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Study on the treatment of protein and mineral removal in Whiteleg shrimp chitin

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Abstract
Crude chitin produced from shrimp shells, crab shells, and other seafood processing by-products often has low purity, which does not meet the requirements of certain high-tech applications. This paper introduces a study on the chemical treatment of chitin to remove proteins and minerals, yielding purified chitin. The research investigated the effects of factors such as chemical concentration, solvent-to-material ratio, and treatment time on the purification efficiency. Experimental planning and process optimization were also conducted.
The results showed that under the conditions of protein removal using a 4.8% NaOH solution for 9.6 hours with a solvent-to-material ratio of 11.4:1, and demineralization using a 3.5% HCl solution for 11.5 hours with a solvent-to-material ratio of 11:1, purified chitin was obtained. The purified chitin contained 0.34% residual protein and 0.42% residual minerals, meeting the requirements of specific chitin processing technologies.

Keywords: Deproteinization, demineralization, purified chitin, raw chitin

Introduction
In nature, chitin primarily exists in arthropods and mollusks such as shrimp, crab, lobster, squid, etc. In these aquatic species, chitin is mainly found in the shell, carapace, and claws. In the seafood processing industry, these parts are utilized to produce chitin after extracting the protein of nutritional value. The technology for producing chitin from these by-products is generally simple. The resultant product, referred to as crude chitin, is of low quality and still contains proteins, minerals, and other impurities, which do not meet the requirements for use as raw materials in certain advanced technologies. This study aims to determine the technological conditions to process crude chitin into high-purity chitin, which can be used as raw material for advanced applications, such as the production of nanochitin.

Research Methods and Materials

1. Research Materials
The crude chitin used for the research was derived from the shells of white-leg shrimp with a moisture content of 9-11%, protein content of 4.0-4.5%, and mineral content of 4.2-5%. This chitin was processed from the fresh shells of white-leg shrimp, which are by-products from the shrimp meat production process of a seafood processing company in Binh Thuan. The chemicals used include hydrochloric acid, sodium hydroxide, sodium carbonate, copper sulfate, and reagents of analytical grade. The equipment utilized includes a UV-VIS spectrophotometer, furnace, thermostatic water bath, and drying oven.

2. Research Methods
2.1. Study on the Protein removal Process
To remove protein, the study applied the following process:

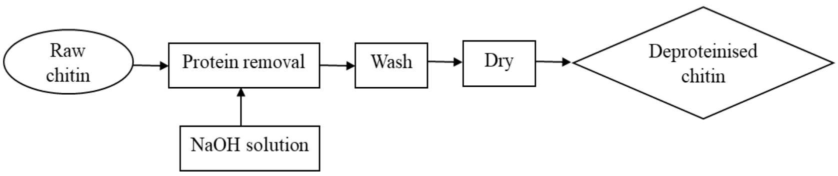


Fig 1: Flowchart of the process for removing protein from raw chitin [5, 7, 8]

According to the process described above, raw chitin is mixed with a NaOH solution at a specific ratio and soaked for a determined period at room temperature with continuous stirring. The mixture is then washed with clean water until all residual NaOH is removed, and the product is collected and dried.
In the raw chitin treatment process, the step of protein removal plays a decisive role in the degree of protein separation from chitin. Therefore, the study focuses on investigating this step.

Single-Factor Experiments
The single-factor experiments were conducted as follows: 1-5 grams of raw chitin samples were mixed with NaOH solution at the designed ratios for each experimental sample. The protein removal process was carried out under various conditions of NaOH concentration, treatment time, and NaOH solution-to-chitin ratio as specified for each sample. After the process, the samples were washed with clean water until all residual NaOH was removed, the products were collected, and then dried. The dried products were analyzed to determine the remaining protein content using the Microbiuret method.
The protein removal step from raw chitin was investigated for the effects of NaOH concentration, treatment time, and solvent-to-material ratio on the protein removal efficiency. The efficiency of the protein removal process was assessed based on the remaining protein content in the product. The

results from the single-factor experiments were used as a basis for experimental design and optimization to determine the optimal conditions.

2.2. Research on the Demineralization Process

To remove minerals, the study applied the following process:

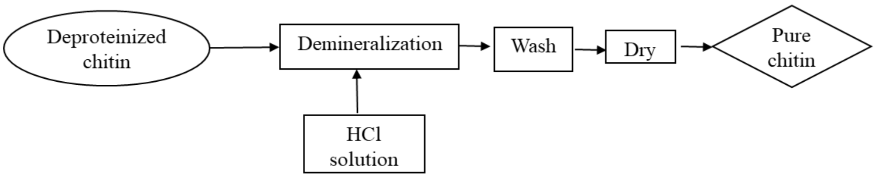


Fig 2: Flowchart of the demineralization process [5, 7, 8]

According to this procedure, the protein-removed chitin was mixed with HCl solution at a specific ratio, and demineralization was carried out for a predetermined time at room temperature with stirring. The mixture was then washed with clean water to remove all excess acid, and the product was collected and dried.

Single-factor experiments

The experiments were conducted as follows: 1-5 grams of chitin samples were mixed with HCl solution at specific ratios designed for each experimental sample. The demineralization process was carried out with varying HCl concentrations, treatment times, and HCl solution-to-chitin ratios. After the process, the samples were washed with clean water to remove excess HCl, the products were collected, and dried. The dried samples were analyzed for ash content following the AOAC method.

The demineralization step was studied to evaluate the effects of HCl concentration, treatment time, and solvent-to-material ratio on demineralization efficiency. The parameter used to assess the efficiency was the ash content of the product. The results of single-factor experiments were used as the basis for experimental planning.

2.3. Analytical Methods

Protein Content Determination: Using the Microbiuret method [4, 7].

Ash Content Determination: Following the AOAC method, 1990 [6].

Moisture Content Determination: Using the drying method to constant weight at 105 °C, based on AOAC, 1990 [6].

2.4. Experimental Design Method

The Box-Behnken experimental design was used to identify influencing factors and optimize parameters.

Results were processed using Design-Expert 11.1 statistical software.

Results and Discussion

1. Study of the protein removal process

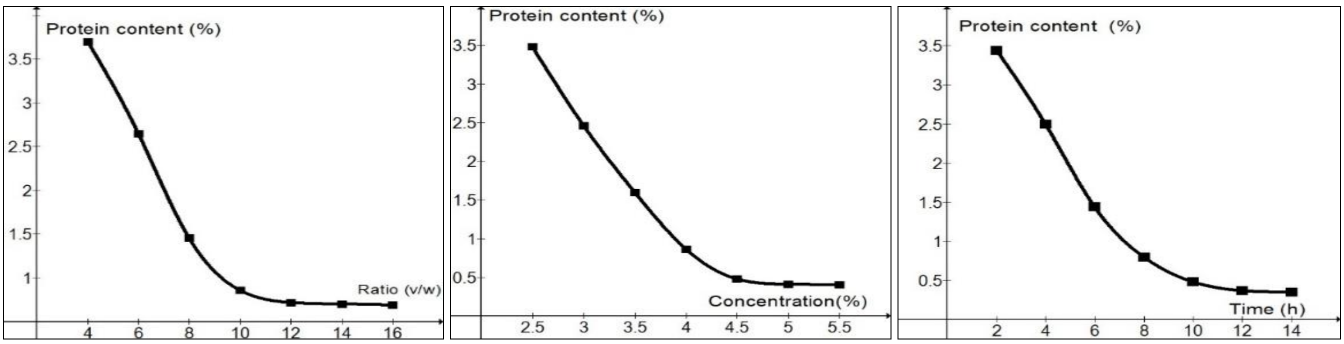
1.1. Single-factor experiments

Effect of NaOH Solution-to-Chitin Ratio on Protein Removal Efficiency

The effect of the NaOH solution-to-chitin ratio on protein removal efficiency was investigated using raw chitin samples mixed with 4% NaOH solution. The solution-to-material ratio was varied from 4:1 to 16:1 in increments of 2:1.

The study was conducted with two fixed parameters: NaOH concentration (4%) and treatment time (10 hours).

The protein removal efficiency was evaluated based on the residual protein content in the product. The results are illustrated in the graph (Figure 3a).



3a ***3b*** ***3c***

Fig 3: The Effects of NaOH Solution-to-Chitin Ratio (3a), NaOH Solution Concentration (3b), and Processing Time (3c) on Protein Content

Observations: Treatment with NaOH solution significantly reduced the protein content compared to the raw material. From a ratio of 4:1 to 10:1, the protein content decreased rapidly, while from 10:1 to 12:1, the decrease slowed down. Beyond a ratio of 12:1, the reduction in protein content was negligible. A ratio of 10:1 was selected for further studies.

Effect of NaOH solution concentration on protein removal efficiency

To study the effect of NaOH solution concentration on protein removal efficiency, experiments were conducted with concentrations ranging from 2.5% to 5.5%, with an increment of 0.5%. Processing time and solution-to-chitin ratio were fixed at 10 hours and 10:1, respectively. The efficiency was evaluated based on the residual protein content in the product. The results are shown in Figure 3b.

Observations: As shown in Figure 3b, increasing the NaOH concentration from 2.5% to 4.0% resulted in a rapid decrease in protein content. From 4.0% to 5.0%, the reduction slowed, and beyond 5.0%, the protein content remained nearly unchanged. A concentration of 4.5% NaOH was chosen for subsequent studies.

Effect of Processing Time on Protein Removal Efficiency
The processing time for chitin in NaOH solution was investigated within a range of 2 to 14 hours, with an increment of 2 hours. The NaOH solution-to-chitin ratio and NaOH concentration were fixed at 10:1 and 4.5%, respectively. The efficiency was evaluated based on the residual protein content in the product. The results are displayed in Figure 3c.

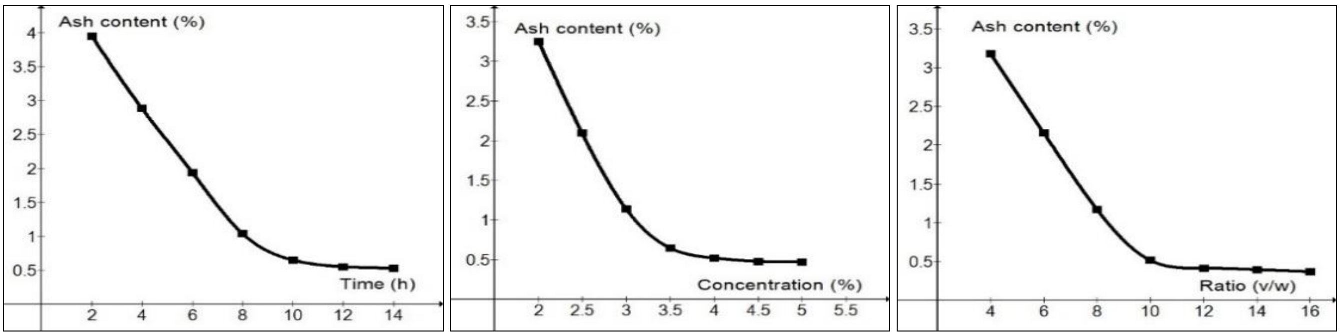
Observations: As shown in Figure 3c, increasing the processing time from 2 to 8 hours led to a rapid decrease in residual protein content. Between 8 and 12 hours, the reduction slowed, and beyond 12 hours, the decrease became negligible. A processing time of 10 hours was selected for further studies.

1.2. Experimental Design and Optimization of the Protein Removal Step
The Box-Behnken design was used to study three factors: NaOH concentration, processing time, and NaOH solution-to-chitin ratio, with 17 experiments, including five replicates at the center point. The objective function was the residual protein content (Y1). The residual protein content in the final chitin product was modeled using a second-order equation as follows:

$$Y_1 = 0.476- 0.2776A - 0.2865B - 0.2664C + 0.1045AB + 0.0998AC + 0.0925BC + 0.1806A^2 + 0.1079B^2 + 0.1536C^2 \quad (1)$$

Optimization was performed using the "desirability function" algorithm proposed by Derringer and Suich. Using Design-Expert 11.1 software, the optimal conditions were determined to be: NaOH concentration of 4.8%, processing time of 9.6 hours, and solution-to-material ratio of 11.4 ml/g. Under these conditions, the residual protein content in the final chitin product was 0.34%.

2. Study on the demineralization step
2.1. Single-factor experiments
Effect of Processing Time with HCl Solution on Demineralization Efficiency
The effect of processing time on demineralization efficiency was investigated within a range of 2 to 14 hours, with an increment of 2 hours. The solution-to-chitin ratio and HCl concentration were fixed at 10:1 and 3.5%, respectively. The efficiency was evaluated based on the residual mineral content in the product. The results are presented in Figure 4a.



4a ***4b*** ***4c***

Fig 4: Effects of Processing Time (4a), HCl Acid Solution Concentration (4b), and HCl Acid Solution to Chitin Ratio (4c) on Residual Mineral Content

Observations: As shown in Figure 4a, when the processing time for chitin increases from 2 hours to 8 hours, the residual mineral content in the final product decreases rapidly. Between 8 hours and 12 hours, the reduction in residual mineral content slows down, and after 12 hours, the decrease becomes negligible. Based on these results, a processing time of 10 hours was selected for subsequent experiments.

Effect of HCl Solution Concentration on Demineralization Efficiency
Experiments to investigate the effect of HCl solution concentration on demineralization efficiency were conducted with HCl concentrations ranging from 2% to 5%, with intervals of 0.5%. The processing time and the solution-to-material ratio were fixed at 10 hours and 10:1 (v/w), respectively, in these experiments. The demineralization efficiency was evaluated based on the residual mineral content in the final product, and the results are illustrated in Figure 4b.

Observations: As shown in Figure 4b, when the HCl solution concentration increases from 2% to 4%, the residual mineral content in the final product decreases significantly. Between 4% and 4.5%, the reduction in residual mineral content slows down, and beyond 4.5%, the decrease becomes nearly negligible. Based on these results, a concentration of 4.0% HCl was selected for further studies.

Effect of HCl Solution to Chitin Ratio on Demineralization Efficiency
To evaluate the effect of the HCl solution-to-chitin ratio on demineralization efficiency, experiments were conducted with solution-to-material ratios ranging from 4:1 to 16:1, with intervals of 2:1. The processing time and HCl concentration were fixed at 10 hours and 4.0%, respectively.

The demineralization efficiency was assessed based on the residual mineral content in the final product, and the results are depicted in Figure 4c.

Observations: As illustrated in Figure 4c, when the HCl solution-to-chitin ratio increases from 4:1 to 8:1, the residual mineral content in the final product decreases significantly. Between 8:1 and 12:1, the reduction slows down, and beyond 12:1, the decrease becomes negligible. Based on these results, a ratio of 10:1 was selected for further experiments.

2.2. Experimental Design and Optimization of the Demineralization Process

The Box-Behnken design was applied to optimize three factors: HCl solution concentration, processing time, and HCl solution-to-chitin ratio, with a total of 17 experiments, including 5 center-point replicates. The response variable was the residual mineral content (Y2). The residual mineral content in purified chitin was modeled using the following second-order polynomial equation:

$$Y_2 = 0.522 - 0.1268A - 0.2125B - 0.4263C + 0.035AB + 0.0625AC + 0.13BC + 0.0402A^2 + 0.1077B^2 + 0.2803C^2 \quad (2)$$

Optimization was performed using the "desirability function" algorithm proposed by Derringer and Suich. Using Design-Expert 11.1 software, the optimal conditions were determined to be: HCl concentration of 3.5%, processing time of 11.5 hours, and solution-to-material ratio of 11:1 (ml/g). Under these conditions, the residual mineral content in the final chitin product was 0.42%.

Conclusion

In the proposed deproteinization and demineralization process, the study determined the optimal technological parameters for deproteinization (processing time: 9.6 hours, solution-to-material ratio: 11.4:1, NaOH concentration: 4.8%) and demineralization (processing time: 11.5 hours, solution-to-material ratio: 11:1, HCl concentration: 3.5%). Under these conditions, the purified chitin had a residual protein content of 0.34% and an ash content of 0.42%. These parameters meet the quality requirements for advanced chitin-based applications such as nanocrystalline chitin and chitosan production [1-3, 9].

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