



## Research Article

### Experimental evaluation of the process of protein removal from waste shrimp shells using papaya and pineapple juice solution

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#### Abstract

This research aims to improve the quality of chitin obtained from process protein extraction for shrimp shells. Previous studies showed that for the synthesis of chitosan, deproteinization, and demineralization is carried out by alkali and acid treatment, combined with heating, which affects the biosecurity of the material. Therefore, this study aims to remove protein from waste shrimp shells to purify chitin using a simple and safe green chemical approach (using pineapple and papaya juice solutions) and safe method. The raw shrimp shells were treated in steps: deproteinization with pineapple and papaya solutions for 12, 24, 36, and 48 h, varying immersion volumes from 10 mL to 60 mL. The experimental results showed that the protein in the shrimp shell was removed at 7.37% for freshwater shrimp (7.14%), higher than that of saltwater shrimp. The pineapple juice solution removed the protein better than the papaya juice.

**Keywords:** deproteinization, shrimp wastes, bromelain, parain, chitin extraction.

#### 1. INTRODUCTION

According to data from the Ministry of Industry and Trade, the amount of shrimp waste in Vietnam is estimated to be around 325,000 tons annually. With the growth of the shrimp processing industry, shrimp waste could exceed 450,000 tons by 2025, corresponding to more than 1,000 tons of waste being discarded daily [1]. This is an abundant seafood waste source; however, it could become a severe environmental problem if not properly treated or reused. Seafood waste contains many organic substances, soluble proteins, and

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carbohydrates. Therefore, these wastes can negatively impact human health, biodiversity, and the ecological environment if not correctly handled. The main components of shrimp heads are protein, minerals, fats, a small amount of carotenoid pigments, and especially a considerable amount of chitin. These components can generate substantial profits if adequately processed.

Chitin is the second most abundant natural polysaccharide after plant-derived cellulose [2]. Chitin has a similar structure to cellulose, but instead of hydroxyl groups, it contains acetamide groups. It exhibits unique characteristics, including high biocompatibility, biodegradability, antimicrobial, anticancer, and antioxidant activities [3]. During seafood processing, chitin undergoes hydrolysis to produce Chitosan ( $C_6H_{11}NO_4$ )<sub>n</sub>, and chitosan can be used to create various essential products in agriculture, industry, medicine, textiles, wastewater treatment, and cosmetics [4].

The primary material for producing chitin is the cuticle of crustaceans, mainly shrimp and crab shells. However, these cuticles have a complex combination of chitin fibers and proteins. Protein impurities must be removed to ensure chitin has high purity and good application potential. Many researchers have developed various methods to reduce the protein content in chitin [5]. The deproteinization and demineralization processes are typically performed using alkaline and acid treatments combined with heating [6]. However, this method has a significant disadvantage because it causes environmental pollution [7]. Therefore, using protease enzymes found in pineapples (bromelain) and papayas (papain) is expected to minimize ecological pollution and residual chemicals after extraction. Pineapples mainly contain water and carbohydrates, and they are important nutrient sources for humans, which include sugars, organic acids, fiber, essential minerals (Cu, Mg, and Mn), and vitamins (A, B, and C). Additionally, pineapples contain the enzyme bromelain, which can break down proteins. Similarly, papayas also contain many nutritional components like pineapples, including vitamins (A, C, B1, and B2), Ca, Fe, K, and fiber, and notably, the enzyme papain is very effective in breaking down proteins.

Based on the above analysis, we conducted this study to extract chitin from shrimp shells using green chemical methods. The main objective is to experimentally evaluate the effectiveness of protein separation to obtain highly pure chitin for chitosan production by using eco-friendly chemicals, such as protease from fruit wastes (pineapple, papaya, etc.). This approach aims to reduce the use of hazardous chemicals, minimize environmental pollution, and create added value for shrimp products.

## 2. MATERIALS AND METHODS

### 2.1. Materials

Shrimp heads were collected at the wholesale markets in Quy Nhon City. Next, they were cleaned of any remaining meat and dried (referred to as dried shrimp shells). They were then dried at 40°C and ground into powder for use in experiments.

The pineapple and papaya used in this study were overripe and of little economic value. They were purchased at the market in Quy Nhon City.

## 2.2. Methods

Preparing pineapple and papaya extract: After removing the leaves, the pineapples and papayas were thoroughly washed, crushed, and then extracted using distilled water at a ratio of pineapple/papaya to water of 1:3. The mixture was stirred evenly on a magnetic stirrer for 1 min before chitin processing.

### 2.2.1. Removing protein in shrimp heads and shells

200 g of grounded dried shrimp heads and shells were soaked 200 g in pineapple or papaya extract, with the different volumes: 20 mL, 30 mL, 40 mL, 50 mL, and 60 mL over periods 12, 24, 36, and 48 h. Next, the hydrolysis was performed on a water bath at 37°C, and the enzymes were not added to the control samples. The weight of the sample for hydrolysis is about 1 kg. After hydrolysis, the hydrolysis solution was heated at 100°C for 15 min to inactivate the enzyme. The hydrolyzate was then filtered and analyzed for the protein content. Finally, after filtration, the residue was dried at 80°C for 24 h.

### 2.2.2. Determination of protein content by Microbiuret method

Protein content in shrimp shells was determined by the Microbiuret method. Shrimp shell materials were incubated in 3% NaOH at 80°C for 6 h. Then, the sample was filtered and washed with 3% NaOH. The filtrate was centrifuged at 4500 rpm/15 min, and the supernatant was reacted with Microbiuret reagent for 15 min before measuring the OD value at 355 nm. The protein content in 1 mL of solution was calculated based on the standard curve. Finally, the content of protein was calculated as follows:

$$\% \text{ protein} = \frac{V.C.100}{m.1000.(100 - Mc)/100}$$

Where: V: Volume of the sample after diluting (ml), C: Protein concentration calculated by Microbiuret standard curve (mg/ml), m: The weight of the sample test (g), Mc: Sample moisture (%).

### 2.2.3. Determination of the chemical composition of shrimp shells

Determination of protein content: Protein content was determined according to the Kjehdal method, TCVN 3705-1990.

Determination of lipid content: Lipid content in raw materials and semi-finished products

products and aquatic products were determined according to TCVN 2703:2009.

Determination of mineral salt content: Mineral salt content was determined according to the method of determining total ash content: TCVN 5105:2009.

Determination of chitin content: Chitin content was determined according to Cho et al. (1998) [9].

## 3. RESULTS AND DISCUSSION

### 3.1. Chemical composition of saltwater and freshwater shrimp shells

The chemical composition of freshwater and saltwater shrimp shells was analyzed using selected analytical methods, and the results are shown in Table 1.

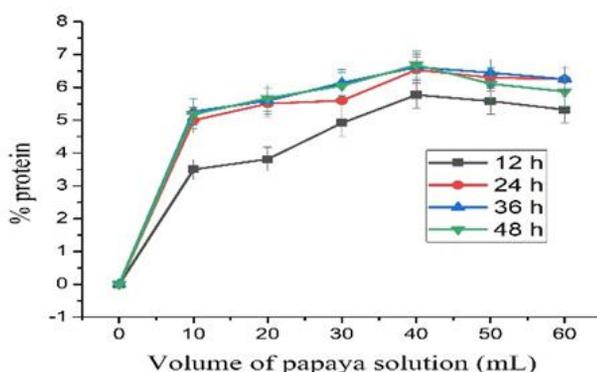
**Table 1.** Chemical composition of shrimp shells

Chemical composition (%)	Saltwater shrimp shells	Freshwater shrimp shells
Chitin	17.14 ± 0.13	16.05 ± 0.09
Mineral	29.14 ± 0.12	27.34 ± 0.14
Protein	36.45 ± 0.21	32.24 ± 0.12
Lipid	1.93 ± 0.16	0.57 ± 0.06

As shown in Table 1, we found that protein is the main component in saltwater and freshwater shrimp shells. The protein in saltwater shrimp shells (36.45% ± 0.21) is higher than those of freshwater shrimp shells (32.24% ± 0.12). Otherwise, lipids only account for a small amount in the composition of shrimp shells and heads (0.16÷1.93%). This result shows similarities with the results of Nguyen Cong Minh (protein 45.2%; mineral 21.5%; chitin 17.2%) [9] and Tran Quoc Huy et al. (protein 38.5%; chitin 17.3%; mineral 30.6%) [11]. In addition, compared to the study of Tran Thi Luyen et al., the chemical composition of dried black tiger shrimp shells is different, specifically mineral salt (45.16%), chitin (27.50%), and protein (23.25%). This difference may be due to the material composition of black tiger shrimp shells being different from saltwater and freshwater shrimp shells.

### 3.2. Protein removal in freshwater shrimp shells by papaya extract

Grounded freshwater shrimp shells were soaked in papaya extract, and the results of protein removal are shown in Figure 1.



**Figure 1.** Efficiency of protein removal from freshwater shrimp shells using papaya extract

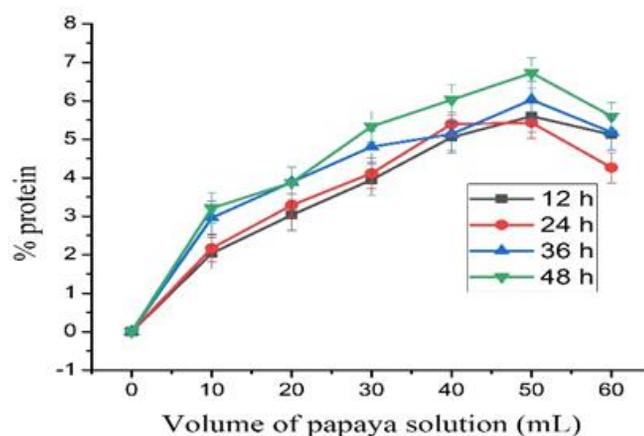
The results showed that the incubation volume affected the efficiency of protein removal in freshwater shrimp shells with papaya extract. The protein removal efficiency was evaluated every 12 h, and the result showed that the volume of papaya extract at 50 mL showed the highest protein removal efficiency (6.72% at 48 h). It could be explained that this volume was suitable for enzyme activity and removing proteins from shrimp shells. Initially, the protein still in shrimp shells will be hydrolyzed by the papain enzyme in papaya extract. Interestingly, when increasing the volume of papaya extract to 60 mL, although the protein removal efficiency is proportional to the incubation time from 12 h to 48 h, the amount of protein removed at each time point was smaller than the amount of protein

removed by 50 mL of papaya extract at the corresponding time points. This result might be related to factors that influence the ability of the papain enzyme in papaya extract. When the volume of the enzyme solution changed, the enzyme concentration, water activity, and pH were also changed, and these factors may have affected the enzyme's ability to decompose protein and extract protein from shrimp shells.

The incubation time with papaya extract also affected the protein separation efficiency. When increasing from 12 h to 48 h, the amount of protein removed rose from 5.59% to 6.72%. This result indicated that the incubation time affects the efficiency of protein removal. Furthermore, when the incubation time is from 12 to 36 h, it might be insufficient for the enzyme to expose and cleave proteins, so the efficiency of protein removal is low. However, when increasing the incubation time to 48 h, the enzyme has enough time to contact and cleave proteins in the sample, so the amount of protein removed increases.

### 3.3. Protein removal in saltwater shrimp shells by papaya extract

The experiment was conducted similarly in Section 3.2, and the result of protein removal in saltwater shrimp shells by papaya extract is shown in Figure 2.

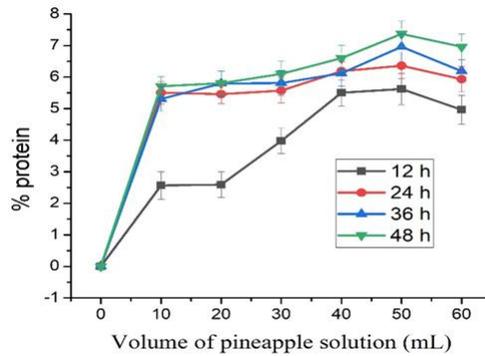


*Figure 2. The efficiency of protein removal from saltwater shrimp shells using papaya extract*

When removing protein in saltwater shrimp shells with papaya extract, we found that only the incubation time of 12 h showed a significant difference. Otherwise, the incubation time was higher than 24 h; there was no difference. In this study, we found that the protein removal efficiency from saltwater shrimp shells only depends on the volume of papaya extract and does not depend on the incubation time. The volume of papaya extract at 40 mL showed the highest protein removal efficiency compared with the remaining volumes; at 12 h, 24 h, 36 h, and 48 h, the amount of protein removed was 5.76%, 6.53%, 6.61%, and 6.68%, respectively.

### 3.4. Protein removal in freshwater shrimp shells by pineapple extract

The experimental steps were carried out similarly, but papaya extract was replaced with pineapple extract. The results of protein removal in freshwater shrimp shells by pineapple extract are shown in Figure 3.



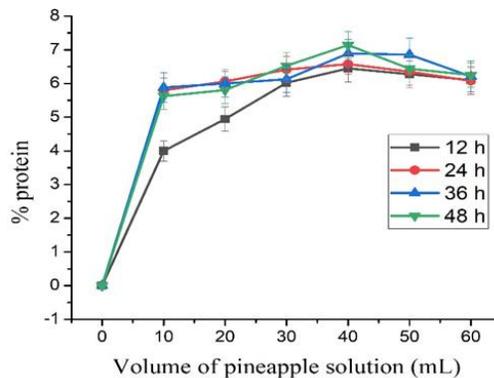
**Figure 3.** The efficiency of protein removal from freshwater shrimp shells using pineapple extract

As a result, the 48-hour incubation time gave the highest protein separation efficiency of 7.37%, followed by 36 hours, which reached 6.96%; 24 hours, which reached 6.36%; and finally, 12 hours, which gave the highest protein removal efficiency of 5.62%. Thus, we found that the 48-hour incubation time had the best protein removal efficiency.

Compared with the treatment results for papaya extract, we found that the efficiency of protein removal from shrimp shells from both pineapple and papaya extracts is affected by the extract volume and incubation time.

### 3.5. Protein removal in saltwater shrimp shells by pineapple extract

The protein removal efficiency in saltwater shrimp shells with pineapple extract was evaluated, and the result is shown in Figure 4.



**Figure 4.** The efficiency of protein removal from saltwater shrimp shells using pineapple extract

For saltwater shrimp shells processed with pineapple extract, we also found that incubation time had little significant effect on protein removal efficiency. The protein removal efficiency from shrimp shells in this study was affected by the volume of pineapple extract. At a volume of 40 mL, the protein removal efficiency was highest at most incubation times. The amount of removal protein at 12 h, 24 h, 36 h, and 48 h was 6.44%, 6.57%, 6.89%, and 7.14%, respectively.

### 3.6. Comparison of the effectiveness of different enzymes

The results showed that the protein removal efficiency by enzymes on freshwater shrimp was higher than that of saltwater shrimp. However, the incubation time of both types of shrimp was the same (which showed the highest efficiency at 48 h). This may be related to the composition and mineral ratio in the two types of shrimp, which affected the protein separation efficiency.

Comparing the protein removal efficiency in two types of saltwater and freshwater shrimp shells using pineapple and papaya extracts showed that pineapple extract exhibits better protein removal efficiency than processing with papaya extract. This can be explained by the higher protease activity in pineapple (bromelain) compared to papaya (papain). This result indicated that using fruit waste containing biological compounds (proteases) to process protein showed a great advantage and potential in chitin purification.

*Table 2. Protein removal efficiency compared with previous studies*

Protein removal efficiency (%)	Conditions	Methods	Sources
10.2	Enzyme SEB Digest F35P at 55°C, with a reaction time of 24 h.	Semi-bio	[10]
30.9	NaOH 5.0 M, at 160°C with a reaction time for a few days	Chemical	[11]
88.1	NaOH 3%, a ratio of material/solution = 1/5 (w/v), at 70°C, with a reaction time of 12 h.	Chemical	[9]
7.37	40 mL pineapple extract, with an incubation time of 48 h.	Enzyme	This study

Compared to other protein removal methods (Table 2), although the protein removal efficiency of this enzyme method is not higher than that of chemical or semi-bio-based methods, utilizing enzymes from fruit waste to remove protein from shrimp shells is considered a green, environmentally friendly, economically viable, and sustainable approach. Among these advantages, we recognize that eliminating and minimizing residual chemicals after extraction is essential. This has scientific meaning and practical implications for implementing green chemistry in pilot and industrial-scale production.

## 4. CONCLUSION

This study has demonstrated the potential for obtaining high-purity chitin from saltwater and freshwater shrimp shells using a green chemical approach by utilizing bioactive compounds from plant wastes (pineapple, papaya). The results have shown the potential of protein removal from shrimp shells to obtain high-purity chitin without using chemical methods. By using protease from plant sources, including bromelain from pineapple and papain from papaya, the experimental results have demonstrated that, with the same soaking time for shrimp shells (saltwater, freshwater: 40 mL) in equal volumes of protease extract (from papaya and pineapple: 48 h), the protein removal efficiency of freshwater shrimp using papaya extract (6.68%) is higher than that of saltwater shrimp

(6.72%). Conversely, when tested with pineapple extract, the protein removal efficiency of freshwater shrimp (7.37%) is higher than saltwater shrimp's (7.14%).

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