Iron-Binding Capacity and Antidiabetic Activity of Baby Clam (*Corbiculidae* sp.) Meat Protein Hydrolysate

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Abstract: *Baby clam (*Corbiculidae *sp.) meat is served as a traditional dish in Vietnam, and the antioxidant activity of its protein extract has been discovered. This study evaluates baby clam meat protein hydrolysate's iron-binding capacity (IBC) and antidiabetic activity. Initially, an analysis of the basic chemical composition of the meat was conducted. Subsequently, Alcalase was employed for hydrolysis. The highest IBC and α-amylase inhibition activity were targets for obtaining the best hydrolysis condition, including the clam meat-to-water ratio, enzyme-to-substrate (E:S) ratio, and time. Under the best condition, the hydrolysates demonstrated the IBC of 1246.20 ± 44.00 μg Fe^{2+}/g protein and α-amylase inhibition activity of 48.33 ± 1.44%, approaching three-quarters of the activity of ethylenediaminetetraacetic acid (EDTA) sodium salt and acarbose, respectively. These results served as preliminary data for the development of the protein hydrolysates as a natural iron chelator or α-amylase inhibitor, which could support the treatment of iron deficiency and diabetes.*

Keywords: *baby clam meat; iron-binding capacity; α-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. 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. Iron deficiency can lead to various health issues, including mood changes, muscle weakness, immunodeficiency, and anemia [1]. While multiple factors contribute to iron deficiency, its low bioavailability is the primary cause. 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 [2]. Non-heme 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 [2]. 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. Despite the potential benefits of fortifying foods with iron metal, challenges such as lipid oxidation (iron generates free radicals, oxidizing lipids) and its low solubility pose obstacles to achieving these

objectives [1]. 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 the neutral aqueous condition of the human intestine [3].

Conversely, iron-chelating peptides have been shown to be effective in preventing iron from binding with water molecules, thereby inhibiting the formation of $Fe(OH)_3$ and increasing iron solubility. This facilitates iron absorption and bioavailability. Three transport pathways have been suggested for chelating peptides that enhance ferrous ion absorption, i.e., Fe^{2+} transport pathway, peptide absorption pathway, and peptide as metal ion transport carrier [4]. An *in vitro* study has shown that the bioavailability of iron in peptide-Fe complexes is considerably higher than that of ferrous sulfate [3]. In addition to promoting iron absorption, these peptides can increase hepatic iron content by upregulating divalent metal transporter 1 and 14. Additionally, the *in vivo* studies on mice have confirmed the safety of iron-binding protein hydrolysates [5].

Furthermore, diabetes mellitus has witnessed a substantial global surge, with projections indicating that it will affect approximately 438 million individuals by 2030 [6]. This disease poses significant cardiovascular risks and is ranked among the top five leading causes of mortality worldwide. 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 [7]. Inhibiting the hydrolysis of starches into oligo- and monosaccharides within the small intestine effectively mitigates glucose absorption and transportation into the bloodstream. Salivary and pancreatic α-amylase and intestinal α-glucosidase play vital roles in the digestion of starch within the gastrointestinal tract. The α-amylase enzymes break down starch into maltose and other oligosaccharides by cleaving α-1,4-glycosidic bonds. Subsequently, α-glucosidase, which is embedded in the epithelial mucosa of the small intestine, hydrolyzes the non-reducing end of oligosaccharides, releasing free glucose molecules. Current therapeutic α-amylase inhibitors, such as acarbose, voglibose, and miglitol, may be associated with adverse effects like bloating, abdominal pain, diarrhea, and flatulence, and may reduce appetite and food intake [8-9]. On the other hand, 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 [6,10].

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/kg (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. Our previous study utilized the baby clam broth to generate an antioxidant protein hydrolysate [11]. While both intact proteins and their peptides can serve as bioactive agents, peptides have been demonstrated to exhibit enhanced bioactivities [12]. 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) [12]. Islam et al. [13] also highlighted the importance of hydrolysis parameters, such as material-to-liquid ratio, enzyme-to-substrate (E:S) ratio, and incubation time, on the bioactivities of the hydrolysates. 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 a starch-digesting enzyme, i.e., α-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 90–95 °C for 5 min. Subsequently, the meat

portions were collected, thoroughly washed, and drained for 10 min. The clam meat was then ground and stored at −20 °C until further processes. Alcalase® 2.5 L, 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 also 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 the Association of Official Agricultural Chemists (AOAC) methods [14]. Additionally, the Cd and Pb contents were analyzed following the AOAC 999.11 procedure [14], while the Hg content was detected using the U.S. Environmental Protection Agency method 7473.

Production of protein hydrolysates from baby clam meat

The baby clam meat was hydrolyzed using Alcalase, following the methodology described in our previous study [1]. 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 min to inactivate endogenous enzymes. The pH of the mixture was then adjusted to 7.5 using either 1 M NaOH or 1 M 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 min 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 [15].

The effects of clam meat-to-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 α-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. [1]. 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 $FeSO_4$ solution (0.2 mM). Following a 30-min incubation in the dark at room temperature, 0.3 mL of ferrozine (5 mM) was added, and the absorbance of the resulting Fe^{2+}-ferrozine complex was measured at 562 nm. The IBC was subsequently calculated using the Eq. (1);

$$\text{IBC } (\mu g Fe^{2+} / g \text{ protein}) = \frac{A_b \times A_s}{A_b} \times \frac{m_{Fe^{2+}}}{m_{protein}} \quad (1)$$

where A_b denotes the absorbance of the blank, A_s is the absorbance of the sample, $m_{Fe^{2+}}$ represents the initial weight of ferrous ion (μg), and $m_{protein}$ is the weight of the protein of the hydrolysate (g).

Determination of α-amylase inhibitory activity of the baby clam meat hydrolysate

The α-amylase inhibitory activity was determined according to the established method of Admassu et al. [16]. 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 α-amylase (1 U/mL, prepared in solution A) at 37 °C for 20 min. 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 min. 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 min (to deactivate the α-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 α-amylase inhibitory activity was calculated using the Eq. (2);

$$\alpha-\text{amylase inhibitory activity (\%)}=\frac{A_c-A_s}{A_c-A_b}\times 100\% \quad (2)$$

where A_c represents the absorbance of the control (a mixture containing enzyme, substrate, and solution A instead of hydrolysate), A_s denotes the absorbance of the tested sample (a mixture containing enzyme, substrate, and hydrolysate), and A_b indicates the absorbance of the blank (a mixture containing hydrolysate, substrate, and solution A instead of enzyme α-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) (Table 1). 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 α-amylase inhibitory activity [1,17-18]. 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 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 α-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. 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 [19]. Furthermore, Athira et al. [20] have confirmed the broad specificity of Alcalase for various amino acid categories, including acidic (Glu), aliphatic (Leu and Ala), hydroxyl (Ser), and basic (Lys) residues. The side chains of these amino acids

Table 1. Proximate and heavy metals composition of baby clam (*Corbiculidae* sp.) meat

Parameter	Content
Moisture	73.34 ± 0.81 (%)
Protein	54.48 ± 2.10 (%, dry basis)
Lipid	15.73 ± 0.53 (%, dry basis)
Carbohydrate	26.16 (%, dry basis)
Ash	3.66 ± 0.18 (%, dry basis)
Cadmium (Cd)	0.20 mg/kg
Lead (Pb)	0.14 mg/kg
Mercury (Hg)	Non-detected (method quantification limit of 0.01 mg/kg)

have been verified to be involved in the coordinate bonds between the containing peptides and ferrous ions [1,19,21]. Alcalase has also been utilized in the production of iron-binding protein hydrolysates/peptides from various sources, such as viscera protein [22], sea cucumber [21], and *Acetes japonicus* [1]. 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. [13] highlighted the importance of hydrolysis conditions, including material-to-liquid ratio, E:S ratio, and incubation time, on the bioactivities of the resulting hydrolysates.

Effect of baby clam meat-to-water ratio

As depicted in Fig. 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. 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 [23]. A similar pattern of solid-to-liquid ratio – IBC of goat milk casein hydrolysates was observed by Shu et al. [24]. Moreover, the material-to-water ratio of 1:7 (w/v) was set in the hydrolysis process of silver carp muscle and rice bran proteins to obtain Fe^{2+} chelating hydrolysates [24-25]. Consequently, a meat-to-water ratio of 1:7 (w/v) was chosen for further experiments.

Effect of E:S ratio

As shown in Fig. 2, the influence of the E:S ratio on the hydrolysate's IBC can be described with 3 distinct stages. At 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 μg Fe^{2+}/g protein. At stage 2, the IBC showed an increasing trend, reaching its maximum value of 1144.9 ± 29.5 μg Fe^{2+}/g protein at an E:S ratio of 30 U/g protein. Lastly, at stage 3, beyond this point, the E:S ratio was further increased to 50 U/g protein, and IBC decreased. The characteristics of Alcalase activity were demonstrated to adhere to Michaelis–Menten kinetics [26]. 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 [1,27], the root cause of the decreased IBC observed in stage 1. Besides, a decline in the hydrolysate's IBC at the E:S ratio of 20 U/g protein might be attributed to the accumulation of large peptides, which probably obstructed the accessibility of Fe^{2+} ions to their binding sites. On the other hand, an excessively high enzyme concentration can hinder the enzyme-substrate

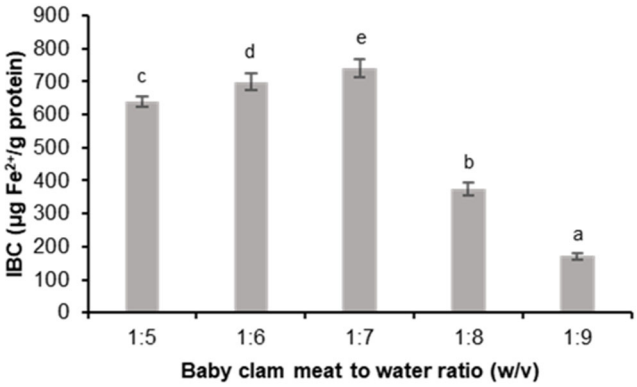

Fig 1. Effect of baby clam meat to water ratio on IBC of the hydrolysate. Bars with different letters indicate significant differences ($p < 0.05$)

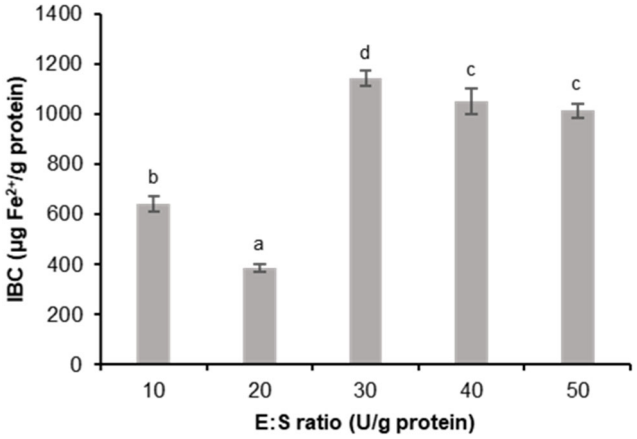

Fig 2. Effect of E:S ratio on IBC of the hydrolysate. Bars with different letters indicate significant differences ($p < 0.05$)

interaction and consequently slow down the hydrolysis process [28], leading to the decline in IBC observed in stage 3. Our previous research recorded the correlation between the E:S ratio and IBC of *Acetes japonicus* hydrolysate, showing the same pattern as at stages 2 and 3 of the current study [1]. Additionally, matching with the findings of Salami et al. [29] and Dhanabalan et al. [30], IBCs of *Garcinia kola* and *Acetes indicus* hydrolysates reached the peaks at certain E:S ratios, beyond which a decline in their IBC appeared. 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 [28]. This facilitates the protein breakdown 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 min, 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. This typical trend of IBC with respect to the duration of hydrolysis has been previously reported in studies conducted by Liu et al. [31] and Vo et al. [1].

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 min, 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 the intact clam meat protein. Zhang et al. [32] proposed that small peptides, due to their simpler spatial structure and increased exposure to 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 $EDTANa_2$, a widely used and effective synthetic iron supplement. Compared to other hydrolysates, the IBC of the clam meat hydrolysate in this study significantly surpassed that of Pacific cod skin gelatin hydrolysate [33], scad processing by-product hydrolysate [34], and *Acetes japonicus* baby shrimp hydrolysate [35], by 1.8, 5.8, and 7.0 folds, respectively. This variation might be due to the differences in molecular weight, spatial distribution, amino acid composition, and sequence of peptides in these hydrolysates. 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.

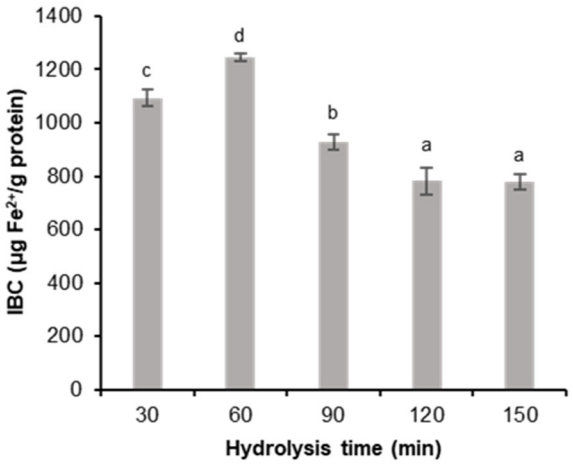

Fig 3. Effect of hydrolysis time on IBC of the hydrolysate. Bars with different letters indicate significant differences ($p < 0.05$)

Effect of Hydrolysis Condition on the α-Amylase Inhibitory Activity of the Baby Clam Meat Hydrolysate

The application of Alcalase in the production of α-amylase inhibitory hydrolysates has been documented [36-38]. 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) [10]. An α-amylase has been found to contain several aromatic residues within its substrate-binding pocket. This increases the likelihood of α-amylase binding with peptides, particularly those containing aromatic residues [6]. Altogether, Alcalase emerges as an appropriate enzyme

for the production of α-amylase inhibitory hydrolysates.

Effect of baby clam meat on water ratio

The ratio of baby clam meat-to-water is crucial in determining the amount of water and substrate for Alcalase hydrolysis. This ratio directly influences enzyme-substrate interactions and reaction rate, impacting the α-amylase inhibitory activity of the resulting hydrolysate. As illustrated in Fig. 4, the α-amylase inhibitory activity of the hydrolysates increased as the ratio of clam meat to water was in the range of 1:2-1:7 (w/v) and decreased afterward. With a fixed enzyme amount, the reaction rate proportionally increases with substrate concentration up to a specific value. Beyond this point, the reaction rate remains unchanged despite further increases in substrate concentration. Conversely, Ding et al. [39] explained that the decline in bioactive peptide content resulted from 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. With the clam meat-to-water ratios from 1:8 to 1:10 (w/v), large amounts of water probably diminished the effectiveness of collision between enzyme and substrate molecules, decreasing α-amylase inhibition activities of the hydrolysates. Our previous study also found an upside-down U-shaped curve, showing the relationship between earthworm to phosphate buffer ratio and α-amylase inhibition activity of earthworm hydrolysates and the highest activity at the ratio of 1:8 (w/v) [40]. On the other hand, a material-to-water ratio of 1:5 (w/v) was used to generate α-amylase inhibition hydrolysates from salmon bone and Asian bullfrog skin collagen [41-42]. The difference in best ratios might be attributed to the variation of protein sources, hydrolysis enzyme types, and different hydrolysis conditions. In this study, the suitable ratio of baby clam meat to water for producing the hydrolysate with maximal α-amylase inhibitory activity was 1:7 (w/v), and this ratio was utilized for further experiments.

Effect of E:S ratio

According to Fig. 5, the α-amylase inhibitory activity of the baby clam meat hydrolysate reached its peak at the E:S ratio of 30 U/g protein. This E:S value was properly sufficient for enzyme-substrate saturation, elevating the release of bioactive peptides contributing to the α-amylase inhibition activity of the clam meat hydrolysate. When E:S ratios were below 30 U/g protein, an inadequate amount of enzyme for the hydrolysis might result in a low release level of bioactive peptides in the hydrolysate, lowering the α-amylase inhibition activity [43]. Subsequently, when the E:S ratio exceeded 30 U/g protein, the inhibitory activity exhibited a decreasing trend. Higher enzyme amounts are believed to degrade the bioactive peptides present, consequently diminishing the bioactivity of the hydrolysate [44].

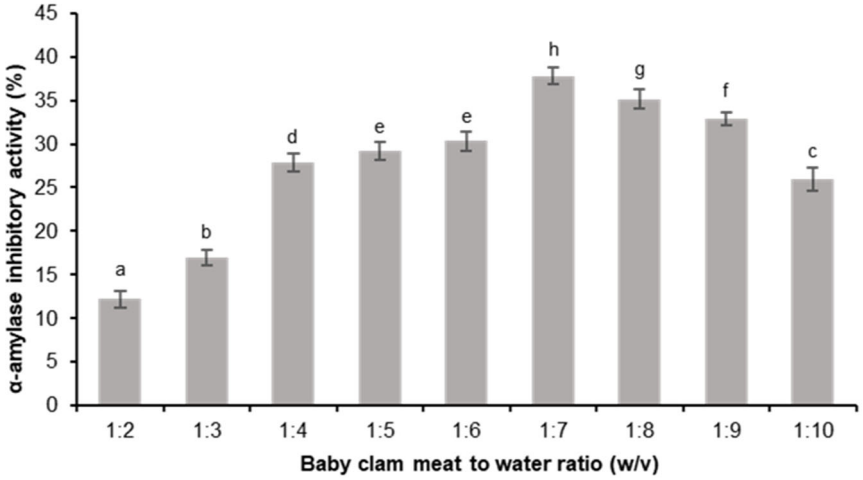

Fig 4. Effect of baby clam meat to water ratio on α-amylase inhibitory activity of the hydrolysate. Bars with different letters indicate significant differences ($p < 0.05$)

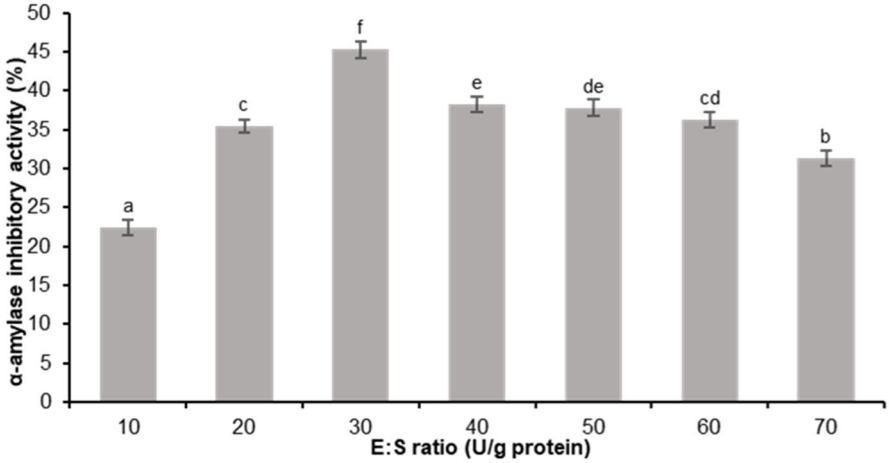

Fig 5. Effect of E:S ratio on α-amylase inhibitory activity of the hydrolysate. Bars with different letters indicate significant differences ($p < 0.05$)

Mudgil et al. [43] reported that within the studied E:S ratio range from 1% to 5%, the α-amylase inhibition activity of camel skin gelatin hydrolysate achieved the maximum at an E:S ratio of 3%. With earthworm protein hydrolysate, Castañeda-Pérez et al. [44] showed the α-amylase inhibition activity of the hydrolysate peaks at the E:S ratio of 600 U/g protein. The difference may be due to various protein sources and hydrolysis agents. 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 α-amylase inhibitory capacity of the baby clam meat hydrolysate is directly proportional to the hydrolysis duration within the initial period (2–4 h). However, over 4 h, an inverse proportion was observed (Fig. 6). This finding was supported by previous observations [37-38,44]. The extended hydrolysis time allowed for more extensive enzymatic breakdown of proteins, leading to an increased number of small peptides in the hydrolysate [45], enhancing binding affinity for the enzyme's active site. Larger peptides face steric hindrances in their interactions with the enzyme [46]. Castañeda-Pérez et al. [44] further suggested that low molecular weight peptides can readily access and inhibit the catalytic site of α-amylase, altering its conformation and disrupting the binding between the enzyme and its natural substrate. However, excessively prolonged hydrolysis (5, 6, and 7 h) may result in a decline in Alcalase activity substrate depletion [45], therefore reducing the α-amylase activity of the hydrolysate.

From these experiments, the suitable condition for Alcalase hydrolysis to obtain a baby clam meat hydrolysate

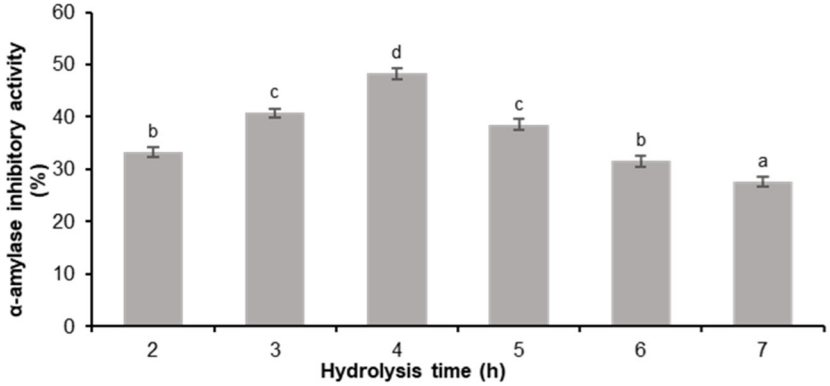

Fig 6. Effect of hydrolysis time ratio on α-amylase inhibitory activity of the hydrolysate. Bars with different letters indicate significant differences ($p < 0.05$)

with the highest α-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 4 h. Under this condition, the hydrolysate exhibited a maximum α-amylase inhibitory activity of 48.33 ± 1.44%, 1.35 times lower than acarbose. Therefore, the baby clam meat hydrolysate could be considered a natural alternative α-amylase inhibitor.

■ CONCLUSION

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 α-amylase inhibitors. It is worth highlighting that the hydrolysates demonstrated a notable IBC of 1246.2 ± 44.0 μg Fe^{2+}/g protein, as well as an α-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 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 their bioactivities.

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■ CONFLICT OF INTEREST

The authors declare no conflict of interest.

■ AUTHOR CONTRIBUTIONS

Conceptualization: Tam Dinh Le Vo; Funding acquisition: Tam Dinh Le Vo; Project administration: Tam Dinh Le Vo; Supervision: Tam Dinh Le Vo; Visualization: Bao Chi Vo; Writing-review and editing: Tam Dinh Le Vo; Writing-original draft preparation: Bao Chi Vo; Data curation: Bao Chi Vo; Resources: Tam Dinh Le Vo; Investigation: Tam Dinh Le Vo, Bao Chi Vo, Thuy Thi Le, An Thi Tuong Tran; Formal analysis: Tam Dinh Le Vo, Bao Chi Vo, Thu Huynh; Validation: Tam Dinh Le Vo, Bao Chi Vo; Methodology: Tam Dinh Le Vo, Bao Chi Vo. All authors agreed to the final version of this manuscript.

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