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A Modified Process for Deprotinization of Green Grab Shells (*Carcinus maenas*) Extraction of Chitin/Chitosan

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ABSTRACT

Chitin/Chitosan is a valuable and renewable biomaterial for development of numerous biomedical scaffolds and devices for regeneration of various tissues. There are so many chemical based extraction processes for isolation of chitin from grab shells. However, the strong chemicals such as Concentrated Hydrochloric acid and Sodium hydroxide solution are used for extraction and it results affecting the quality of chitin polymer and also environment related problems. Therefore, the eco-friendly modified chemical based extraction of Chitin/Chitosan is developed from the green crabs *Carcinus maenas*. The chemical based extraction of chitosan from green crab shells is developed and the two integral steps in the extraction such as demineralization and deproteinization are optimized. The optimized time for demineralization and deprotinization of green grab shells are 6-7 hours, and 1.5 hrs., respectively. In the step of deprotinization, 1 M concentration of sodium hydroxide solution is used. Furthermore, the percentage removal of protein from the crab shell is calculated based on the dry weight of the crab shell. The optimized temperature of deprotinization is found to be 45°C. The various limitations and recommendations to improve the process are mentioned. The chemical based extractions of chitosan from green crab shells are developed. The demineralization and deproteinization are optimized and controlled for efficient removal of calcium and proteins from the shells. In NaOH treatment, 1 M as reactive, the deproteinization process is satisfactory and 1-2 hours are necessary to perform a satisfying deproteinization. Moreover, to improve the extraction process, various recommendations are suggested.

KEY WORDS: Chitin/Chitosan, Demineralization, Deprotinization and Green crab shells.

1. INTRODUCTION

Green Crab shells are utilized as raw materials for the production of chitin and chitosan as a biomaterial for development of medical devices and scaffolds. The chitosan is produced by chemical based extraction from crab shells which has various sequence steps namely demineralization, deprotenization, deacetylation and decoluration/ depigmentation. Deprotenization is the crucial step in extraction process. Because it remains obscure about binding of proteins with chitin in the crab shells. As a consequence, the deproteinization is a complex process and lack of information of about interaction between protein and chitin and its chemistry in the literature. Deprtotenization by alkali method such as sodium hydroxide is a common method for removal of proteins from the shrimp shells. Based on the literature review, Sodium hydroxide is chosen as a deprotinization agent for this process. This report explains the optimized concentration of sodium hydroxide, contact time and temperature of the deproteinization process of crab shells and its limitations such process problems.

The exoskeleton of the crab shells contains three distinct layers namely epicutile, exocuticle and endocuticle. Generally, the exoskeleton has a high degree of mineralization, typically calcium carbonate as main constituent, in some case calcium phosphate. In exoskeleton, chitin fibrils are wrapped with proteins forms a form of fibers which is assembled further into a bundle of fibers in the exoskeleton. In addition to that, the calcium carbonate in the form of calcite deposited in the chitin–protein matrix.

Most of the chemical based extraction processes for chitosan from grabs shells are involved with harsh chemicals with high concentration and temperature. For example, 2M concentration of Hydrochloric acid used for demineralization process and 2M sodium hydroxide solution for deprotenization at 100^0 C and exposed to 24 hours, and these prolonged exposure of chitin and chitosan with harsh chemicals affect the quality of the polymer in terms of Molecular weight and degree of acetylation. Therefore, The eco-friendly and diluted concentration of Hydrochloric acid and NaOH chemical based extraction is developed for the isolation of chitosan from green crab shells.

Figure.1.Chemical Based Extraction of Chitin/Chitosan

2. EXPERIMENTAL METHODS

2.1. Demineralization: The demineralization process is carried out in 0.1 M hydrochloric acid and it has taken 6-7 hrs., to neutralize the calcium carbonate or calcite in the crab shells. This process has been developed and contributed by Dr. John Young, Professor Emeritus, Advanced Inorganic Chemistry, SMU, Halifax, NS, Canada. In my point of

view, after completion of demineralization of green crab shells, the crab shells are filmy and lost its brittleness. Therefore, it is confirmed that the demineralization of crab shells are completed.

2.2. Deprotenization: Deprotenization is the crucial step in extraction process. Because it remains obscure about binding of proteins with chitin in the crab shells. As a consequence, the deproteinization is a complex process and lack of information of about interaction between proteins and chitin and its chemistry in the literature. Deprotenization by alkali method such as sodium hydroxide is a common method for removal of proteins from the shrimp shells. Based on the literature review, Sodium hydroxide is chosen as a deprotinization agent for this process. This step explains the optimized concentration of sodium hydroxide, contact time and temperature of the deproteinization process of crab shells and its limitations such process problems.

2.3. Absorption Assay: The protein in the solution absorb at 280 nm due to the presence of aromatic amino acids in the proteins. Therefore, the quantification of the amount of protein in a solution is possible in a simple UV-Visible spectrometer. Absorption of radiation in the near UV by proteins depends on the Tyr and Trp content (and to a very small extent on the amount of Phe and disulfide bonds). Therefore, Absorbance at 280 nm varies greatly between different proteins. Total protein content in the sample is determined by standard curve of BSA proteins.

3. RESULTS

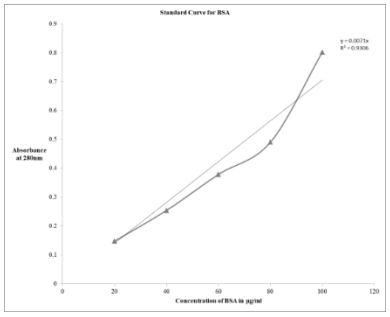
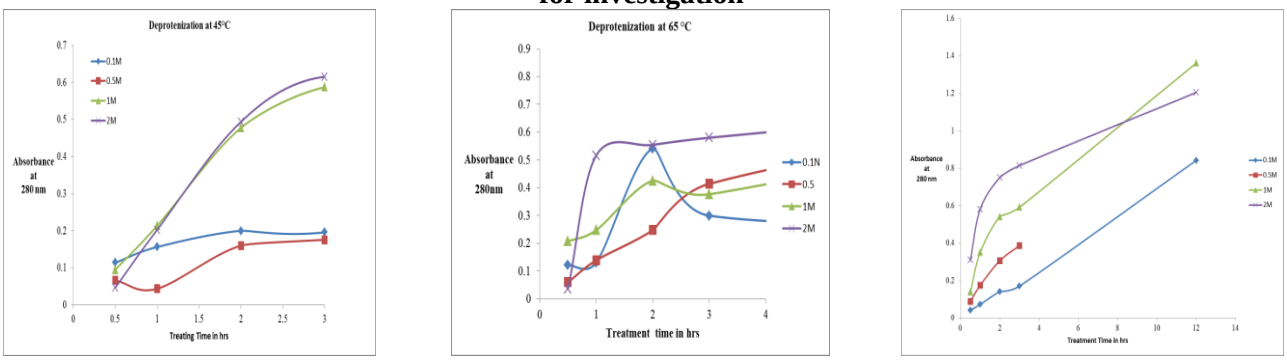


Figure.2.Standard Curve for BSA (Bovine Serum Albumin). In the graph, $R^2 = 0.9306$. The data is valid for investigation



Deprotinization at 45 °C **Deprotinization at 65 °C** **Deprotinization at 85 °C**

Figure.3.Deprotinization of green crab shells at different temperature and different concentrations of NaOH

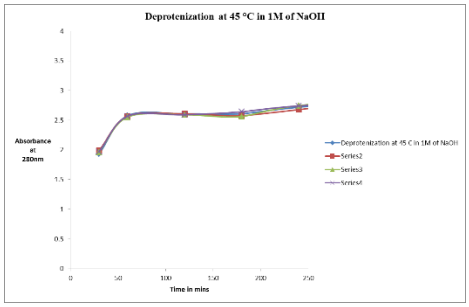


Figure.4.In this experiment, the absorbance is almost constant after 60 mins. So, mixing is decreased the treatment time. The equilibrium for protein release from crab shell was reached in 60 mins at Lab scale reactor

Influence of Temperature on Deproteinization:

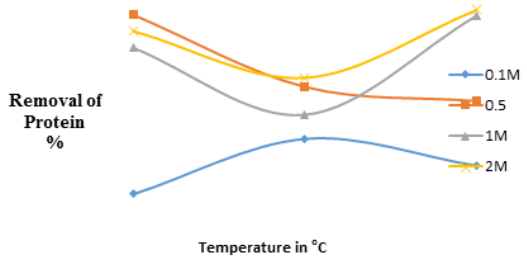


Figure.5.Temperature Vs Removal of Proteins in % for 3 hours treatment with various concentration of NaOH. In this figure, Temperature 45 °C favors high deprotenization and also high temperature deprotinization may cause a decrease in quality of chitin/chitosan polymer

DISCUSSION

The performance of deprotenization by chemical method such as sodium hydroxide depends the crab shell thickness, concentration of NaOH, treatment time, temperature and effective mixing. Based on these investigations, 1 M concentration of NaOH at temperature of 45 °C is suitable for deprotenization of green crab shells for treatment time of 1-2 hrs. However, the thickness of the shell and its protein content (usually 10%) plays major in performance. The literature reported that deprotenization of shrimp shell is performed with 1M NaOH for treatment time of 24 hrs. But these experiments are performed in 200ml beakers and effective mixing doesn't influence on the deprotenization process. Moreover, Shrimp shells are flimsy in nature and thinner than crab shell. In our case with thick crab shell, Good contact with NaOH solution is required and it can be provided only by effective mixing.

Limitations and Recommendations:

a) Absorption assay at 280nm is a simple method for finding protein releases from the crab shell. BSA (Bovine Serum Albumin) is not a suitable marker for evaluation of crab shell proteins in the solution. But it used to find the total protein content in the solutions. Further, micro syringe is used for preparation of working standard solution of BSA. I am not sure about how precise is it?. Micro pipette is highly recommended for protein analysis.

b) Active Mixing or stirring is provided for deprotenization process to minimize the treatment time. In addition, sometime there is a fluctuation in temperature in Hot air oven.

c) The complete chemical analysis of grab shell is highly recommended for various analytical purposes.

d) Various scientific approach on deprotenized shell such as SEM (Scanning Electron Microscopy), Nitrogen estimating method should be performed.

4. CONCLUSION

The chitin could be extracted from the green crab shells using a modified process of chemical extraction where the low concentration of harsh chemical involved. As the result, the eco –friendly process is developed and the further work on this investigation is to develop kinetic models on deproteinization and demineralization and then conceptual process design for feasibility analysis.

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