The Annals of the University Dunarea de Jos of Galati
Fascicle VI – Food Technology (2025), 49(1), 43-57

ORIGINAL RESEARCH PAPER

INVESTIGATIONS ON THE ZINC-BINDING CAPACITY AND FUNCTIONAL PROPERTIES OF THE BABY CLAM (*CORBICULIDAE SP.*) MEAT PROTEIN HYDROLYSATE

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Received on 24 December 2024
Revised on 14 March 2025

Abstract

In this research, Alcalase was employed to create a zinc-binding protein hydrolysate from baby clam (*Corbiculidae sp.*) meat. The hydrolysis conditions involved a ratio of 1:7 (w/v) for clam meat to water, an enzyme to substrate (E:S) ratio of 50 U/g protein, and a hydrolysis duration of 5 h. These conditions yielded a protein hydrolysate with 27.55±0.26% degree of hydrolysis (DH) and 1277.44±12.19 μg Zn^{2+}/g protein zinc-binding capacity (ZnBC), the latter being 1.80 times lower than that of ethylenediaminetetraacetic acid disodium salt (Na_2EDTA). The hydrolysate had a high concentration of hydrophobic amino acids, making up 87.43% of the total amino acids, which enhanced its emulsifying properties. In the pH range 3.0-8.0, the emulsifying activity index (EAI) and emulsifying stability index (ESI) were in ranges 61.04-72.64 m^2/g and 19.63-67.46 min, respectively. The hydrolysate also demonstrated moderate foaming properties with a maximum foaming capacity (FC) and foaming stability (FS) of 21.67±1.44% and 15.83±1.44%, respectively. These findings indicate the potential for the development of a novel food supplement sourced from baby clam meat, having zinc-binding capacity, amino acids, as well as foaming and emulsifying properties.

Keywords: baby clam meat, emulsifying property, foaming property, protein hydrolysate, zinc-binding capacity

https://doi.org/10.35219/foodtechnology.2025.1.03

Introduction

The *Corbiculidae sp.*, a bivalve mollusc commonly referred to as baby clam, is widely distributed in the brackish water and freshwater habitats of Vietnam. As reported by the Vietnam Fisheries Association, this clam species is harvested from the wild throughout the year, however, it is purchased at a relatively low price of around 20,000 VND per kilogram (0.8 USD/kg) (Vo *et al.*, 2025). Despite its low market value, the high protein content of 54.48 ± 2.10% on a dry weight basis makes the baby clam an attractive subject for further research (Vo *et al.*, 2025). The present study aims to explore ways to add value to this protein-rich baby clam by utilizing its protein effectively.

In recent times, there has been a growing interest in exploring alternative natural sources of zinc supplements to address the widespread issue of zinc deficiency, which affects over 2 billion people globally (Katimba *et al.*, 2023). Zinc is recognized as a crucial micronutrient due to its multifaceted roles in the human body. It serves as a cofactor for over 300 enzymes, contributes to the stabilization of cell membranes and protein structures, and acts as a stimulant for zinc-sensing receptors, triggering intracellular signaling pathways (Katimba *et al.*, 2023). Zinc deficiency can lead to severe consequences, including impaired growth, loss of appetite, impairment of taste and smell, disruption of various organ systems, and compromised humoral and cell-mediated immune function (Zheng *et al.*, 2023). The causes of zinc deficiency can be attributed to mutations in zinc transporter genes or inadequate absorption of zinc (Shi *et al.*, 2024). The latter may arise from the presence of food antinutrients such as phytate, which forms insoluble complexes with zinc ions in the gastrointestinal tract, the low solubility of zinc ions in the slightly alkaline environment of the small intestine, and certain diseases that lead to zinc loss (Katimba *et al.*, 2023).

To address zinc deficiency, inorganic zinc compounds such as zinc sulfate, zinc chloride, and zinc oxide, as well as organic weak acid zinc salts like zinc gluconate and zinc citrate, have been utilized as zinc supplements (Shi *et al.*, 2024). However, their use in foods is limited due to their tendency to generate an unpleasant metallic off-flavor, potential damage to the gastrointestinal mucosa, and impaired intestinal absorption (Katimba *et al.*, 2023). Furthermore, Wang *et al.* (2023) demonstrated the toxicity of zinc gluconate to cells via an *in vivo* study. Additionally, a clinical trial conducted by Fallah *et al.* (2015) revealed that children experienced nausea and cramping when consuming a single dose of 50 mg of zinc in the form of zinc sulfate. Recently, there has been growing interest in exploring food-derived zinc-binding peptides as potential new zinc supplements due to their ability to form stable and soluble peptide-Zn^{2+} complexes, which can prevent precipitation of zinc ions and thereby enhance the efficiency of zinc absorption (Katimba *et al.*, 2023).

Zinc-chelating peptides are initially inactive within the intact protein structure but become active upon release from the protein matrix during processes such as enzymatic hydrolysis, fermentation, and chemical hydrolysis (Vo *et al.*, 2023b). Among these methods, enzymatic hydrolysis is favored in the food industry due to its low cost, safety, simplicity, ease of control, and environmental friendliness

(Cejudo-Bastante *et al.*, 2022). The resulting protein hydrolysates exhibit advantages such as non-toxicity, absence of side effects, and ready availability (Cejudo-Bastante *et al.*, 2022). The enzymatic hydrolysis of proteins leads to a reduction in molecular weight and exposure of ionizable and hydrophobic groups, thereby altering the bioactivities and functional properties of the hydrolysates (Thirulogasundar *et al.*, 2024). Owing to the specificity of proteases, different proteases generate hydrolysates with varying characteristics (Wu *et al.*, 2024). One of the most commonly used proteases in the food industry is Alcalase, a commercial liquid enzyme preparation derived from *Bacillus licheniformis* (Eberhardt *et al.*, 2021). Alcalase is known to hydrolyze a broad range of potential sites, including Phe, Trp, Tyr, Glu, Met, Leu, Ala, Ser, Lys, and preferentially for large uncharged residues such as Gln and Asn (Halavach *et al.*, 2024). The side chains of these amino acids have been confirmed to participate in the coordination bonds between the peptides and zinc ions (Katimba *et al.*, 2023). This enzyme preparation has been employed to produce zinc-binding hydrolysates from oyster (Z. Wang *et al.*, 2021) and featherback skin gelatin (Vo *et al.*, 2023b).

In addition to the choice of protease, Islam *et al.* (2022) highlighted the significance of hydrolysis conditions, such as the material-to-liquid ratio, E:S ratio, and incubation time, on the amino acid profiles and bioactivities of the resulting hydrolysates.

Concurrently, foaming and emulsifying properties are two crucial attributes of protein hydrolysates, particularly for their incorporation into various food products such as desserts, sauces, beer, bread, coffee, meringues, cakes, ice cream, milk beverages, toppings, and mousses (Mohammadi *et al.*, 2022; Thirulogasundar *et al.*, 2024). Protein hydrolysates derived from grass turtle (Islam *et al.*, 2021), *Acetes japonicus* (Vo *et al.*, 2020), and featherback skin (Vo *et al.*, 2023a) showed promising potential as foaming and emulsifying agents.

Taking these factors into consideration, this study aims to explore the effect of hydrolysis condition (baby clam meat-to-water ratio, E:S ratio, and hydrolysis time) on the ZnBC of the clam meat hydrolysate. Additionally, its characteristics, including DH, amino acid composition, and foaming and emulsifying properties, were studied. This study indicates clam meat hydrolysate could be used as a ZnBC ingredient, supporting minor mineral nutrition. Furthermore, based on the hydrolysate's amino acid profile, it could be beneficial for food formulations. Besides, it could be added to food products to improve their functional features. The preliminary data of this research would boost the hydrolysate's application in related industries.

Materials and methods

Materials

Fresh baby clams were obtained from a market in Ho Chi Minh City, Vietnam, and transported on ice to the laboratory at Ho Chi Minh City University of Technology - Vietnam National University Ho Chi Minh City. The clams were blanched at 90-

95°C for 5 min, and the meat was recovered, washed, and drained for 10 min. The meat was then ground and stored at -20°C until further use.

Alcalase® 2.5L with optimal pH of 7.5 and optimal temperature of 55ºC was procured from Novozymes (Denmark). All reagents used in the experiments were of analytical grade, purchased from Sigma-Aldrich and Merck. Double-distilled water was utilized throughout the experiments.

Analysis of chemical composition

The proximate composition including moisture, protein, lipid, carbohydrate and ash of the baby clam meat was determined using the (AOAC, 2023): AOAC 950.46B, AOAC 990.03, AOAC 920.39, AOAC 986.25, and AOAC 920.15, respectively. Furthermore, its Cd and Pb contents were analyzed following the AOAC 999.11 procedure (AOAC, 2023) while Hg content was detected according to the method 7473 of U.S. Environmental Protection Agency.

Preparation of protein hydrolysates from baby clam meats

The hydrolysis of clam meat using Alcalase was carried out following the procedure described by Vo *et al.* (2020). The clam meat was combined with distilled water to achieve the desired clam meat-to-water ratio. This mixture was then heated at 90°C for 10 min to inactivate any endogenous enzymes present. Subsequently, the pH of the mixture was adjusted to 7.5 using either 1M NaOH or 1M HCl solution, followed by heating to 55°C. Alcalase (activity of 1464.60±63.19 U/ml, measured using casein as the substrate) was then added at the appropriate E:S ratio. After the specified hydrolysis time, the mixture's temperature was raised to 90°C for 10 min to deactivate the Alcalase enzyme. The resulting mixture was centrifuged, and the supernatant was collected. The soluble protein content and DH of the supernatant were determined following the guidelines provided by Nwachukwu and Aluko (2019). A control sample was prepared the same with no Alcalase addition and its ZnBC was then determined.

The effects of clam meat:water ratio, E:S ratio and hydrolytic time on the ZnBC of the clam meat hydrolysate

To investigate the effect of the clam meat-to-water ratio, the clam meats were hydrolyzed under fixed conditions, including an E:S ratio of 40 U/g protein and a hydrolysis duration of 4 h. The clam meat-to-water ratios varied from 1:2 to 1:10 (w/v). The resulting hydrolysates were analyzed for their ZnBC, and based on these results, an optimal clam meat-to-water ratio was selected.

A similar procedure was followed to evaluate the influence of the E:S ratio and hydrolysis time on the ZnBC of the hydrolysate. In these experiments, the E:S ratios varied within the range of 20 to 70 U/g protein, while the hydrolysis time from 2 to 7 h.

Determination of ZnBC

The ZnBC was evaluated using a modified version of the procedure described by Vo *et al.* (2023b). Initially, the hydrolysates were demineralized using a macroporous resin (Amberlite IRC-748I sodium form, Acros) and diluted to a soluble protein content of 1 mg protein/mL using 4-(2-hydroxyethyl)-1-piperazineethanesulfonic

acid (HEPES)-KOH buffer (40 mM, pH 7.5). A mixture consisting of 2 mL of the diluted hydrolysate, 1 mL of 8 mM dithiothreitol (DTT) solution, and 1 mL of 0.1 mM $ZnSO_4$ solution was incubated at 50-60°C for 30 min. Subsequently, 0.1 mL of 2 mM 4-(2-pyridylazo) resorcinol solution was added to the mixture, and the absorbance at 500 nm was recorded using a UV-vis spectrometer (UV-vis 752, China). The ZnBC of the sample was calculated using the equation 1.

$$\text{ZnBC}\left(\mu\text{g } Zn^{2+}/\text{g protein}\right)=\frac{A_b - A_s}{A_b} * \frac{m_{Zn^{2+}}}{m_{protein}} \quad (1)$$

where A_s and A_b are the sample and blank absorbances, respectively. In the blank sample, 2 ml of HEPES-KOH buffer (40 mM, pH 7.5) was used instead of 2 ml of the test sample. m_{Zn2+} indicates the initial weight of Zn^{2+}; $m_{protein}$ represents the weight of protein of the hydrolysate. A 1 mg/ml solution of Na_2EDTA in HEPES-KOH buffer (40 mM, pH 7.5) served as comparison standard.

Amino acid composition analysis

The amino acid composition of the baby clam meat hydrolysate was analyzed following the procedure described in our previous study (Vo *et al.*, 2020). Briefly, the hydrolysate was subjected to complete hydrolysis using a 6M HCl solution for 23 h at 110 ± 2°C. Subsequently, the amino acids in the resulting solution were separated using ion-exchange chromatography, followed by reaction with Ninhydrin for detection. For the determination of free amino acids present in the sample, standard amino acid solutions were used, with the absorbance measured at 440 nm for proline (Pro) and 570 nm for the remaining amino acids.

Determination of FC and FS

To assess the foaming property of the hydrolysate, the approach outlined by Vo *et al.* (2020) was adopted. A handheld whisk-type frother (RoHS, China) was used to froth 20 mL of the hydrolysate (10 mg protein/ml) in a 100 ml beaker for 1 min. The total volume of the frothed hydrolysate was measured after 30s, and the FC was determined using the equation provided below:

$$\text{FC (\%)}= \frac{A - B}{B} * 100 \quad (2)$$

where A indicates the volume of the frothed hydrolysate (mL); B represents the initial volume of the hydrolysate (mL).

The frothed sample was allowed to stand at 20°C for 3 min, and its volume was then recorded. The FS was calculated using the following equation:

$$\text{FS (\%)}= \frac{A_t - B}{B} * 100 \quad (3)$$

where A_t expresses the volume of the hydrolysate after standing (mL); B depicts the initial volume of the hydrolysate (mL). An albumin solution (10 mg/mL, in distilled water) was used as a standard, and the preparation of the standard before determination of its FC and FS was the same as the procedure for sample preparation.

Determination of EAI and ESI

The emulsifying property of the hydrolysate was assessed according to the protocol described by Vo *et al.* (2020). A mixture consisting of 5 mL of vegetable oil and 15 mL of the hydrolysate (10 mg protein/ml) was prepared. The pH of this mixture was adjusted to 3.0, 4.0, 5.0, 6.0, 7.0, or 8.0 using 0.1M NaOH or 0.1M HCl solution, followed by homogenization for 1 min. After that, at 0 and 10 min, a 50 µL of the emulsion was drawn from the bottom of the container and mixed with 4.95 mL of a 1 mg/mL sodium dodecyl sulfate solution, and the absorbance of the resulting mixture was measured at 500 nm (UV-vis 752, China). The EAI and ESI of the hydrolysate were calculated using the equation below:

$$\text{EAI}\left(m^2/g\right)= \frac{2*2.303*A_0}{0.25*\text{protein weight (g)}} \quad (4)$$

$$\text{ESI (min)}= \frac{A_0*\Delta t}{A_0 - A_{10}}*100 \quad (5)$$

where $\Delta t = 10$ min; A_0 and A_{10} are the absorbances of the samples taken at 0 min and 10 min after homogenization, respectively; $2*2.303*A_0/1$ (m^2) represents oil-water interface area; 1 (cm) denotes the pathlength of the cuvette; 0.25 is volumetric ratio of oil phase in the emulsion. The same procedure was applied to prepare the sodium caseinate standard (10 mg/mL, in distilled water) before determining its EAI and ESI.

Data analysis

The data were presented as means ± standard deviations obtained from triplicate experiments. One-way analysis of variance (ANOVA) was performed on the data and the significance was determined using Tukey method ($p < 0.05$), which was conducted using the Statgraphics Centurion 18 software.

Results and discussion

Chemical composition of the baby clam meat

Profile of chemical composition of the baby clam meat included 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). The protein content of the baby clam meat was nearly equal to that of oyster freeze-dried powder and tilapia skin, which have been utilized as protein sources for producing zinc-binding peptides in the literature (Meng *et al.*, 2021; Z. Wang *et al.*, 2021).

Alternatively, as a benthos, the accumulation of heavy metals in the clam was a major concern for consumers. However, in this study, the Cd and Pb contents of the clam meat were 0.20 mg/kg and 0.14 mg/kg, respectively. These were significantly lower than the maximum limits set by the Vietnamese Technical Regulations 8-2:2011/Ministry of Health (Vietnam) (2.0 mg/kg for Cd and 1.5 mg/kg for Pb) and Commission Regulation (Europe) 2023/915 (1.0 mg/kg for Cd and 1.5 mg/kg for

Pb). Additionally, no detectable levels of mercury (Hg) were observed in the baby clam meat with the method quantification limit of 0.01 mg Hg/kg.

Based on these results, the baby clam meat could be considered as a safe and competitive material to obtain zinc-binding protein hydrolysates.

Effect of baby clam meat:water ratio on the ZnBC of the clam meat hydrolysate

The clam meat:water ratio determines the amount of water and substrate amount for the Alcalase hydrolysis, influencing the enzyme-substrate interactions and reaction rate, and thus impacting the ZnBC of the resulting hydrolysate. As shown in Figure 1, the ZnBC of the hydrolysates increased as the clam meat-to-water ratio increased from 1:2 to 1:7 (w/v), followed by a subsequent decrease. This result is consistent with the theory of enzyme-catalyzed reaction rates, which states that at a constant enzyme quantity, the reaction rate proportionally augments with the substrate concentration up to a certain value before stabilizing despite further increases in substrate concentration (Megrous *et al.*, 2024). Similar findings were reported by Eberhardt *et al.* (2021) and Ding *et al.* (2020), who assumed that the decrease in bioactive peptide content is caused by substrate inhibition at high substrate concentrations. Additionally, the clam meat-to-water ratio of 1:7 (w/v) may have provided sufficient water for effective enzyme-substrate interactions and optimal viscosity for enzymatic reactions, thereby enhancing the bioactivity of the hydrolysate (Vo *et al.*, 2020). Consequently, the clam meat-to-water ratio of 1:7 (w/v) was selected for further investigations.

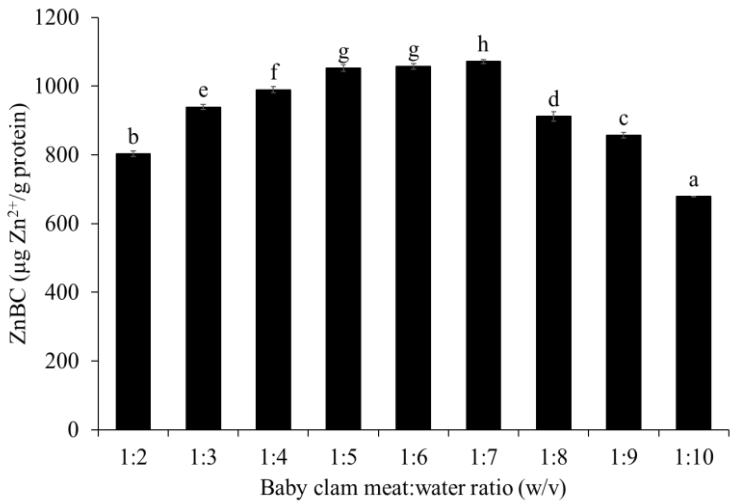

Figure 1. Effect of clam meat:water ratio on ZnBC of the hydrolysate. Bars with different letters indicate significant differences ($p<0.05$).

Effect of E:S ratio on the ZnBC of the clam meat hydrolysate

The study observed a basic upside-down U-shaped relationship between the E:S ratio, ranging from 20 to 70 U/g protein, and the ZnBC of the hydrolysate (Figure 2). Similar impacts of the E:S ratio on the ZnBC of hydrolysates derived from octopus scraps and featherback skin gelatin were reported by Fang *et al.* (2019) and Vo *et al.* (2023b), respectively. This can be attributed to the fact that, at a fixed quantity of clam meat, an increase in enzyme amount facilitates its combination with the substrate, improving the efficiency of enzymatic hydrolysis and therefore, enhancing the bioactivity of the hydrolysate (Wu *et al.*, 2024). However, when the E:S ratio exceeds a certain threshold, competitive inhibition caused by the excessive amount of enzyme hinders the formation of Alcalase-clam meat protein complexes and decelerates the hydrolysis process (Ding *et al.*, 2020). Additionally, it is presumed that a high enzyme amount may lead to an overdone hydrolysis, potentially causing damage to the zinc-binding peptides released during the early stages of hydrolysis, reducing the ZnBC of the hydrolysate (Vo *et al.*, 2021). Consequently, an appropriate E:S ratio of 50 U/g protein was chosen for further experiments.

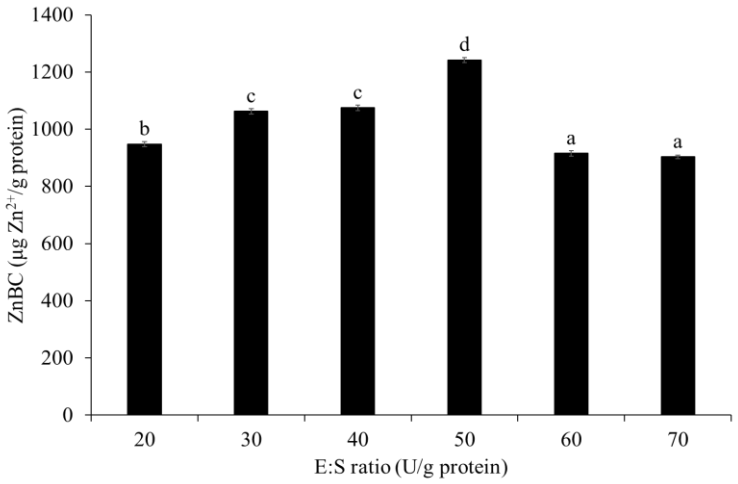

Figure 2. Effect of E:S ratio on ZnBC of the hydrolysate. Bars with different letters indicate significant differences ($p<0.05$).

Effect of hydrolysis time on the ZnBC of the clam meat hydrolysate

As illustrated in Figure 3, the ZnBC of the clam meat hydrolysate reached a maximum of 1272.80 ± 6.14 µg Zn^{2+}/g protein at a hydrolysis time of 5h. It is believed that during the early stages of Alcalase hydrolysis, the rate of hydrolysis is high, resulting in the release of numerous bioactive peptides from intact clam meat proteins into the hydrolysate (Kumari *et al.*, 2023). However, when the hydrolysis duration was extended to 7 h, a decline of ZnBC of the hydrolysate was observed (Figure 3). This decrease can be attributed to factors such as substrate depletion, product inhibition, or further hydrolysis of peptides into inactive fragments or amino

acids (Wali *et al.*, 2020). Similar effects of hydrolysis time on ZnBC have also been reported by Ke *et al.* (2021) and Vo *et al.* (2023b) for tilapia skin collagen, rapeseed, and featherback skin gelatin, respectively.

Under the selected hydrolysis condition, the ZnBC of the hydrolysate increased by 2.84 times compared to that of the unhydrolysed clam meat proteins (ZnBC = 450.12 ± 11.37 µg Zn^{2+}/g protein). This can be attributed to the generation of small peptides and an increase in ionizable amino and carboxyl groups during protein hydrolysis, providing additional binding sites on peptide molecules for zinc ions (Thirulogasundar *et al.*, 2024).

Compared to the ZnBC of Na_2EDTA, a popular zinc carrier, the hydrolysate's ZnBC was 1.81 times lower. Although Na_2EDTA exhibits high ZnBC, EDTA-Zn complexes have been reported to be unable to cross the basolateral membrane in an animal model, potentially reducing zinc bioavailability (Hall and King, 2023). In contrast, peptide-Zn complex has been shown to be cotransported with lipids through the plasma membrane (Hall and King, 2023).

Furthermore, the clam meat hydrolysate's ZnBC was similar to that of the featherback skin gelatin hydrolysate (Vo *et al.*, 2023b). However, it was 3.83 and 4.18 folds lower than the ZnBC of Alcalase hydrolysates derived from tilapia skin collagen (Ke *et al.*, 2021) and oyster (Z. Wang *et al.*, 2021), respectively. This difference could be attributed to the high DH value of the clam meat hydrolysate (DH = 27.55±0.26%), which may have comprised more small peptides with reduced zinc-chelating capabilities (Vo *et al.*, 2023b).

Overall, the clam meat hydrolysate showed promising potential as an alternative zinc supplement.

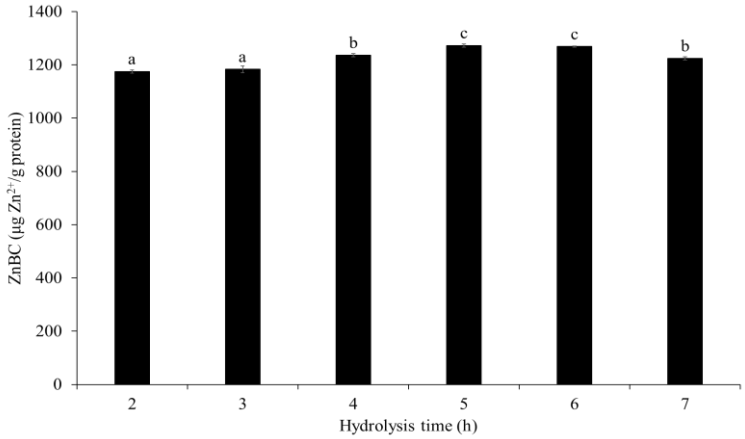

Figure 3. Effect of hydrolysis time on ZnBC of the hydrolysate. Bars with different letters indicate significant differences ($p<0.05$).

Amino acid composition of the baby clam meat hydrolysate

The amino acid composition of the clam meat hydrolysate, presented in Table 1, plays a crucial role in determining the bioactivity of the hydrolysate. Phe was found

to be the predominant amino acid in the hydrolysate, constituting 61.57% of the total amino acids. The binding of Phe with zinc ions has been reported by Z. Wang *et al.* (2021). Additionally, the presence of Phe and other hydrophobic amino acids in the hydrolysate such as Gly, Ala, Val, Leu and Ile, which make up of 87.43% of the total amino acids, could form a barrier that shields peptide-zinc complexes from water molecules (Vo *et al.*, 2021). Meanwhile, carboxyl group of Asp and Glu, imidazole ring of His and amino group of Lys in the containing peptides contributed mainly to the chelation of zinc ions (Vo *et al.*, 2023b). From a nutritional perspective, the hydrolysate could provide 7 essential amino acids, accounting for 31.69% of the total amino acids.

Table 1. Amino acid profile of the clam meat hydrolysate.

Essential Amino acids	Content (mg/100 ml)	Non-essential Amino acids	Content (mg/100 ml)
His	0.46 ± 0.21	Ala	3.5 ± 0.99
Ile	0.14 ± 0.06	Asp	0.75 ± 0.34
Leu	0.96 ± 0.44	Glu	2.14 ± 0.61
Lys	0.53 ± 0.24	Gly	2.27 ± 0.64
Phe	25.48 ± 3.98	Ser	1.03 ± 0.29
Thr	0.29 ± 0.13		
Val	3.83 ± 0.67		

Emulsifying property of the baby clam meat hydrolysate

Proteins, owing to their amphiphilic feature, exhibit significant emulsifying properties that could be further enhanced by enzymatic degradation, which exposes their hidden hydrophobic groups (Mohammadi *et al.*, 2022). Understanding the emulsifying properties of a hydrolysate is crucial for its potential application as a natural emulsifying agent in the food industry (Mohammadi *et al.*, 2022). The emulsifying property is typically assessed through EAI and ESI, which represent the protein's ability to promote the dispersion of the oil phase in water and the stability of the emulsion against creaming, flocculation, and coalescence, respectively (Vo *et al.*, 2020). These indexes are sensitive to the pH of the hydrolysate, which can alter the solubility, surface charge, and surface hydrophobicity of the peptides (Kumari *et al.*, 2023). In this study, within the tested pH range from 3.0 to 8.0, EAI of the clam meat hydrolysate reached its peak of 72.64 ± 1.63 m^2/g at pH 8.0 while its ESI reached the highest value of 67.46 ± 3.76 min at pH 4.0 (Figure 4). The highest EAI at alkaline pH was also reported by Kumari *et al.* (2023) and Vo *et al.* (2023a) for hydrolysates from pink perch by-products and featherback skin gelatin, respectively. They highlighted that the unfolding of polypeptides and the formation of negative charges at alkaline pH facilitated the orientation of hydrophilic and hydrophobic peptides at the oil-water interface (Kumari *et al.*, 2023; Vo *et al.*, 2023a). On the other hand, the peak ESI at pH 4.0 may be attributed to increased hydrophobic interactions between peptides and the lipid core (Vo *et al.*, 2023a). The maximum EAI and ESI values of the clam meat hydrolysate were found to be 2.55 and 1.02

folds higher than those of sodium caseinate, a widely used commercial protein-based emulsifier (Vo *et al.*, 2023a). The high emulsifying property of the hydrolysate was facilitated by its high content of hydrophobic amino acids (as shown in Table 1) and the appropriate amount of small peptides (with a DH of 27.55±0.26%). Islam *et al.* (2021) has underlined the role of hydrophobic peptides in enhancing the stability of emulsions. Meanwhile, small peptides have been highlighted for their high mobility and capacity for adsorption onto the surface of oil droplets, thereby lowering the interfacial tension and preventing droplet aggregation (Vo *et al.*, 2020). These findings lead to a conclusion that the baby clam meat hydrolysate could serve as a natural emulsifier in foods.

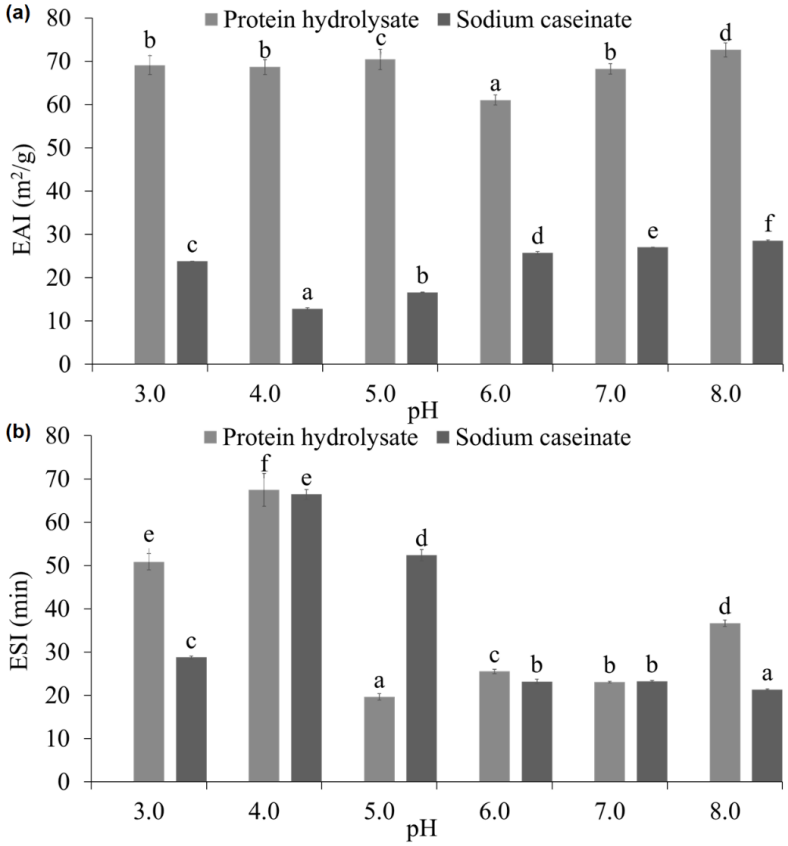

Figure 4. Emulsifying property of the baby clam meat hydrolysate. The same color bars with different letters indicate significant differences ($p<0.05$).

Foaming property of the baby clam meat hydrolysate

FC and FS are two critical parameters when evaluating the foaming property of protein hydrolysates (Thirulogasundar *et al.*, 2024). FC indicates the volume of generated foam relative to the initial volume of the sample, while FS depicts the

percentage of foam remaining after a defined time. The FC of a protein/peptide is determined by their ability to relocate to the air-water interface, along with its unfolding and reshaping at the interface (Thirulogasundar *et al.*, 2024). Meanwhile, formation of an indestructible protein layer around air bubbles due to interactions between hydrophobic parts of the proteins led to a steady rise in FS (Vo *et al.*, 2020). Additionally, it was highlighted that the environmental pH greatly impacted the foaming property of hydrolysates by altering the ionization ability of peptides (Vo *et al.*, 2021). In this study, the highest FC and FS values of the clam meat hydrolysate were obtained at pH 3.0, reaching 21.67±1.44% and 15.83 ± 1.44%, respectively (Figure 5). Whereas, the minimum FC and FS values of the hydrolysate were observed at pH 8.0. These findings align with the results reported by Huang *et al.* (2024) and Dong *et al.* (2024), who explained that the excessive increase in net charge of the protein at high pH values weakens the protein–protein hydrophobic interaction, resulting in decreased FS.

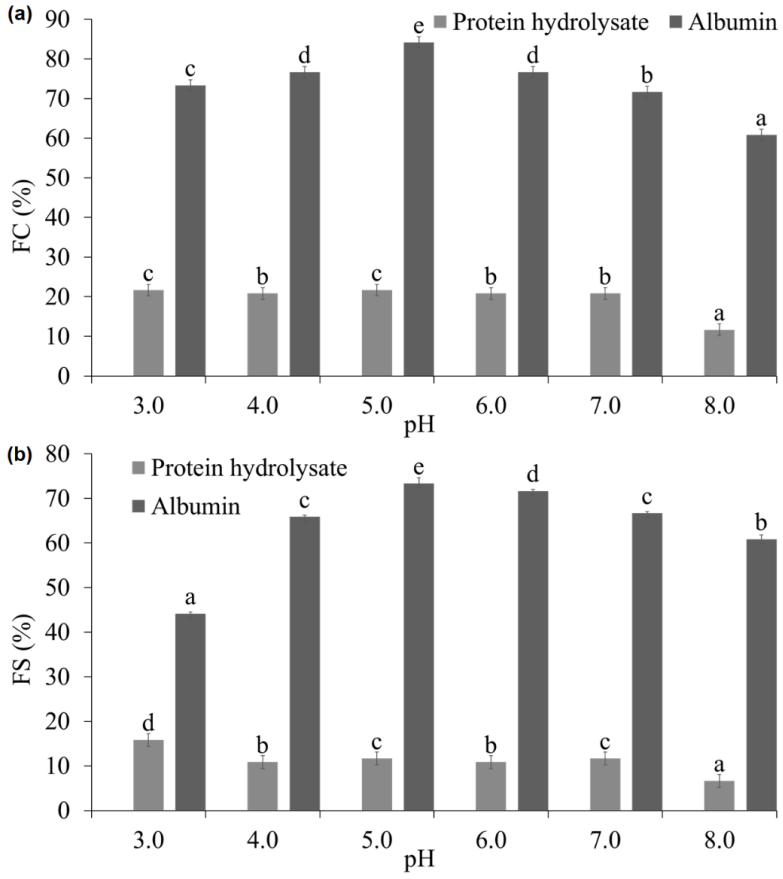

Figure 5. Foaming property of the baby clam meat hydrolysate. The same color bars with different letters indicate significant differences ($p<0.05$).

When compared to albumin, which is the most widely used foaming agent in foodstuff, the clam meat hydrolysate exhibited lower FC and FS within the tested pH range of 3.0-8.0. Specifically, the FCs and FSs of the hydrolysate were 2.81 – 7.21 and 2.78 – 11.00 times lower than those of albumin, respectively (Figure 5). However, the hydrolysate in this study demonstrated superior foaming properties compared to the *Acetes japonicus* hydrolysate reported in our previous study (Vo *et al.*, 2020). Overall, the hydrolysate derived from baby clam meat could be considered as a foaming enhancer for some food products.

Conclusions

This study identified the optimum Alcalase hydrolysis condition for producing a baby clam meat hydrolysate with desirable properties. The condition includes a clam meat:water ratio of 1:7 (w/v), E:S ratio of 50 U/g protein and a hydrolysis duration of 5 h. Under this condition, the resulting baby clam meat hydrolysate achieved the value of ZnBC of 1277.44 ± 12.19 μg Zn^{2+}/g protein, which is 1.80 times lower than that of Na_2EDTA. The hydrolysate also displayed superior emulsifying properties compared to sodium caseinate. Furthermore, it showed moderate foaming properties. Another noteworthy aspect of the hydrolysate is its ability to supply 7 essential amino acids for human nutrition. The findings from this study serve as a foundation for subsequent investigations, including identifying peptide sequence, peptide action mechanisms within a cell or an animal, conducting clinical trials, or exploring potential applications in functional foods or nutraceuticals.

Acknowledgments

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.

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