Antioxidant Bioactive Peptides from By-Product Canned Yellowfin Tuna (*Thunnus* sp.) through an Enzymatic Membrane Reactor (EMR)

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ABSTRACT

As a by-product of the pre-cooking phase, canned yellowfin tuna (*Thunnus sp*.) is processed into body juice. A bioactive peptide derived from an organism's body juice was developed to create a functional food. Hence, the purpose of the research was to evaluate the biological activity of antioxidant bioactive peptides prepared from body juice. Three stages were conducted to determine the substrate-to-enzyme and enzyme-to-substrate ratios for the production of antioxidative bioactive peptides in a batch reactor. These stages include the production of antioxidative bioactive peptides in an automated EMR with a constant flux and residence time, as well as the production of bioactive peptides using ultrafiltration with membranes of 10, 5, 4, and 2 kDa. The process was conducted at a temperature of 50°C, pH level of 7, constant flux of 8.08 L/m^2/h, enzyme concentration of 15%, and substrate concentration of 2%. Furthermore, the optimal treatment implemented an automated EMR and filtered through a membrane of size 4 kDa, generating an IC_{50} value of 0.604 mg/mL for antioxidant activity. The LC–MS analysis results indicated that the bioactive peptide, which passed through a 4 kDa membrane, contained the amino acid phenylalanine. Therefore, it can be inferred that an automated EMR could potentially be applied to create bioactive peptides from the body juice of by-products of canned yellowfin tuna (*Thunnus sp*.) that exhibit antioxidant activity.

Keywords: body juice; pre-cooking; amino acid; free radicals; oxidation

Introduction

Reactive oxygen species (ROS) and free radicals are crucial contributors to degenerative diseases. Both free radicals and reactive oxygen species (ROS) are highly unstable and easily react with other substances owing to the presence of unpaired electrons. This can result in oxidative stress, leading to cellular or tissue damage in the human body (Chen et al., 2022). The assessment of antioxidative activities was examined using colorimetric methods, which included the DPPH radical scavenging activity assay, FRAP assay, and ABTS assay (Henriques et al., 2021; Ryan et al., 2011).

Proteins play a crucial role in human nutrition and serve as an essential source of nutrients, including fish proteins. The popularity of fish intake in everyday human meals stems from its reputation as a rich source of polyunsaturated fatty acids. In recent years, significant research has been conducted on fish proteins, particularly regarding their potential as a source of bioactive peptides. Peptides with bioactive properties play a crucial role in enhancing human health, particularly in degenerative diseases. The production of bioactive peptides from fish involves the conversion of fish proteins into peptides with biological effects. Previous research (Chen et al., 2022) demonstrated that bioactive peptides with molecular weights ranging from 270-1800 Da exhibited strong antioxidant activity. Hydrophobic acid amino acids and one or more residues of Histidine, Proline, Methionine, Cysteine, Tyrosine, Tryptophan and Phenylalanine can increase the activities of the antioxidant peptides (Chalamaiah et al., 2012).

Fish production is often used for direct human consumption, such as yellowfin tuna (*Thunnus sp*.), which is processed in canned factories. Nevertheless, the environmental impact of by-products from canned factories, such as body juice from the precooking stages, should not be overlooked. Consequently, fish are typically discarded or traded commercially as animal feed. Elaborating further, prior studies (Henriques et al., 2021; Moosavi-Nasab, 2018; Nilsang et al., 2005; Urakova et al., 2012; Venkatesan et al., 2017) reported that by-products from fish can be transformed into high-value goods, such as fish protein hydrolysate containing bioactive peptides. Peptides have the ability to function as unpaired electron captors or antioxidants as reported by a study (Tacias-Pascacio et al., 2021). Several research have focused on the examination of the antioxidant activity of protein derived from fish and fish by-products, including the dark muscles of skipjack tuna (*Katsuwonus pelamis*) (Chi, Hu, et al., 2015), Bluefin leatherjacket (*Navodon septentrionalis*) (Chi, Wang, et al., 2015), Gelatin derived from the skin of Nile Tilapia (Zheng et al., 2018), cooking juice (*Bacillus subtilis*) (Hsu et al., 2009), Alaska pollack skeleton *Theragra chalcogramma* (Je et al., 2005), Hoki-derived protein structure (*Johnius belengerii*) (Kim et al., 2007), and croceine croaker (*Pseudosciaena crocea*) (Cai et al., 2015).

Bioactive peptides have been synthesized from fish proteins via enzymatic or acid hydrolysis, with emphasis on peptides with a molecular weight of lesser than 3kDa (<3kDa). One of the benefits of enzymatic hydrolysis is its ability to quickly achieve (requiring a shorter time) a significant level of hydrolysis. Short peptides are believed to be more resistant to gastrointestinal digestion than intact proteins are. The peptides produced during enzymatic hydrolysis depend on the specificity of the protease enzyme, as well as factors such as temperature, pH, and the ratio of enzyme to substrate. Previous studies (Sharkey et al., 2020; Zamora-sillero et al., 2018) additionally suggests that the effectiveness of the peptides is not influenced by their molecular weight, but rather by their specific sequence of amino acids. Furthermore, bioactive peptides are distinguished by their composition of 2-20 amino acids. Two methods can be used to generate bioactive peptides: hydrolysates, which are mixtures of amino acids and peptides, or bioactive purified peptides (Tacias-Pascacio et al., 2021; Urakova et al., 2012). The process of breaking down fish soluble protein can be accomplished by utilising either endo or exo-peptidase enzymes through hydrolysis (Nilsang et al., 2005). The hydrolysates derived from fish using the enzymes Cyrotin-F, Protease A, Protease N, Flavourzyme, and Neutrase exhibit a significant capacity to eliminate reactive oxygen species (ROS) and decrease ferric ions (Ryan et al., 2011).

However, peptide fractionation may be challenging owing to its dimensions, electrical charge, and polarity. Membrane separation has emerged as the prevailing approach for separating and purifying hydrolysates based on their molecular weight (Gao et al., 2021). Membrane technology has several benefits, including sustainability and lack of reliance on solvents or sorbents. For instance, ultrafiltration (UF) offers an extensive array of molecular weight cut-offs (MWCOs) that are suitable for the purification of proteins and peptides (Ghalamara et al., 2020). Continuous or batch reactions can facilitate enzyme-catalysed biotransformations. Designed reactors, referred to as automated enzymatic membrane reactor (EMR), enable continuous enzyme-catalyzed biotransformations, such as the hydrolysis of large molecules, while also facilitating separation and catalytic reaction (Sitanggang et al., 2022). Ultrafiltration (UF) membranes are frequently employed in different configurations of enzymatic membrane reactor (EMR) systems due to the fact that enzymes typically have molecular weights ranging from 10 to 150 kilodaltons (kDa) (Sitanggang et al., 2023).

In particular, EMR was utilised in this study to perform better extraction of bioactive peptides (Sitanggang et al., 2022). Further, this study employed EMR to continuously hydrolyze the by-product of canned yellow fin tuna, adopting a constant flux and residence duration operation. Accordingly, the novelty of this study lies in its ability to assess the antioxidant activity of bioactive peptides derived from canned yellowfin tuna (*Thunnus sp*.) body juice by-products generated through an automated enzymatic membrane reactor (EMR).

Material and Methods

Materials

Body juice as a substrate, a by-product of canned yellowfin tuna (*Thunnus sp.*), was acquired from the Fish Canning Industry PT. Aneka Tuna Indonesia, Gempol, East Java on March 2023. The batch number (SJ 32.33.34.35.36), production date (2023.3.2 – 3.7), and filling date (FJ-230314) are provided. The polyethersulfone (PES) membranes constructed by employing molecular weight cut-offs of 5-(NADIR® up005), 4- (NADIR® UH004) and 2-kDa (TRISEP®SBNF) were purchased from MANN + HUMMEL (Ludwigsburg, Germany). Meanwhile, the protease enzyme Nutrease® 0.8 L (EC 3.4.24.28) was obtained from Novozymes A/S (Bagsvaerd, Denmark),

with enzyme activity Neutrase® 0.8 L (EC 3.4.24.28, ~12.000 U/mL). Tri chloroacetic acid, folin-Ciocalteu, methanol, ethanol, $FeCl_3$, NaOH, acetate buffer (pH 3.6), acetic acetate, sodium acetate, HCl 37%, KH_2PO_4, Na_2HPO_4, and Na_2CO_3 were purchased from Merck KGaA (Darmstadt, Germany). TCA, 2,2-diphenyl-1-picrylhydrazyl (DPPH), ABTS, potassium persulfate, TPTZ, tyrosine, Trolox, and BSA were obtained from Sigma-Aldrich Corporation (St.Louis, MO, USA). Louis, MO, USA). LouisUSA) (. Loius, MO, USA). Filter paper of 3 mm and filter paper no. 1 were purchased from Toyo (Japan) and Hawach Scientific (China), respectively.

Experimental Design

This study was conducted in four steps according to Sitanggang *et al* with slightly modification: determination of the substrate-enzyme ratio, determination of the enzyme-substrate ratio, threshold flux, and production of antioxidative peptides using continuous EMR (Sitanggang et al., 2021). The first step was to determine the substrate-enzyme ratio with two substrate concentrations (1 and 2% (w/v)) and an enzyme concentration of 15% (w/v). To determine the enzyme substrate, two enzyme concentrations (10 and 20 %) were used. For threshold flux, three different flux values were evaluated including 10.38 08 L/h/m^2 (retention time 7 hours), 8.08 08 L/h/m^2 (retention time 9 hours) and 6.07 L/h/m^2 (retention time 12 hours). The last part was the production of antioxidative peptides using automated continuous EMR. The best results from the first and second steps were determined based on the antioxidant capacity analysis.

Production of Bioactive Peptides in a Batch Reactor

Production of bioactive peptides in a batch reactor was conducted based on Sitanggang *et al* (Sitanggang et al., 2021). The substrates, with concentrations of 1% and 2% (w/v), respectively, were dissolved in a 0.01 M phosphate buffer with a pH of 7.0. The initial step involved filtration using pure water in a batch reactor. This process was carried out to ensure the cleanliness of the reactor and to verify its correct positioning. The substrate was reacted with a 15% neutrase (w/v) solution for 7 h at 50°C. The reaction mixture was stirred at an agitation speed (N) of 300 rpm. Samples were obtained at multiple time points, specifically at 1, 2, 4, 5, and 7 h. The antioxidant activities of the permeates were assessed using the2,2-Diphenyl-1-picrylhydrazyl(DPPH), ferric reducing antioxidant power (FRAP), and 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) methods. Finally, the enzyme to substrate ratio was investigated by reacting three different enzyme concentrations (10%, 15%, and 20%) with 2% substrate. The reaction was performed in a batch reactor for 7 h at 50°C and pH 7.

Production of Bioactive Peptides in An automated EMR

In accordance with the preceding procedure (Sitanggang et al., 2021), bioactive peptides were generated through an automated EMR. First, the residence time was determined by applying three different durations of 7, 9, and 12 h. Subsequently, optimal treatment was determined using a continuous reactor. The residence time was analysed using various fluxes. The enzyme neutrase was used for substrate hydrolysis. A continuous membrane reactor was used to carry out the reaction with a substrate concentration of 2% and an enzyme concentration of 15%. The reaction was conducted for 7 h at 50°C with stirring at an agitation speed (N) of 300 rpm (Sitanggang et al., 2023). A membrane with a molecular weight cutoff of 10 kDa was used in the reactor. The flux was maintained at a constant level by adjusting the pressure, density, and flux of the automated PID controller. Variations in the fluxes resulted in differences in the flux values. The optimal conditions for producing bioactive peptides were determined to be a substrate concentration of 2%, enzyme concentration of 15%, membrane with a molecular weight cut-off of 10 kDa, and residence time of 9 h. The reactor was then operated for 7 h at 50°C. The mixture was stirred at an agitation speed of 300 rpm while the flux remained constant. Lastly, the permeates were filtered using membranes of different sizes, specifically 2, 4, and 5 kDa, from which the antioxidant activities of each permeate were determined using the DPPH, ABTS, and FRAP methods (Kurniadi et al., 2023).

Antioxidant Activity

The antioxidant activities of the permeates were assessed using DPPH, ABTS, and FRAP assays. The DPPH assay was carried out following the methodology described by Sitanggang et al. (2021), whereas the ABTS and FRAP assays were performed using their respective protocols (Benzie & Strain, 1996; Puchalska et al., 2014). The antioxidant capacity of the hydrolysates was evaluated using DPPH, FRAP, and ABTS assays. The DPPH assay was conducted with Trolox used as the standard. In the FRAP assay, 150 mL of hydrolysate was reacted with 4.5 mL of FRAP reagent, and Trolox was used as the standard (Benzie & Strain, 1996). The antioxidant capacity was

expressed as ìM TE/mL, with values calculated based on the standard curves of Trolox: y = -0.0024x + 0.949 (R^2 = 0.9992) for the DPPH assay and y = 0.0025x – 0.0053 (R^2 = 0.9999) for the FRAP assay. The ABTS assay was used to measure antioxidant capacity, which was expressed as a percentage inhibition.

LC-MS/MS Analysis

LC-MS/MS was performed using a UPLC consisting of an LC Acquity UPLC H-Class System (Waters, USA) and mass spectrometry XEVO G2-S QTof (Waters, USA). C18 Acquity UPLC HSS (1.8 ìm 2.1x100 mm) (Waters, USA) was used as the stationary phase at a temperature of 50°C (coloumn) and 25°C (room). The mobile phase comprised ammonium formic acid (5 mM, A) in water and 0.05% formic acid in acetonitrile (B), at a flow rate of 0.2 mL/ min (step gradient) running for 23 minutes and an injection volume of 5 ìL. Mass spectrometry was performed using electrospray ionisation (ESI) with a positive charge, with m/z 50-1200. The temperature source and desolvation temperature were 100 °C and 350°C, respectively. Cone and desolvation gas flow rates of 0 L/hr and 793 L/hr were also used correspondingly, while the collision energy varied between 4 and 60eV. Data analysis was performed using MassLynx version 4.1 (Waters, USA) (Ismed et al., 2021).

Statistical Analysis

Statistical analysis was performed using GraphPad Prism software, version 10.4.1 (Boston, MA, USA), and all experiments were conducted in triplicates.

Results and Discussion

When analysing the hydrolysis of body juice, it is important to consider certain parameters, including pH, temperature, and reaction duration. An experiment was conducted to optimize the enzyme reaction by carrying out hydrolysis using endopeptidase enzymes (Neutrase) at a pH of 7 and a temperature of 50°C (Meng et al., 2018).

Substrate-to Enzyme Ratio

To determine the substrate-to-enzyme ratio, we employed substrate concentrations of 1% (S1) (w/v) and 2% (S2) (w/v), along with a 15% neutrase (w/v) solution. The substrate, which was diluted in phosphate buffer (pH 7), served as the medium for body juice. The substrate was reacted with the neutrase enzyme for 7 h, during which sampling was conducted at intervals of 1, 2, 4, 5, and 7 h. The antioxidant activity of the permeate was assessed, as shown in **Figure 1**. Antioxidant analysis proved that the antioxidant capacity of the hydrolysate was significantly higher than that of the substrate and was also higher at higher concentrations. Based on its antioxidant activity, the optimal substrate was found to be 2%. The corresponding values for DPPH, FRAP, and ABTS were 346.7 µM TE/mL, 575.6 µM TE/mL, and 93.80%, respectively. According to a previous study (Sendjaya, 2020), a substrate concentration of 2% was found to be the optimal treatment, exhibiting the highest antioxidant activity when compared to a substrate concentration of 1%. Meanwhile, the substrate with a concentration of 1% exhibited the lowest antioxidant activity, marking that the antioxidant activity of the peptide increased as the concentration of its substrate increased, which aligned with the findings of a previous study (Hosseini et al., 2017). However, an increase in the concentration of the substrate will lead to an increase in antioxidant activity owing to the lack of compatibility between the enzymes and substrate, as well as an insufficient amount of substrate for hydrolysis by the enzyme (Vitolo, 2020). This also indicated that neutrase was saturated by the body juice extract as a substrate and produced more peptides. The FRAP assay showed a higher antioxidant capacity than the DPPH assay. It can be assumed that the hydrolysate of body juice functions as a metal-reducing antioxidant, not as a radical scavenger (Kardinan et al., 2024). The substrate 2% (w/v) was selected for further analysis.

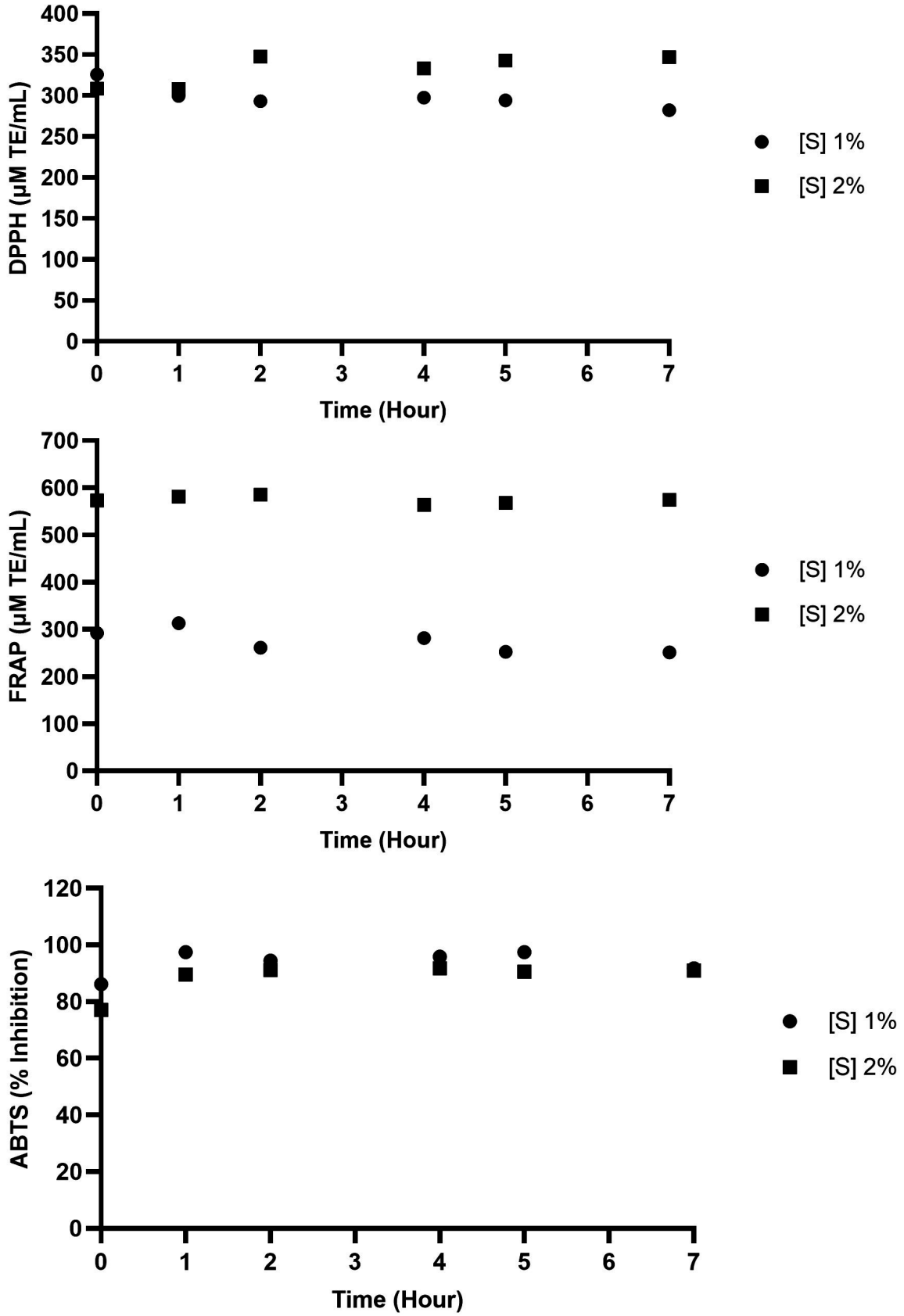


Figure 1. Effect of substrate to enzyme ratio [S]/[E] on antioxidant activity [DPPH assay (a), FRAP assay (b), ABTS assay (c)]

Enzyme-to-Substrate Ratio

Continuing from the previous procedure, the enzyme-to-substrate ratio was established using a 2% (w/v) substrate and enzyme concentrations of 10, 15, and 20% (w/v). A batch reactor was employed to conduct the reaction for 7 h, and the permeate was collected at intervals of 1, 2, 4, 5, and 7 h. **Figure 2** illustrates the mechanism of the DPPH, ABTS, and FRAP assays to assess the antioxidant activity of the permeate. As determined by this assay, an enzyme concentration of 15% exhibited the highest antioxidant activity. Hydrolysis caused an increase in the antioxidant capacity, and it was proved that the capacity of the permeate was higher than that of the substrate. The antioxidant capacity at the mid-point of [E]/[S] 15% w/v was higher. At concentration enzyme 15% (w/v), the antioxidant capacity was the lowest due to insufficient enzyme present, resulting in enzyme saturation by the substrate. At concentration enzyme 20%, excessive hydrolysis generating free amino acids induces protein aggregation and lowers the antioxidant capacity (Kardinan et al., 2024).

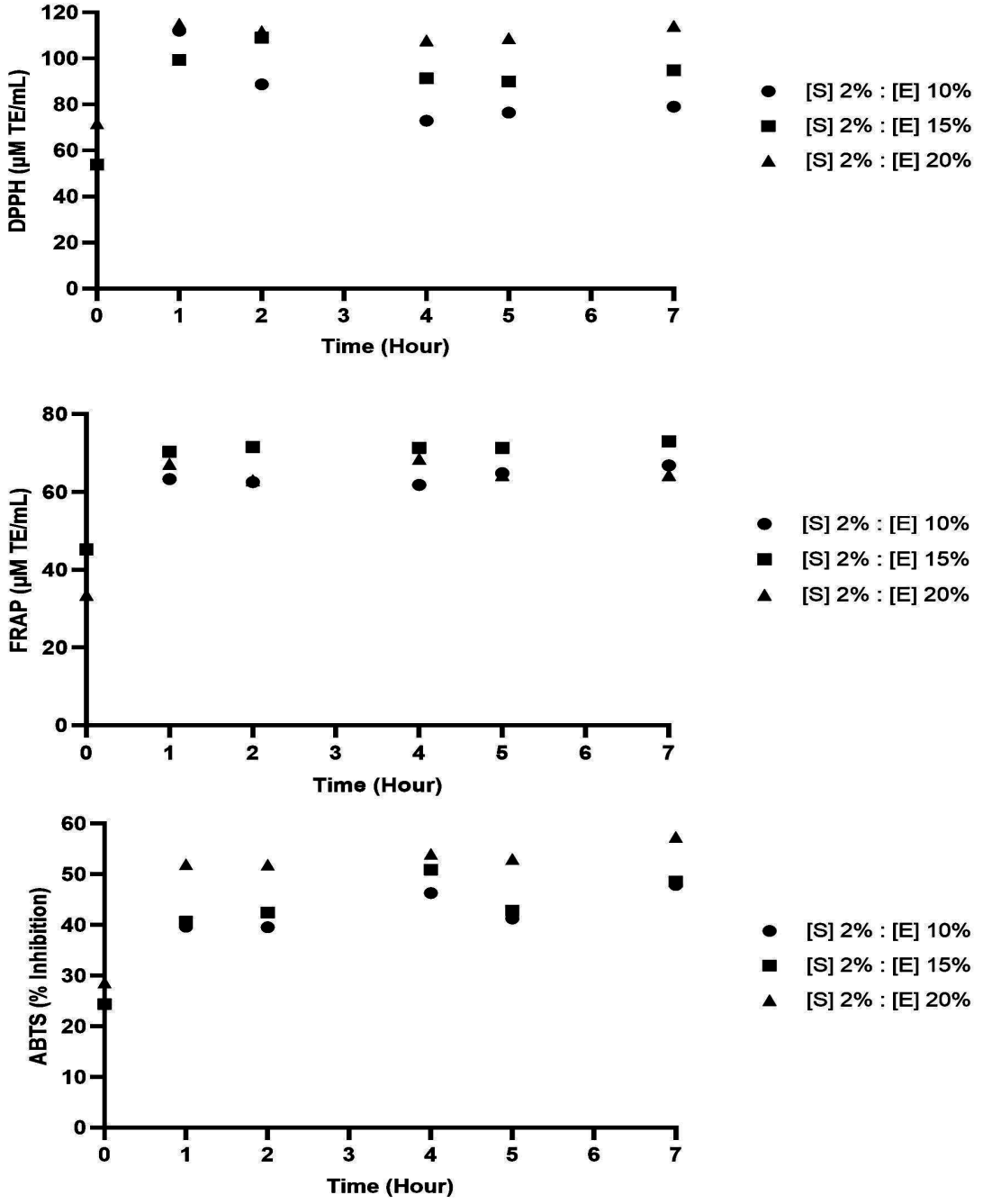
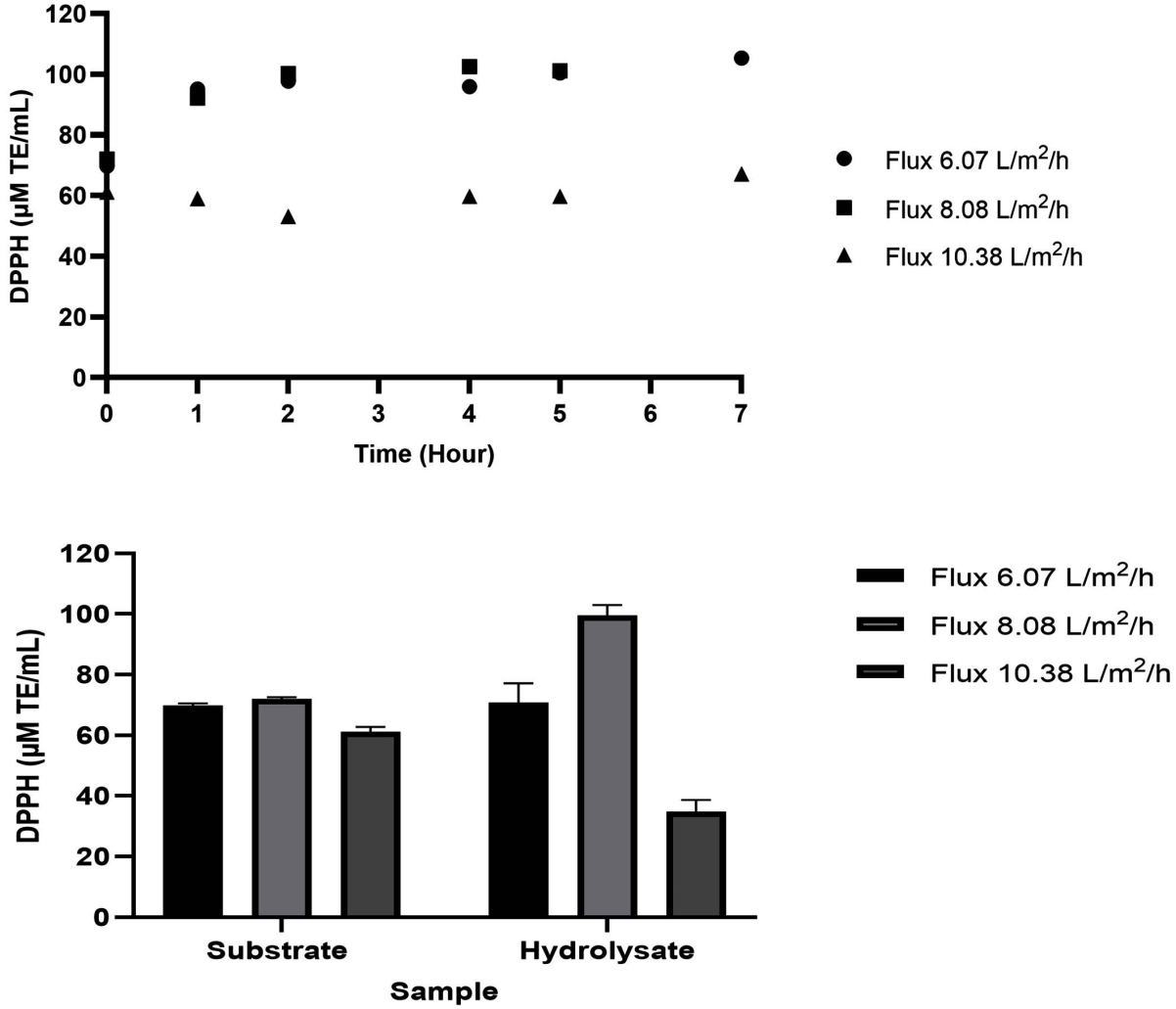


Figure 2. Effect of enzyme to substrate ratio [E]/[S] on antioxidant activity [DPPH assay (a), FRAP assay (b), ABTS assay (c)]

Treshold Flux

The threshold flux was determined using an automated EMR by applying a substrate concentration of 2% and enzyme neutrase concentration of 15%. Measurements were performed at flux rates of 10.38 L/m^2/h, 8.08 L/m^2/h, and 6.07 L/m^2/h. The determination was based on residence durations of 7, 9, and 12 h as well as the volumetric flow rate, volume of the reactor, and membrane area. The hydrolysis process was performed for 7 h using an automated EMR system at a temperature of 50°C, pH of 7, flow rate of 300 rpm, and constant flux. The antioxidant activities of the permeates were assessed using DPPH, ABTS, and FRAP assays, as illustrated in **Figure 3**. The optimal treatment exhibited the highest antioxidant activity, with a substrate concentration of 2%, enzyme concentration of 15%, and a flux of 8.08 L/m2/h. The corresponding antioxidant activity values for the substrate were 72.08 µM TE/mL (DPPH assay), 44.22 µM TE/mL (FRAP assay), and 29.97% (ABTS assay). The antioxidant activity values were 99.58 µM TE/mL (DPPH assay), 63.820 TE/mL (FRAP assay), and 86.66% for DPPH, FRAP (and ABTS assays).

(a)



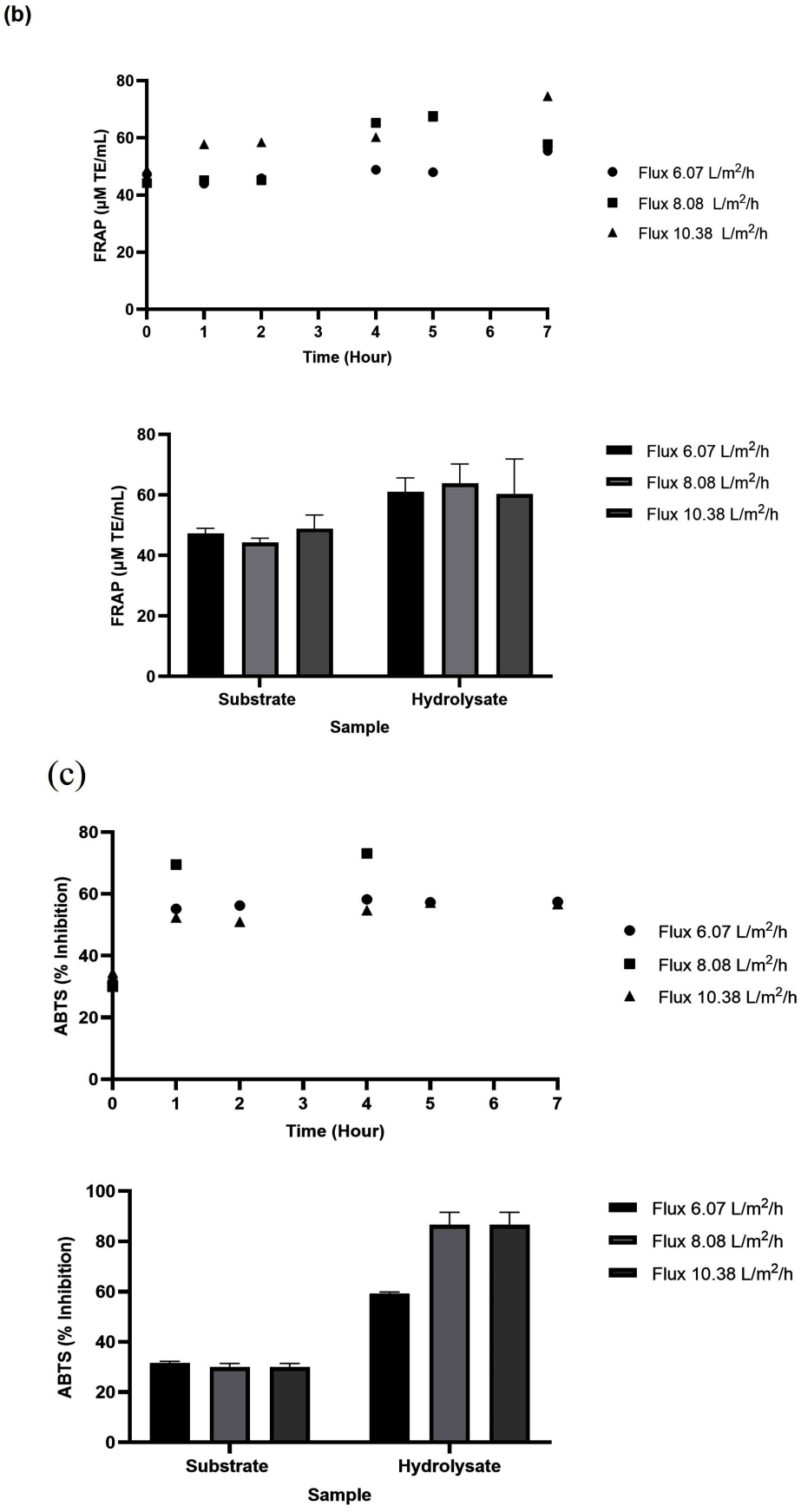


Figure 3. Effect of threshold flux on the antioxidant activity [DPPH assay (a), FRAP assay (b), ABTS assay (c)]

Bioactive Peptide Production

As determined by measuring antioxidant activity, the DPPH method was the most accurate. A bioactive peptide was generated using a 2% substrate concentration, 15% enzyme concentration, 8.08 L/m^2/h flux, and 10 kDa membrane. The reaction was conducted for 7 hours using an automated EMR system. The reaction was carried out at a temperature of 50°C, a velocity of 300 rpm, and constant flux. The hydrolysate underwent filtration using membranes of various sizes, including 5, 4, and 2 kDa, in order to allow the target peptides to permeate (Ghalamara et al., 2020). Antioxidant activity was determined for the hydrolysate generated from a membrane with sizes of 5, 4, and 2 kDa, the substrate, and the batch using the DPPH assay (**Figure 4**). The substrate, batch, and membrane size of 5, 4, and 2 kDa respectively, demonstrated radical-scavenging activity at concentrations of 1.085 mg/mL (1085 ppm), 0.873 mg/mL (873 ppm), 0.671 mg/mL (671 ppm), 1.444 mg/mL (1444 ppm), 0.604 mg/mL (604 ppm), and 0.838 mg/mL (838 ppm).

The highest antioxidant activity, as determined by DPPH measurement, was observed in the sample with a concentration of 0.604 mg/mL and a membrane size of 4 kDa. It can be observed that the mechanisms of peptides as antioxidant by scavenging free radicals instead of ferric-reducing antioxidant, indicating an increase in antioxidant capacity on the DPPH and ABTS measurement compared to FRAP analysis. Hydrolysis increased the antioxidant capacity observing that the capacity of antioxidant the hydrolysate was higher than that of substrate (Kardinan et al., 2024; Prasetyo et al., 2024). Peptides with higher bioactivity are found in peptides with lower molecular weights (Kurniadi et al., 2023). The antioxidative peptide derived from the common carp (*Cyprinus carpio*) roe (egg) was subjected to hydrolysis using alcalase, trypsin, and pepsin enzymes. The concentration of the antioxidative peptide was determined using the DPPH assay, resulting in concentrations of 1.151, 1.158, and 2.255 mg/mL for the alacalase, trypsin, and pepsin hydrolysates, respectively (Chalamaiah et al., 2015). The antioxidant activity of common carp peptides was shown to be less potent than the antioxidative peptide activity observed in the present study, which involved hydrolysis by nutrease and experimentation in an EMR. Variations in hydrolysis conditions, such as the choice of enzyme, temperature, and sample preparation, affect the antioxidant properties of peptide mixtures. In addition, a previous study (Wang & Mejia, 2005) concluded that tyrosine, tryptophan, methionine, lysine, cysteine, proline, and histidine increased antioxidant activity by contributing to the exchange of protons and hydrogen atoms. In addition, peptides of fish protein hydrolysate with molecular weight between 1-4 kDa have more interesting biological activity (Sierra-Lopera & Zapata-Montoya, 2021). Tuna cooking juice contains water-soluble proteins in a group of sarcoplasmic proteins. Therefore, it is easily hydrolysed to small peptides (Kasiwut et al., 2019).

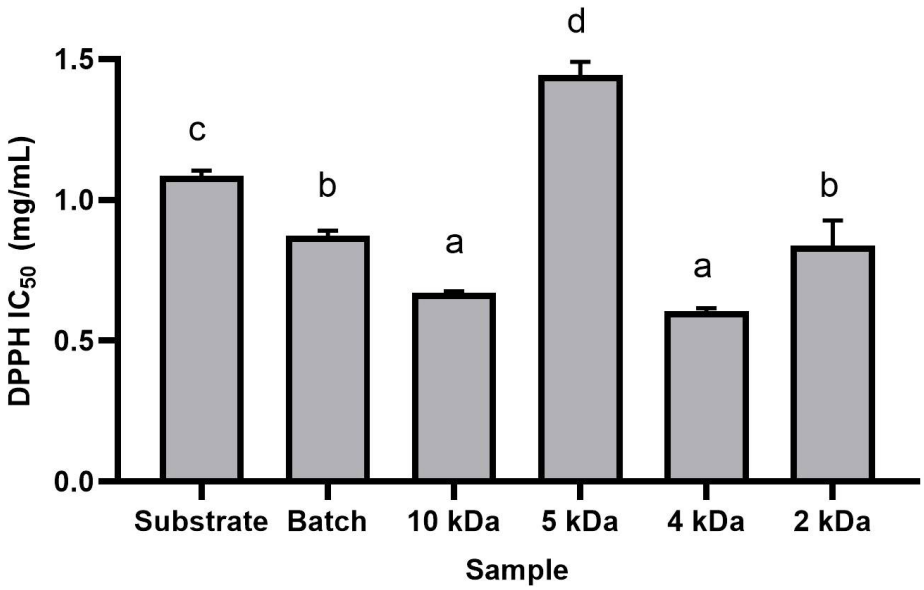


Figure 4. IC_{50} values of antioxidant activity. Different superscripts letters show a significant difference with a confidence level of 95%

LC-MS/MS analysis

Protein extracted from fish possessed antioxodative properties (Chen et al., 2022). Antioxidative peptides found in hydrolysed fish proteins consist of 2-16 amino acid residues. Antioxidative peptides are composed of free amino acids and short-chain peptides. The precursor protein sequence is initially inactive in terms of oxidation. However, its antioxidant activity can be activated through hydrolysis (Chalamaiah et al., 2012)**.** Peptides extracted from cooking juice tuna showed DPPH radical scavenging properties. Peptide fractions ranging from 400-1500 Da showed the highest antioxidative activity (Hsu et al., 2009). Derived hydrolysates and fractions from dark muscle skipjack tuna (*Katsuwonus pelamis*) could prevent oxidative reactions due to its high content of hydrophobic and aromatic amino acid residues, small molecular sizes and low molecular weights (Chi et al., 2015). The results of LC MS/MS analysis revealed that a bioactive peptide derived from a 4kDa membrane contains phenylalanine with a retention time of 2.99. (molecular formula of $C_9H_{11}NO_2$, $[M+H]^+$ *m/z* 166.1895). Antioxidative peptides were increased by hydrophobic amino acids, including histidine, proline, methionine, cysteine, tyrosine, tryptophan, and phenylalanine. The aromatic amino acids, tyrosine and phenylalanine, functioned as radical scavengers, as reported by other studies (Chalamaiah et al., 2015; Zou et al., 2016)**.** Hydrophobic properties, such as amino acids, play a pivotal role in scavenging lipid-derived radicals because they interact with lipid molecules and can be scavenged by donating protons to lipid-derived radicals (Je et al., 2007; Jun et al., 2004). Phenylalanine as part of hydrophobic amino acids have a role as antioxidant bioactive of tuna body juice. In addition, Neutrase has specificity for leucine and phenylalanine. Hydrolysis can maintain the quality of the amino acids. High protein content in hydrolysate is affected by the solubility of proteins during the hydrolysis process and the removal of insoluble solid material through separation by filtration (Martosuyono et al., 2019).

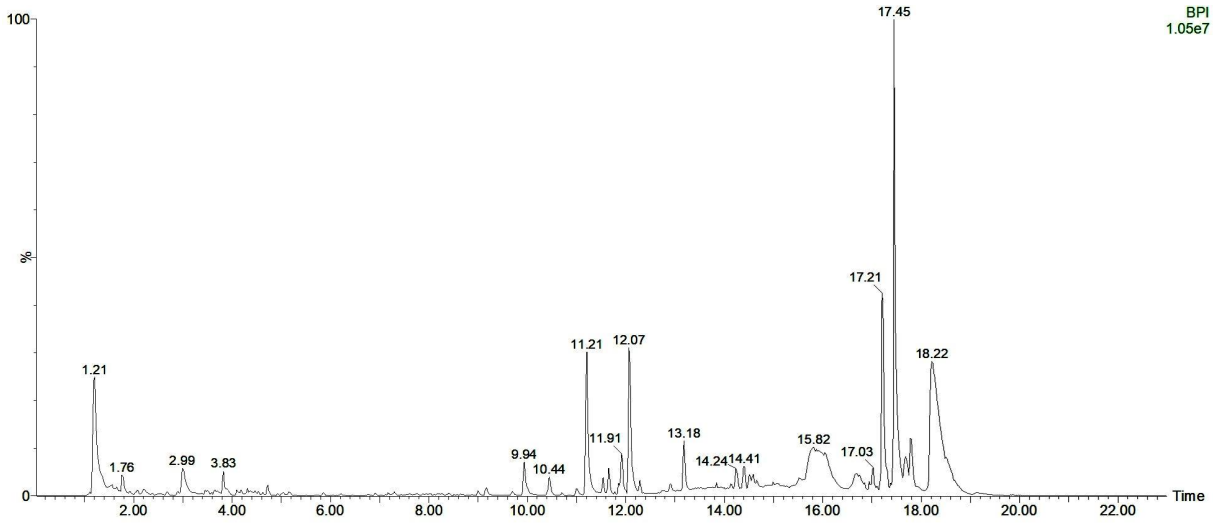


Figure 5. Chromatogram of bioactive peptide filtrated from membrane 4kDa

Conclusion

In conclusion, a bioactive peptide was generated from the body juice of yellow fin tuna (*Thunnus sp.*) using an automated EMR system. In this study, the process was conducted at a temperature of 50°C, pH level of 7, constant flux of 8.08 L/h/m^2, and enzyme to substrate ratios of 15% and 2%. The antioxidant activity of the optimum treatment generated by automated EMR was measured to have an IC_{50} concentration of 0.604 mg/mL (604 ppm), obtained after filtration with a 4 kDa membrane. Currently, antioxidant peptide sequences of protein hydrolysates remain not well established. Much future research on antioxidant peptides of body juices is still required to realize their potential application in human health.

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