

Demineralization Optimization for Chitosan Synthesis from Crab Shell Waste (*Portunus pelagicus*)

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ABSTRACT

Chitosan, gelatin, albumin, and sodium alginate are examples of natural polymers that are often utilized as a basis material for polymeric nanoparticles. The deacetylation of chitin molecules produces the formation of chitosan. Chitin, protein, CaCO₃, MgCO₃, and astaxanthin pigment are all found in crab shells. Crab shell is an undervalued potential waste. Despite the fact that copious crab shell waste can be used to produce raw materials and industrial products. The previous research on the synthesis of chitosan from crab shells got a yield of 70.4%, and the following analysis showed that this high yield is due to the amount of calcium. The purpose of this study was to obtain the optimum concentration of demineralization (calcium removal) in the synthesis of chitosan from crab shells. The calcium content was measured after optimization with various solvent concentration variations using an atomic absorption spectrophotometer. The best demineralization optimization results use 3 M hydrochloric acid, which reduces calcium content by 97.75%.

Keywords: Demineralization, chitosan, portunus

Introduction

Crab shell is an undervalued potential waste, on the other hand, copious crab shell waste can be used to make raw materials and industrial products. Chitosan compounds, which have a high economic value, are found in crab shell waste, and the processed products can be used for a variety of uses (Zaghib et al., 2022; Perez et al., 2022). Chitosan can be used in a variety of modern sectors, including pharmaceutical, biochemical, cosmetic, food, and pharmaceutical (Liu et al., 2023; Yanat and Schoroen, 2021; Ismik et al., 2020).

Chitosan and its derivatives have been extensively studied as excipients in drug formulations and drug delivery systems in the pharmaceutical industry (Tissera et al., 2021). In addition, chitosan possesses significant inherent characteristics such as mucoadhesion, permeability enhancement, and antibacterial capabilities. Chitosan and its derivatives have the potential to be utilized in various forms such as solutions, gels, pills, capsules, fibers, films, and sponges. Significant advancements were occurring in the fields of biomedicine, nutraceuticals, and cosmeceuticals. Pharmaceutical formulations that include chitosan and its derivatives are suggested for use in slimming, managing body weight, and as cosmetics to improve the effectiveness of skincare, among other applications (Ali and Ahmed, 2018; Badwan et al., 2015; Morin-Crini et al., 2019).

From the skin of marine crustaceans or cephalopods, chitosan is extracted. Demineralization and deproteination are the processes used in separation to obtain chitin. After chitin is obtained, large quantities of NaOH or KOH are used in a deacetylation procedure to produce chitosan. The results of research conducted by Rahayu and Purnavita showed that the chitosan produced from crab shell powder was around 15-30% of its dry weight. Based on the results of their research, chitosan was isolated with a yield of 70.71% and a deacetylation degree of 76.6% (Mohadi et al., 2014; Ahyat et al., 2017). Previous research related to the optimization of chitosan synthesis has been with a variety of bases at the deacetylation stage. The results

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of this study showed that KOH reagent with a concentration of 50% gave chitosan results with the best characteristics (Indriani, 2022). Another study on the synthesis of chitosan from crab shells, obtained a yield of 70.4% and after further investigation, this large yield comes from the amount of calcium (Abirami et al., 2021; Hosney et al., 2022; Al Shaqsi et al., 2020). So it is necessary to optimize demineralization (calcium removal) in the synthesis of chitosan from crab shells.

Material and Method

Materials

The material used for this research is crab (*Portunus pelagicus*) shell waste obtained from PT Siger Jaya Abadi (Jalan Raya Tanjung Bintang No. 99, Serdang Village, Kec. Tanjung Bintang, South Lampung, Indonesia 35361) which has been identified as crab or crab shell waste (*Portunus pelagicus*).

Methods

Preparation of Materials

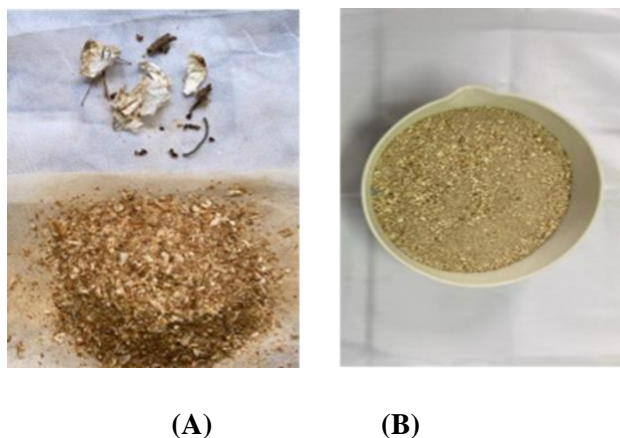


Figure 1. (A) Crab shell waste, (B) Crab shell powder

The process of preparing this material goes through 3 steps, namely the process of cleaning crab shell waste from feathers or dirt attached to the crab shell by rinsing using running water. Then proceed with the drying process using an oven at 37°C until dry and continue with the grinding process which aims to smooth or reduce the size of the shell waste obtained to make it easier in the process of making chitosan powder and sieved using mesh no.80 sieve which aims to equalize the particle size to speed up the reaction (Sidauruk, 2018).

After that, the calcium content of the crab shell waste powder was analyzed using an atomic absorption spectrophotometer (SSA) instrument.

Demineralization optimization

Crab shells weighing up to 100 g that have gone through a mesh no. 80 sieve are added in 2000 mL beaker glass, and the mineral removal process (demineralization) is carried out by adding HCl solution at various concentrations (1 M, 2 M, and 3 M). At room temperature, the mixture was agitated using a magnetic stirrer for 3 hours. The mixture was then filtered via the Whatman filter paper, and the residue in the filter paper was neutralized. Following that, the pH-neutralized residue is dried in an oven at 70°C to dry with a constant weight.

Result and Discussion

Chitosan is produced from chitin and has a chemical structure that is almost the same as chitin. The distinction between chitin and chitosan is that each ring of the chitin molecule has an acetyl group (-CH₃-CO) on the second carbon atom, whereas chitosan has an amine group (-NH) (Imtihani et al., 2020).

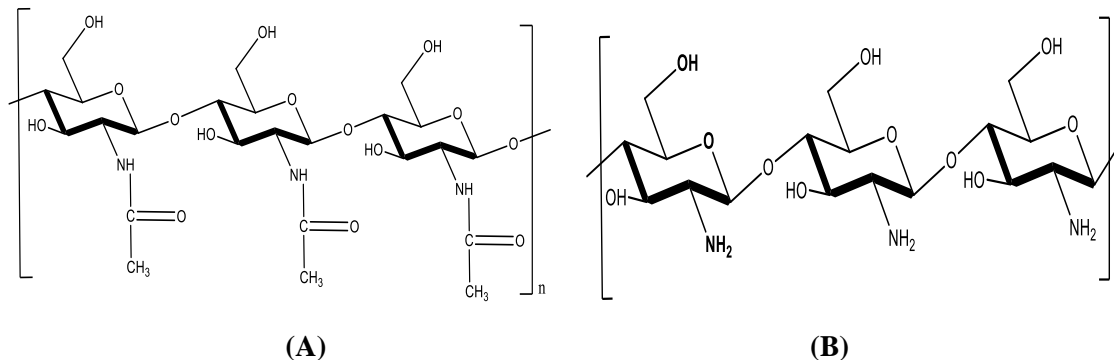


Figure 2. Structures of (A) Chitin, (B) Chitosan

Chitin and chitosans are prevalent in the biosphere as exoskeleton components and seafood industry waste, such as crab (*Portunus pelagicus*). A crab shell contains chitin, protein, CaCO₃, a little MgCO₃, and astaxanthin. The previous research indicated that deproteinization, demineralization, and decolorization are sequential stages in the chemical extraction process used to isolate chitin. Deacetylation is necessary to convert chitin into chitosan (Iber et al., 2022).

Several ways for producing chitin have been developed over the decades. The previous research on the synthesis of chitosan from crab shells got a yield of 70.4%, and the following analysis showed that this high yield is due to the amount of calcium (Indriani, 2022). Almost all marine waste samples were demineralized using acidic medium (HCl, HNO₃, HCOOH) at 90-100°C. Hydrochloric acid was selected for this study due of its ready accessibility and familiarity as a solvent. In addition, the acid requires the presence of two HCl molecules to convert calcium carbonate into calcium chloride, thus achieving the demineralization process. A comparable or even greater amount of acid is required to extract minerals from an identical amount of the same mineral (Al Shaqsi et al., 2020).

The demineralization process aims to remove the mineral content contained in the crab shell waste powder. In this study, the same acid method was used with slight modifications. The procedure of demineralization optimization was carried out by utilizing varying concentrations of hydrochloric acid (HCl) while keeping the temperature at a constant, namely 1 M, 2 M, and 3 M. The best optimization results are seen from the decrease in calcium levels between the crab shell powder and the demineralization results. The calcium content of the crab shell waste powder was analyzed using an atomic absorption spectrophotometer (SSA) instrument. Calcium content before demineralization was obtained at 46,942.45 mg/100g.

Table 1. Calcium content on demineralization optimization

HCl Concentration	Calcium Content (mg/100g)	
	Before Demineralization	After Demineralization
1 M		19.694,53
2 M	46.942,45	22.365,15
3 M		1052,70

The results of the decrease in calcium levels between the crab shell powder and the demineralization results can be seen in **table 1**. The demineralization process with 3 M hydrochloric acid showed the most optimal result of 97.75%. Prior studies have indicated that elevating the concentration of hydrochloric acid

enhances the efficacy of demineralization. Furthermore, a significant rise in concentration will have an impact on the viscosity of the end product, specifically resulting in a decrease in the viscosity of the chitosan solution.

The following is the demineralization reaction mechanism:



During the demineralization process, the calcium content in the crab shell waste will react with Hydrochloric Acid which forms water-soluble calcium salts and which will be characterized by the formation of large froth or bubbles. This large froth or bubble is due to the formation of gases, namely water (H₂O) and carbon dioxide (CO₂). The indicator to signify that the mineral content has disappeared is the disappearance of the froth or bubbles. The residue after the process of removing minerals result in a dark brown powder with a rough texture.

Following this stage, the process of chitin extraction can continue by removing pigments and converting chitin into chitosan, which is known as decolorization and deacetylation, respectively. Alcohol, namely ethanol, can be used for decolorization, and the deacetylation procedure requires the use of a strong alkali like NaOH.

Conclusion

Determination results showed that the shell waste is the shell of crab or blue swimming crab (*Portunus pelagicus*), the demineralization optimization procedure involved using different concentrations of hydrochloric acid while maintaining a constant temperature. The best demineralization optimization was achieved using 3 M hydrochloric acid, with a decrease in calcium content of 97.75%.

References

- Abirami, S., Nagarajan, D., Samrot, A. V., Varsini, A. M., Sugasini, A., & Anand, D. A. (2021). Extraction, characterization, and utilization of shrimp waste chitin derived chitosan in antimicrobial activity, seed germination, preservative, and microparticle formulation. *Biointerface Res. Appl. Chem*, 11(2), 8725-39.
- Ahyat, N. M., Mohamad, F., Ahmad, A., & Azmi, A. A. (2017). Chitin and chitosan extraction from *Portunus pelagicus*. *Malaysian Journal of Analytical Sciences*, 21(4), 770-777.
- Al Shaqsi, N. H. K., Al Hoqani, H. A. S., Hossain, M. A., & Al Sibani, M. A. (2020). Optimization of the demineralization process for the extraction of chitin from Omani *Portunidae segnis*. *Biochemistry and Biophysics Reports*, 23, 100779.
- Ali, A., & Ahmed, S. (2018). A review on chitosan and its nanocomposites in drug delivery. *International journal of biological macromolecules*, 109, 273-286.
- Badwan, A. A., Rashid, I., Al Omari, M. M., & Darras, F. H. (2015). Chitin and chitosan as direct compression excipients in pharmaceutical applications. *Marine drugs*, 13(3), 1519-1547.
- Hosney, A., Ullah, S., & Barčauskaitė, K. (2022). A review of the chemical extraction of chitosan from shrimp wastes and prediction of factors affecting chitosan yield by using an artificial neural network. *Marine Drugs*, 20(11), 675.
- Iber, B. T., Kasan, N. A., Torsabo, D., & Omuwa, J. W. (2022). A review of various sources of chitin and chitosan in nature. *Journal of Renewable Materials*, 10(4), 1097.

- Imtihani, H. N., Wahyuono, R. A., & Permatasari, S. N. (2020). *Biopolimer kitosan dan penggunaannya dalam formulasi obat*. Penerbit Graniti: Gresik.
- Indriani, Alya. Pembuatan dan Karakterisasi Kitosan dari Limbah Cangkang Rajungan dengan Tiga Jenis Pereaksi Basa. (*Skripsi*). 2022.
- Ismik, D., Mansuroglu, D. S., Buluş, E., & Sahin, Y. M. (2020). The use of chitosan nanoparticles obtained by ionic gelation method as a drug delivery system. *Journal of Materials and Electronic Devices*, 5(1), 6-11.
- Liu, R., Luo, C., Pang, Z., Zhang, J., Ruan, S., Wu, M. & Gao, H. (2023). Advances of nanoparticles as drug delivery systems for disease diagnosis and treatment. *Chinese chemical letters*, 34(2), 107518.
- Mohadi, R., Kurniawan, C., Yuliasari, N., & Hidayati, N. (2014, October). Karakterisasi Kitosan dari Cangkang Rajungan dan Tulang Cumi dengan Spektrofotometer FT-IR Serta Penentuan Derajat Deasetilasi Dengan Metode Baseline. *In Seminar Nasional FMIPA UNSRI* (Vol. 2, pp. 1-10).
- Morin-Crini, N., Lichtfouse, E., Torri, G., & Crini, G. (2019). Applications of chitosan in food, pharmaceuticals, medicine, cosmetics, agriculture, textiles, pulp and paper, biotechnology, and environmental chemistry. *Environmental Chemistry Letters*, 17(4), 1667-1692.
- Pérez, W. A., Marín, J. A., López, J. N., Burgos, M. A., & Rios, L. A. (2022). Development of a pilot-ecofriendly process for chitosan production from waste shrimp shells. *Environmental Processes*, 9(3), 55.
- Sidauruk, S. W. (2018). Karakteristik Rajungan (*Portunus pelagicus*) dan Potensinya Di Bidang Pangan Dan Kesehatan. *Jurnal Perikanan*. Departemen Perikanan dan Ilmu Kelautan.
- Tissera, W. M. J. C. M., Rathnayake, S. I., Abeyrathne, E. D. N. S., & Nam, K. C. (2021). An improved extraction and purification method for obtaining high-quality chitin and chitosan from blue swimmer (*Portunus pelagicus*) crab shell waste. *Food Science and Biotechnology*, 30(13), 1645-1655.
- Yanat, M., & Schroën, K. (2021). Preparation methods and applications of chitosan nanoparticles; with an outlook toward reinforcement of biodegradable packaging. *Reactive and Functional Polymers*, 161, 104849.
- Zaghib, I., Arafa, S., & Hassouna, M. (2022). Biological, functional and physico-chemical characterization of chitosan extracted from blue crab (*Portunus segnis*) shell waste by chemical method. *Am. Acad. Sci. Res. J. Eng. Technol. Sci.*, 85, 100-115.