyr-Gly-Pro (LSGYGP) from tilapia (*Oreochromis niloticus*) skin gelatin hydrolysates, which showed an IC_{50} of 2.577 μmol/L and effectively reduced blood pressure in spontaneously hypertensive rats within six hours, while remaining stable during simulated digestion for up to four hours.

The newly identified peptide DKQTPSGY, with its high ACE-inhibitory potency and novel sequence, demonstrates strong potential as a functional ingredient in health-promoting food products and nutraceutical formulations. Its effectiveness at low concentrations suggests that it could provide therapeutic benefits even in small doses, making it ideal for incorporation into food-based delivery systems. Additionally, like other marine-derived peptides, its anticipated stability under processing and digestive conditions further supports its practicality for real-world applications in hypertension management.

Mode of Inhibition of a Purified Peptide from Red Bigeye Muscle Hydrolysate Towards ACE

The inhibitory mechanism of the purified peptide against ACE was analysed using Lineweaver–Burk plots to determine how peptide binding influences enzymatic activity. Enzyme inhibition generally occurs through two primary modes: competitive and non-competitive. As described by Ghassem *et al*. (2014), competitive inhibitors temporarily bind to the enzyme's active site, preventing the substrate from accessing it. In contrast, non-competitive inhibitors attach

to different regions of the enzyme, causing conformational changes that reduce its catalytic performance.

Based on the kinetic analysis illustrated in Figure 5, the peptide DKQTPSGY demonstrated a competitive mode of inhibition. This indicates that the peptide interacts directly with the enzyme's active site, competing with the substrate for binding. A similar pattern has been reported for peptides derived from shortfin scad (*Decapterus macrosoma*) hydrolysates, which also act as competitive ACE inhibitors. For instance, the peptide RGVGPVPAA exhibited an IC_{50} value of 0.20 mg/mL, attributed to strong hydrogen bonding interactions within the ACE active site (Ishak *et al*., 2021). These findings suggest that DKQTPSGY follows a comparable competitive inhibition mechanism, effectively blocking substrate access to ACE's catalytic centre.

Peptides isolated from oyster hydrolysates, such as AEYLCEAC, have shown strong competitive inhibition of ACE, with an IC_{50} of 4.287 mM, attributed to their ability to form hydrogen bonds within the enzyme's active site (Chen *et al*., 2022). This mechanism is similar to the interaction likely exhibited by DKQTPSGY. Both marine- and fish-derived peptides appear to share a comparable competitive inhibition mechanism, characterised by binding to ACE's active sites and interacting with key residues through hydrogen bonding and hydrophobic interactions. These findings further highlight the promise of marine-derived bioactive peptides as natural agents for managing hypertension.

These findings indicate that the peptide DKQTPSGY inhibits ACE activity by directly competing with the substrate Hip-His-Leu (HHL) for binding to the enzyme's active site. The kinetic analysis confirms that DKQTPSGY acts as a competitive inhibitor, effectively preventing HHL from interacting with ACE. Comparable results have been observed in other marine-derived peptides, such as those isolated from shortfin scad, and fire fish, which also display competitive inhibition behaviour (Ishak *et al*., 2021; Hu *et al*., 2022). For instance, the peptide DLTAGLLE, identified from fire fish, was reported to exhibit competitive ACE inhibition with an IC_{50} value of 0.13 mg/mL (Hu *et al*., 2022).

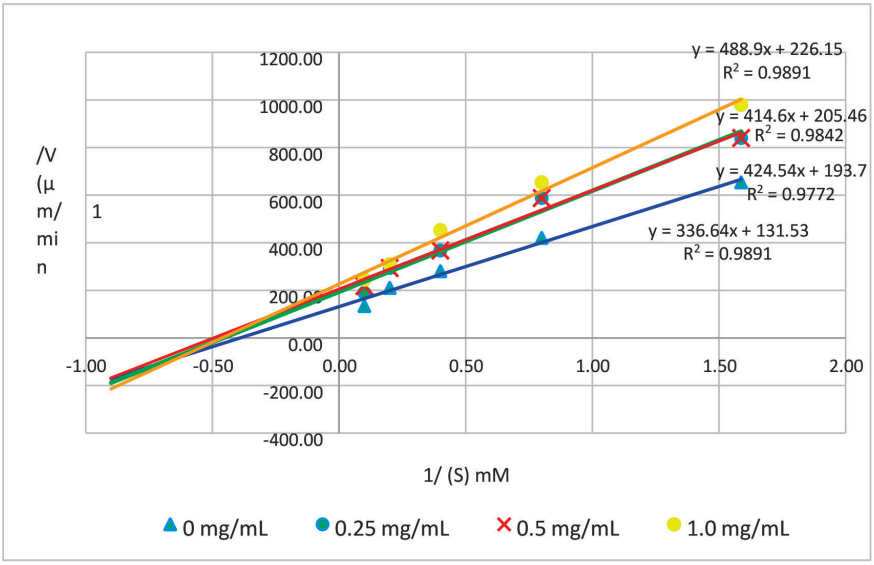

Figure 5: The mode of inhibition of the purified peptide (DKQTPSGY)

Stability of Purified Peptide (F3) Towards Different pH, Temperature, and In Vitro Gastrointestinal Digestion

Figure 6 shows the impact of pH on the stability of the ACE inhibitory peptide DKQTPSGY over a 60-minute period, across a pH range from 2 to 12. The peptide maintained high inhibitory activity across a broad pH range, with the highest activity observed at neutral to slightly alkaline pH levels (6–8), where its ACE inhibitory activity reached a maximum of 86.12% at pH 8. Activity significantly declined at extreme pH values, both acidic (pH 2) and highly alkaline (pH 12), with the lowest activity recorded at 35.58%. The peptide's ACE inhibitory activity notably diminished under alkaline conditions, particularly at pH 10 and 12. This finding is critical for applications in functional foods and the gastrointestinal environment, where pH conditions vary. Similar results were observed in studies on ACE inhibitory peptides derived from firefish (*Lepidotrigla microptera*) (Hu *et al*., 2022), which demonstrated increased activity from pH 3.0 to 9.0 and a decrease at pH 11.

This pattern of pH stability corresponds with the findings of Zaliha *et al*. (2021), where the ACE inhibitory activity in mud crab hydrolysate remained stable within a pH range of 6 to 10, showing values between 95.13% and 86.87%. However, at pH 12, there was a notable decrease in ACE inhibitory activity, falling to 44.90%. These observations highlight that marine-derived peptides, such as DKQTPSGY, are well-suited for functional applications that require stability under moderate pH conditions. Zuo *et al*. (2017) reported that peptides from dried-cured ham maintained stable ACE inhibitory activity between pH 3 and 7 (38-45%), with significant reductions at pH 11 (25%). The decline in ACE inhibitory activity at elevated pH levels may be due to peptide deamination and racemisation reactions, which can alter the peptide's structure and conformation, ultimately reducing its bioactivity (Zhu *et al*., 2016). These results indicate that the peptide's ACE inhibitory activity is most effective at moderate pH levels, while highly acidic or alkaline conditions may result in its deactivation. Overall, these studies confirm that both highly acidic and alkaline environments negatively impact the stability of ACE-inhibitory activity in peptides. Singh and Vij (2018) found that peptides exhibiting stability across a broad pH range are likely to demonstrate high bioavailability, as they remain active through various stages of digestion, including highly acidic conditions (such as pepsin) and alkaline environments (such as pancreatic enzymes).

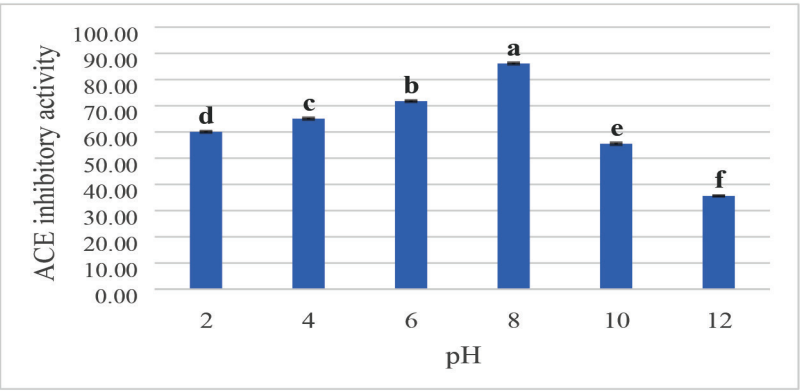

Figure 6: Effect of pH on the stability of purified ACE inhibitory peptide (DKQTPSGY)

Figure 7 depicts the impact of temperature on the stability of DKQTPSGY. The peptide retained over 60% activity at temperatures up to 70°C but exhibited a decrease in activity at 90°C, demonstrating good thermal stability. The highest ACE activity was observed at 70°C, with an inhibitory percentage of 70.12%. This characteristic is beneficial for applications in food systems that undergo moderate heat processing. Similarly, peptides derived from rohu fish waste exhibited ACE inhibitory activity up to 75°C, with a significant decrease in activity at 121°C (Kumar *et al*., 2023). Additionally, peptides from *Lepidotrigla microptera* demonstrated stability up to 100°C, further reinforcing the robustness of marine-derived peptides for thermal applications (Hu *et al*., 2022).

Similarly, Zaliha (2021) reported that the ACE inhibitory activity from mud crabs remained stable at elevated temperatures up to 70°C (87.31%), but significantly declined at 90°C (50.11%) during a 10-minute incubation. The peptide also displayed considerable sensitivity to temperature fluctuations. These results are consistent with the thermal stability of DKQTPSGY and underscore its potential for application in heat-processed functional foods or nutraceuticals.

Additionally, an *in vitro* gastrointestinal digestion experiment utilising pepsin and a combination of pepsin with pancreatin enzymes is presented in Figure 8. The pure peptide without any enzyme treatment (control) exhibited a residual ACE inhibitory activity of 65.21%. When treated with pepsin, the pure

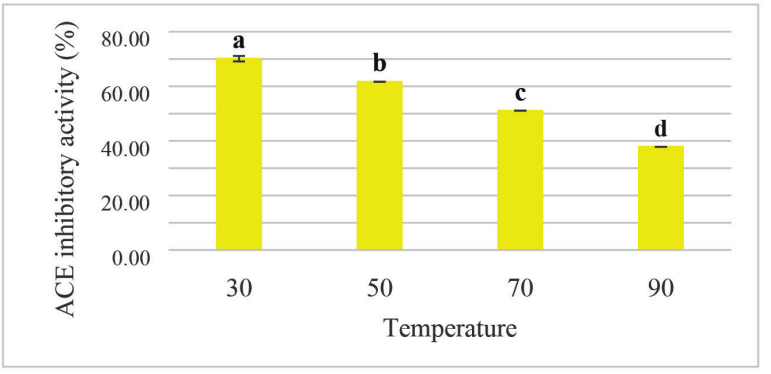

Figure 7: Effect of temperature on the stability of purified ACE inhibitory peptide (DKQTPSGY)

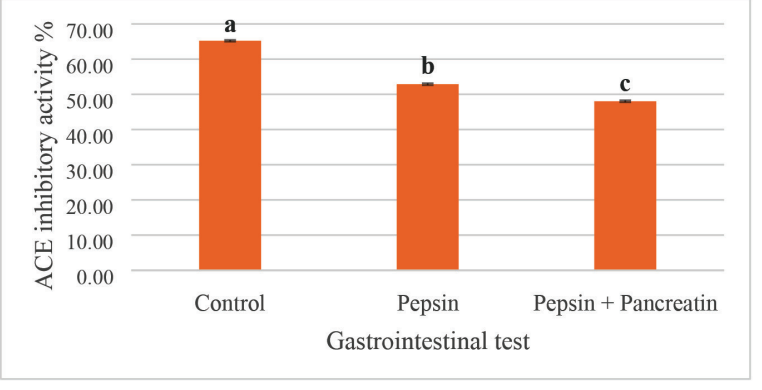

Figure 8: *In vitro* gastrointestinal digestion of purified peptide (DKQTPSGY) using pepsin treatment alone, as well as pepsin followed by pancreatin treatment

peptide showed a residual activity of 54.1%, reflecting an 11.11% decrease compared to the control. In contrast, the pure peptide treated with pepsin followed by pancreatin yielded a residual activity of 48.02%, indicating a 17.19% decrease compared to the control sample. Although the ACE inhibitory activity decreased after digestion, the peptide retained significant activity, indicating partial resistance to enzymatic hydrolysis and the presence of active fragments post-digestion. This resilience is crucial for its therapeutic potential in oral applications.

Similarly, rohu fish peptides maintained their activity during digestion with pepsin and trypsin (Kumar *et al*., 2023). Zaliha (2021) also observed that ACE inhibitory activity decreased to 43.74% after treatment with pepsin and pancreatin enzymes, compared to a control value of 76.32%. The characteristics of pepsin's catalytic activity at pH 2 may account for the decrease in ACE inhibitory activity of the DKQTPSGY peptide. These findings suggest that marine-derived peptides like DKQTPSGY are well-suited for oral delivery, as they retain functionality despite enzymatic degradation during digestion. This result highlights the potential antihypertensive properties of this peptide for use as a functional food ingredient, paving the way for further research and development in the field of functional foods.

In conclusion, DKQTPSGY demonstrates remarkable stability across a broad pH spectrum, moderate thermal conditions, and gastrointestinal digestion. These characteristics highlight its potential for use in both functional food formulations and therapeutic applications. Comparisons with research on fish-derived peptides underscore the resilience of marine-sourced ACE inhibitory peptides, including DKQTPSGY, under comparable environmental and physiological conditions. Moreover, the peptide's robust performance in stability tests reinforces its viability as a practical intervention for hypertension management. This strengthens its appeal as a bioactive ingredient that can be incorporated into dietary regimens or developed as a pharmacological agent, offering a promising solution for hypertension-related health challenges.

Further research should explore the mechanism of action of DKQTPSGY in detail, including its binding dynamics with ACE through molecular docking and simulation studies. Evaluating its stability under various physiological and processing conditions, as well as its bioavailability and efficacy in in vivo models, will provide critical insights into its potential as a therapeutic agent. Additionally, investigations into its antioxidant or synergistic properties with other bioactive compounds could expand its applications in managing cardiovascular and other health conditions.

In conclusion, DKQTPSGY from red bigeye is a novel and potent ACE inhibitory peptide with significant therapeutic potential. Its high inhibitory activity, unique sequence, and functional attributes underscore its value as a promising bioactive molecule for addressing hypertension and advancing functional food technologies.

Conclusions

In summary, this study successfully isolated a novel ACE inhibitory peptide, DKQTPSGY, from red bigeye muscle hydrolysate. The purified peptide exhibited strong ACE inhibition (93.2%) with an IC_{50} value of 0.163 mg/mL, demonstrating its high potency. The results confirmed that DKQTPSGY inhibits ACE through a competitive mechanism. Its stability under neutral to mildly alkaline pH, tolerance to moderate heat, and substantial retention of activity after simulated gastrointestinal digestion indicate its suitability for oral intake and its potential as a functional bioactive ingredient.

Comparisons with other fish-derived peptides further support the stability and robustness of DKQTPSGY across various environmental and processing conditions, aligning with trends observed in other marine peptides. Collectively, these characteristics highlight its promise for managing hypertension,

particularly through its application in antihypertensive functional foods and nutraceuticals. Additionally, this research opens avenues for exploring the broader implications of DKQTPSGY in healthcare. Investigations into its clinical efficacy, long-term safety, and bioavailability could enhance its utility and pave the way for its integration into global strategies aimed at combating hypertension. Such studies could also shed light on its potential synergistic effects with other bioactive compounds, making it a cornerstone in the development of innovative therapeutic solutions for cardiovascular health.

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Conflict of Interest Statement

The authors declare that they have no conflicts of interest.

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