Plagiarism Checker X Originality Report

Similarity Found: 11%

Date: Saturday, August 03, 2024
Statistics: 617 words Plagiarized / 5381 Total words
Remarks: Low Plagiarism Detected - Your Document needs Optional Improvement.

Iron-binding capacity and antidiabetic activity of baby clam (Corbiculidae sp.) meat protein hydrolysate Tam D. L. Vo1,2,*, Thu Huynh2,3, Thuy T. Le1,2, An T. T. Tran1,2, Bao C. Vo1,2 1Department of Food Technology, Faculty of Chemical Engineering, Ho Chi Minh City University of Technology, 268 Ly Thuong Kiet Street, District 10, Ho Chi Minh City, Vietnam 2Vietnam National University Ho Chi Minh City, Linh Trung Ward, Thu Duc City, Ho Chi Minh City, Vietnam 3Department of Biotechnology, Faculty of Chemical Engineering, Ho Chi Minh City University of Technology, 268 Ly Thuong Kiet Street, District 10, Ho Chi Minh City, Vietnam * Corresponding author, tel/: +84-79 896 7699, email: vdlt@hcmut.edu.vn ABSTRACT This study focuses on evaluating the iron-binding capacity (IBC) and antidiabetic activity (?-amylase inhibition) of baby clam (Corbiculidae sp.) meat protein hydrolysate.

Initially, an analysis of the approximate composition of the clam meat was conducted, including moisture, protein, lipid, carbohydrate, ash, and heavy metal (Hg, Cd, and Pb). Subsequently, Alcalase, a common protease for protein hydrolysis, was employed to hydrolyze the clam meat. The highest IBC and ?-amylase inhibitory activity were targets for obtaining the best hydrolysis condition, including the clam meat-to-water ratio, enzyme-to-substrate (E:S) ratio, and hydrolysis time.

The clam meat was found to be a suitable source for the bioactive protein hydrolysate production, as it contained a high protein content (54.48±2.10% dry weight basis) and heavy metal levels that were within the safe limits according to Vietnamese regulations (8-2:2011/ Ministry of Health (Vietnam)). Under the best hydrolysis condition, the protein hydrolysates demonstrated the IBC of 1246.20±44.00 μgFe2+/g protein and ?-amylase nhibitory activity of 48.33±1.44%, approaching three-quarters of the activity of ethylenediaminetetraacetic acid (EDTA) sodium salt and acarbose, respectively.

The results of this study serve as a foundation for further in-depth investigations into the bioactivities of clam meat hydrolysates, specifically focusing on IBC and ?-amylase inhibitory activity. Keywords: Baby clam meat, iron-binding capacity, ?-amylase inhibitory activity, protein hydrolysate. INTRODUCTION Iron is a crucial micronutrient involved in various physiological processes, including oxygen transport, electron transfer reactions, gene regulation, cell growth, and differentiation [1].

Iron deficiency, a prevalent global health issue impacting at least one-fifth of the population, primarily results from malnutrition or inadequate dietary intake of iron-rich foods [2]. Iron deficiency can lead to various health issues, including mood changes, muscle weakness, immunodeficiency, and anemia [3]. While multiple factors contribute to iron deficiency, its low bioavailability is the primary cause [4]. Although oral iron supplementation can be obtained from traditional food sources, the efficiency of dietary iron absorption is generally low, ranging from 5 to 20% in a mixed diet [5].

Nonheme iron, commonly found in fruits and vegetables, can form insoluble complexes with other anions in the duodenum (the primary site of iron absorption), hindering its transport across enterocytes. In contrast, heme iron from animal tissues exhibits higher bioavailability due to its transport by a protoporphyrin ring ligand. However, accessibility to heme iron is limited for populations without regular access to animal-derived food products and this may be associated with health risks, such as an increased risk of certain cancers [5].

To address these challenges, novel approaches to fortifying foods with iron have been explored to increase human iron intake as well as develop alternative sources of iron with enhanced bioavailability [6]. Despite the potential benefits of fortifying foods with iron metal, challenges such as lipid oxidation (iron generates free radical, oxidizing lipid) and its low solubility pose obstacles in achieving these objectives [3]. Iron sulfate, commonly used in clinical treatments and dietary supplements due to its high solubility, undergoes oxidation from ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}) and subsequent reaction to form insoluble ferric hydroxide ($Fe(OH)_3$) under neutral aqueous condition of the human intestine [4]. Conversely, iron-chelating peptides have been shown to be effective in preventing iron from binding with water molecules, thereby inhibiting the formation of ferric hydroxides and increasing iron solubility.

This facilitates iron absorption and bioavailability [3]. Three transport pathways have been suggested for chelating peptides that enhance ferrous ion absorption: ferrous ion transport pathway, peptide absorption pathway, and peptide as metal ion transport carrier [7]. An in vitro study has shown that the bioavailability of iron in peptide-Fe

complexes is considerably higher than that of ferrous sulfate [4].

In addition to promoting iron absorption, these peptides can also increase hepatic iron content by upregulating divalent metal transporter 1 and 14 [6]. Additionally, the in vivo studies conducted by Xue et al. [6] on mice have confirmed the safety of iron-binding protein hydrolysates. Diabetes mellitus has witnessed a global substantial surge, with projections indicating that it will affect approximately 438 million individuals by 2030 [8]. This disease poses significant cardiovascular risks and is ranked among the top five leading causes of mortality worldwide [8].

Hyperglycemia, the primary pathogenic characteristic of type 2 diabetes mellitus, plays a critical role in the development of various secondary microvascular and macrovascular complications associated with this disease [9]. Inhibiting the hydrolysis of starches into oligo- and monosaccharides within the small intestine effectively mitigates glucose absorption and transportation into the bloodstream [10]. Salivary and pancreatic ?-amylase, along with intestinal ?-glucosidase, play vital roles in the digestion of starch within the gastrointestinal tract.

The ?-amylase enzymes break down starch into maltose and other oligosaccharides by cleaving ?-1,4 glycosidic bonds. Subsequently, ?-glucosidase, which is embedded in the epithelial mucosa of the small intestine, hydrolyzes the non-reducing end of oligosaccharides, releasing free glucose molecules [11]. Current therapeutic ?-amylase inhibitors, such as acarbose, voglibose, and miglitol, may be associated with adverse effects like bloating, abdominal pain, diarrhea, and flatulence [12], and may reduce appetite and food intake [10].

Peptides have shown promise as a versatile platform for developing novel inhibitors of these enzymes, potentially binding to the substrate-binding sites and hindering carbohydrate interactions through steric hindrance [8, 13]. In Vietnam, the baby clam (Corbiculidae sp.), a bivalve mollusk thriving in brackish and freshwater environments, is harvested throughout the year but commands a relatively low market value of approximately 20,000 VND per kilogram (0.8 USD/kg), according to the Vietnam Fisheries Association. Despite this, its high protein content of 54.48 ± 2.10% on a dry weight basis presents an opportunity to add value to this resource.

While both intact proteins and their peptides can serve as bioactive agents, peptides have been demonstrated to exhibit enhanced bioactivities [14]. The sequence, size, and structure of peptides in hydrolysates can vary based on the type of peptidases (specificity and selectivity) and substrates (type and sequence of amino acid residues) [14]. Islam et al. [15] also highlighted the importance of hydrolysis parameters, such as

material-to-liquid ratio, E:S ratio, and incubation time, on the bioactivities of the hydrolysates.

Taken together, this study aims to explore the potential of utilizing baby clam protein to enhance their economic value by producing protein hydrolysates possessing the capacity to chelate ferrous ions or inhibit starch-digesting enzyme, ?-amylase, through enzymolysis. EXPERIMENTAL SECTION Materials Fresh baby clams were purchased from a market in Ho Chi Minh City, Vietnam, and transported on ice to the laboratory of Ho Chi Minh City University of Technology - Vietnam National University Ho Chi Minh City. The clams were subjected to a blanching process at a temperature of 90-95°C for a duration of 5 minutes. Subsequently, the meat portions were collected, thoroughly washed, and drained for 10 minutes.

The clam meat was then ground and stored at -20°C until further processes. Alcalase® 2.5L, an enzyme with an optimal pH of 7.5 and an optimal temperature of 55°C, was procured from Novozymes (Denmark). All analytical-grade reagents were obtained from Sigma-Aldrich and Merck. Double-distilled water was utilized in the experiments. Instrumentation In this study, a UV-VIS spectrometer (UV-VIS 752, China), a water bath (Memmert WB14- Germany), and a freeze-dryer (Alpha 1-2/Ldplus, UK) were utilized.

Procedure Analysis of chemical composition The proximate composition of the baby clam meat, including moisture, protein, lipid, carbohydrate, and ash content, was assessed utilizing AOAC methods [16]. Additionally, the Cd and Pb contents were analyzed following the AOAC 999.11 procedure [16], while the Hg content was detected using the U.S. Environmental Protection Agency method 7473. Production of protein hydrolysates from baby clam meat The hydrolysis of the baby clam meat was carried out using Alcalase, following the methodology described in our previous study [3].

Initially, the clam meat was added with distilled water to achieve the desired clam meat-to-water ratio. This mixture was heated to 90°C for 10 minutes to inactivate endogenous enzymes. The pH of the mixture was then adjusted to 7.5 using either 1M NaOH or 1M HCl solution, and the temperature was maintained at 55°C. Alcalase, with an activity of 1464.60±63.19 U/ml (using casein as the substrate), was added at the appropriate E:S ratio. After the specified hydrolysis period, the mixture's temperature was raised to 90°C for 10 minutes to deactivate the Alcalase.

The supernatant was obtained by centrifuging the mixture, and its soluble protein content was measured according to the guidelines provided by Nwachukwu and Aluko [17]. The effects of clam meat:water ratio, E:S ratio and hydrolysis time on the bioactivities of the baby clam meat hydrolysate To investigate the impact of clam meat

to water ratio, E:S ratio, and hydrolysis time on the IBC and ?-amylase inhibitory activity of clam meat hydrolysate, each parameter was in turn varied with at least 5 levels while the rest parameters were fixed. The resulting hydrolysates were evaluated for their bioactivities, and the best level of each factor was selected based on these results.

Determination of IBC of the baby clam meat hydrolysate The iron-binding assay was assessed based on the method outlined by Vo et al. [3]. Briefly, the protein hydrolysate was first subjected to demineralization using a macroporous resin (Amberlite IRC-748I sodium form, Acros). Next, 1 mL of the demineralized hydrolysate was sequentially combined with 2.5 mL of acetate buffer (0.1 M, pH 5) and 0.6 mL of FeSO4 solution (0.2 mM). Following a 30-minute incubation in dark at room temperature, 0.3 mL of Ferrozine (5 mM) was added, and the absorbance of the resulting Fe2+-Ferrozine complex was measured at 562 nm.

The IBC was subsequently calculated using the provided formula: (1) Where: Ab denotes the absorbance of the blank; As is the absorbance of the sample; mFe2+ represents the initial weight of ferrous ion, μg; mprotein is the weight of protein of the hydrolysate, g. Determination of ?-amylase inhibitory activity of the baby clam meat hydrolysate The ?-amylase inhibitory activity was determined according to the established method of Admassu et al. [18]. Briefly, 0.5 mL of the hydrolysate (5 mg protein/mL, prepared in solution A (sodium phosphate buffer (0.02 M, pH 6.9), with 6.7 mM NaCl)) was incubated with 0.25 mL of ?-amylase (1 U/mL, prepared in solution A) at 37°C for 20 minutes. Subsequently, 0.25 mL of 1% (w/v) starch solution (prepared in solution A) was added, and the mixture was further incubated at 37°C for 10 minutes. Afterward, it was mixed with 0.5

mL of dinitrosalicylic acid reagent (solution including 1% (w/v) dinitrosalicylic acid, 30% (w/v) potassium sodium tartrate and 1.6% (w/v) NaOH) before being boiled for 5 minutes (to deactivate the ?-amylase) and cooled to room temperature. The cooled mixture was then diluted to 10 mL with distilled water, and its absorbance was measured at 540 nm. The ?-amylase inhibitory activity of the protein hydrolysate was calculated using the provided formula: (2) Where Ac represents the absorbance of the control (a mixture containing enzyme, substrate, and solution A instead of hydrolysate); As denotes the absorbance of the tested sample (a mixture containing enzyme, substrate, and hydrolysate); and Ab indicates the absorbance of the blank (a mixture containing hydrolysate, substrate, and solution A instead of enzyme ?-amylase).

Statistical Analysis The data was presented as means ± standard deviations of triplicate experiments. Statistical analysis was performed on the data using Statgraphics Centurion 18 software, employing a one-way analysis of variance (ANOVA). RESULTS AND

DISCUSSION Chemical composition of the baby clam meat The baby clam meat is composed of 73.34 ± 0.81% moisture, 54.48±2.10% protein, 15.73±0.53% lipid, 26.16% carbohydrate, and 3.66±0.18% ash (on a dry weight basis). A protein content in the range of 11.5 - 72.8% (on dry weight basis) was considered appropriate for producing protein hydrolysates or peptides exhibiting bioactivities, specifically IBC and ?-amylase inhibitory activity [3, 19-20].

As a benthic organism, the accumulation of heavy metals in the baby clam was a primary concern for consumers. However, this study found that the concentrations of cadmium (Cd) and lead (Pb) in the baby clam meat were 0.20 mg/kg and 0.14 mg/kg, respectively, which are well below the maximum limits set in Vietnamese Technical Regulations 8-2:2011/Ministry of Health (Vietnam). Specifically, the Cd and Pb levels were 10.00 and 10.71 times lower than the limits, respectively. Furthermore, Mercury (Hg) was not detected in the baby clam meat, with a method quantification limit of 0.01 mg Hg/kg.

Based on these findings, it can be concluded that baby clam meat presents itself as a safe and competitive raw material for the production of iron-binding and ?-amylase inhibitory protein hydrolysates. Effect of hydrolysis condition on the IBC of the baby clam meat hydrolysate Proteases exhibit distinct hydrolytic activities and selectivities, resulting in limited cleavage of specific substrates at precise sites, which varies depending on the protease class [14]. Alcalase, derived from Bacillus subtilis, primarily contains subtilisin (EC 3.4.21.62), an endoprotease capable of cleaving a diverse range of peptide bonds, with a preference for those involving aromatic and methionine residues [2].

Furthermore, Athira et al. [21] have confirmed the broad specificity of Alcalase for various amino acid categories, including acidic (glutamic acid), aliphatic (leucine and alanine), hydroxyl (serine), and basic (lysine) residues. The side chains of these amino acids have been verified to be involved in the coordinate bonds between the containing peptides and ferrous ions [2-3, 22]. Alcalase has also been utilized in the production of iron-binding protein hydrolysates/peptides from various sources, such as viscera protein [23], sea cucumber [22] and Acetes japonicus [3].

Therefore, this enzyme preparation was employed in this study with the expectation of transforming the baby clam meat into a protein hydrolysate with IBC. In addition to the type of protease, Islam et al. [15] highlighted the importance of hydrolysis condition, including material-to-liquid ratio, E:S ratio, and incubation time, on the bioactivities of the resulting hydrolysates. Effect of clam meat:water ratio As depicted in Figure 1, the IBC of the hydrolysate reached a peak at a clam meat-to-water ratio of 1:7 (w/v).

Lower IBCs observed at other meat-to-water ratios may be attributed to negative impacts on enzyme-substrate interaction due to dilution (in cases of excessive water) or high viscosity (insufficient water) of the mixture, resulting in a low amount of bioactive peptides in the hydrolysates [24]. Conversely, an adequate water quantity not only enhances protein solubility but also effectively disperses hydrolysis products, mitigating feedback effects and augmenting the bioactivity of the protein hydrolysate [24]. Consequently, a meat-to-water ratio of 1:7 (w/v) was chosen for further experiments. Fig. 1. Effect of clam meat:water ratio on IBC of the hydrolysate. Bars with different letters indicate significant differences ($p<0.05$) Effect of E:S ratio As shown in Fig. 2, the influence of the E:S ratio on the hydrolysate's IBC can be categorized into three distinct stages: Stage 1: As the E:S ratio increased from 10 to 20 U/g protein, the IBC decreased from 642.4 ± 30.1 to 386.2

± 13.5 ?g Fe2+/g protein. Stage 2: Subsequently, the IBC showed an increasing trend, reaching its maximum value of 1144.9 ± 29.5 ?g Fe2+/g protein at an E:S ratio of 30 U/g protein. Stage 3: Beyond this point, the E:S ratio was further increased to 50 U/g protein, a decrease in IBC was observed. Fig. 2. Effect of E:S ratio on IBC of the hydrolysate. Bars with different letters indicate significant differences ($p<0.05$) The characteristics of Alcalase activity were demonstrated to adhere to Michaelis-Menten kinetics [25].

At E:S ratios ranging from 10 to 20 U/g protein, which may be below the saturation point, the enzyme may primarily solubilize intact proteins from the clam meat and partially cleave them into smaller peptides. However, long-chain peptides and non-hydrolyzed proteins, characterized by their complex structures and inter/intramolecular bonds, generally display low metal-chelating activity [3, 26], being the root cause of the decrease in IBC observed in Stage 1. On the other hand, an excessively high enzyme concentration can hinder the enzyme-substrate interaction and consequently slow down the hydrolysis process [27], leading to the decline in IBC observed in Stage 3.

Considering the results from all three stages, an E:S ratio of 30 U/g protein was identified as the best condition for maximizing IBC and was selected for use in subsequent experiments. Effect of hydrolysis time In the early stage of enzymatic hydrolysis, higher substrate and enzyme concentrations promote efficient enzymolysis of the baby clam meat protein [27]. This facilitates the breakdown of the protein into smaller peptides, including those with iron-binding properties. Consequently, more iron-binding peptides are released into the hydrolysate, leading to an increase in its IBC.

However, when the hydrolysis duration extends past 60 minutes, a decrease in the IBC of

the hydrolysate is observed (Fig. 3). This decline can be ascribed to several contributing factors, such as enzyme denaturation, enzyme inhibition by reaction products and substrate saturation of the enzyme [15]. This typical trend of IBC with respect to the duration of hydrolysis has been previously reported in studies conducted by Liu et al. [1] and Vo et al. [3]. Fig. 3. Effect of hydrolysis time on IBC of the hydrolysate.

Bars with different letters indicate significant differences ($p<0.05$) Employing the best Alcalase hydrolysis condition, which included a meat-to-water ratio of 1:7 (w/v), an enzyme-to-substrate (E:S) ratio of 30 U/g protein, and a hydrolysis duration of 60 minutes, resulted in a clam meat hydrolysate with an IBC of 1246.2±44.0 μg Fe^{2+}/g protein. Notably, this IBC value was 1.5 times higher than that of the intact clam meat protein. Zhang et al.

[28] proposed that small peptides, due to their simpler spatial structure and increased exposure of metal-binding sites, exhibit higher metal-chelating rates compared to more intact proteins. Remarkably, the IBC of the hydrolysate was found to be approximately 75% of that of EDTANa2, a widely used and effective synthetic iron supplement. To summarize, the protein hydrolysate obtained from baby clam meat through suitable enzymatic hydrolysis exhibits a promising potential as a natural and effective enhancer for improving iron absorption.

Effect of hydrolysis condition on the ?-amylase inhibitory activity of the baby clam meat hydrolysate The application of Alcalase in the production of ?-amylase inhibitory hydrolysates has been documented [29-31]. This enzyme's broad specificity allows it to effectively generate small molecular weight peptides, particularly those containing aromatic amino acid residues (Phe, Trp, and Tyr) and hydrophobic amino acid residues (Leu, Ile, Val, and Met) [13]. ?-amylase has been found to contain several aromatic residues within its substrate-binding pocket. This increases the likelihood of ?-amylase binding with peptides, particularly those containing aromatic residues [8].

Altogether, Alcalase emerges as an appropriate enzyme for the production of ?-amylase inhibitory hydrolysates. Effect of baby clam meat:water ratio The ratio of baby clam meat to water is a crucial factor in determining the amount of water and substrate for Alcalase hydrolysis. This ratio directly influences on enzyme-substrate interactions and reaction rate, impacting the ?-amylase inhibitory activity of the resulting hydrolysate. As illustrated in Fig.

1, the ?-amylase inhibitory activity of the hydrolysates increased as ratio of clam meat to water was in the range of 1:2-1:7 (w/v), and decreased afterwards. With fixed enzyme amount, the reaction rate proportionally increases with substrate concentration upto a

specific value. Beyond this point, despite further increases in substrate concentration, the reaction rate remains unchanged [32]. Conversely, Ding et al. [33] explained that the decline in bioactive peptide content resulted from the substrate inhibition at higher substrate concentrations.

Notably, the ratio of baby clam meat to water of 1:7 (w/v) could provide sufficient water for effective enzyme-substrate interactions and proper viscosity for enzymatic reactions, thereby enhancing the bioactivity of the hydrolysate. In this study, the suitable ratio of baby clam meat to water for producing the hydrolysate with maximal ?-amylase inhibitory activity was 1:7 (w/v), and this ratio was utilized for further experiments. In this study, the suitable ratio of baby clam meat to water for producing the hydrolysate with maximal ?-amylase inhibitory activity was 1:7 (w/v), and this ratio was utilized for further experiments. Fig. 4.

Effect of baby clam meat:water ratio on ?-amylase inhibitory activity of the hydrolysate. Bars with different letters indicate significant differences ($p<0.05$) Effect of E:S ratio According to Fig. 5, the ?-amylase inhibitory activity of the baby clam meat hydrolysate reached its peak at E:S ratio of 30 U/g protein. Subsequently, when the E:S ratio exceeded this value, the inhibitory activity exhibited a decreasing trend. It is believed that higher enzyme amounts can degrade the bioactive peptides present, consequently diminishing the bioactivity of the hydrolysate [34]. Based on these findings, subsequent experiments were performed using a fixed E:S ratio of 30 U/g protein.

Effect of hydrolysis time This study revealed that the ?-amylase inhibitory capacity of the baby clam meat hydrolysate is directly proportional to the hydrolysis duration within the initial period (2-4 hours). However, over 4 hours, an inverse proportion was observed (Fig. 6). This finding was supported by previous observations reported by Feng et al. [30], Mojica and Mejía [31] and Castañeda?Pérez et al. [34]. The extended hydrolysis time allowed for more extensive enzymatic breakdown of proteins, leading to an increased number of small peptides in the hydrolysate [35], which enhances binding affinity for the active site of the enzyme.

Larger peptides face steric hindrances in their interactions with the enzyme [36]. Castañeda?Pérez et al. [34] further suggested that low molecular weight peptides can access and inhibit the catalytic site of ?-amylase more readily, altering its conformation and disrupting the binding between the enzyme and its natural substrate. However, excessively prolonged hydrolysis (5, 6, and 7 hours) may result in a decline in Alcalase activity , substrate depletion [35], and therefore reducing the ?-amylase activity of the hydrolysate Fig. 5.

Effect of E:S ratio on ?-amylase inhibitory activity of the hydrolysate. Bars with different letters indicate significant differences ($p<0.05$) Fig. 6. Effect of hydrolysis time ratio on ?-amylase inhibitory activity of the hydrolysate. Bars with different letters indicate significant differences ($p<0.05$) From these experiments, the suitable condition for Alcalase hydrolysis to obtain a baby clam meat hydrolysate with the highest ?-amylase inhibitory activity included: a baby clam meat:water ratio of 1:7 (w/v), an E:S ratio of 30 U/g protein, and a hydrolysis duration of 4h. Under this condition, the hydrolysate exhibited a maximum ?-amylase inhibitory activity of 48.33±1.44%, which was 1.35 times lower than that of the drug Acarbose.

Therefore, the baby clam meat hydrolysate could be considered as a natural alternative inhibitor of ?-amylase. CONCLUSION Essentially, this research uncovers the promising potential of baby clam meat as a novel material source to generate bioactive hydrolysates or peptides. Through enzymatic hydrolysis using Alcalase, the resulting hydrolysates demonstrated remarkable activities that could position them as natural alternatives to synthetic iron supplements or ?-amylase inhibitors.

It's worth highlighting that the hydrolysates derived from baby clam meat demonstrated a notable iron-binding capacity of 1246.2±44.0 μg Fe^{2+}/g protein, as well as an ?-amylase inhibitory activity of 48.33±1.44%. The findings serve as launching points for upcoming investigations, including the identification of specific bioactive peptide sequences, further refinement of the hydrolysates, and their potential applications across the food, functional food, and even pharmaceutical industries.

Moreover, the integration of emerging advanced technologies (such as ultrasonication, high hydrostatic pressure, and pulsed electric field treatments) with enzymatic hydrolysis holds promise for further enhancement of the bioactivities of these hydrolysates. ACKNOWLEDGEMENTS This research is funded by Vietnam National University HoChiMinh City (VNU-HCM) under grant number: B2024-20-24. We acknowledge Ho Chi Minh City University of Technology (HCMUT), VNU-HCM for supporting this study. CONFLICT OF INTEREST The authors declare no conflict of interest. AUTHOR CONTRIBUTIONS Conceptualization: Tam D. L. Vo; Funding acquisition: Tam D. L. Vo; Project administration: Tam D. L. Vo; Supervision: Tam D. L. Vo; Visualization: Bao C.

Vo; Writing-review and editing: Tam D. L. Vo; Writing-original draft preparation: Bao C. Vo; Data curation: Bao C. Vo; Resources: Tam D. L. Vo; Investigation: Tam D. L. Vo, Bao C. Vo, Thuy T. Le, An T. T. Tran; Formal analysis: Tam D. L. Vo, Bao C. Vo, Thu Huynh; Validation: Tam D. L. Vo, Bao C. Vo; Methodology: Tam D. L. Vo, Bao C. Vo. All authors agreed to the final version of this manuscript. REFERENCES [1] Liu, Y., Wang, Z., Kelimu, A., Korma, S. A., Cacciotti, I., Xiang, H. and Cui, C., 2023, Novel iron-chelating peptide

from egg yolk: Preparation, characterization, and iron transportation, Food Chem.: X, 18, 100692. [2] Berraquero-García, C., Almécija, M. C., Guadix, E. M. and Pérez-Gálvez, R.,

2022, Valorisation of blood protein from livestock to produce haem iron-fortified hydrolysates with antioxidant activity, Int. J. Food Sci. Technol., 57, 2479-2486. [3] Vo, T. D. L., Pham, K. T., Le, V. M. V., Lam, H. H., Huynh, O. N. and Vo, B. C., 2020, Evaluation of iron-binding capacity, amino acid composition, functional properties of Acetes japonicus proteolysate and identification of iron-binding peptides, Process Biochem., 91, 374-386. [4] Gómez-Grimaldos, N. A., Gómez-Sampedro, L. J., Zapata-Montoya, J. E., López-García, G., Cilla, A. and Alegría-Torán, A., 2020, Bovine plasma hydrolysates' iron chelating capacity and its potentiating effect on ferritin synthesis in Caco-2 cells, Food Funct, 11, 10907-10912. [5] Zhang, Y. Y.,

Stockmann, R., Ng, K., Broadbent, J. A., Stockwell, S., Suleria, H., Shaik, N. E. K., Unnithan, R. R. and Ajlouni, S., 2023, Characterization of Fe(III)-binding peptides from pea protein hydrolysates targeting enhanced iron bioavailability, Food Chem., 405, 134887. [6] Xue, D., Jiang, S., Zhang, M., Shan, K., Lametsch, R. and Li, C., 2024, The efficiency and safety evaluation of hemoglobin hydrolysate as a non-heme iron fortifier, Food Sci. Hum. Wellness, 13, 999-1010. [7] Fan, C., Ge, X., Hao, J., Wu, T., Liu, R., Sui, W., Geng, J. and Zhang, M., 2023, Identification of high iron-chelating peptides with unusual antioxidant effect from sea cucumbers and the possible binding mode, Food Chem., 399 133912. [8] Qiao, H., Bi, X., Zhang, Y., Liu, M.,

Zu, S., Jia, N., Jiang, S., Lu, Q., Zu, Y. and Bao, Y., 2020, Enzymic polypeptide antioxidant activity and inhibitory activity on ?-glucosidase and ?-amylase from Paeonia ostii cake, Ind. Crops Prod., 146 112158. [9] Zhao, Q., Wei, G., Li, K., Duan, S., Ye, R. and Huang, A., 2022, Identification and molecular docking of novel ?-glucosidase inhibitory peptides from hydrolysates of Binglangjiang buffalo casein, LWT - Food Sci. Technol., 156, 113062. [10] González-Montoya, M., Hernández-Ledesma, B., Mora-Escobedo, R. and Martínez-Villaluenga, C., 2018, Bioactive Peptides from Germinated Soybean with Anti-Diabetic Potential by Inhibition of Dipeptidyl Peptidase-IV, ?-Amylase, and ?-Glucosidase Enzymes, Int. J. Mol. Sci., 19, 2883. [11] Ibrahim, M.

A., Bester, M. J., Neitz, A. W. and Gaspar, A. R., 2018, Structural properties of bioactive peptides with ?-glucosidase inhibitory activity, Chem. Biol. Drug Des., 91 (2), 370-379. [12] Siahbalaei, R., Kavoosi, G. and Noroozi, M., 2021, Protein nutritional quality, amino acid profile, anti-amylase and anti-glucosidase properties of microalgae: Inhibition and mechanisms of action through in vitro and in silico studies, LWT - Food Sci. Technol., 150, 112023. [13] Balderas-León, I., Baigts-Allende, D. and Cardador-Martínez, A., 2021, Antioxidant, angiotensin-converting enzyme, and ?-amylase inhibitory activities of

protein hydrolysates of Leucaena leucocephala seeds, CYTA J. Food, 19 (1), 349-359. [14] Karami, Z., Butkinaree, C.,

Yingchutrakul, Y., Simanon, N. and Duangmal, K., 2022, Comparative study on structural, biological and functional activities of hydrolysates from Adzuki bean (Vigna angularis) and mung bean (Vigna radiata) protein concentrates using Alcalase and Flavourzyme, Food Res. Int., 161, 111797. [15] Islam, M. S., Wang, H., Admassu, H., Sulieman, A. A. and Wei, F. A., 2022, Health benefits of bioactive peptides produced from muscle proteins: Antioxidant, anti-cancer, and anti-diabetic activities, Process Biochem., 116, 116-125.

[16] Latimer, D. G. W., 2023, Official Methods of Analysis, 22nd Edition, Oxford University Press, UK, p. 3750. [17] Nwachukwu, I. D. and Aluko, R. E., 2019, A systematic evaluation of various methods for quantifying food protein hydrolysate peptides, Food Chem., 270, 25-31. [18] Admassu, H., Gasmalla, M. A. A., Yang, R. and Zhao, W., 2018, Identification of Bioactive Peptides with ?-Amylase Inhibitory Potential from Enzymatic Protein Hydrolysates of Red Seaweed (Porphyra spp), J. Agric. Food Chem., 66 (19), 4872-4882. [19] Pantoa, T., Kubota, M., Suwannaporn, P. and Kadowaki, M.,

2020, Characterization and bioactivities of young rice protein hydrolysates, J. Cereal Sci., 95, 103049. [20] Arise, R. O., Idi, J. J., Mic-Braimoh, I. M., Korode, E., Ahmed, R. N. and Osemwegie, O., 2019, In vitro Angiotesin-1-converting enzyme, ?-amylase and ?-glucosidase inhibitory and antioxidant activities of Luffa cylindrical (L.) M. Roem seed protein hydrolysate, Heliyon, 5, e01634. [21] Athira, S., Mann, B., Sharma, R., Pothuraju, R. and Bajaj, R. K., 2021, Preparation and characterization of iron-chelating peptides from whey protein: An alternative approach for chemical iron fortification, Food Res. Int., 141, 110133. [22] Senadheera, T. R. L., Dave, D. and Shahidi, F.,

2021, Antioxidant potential and physicochemical properties of protein hydrolysates from body parts of North Atlantic sea cucumber (Cucumaria frondosa), Food Prod. Process. Nutr., 3, 3. [23] Wu, W., Jia, J., Wen, C., Yu, C., Zhao, Q. and Hu, J., 2021, Optimization of ultrasound assisted extraction of abalone viscera protein and its effect on the iron-chelating activity, Ultrason. Sonochem., 77, 105670. [24] Shu, G., Zhang, B., Zhang, Q., Wan, H. and Li, H., 2017, Effect of Temperature, pH, Enzyme to Substrate Ratio, Substrate Concentration and Time on the Antioxidative Activity of Hydrolysates from Goat Milk Casein by Alcalase, Acta Univ. Cibiniensis, Ser. E: Food Technol., 20 (2), 29-38. [25] Alves, F.

E. d. S. B., Carpiné, D., Teixeira, G. L., Goedert, A. C., Scheer, A. d. P. and Ribani, R. H., 2021, Valorization of an Abundant Slaughterhouse By?product as a Source of Highly Technofunctional and Antioxidant Protein Hydrolysates, Waste Biomass Valor., 12,

263-279. [26] Vo, T. D. L., Pham, K. T. and Doan, K. T., 2021, Identification of Copper-Binding Peptides and Investigation of Functional Properties of Acetes japonicus Proteolysate, Waste Biomass. Valor., 12, 1565-1579. [27] Wu, D., Cao, Y., Su, D., Karrar, E., Zhang, L., Chen, C., Deng, N., Zhang, Z., Liu, J., Li, G. and Li, J., 2024, Preparation and identification of antioxidant peptides from Quasipaa spinosa skin through two-step enzymatic hydrolysis and molecular simulation, Food Chem.,

445, 138801. [28] Zhang, Y., Ding, X. and Li, M., 2021, Preparation, characterization and in vitro stability of iron-chelating peptides from mung beans, Food Chem., 349, 129101. [29] Wang, J., Wu, T., Fang, L., Liu, C., Liu, X., Li, H., Shi, J., Li, M. and Min, W., 2020, Anti-diabetic effect by walnut (Juglans mandshurica Maxim.)-derived peptide LPLLR through inhibiting ?-glucosidase and ?-amylase, and alleviating insulin resistance of hepatic HepG2 cells, J. Funct. Foods, 69, 103944. [30] Feng, J., Ma, Y.-L., Sun, P., Thakur, K., Wang, S., Zhang, J.-G. and Wei, Z.-J., 2021, Purification and characterization of ?-glucosidase inhibitory peptides from defatted camellia seed cake, Int. J. Food Sci. Technol., 56 (1), 138-147. [31] Mojica, L.

and Mejía, E. G. d., 2016, Optimization of enzymatic production of anti-diabetic peptides from black bean (Phaseolus vulgaris L.) proteins, their characterization and biological potential, Food Funct., 7 (2), 713-727. [32] Megrous, S., Zhao, X., Al-Dalali, S. and Yang, Z., 2024, Response surface methodology and optimization of hydrolysis conditions for the in vitro calcium-chelating and hypoglycemic activities of casein protein hydrolysates prepared using microbial proteases, J. Food Meas. Charact., 18, 3069-3084. [33] Ding, J., Liang, R., Yang, Y., Sun, N. and Lin, S., 2020, Optimization of pea protein hydrolysate preparation and purification of antioxidant peptides based on an in silico analytical approach, LWT-Food Sci. Technol., 123, 109126.

[34] Castañeda?Pérez, E., Jiménez?Morales, K., Quintal?Novelo, C., Moo?Puc, R., Chel?Guerrero, L. and Betancur?Ancona, D., 2019, Enzymatic protein hydrolysates and ultrafiltered peptide fractions from Cowpea Vigna unguiculata L bean with in vitro antidiabetic potential, J. Iran. Chem. Soc., 16, 1773-1781. [35] Nurdiani, R., Firdaus, M., Prihanto, A. A., Jaziri, A. A., Jati, M. R., Abdurrahman, T. R., Ifilah, S., Debataraja, E. R. and Huda, N., 2024, Enzymatic Hydrolysis of Protein Hydrolysate from Pangasius sp. by-Product using Bromelain, Curr. Res. Nutr. Food Sci., 12 (1). [36] Jiang, M., Yan, H., He, R. and Ma, Y.,

2018, Purification and a molecular docking study of ?-glucosidase-inhibitory peptides from a soybean protein hydrolysate with ultrasonic pretreatment, Eur. Food Res. Technol., 244, 1995-2005.

INTERNET SOURCES:

<1% - https://en.wikipedia.org/wiki/Ho_Chi_Minh_City_University_of_Technology
<1% - https://en.wikipedia.org/wiki/Ho_Chi_Minh_City_University_of_Technology_and_Education
<1% - https://www.sciencedirect.com/science/article/pii/S2212429223009756
<1% - https://www.researchgate.net/publication/344778363_Use_of_Alcalase_in_the_production_of_bioactive_peptides_A_review
<1% - https://www.sciencedirect.com/science/article/pii/S0098299720300820
<1% - https://pubmed.ncbi.nlm.nih.gov/32650997/
<1% - https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7509542/
<1% - https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5701707/
<1% - https://www.sciencedirect.com/science/article/pii/S0924224419307290
<1% - https://www.researchgate.net/publication/341192067_Peptide-metal_complexes_obtention_and_role_in_increasing_bioavailability_and_decreasing_the_pro-oxidant_effect_of_minerals
<1% - https://www.nature.com/articles/s41419-021-04490-1
<1% - https://link.springer.com/content/pdf/10.1007/978-3-319-20797-1_13-2.pdf
<1% - https://link.springer.com/chapter/10.1007/978-94-007-5899-5_20
<1% - https://microbenotes.com/starch-digestion/
<1% - https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/amylase
<1% - https://www.sciencedirect.com/science/article/pii/S2212429223006053
<1% - https://pubmed.ncbi.nlm.nih.gov/34173346/
<1% - https://www.seafoodsource.com/news/premium/supply-trade/vietnam-sets-higher-seafood-target-this-year-for-its-export-value-despite-potential-challenges
<1% - https://orbi.uliege.be/bitstream/2268/203987/1/NGO%20THI%20THU%20Hang%20et%20al_SUSTAINABILITY.pdf
<1% - https://wanderlog.com/list/geoCategory/1536924/best-seafood-buffets-in-ho-chi-minh-city
<1% - https://www.sciencedirect.com/science/article/pii/S2214317316300919
<1% - https://pubs.acs.org/doi/pdf/10.1021/acsomega.3c00715
<1% - https://link.springer.com/content/pdf/10.1007/978-3-319-26811-8_5.pdf

<1% - https://link.springer.com/article/10.1007/s42729-023-01498-8
<1% -
https://chem.libretexts.org/Bookshelves/Analytical_Chemistry/Molecular_and_Atomic_Spectroscopy_(Wenzel)/1%3A_General_Background_on_Molecular_Spectroscopy/1.2%3A_Beers_Law
<1% -
https://www.researchgate.net/publication/343594210_In_vitro_Studies_on_a-Amylase_and_a-Glucosidase_Inhibitory_Activity_of_Some_Bioactive_Extracts
<1% - https://bmcchem.biomedcentral.com/articles/10.1186/s13065-018-0445-y
<1% - https://www.sciencedirect.com/science/article/pii/S1878818122000032
<1% -
https://www.semanticscholar.org/paper/Variations-in-gross-biochemical-composition-in-to-Suja-Muthiah/5580237d00909e258b2360c31f54f8ab9acbe0dd
<1% - https://pubmed.ncbi.nlm.nih.gov/22551849/
<1% -
https://quizlet.com/552494443/segment-1-exam-study-cards-part-1-ebiology-flash-cards/
<1% - https://iubmb.qmul.ac.uk/enzyme/EC3/4/21/62.html
<1% - https://dash.harvard.edu/handle/1/41483496
<1% - https://onlinelibrary.wiley.com/doi/full/10.1002/fbe2.12057
<1% - https://link.springer.com/article/10.1007/s10499-022-00889-5
<1% - https://papers.xtremepape.rs/CAIE/IGCSE/Biology%20(0610)/0610_s12_qp_32.pdf
<1% - https://www.nature.com/articles/s41467-023-40471-y
1% -
https://www.researchgate.net/figure/different-letters-indicate-significant-differences-at-p-005-revealed-using-a_fig2_355489964
<1% -
https://www.academia.edu/84353827/Optimization_of_enzymatic_hydrolysis_condition_of_Angelwing_Clam_Pholas_orientalis_meat_using_Alcalase_to_obtain_maximum_degree_of_hydrolysis
<1% - https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8535599/
<1% - https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5109804/
<1% - https://www.sciencedirect.com/science/article/pii/S2214799321000527
<1% - https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5430178/
<1% - https://www.mdpi.com/2076-3921/13/4/479
<1% - https://www.tandfonline.com/doi/full/10.1080/15623599.2023.2274717
<1% -
https://www.researchgate.net/publication/349607238_Conflict_of_interest_the_authors_declare_no_conflict_of_interest
<1% -

https://mdpi-res.com/d_attachment/ijms/ijms-22-01960/article_deploy/ijms-22-01960-s001.pdf?version=1613480525
<1% - https://pubs.acs.org/doi/10.1021/jf0109165
<1% - https://research.ku.dk/search/result/?pure=en%2Fpersons%2Frene-lametsch(70bd350a-6869-4a4a-b9bc-56a756376784)%2Fpublications.html&pageSize=25&page=0
<1% - https://www.mdpi.com/2304-8158/12/1/226
<1% - https://www.mdpi.com/2304-8158/12/1/183
<1% - https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10381244/
<1% - https://www.researchgate.net/figure/SDS-PAGE-patterns-of-protein-hydrolysates-from-using-Alcalase-Protamex-and-Flavourzyme_fig3_287839632
<1% - https://scholar.google.com/citations?user=-c8RebgAAAAJ
<1% - https://academic.oup.com/aoac-publications/book/45491/chapter/392327022
<1% - https://sci-hub.se/10.1016/j.foodchem.2018.07.054
<1% - https://pubs.acs.org/toc/jafcau/66/19
<1% - https://www.semanticscholar.org/paper/Young-rice-protein-as-a-new-source-of-low-protein-Pantoa-Baricevic-Jones/a8fbf7ae9e2bf388a6fa49ddc15157bffdad7f73
<1% - https://sci-hub.se/10.1016/j.heliyon.2019.e01634
<1% - https://www.semanticscholar.org/paper/Preparation-and-characterization-of-iron-chelating-Athira-Mann/3404e1b9023849a2e027dd3335cc3b2149bf3bf8
<1% - https://www.researchgate.net/publication/348941487_Antioxidant_potential_and_physicochemical_properties_of_protein_hydrolysates_from_body_parts_of_North_Atlantic_sea_cucumber_Cucumaria_frondosa
<1% - https://reference.medscape.com/medline/abstract/34304120
<1% - https://www.semanticscholar.org/paper/Effect-of-Temperature%2C-pH%2C-Enzyme-to-Substrate-and-Shu-Zhang/5bad7582bd459ef4e883151a9fb1b0f349aa3a12/figure/0
<1% - https://library.lincoln.ac.uk/items/eds/cmedm/38387316
<1% - https://europepmc.org/article/MED/31143910
<1% - https://link.springer.com/article/10.1007/s11694-024-02388-y
<1% - https://pubs.acs.org/doi/10.1021/jf0300588
<1% - https://pubs.acs.org/doi/10.1021/jf202686m