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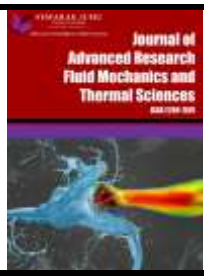

The Effect of Depolymerization Treatment on Modified Properties of Chitosan Derived from Crab Shells as a Candidate for Bioabsorbable Screw Materials

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ABSTRACT

The increase in crab meat exports increased the amount of crab shells discharged into the environment. Improper crab shell waste management can cause environmental concerns, public health issues, and increased oxygen requirements. This research aims to utilize crab shells from crab meat processing businesses in Indonesia into chitosan using demineralization, deproteination and deacetylation processes, with depolymerization as an additional process. Crab shell powder will be deproteinized with 3% NaOH and demineralized using an HCL solution. Furthermore, the deacetylation process was carried out for 8 hours at 140°C with 50% NaOH to produce chitosan. The resulting chitosan was then depolymerized using H_2O_2 at a concentration of 13%. The chitosan properties obtained by the deacetylation and depolymerization procedures were evaluated using X-ray diffraction (XRD), Fourier Transform Infrared (FTIR), and scanning electron microscope (SEM) tests. The results show that the depolymerization process exerts a beneficial effect on the chitosan product. The depolymerization treatment process involves the fragmentation of extended chitosan chains, which leads to the formation of smaller fragments and subsequently causes a decrease in the crystallinity, and particle size of the chitosan obtained. In additions, the results of this study indicate that the depolymerization process causes an increase in the degree of deacetylation of chitosan. The chitosan produced from the deacetylation and depolymerization processes produced chitosan with a degree of deacetylation of 81% and 91%, respectively. Increased the degree of deacetylation (DD, %) leads to reduced molecular weight, improved antimicrobial properties, higher water solubility, improved mechanical properties, and indicates better chitosan purity.

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1. Introduction

The crab industry frequently prioritizes the extraction of crab meat while disregarding the shells as a byproduct. Approximately 57% of the waste by-products in crab meat processing are comprised of shells [1-4]. In the year 2023, the export quantity of crab meat reached a total of 1,019 metric tons, being distributed to many nations [5]. The data indicates that a total of 1351 metric tons of crab shells are generated as waste. Improper management of crab shell waste can give rise to environmental concerns and compromise public health standards, manifesting in issues such as malodorous or disagreeable smells, water contamination, and heightened levels of biological and chemical oxygen requirements [1-4]. The utilization of crab shell waste can yield items of significant economic worth, including productions of chitosan, animal feed, cracker, natural food flavor, and the development of adsorbents for heavy metals [6-8].

The processing of crab shell waste has the potential to produce chitosan, a multifunctional biopolymer that finds applications in the pharmaceutical and cosmetic industries [1,7]. Chitosan is typically derived from the chitin component found in crab shells through a series of procedures including deproteination, demineralization, and deacetylation. The unique macromolecular structure of chitosan has garnered significant attention from both researchers and the industrial sector. Furthermore, chitosan exhibits remarkable attributes such as superior biocompatibility, biodegradability, antibacterial capabilities, and various other intrinsic functional characteristics. The aforementioned benefits contribute to the extensive utilization of chitosan as a promising natural antibacterial agent in various sectors, including pharmaceuticals, cosmetics, agriculture, and food production. Furthermore, chitosan finds utility in several domains of tissue engineering and regenerative medicine, including wound healing, bone regeneration, cartilage regeneration, tooth regeneration, cardiac regeneration, nerve regeneration, and drug delivery systems [9-13].

During the chitosan synthesis procedure, deacetylation is employed as the stage whereby acetyl groups are eliminated through the utilization of a basic solution, such as sodium hydroxide (NaOH) solution. The aforementioned procedure produces chitosan, which is characterized by an elongated polymer chain [14]. Several studies have included a depolymerization method for chitosan derived from crab and shrimp shells. Depolymerization is a chemical procedure employed to decrease the length of the chitosan polymer chain. Depolymerization can be achieved by a variety of methodologies, including thermal treatment and enzymatic processes. The objective of this procedure is to cleave or fragment the chitosan molecule into shorter segments. The process of depolymerization has been found to have several beneficial effects on chitosan products. These include the improvement of solubility, enhancement of bioactivity, enhancement of functionality, and an increase in the adsorption capacity. The enhanced characteristics of chitosan render it a more viable option for biomedical applications and as an adsorbent for the elimination of contaminants from wastewater [15-18].

This study aims to convert crab shells obtained from the crab meat processing business in Pati, Central Java, Indonesia, into chitosan. This conversion will be achieved by the sequential procedures of demineralization, deproteination, and deacetylation. Afterwards, the chitosan obtained undergoes depolymerization as an additional procedural phase. The objective of this study is to employ waste crab shells for the production of chitosan, a material with potential applications in the medical field. Moreover, this investigation aimed to assess the influence of the depolymerization procedure on modifications in the properties of chitosan derived from crab shells. In addition to environmental factors, the utilization of shell wastes can be used to reduce production costs in the future because they are cheap, plentiful, and easily available [19-21]. Furthermore, this study has the

potential to make a valuable contribution towards the advancement of sustainable waste management and recycling practices within the crab meat processing business.

2. Materials and Methodology

In this study, crab shells obtained from the crab meat processing industry were subjected to drying under the sun (as depicted in Figure 1(a)), followed by crushing and then sieving through a 100-mesh sieve to get crab shell powder (as illustrated in Figure 1(b)). The deproteination procedure involved the dissolution of 100 grams of crab shell powder in a 3% NaOH solution using a volume-to-weight ratio of 1:10 (v/w). In this experimental procedure, agitation was performed utilizing a magnetic stirrer at a temperature of 90°C for 1 hour to extract the protein present in the crab shells. Afterwards, a washing process was conducted to achieve a neutral pH, followed by filtration of the solution. The resulting residue was thereafter subjected to drying in an oven.

(a) (b)

Fig. 1. (a) Crab shells, and (b) Crab shell powder

The deproteinization product is then subjected to a demineralization process. This process is used to remove minerals or inorganic compounds present in crab shell powder. In this study, the demineralization process was conducted utilizing a hydrochloric acid (HCl) solution with a weight-to-volume ratio of 1:7. In this experimental procedure, agitation was conducted using a magnetic stirrer at a temperature of 90°C for 1 hour. Subsequently, a washing process was conducted until achieving a neutral pH, followed by filtration of the resulting mixture. Subsequently, the obtained residue was subjected to drying in an oven. 20 grams of demineralized product were subjected to dissolution in a 50% sodium hydroxide solution with a weight-to-volume ratio of 1:20 (w/v) for 8 hours at a temperature of 140°C. The process under consideration is commonly referred to as deacetylation.

It involves the cleavage of the chemical linkage connecting the acetyl group and the nitrogen atom, resulting in the formation of an amine group ($-NH_2$). This transformation ultimately yields the end product known as chitosan. The chitosan obtained was subsequently subjected to depolymerization using a 25-ml solution of 13% hydrogen peroxide (H_2O_2). The chitosan material is immersed in a hydrogen peroxide (H_2O_2) solution for 10 minutes, followed by a baking process in an oven at a temperature of 40°C for 4 hours. Subsequently, the solution underwent a cooling process followed by filtration using the Whatman 42 filter paper. The solid obtained from the filtration procedure was subjected to a washing step using distilled water, followed by drying at a temperature of 60°C for 3 hours.

The X-ray diffraction (XRD) technique was employed to identify the crystalline composition and crystallinity index found in chitosan. The experimental procedure involves the utilization of a Shimadzu XRD-7000 diffractometer, operating at a voltage of 40 kV and a current of 30 mA [20,22]. The diffractometer employs Cu Kα radiation with a wavelength of 0.15406 nm. The diffraction pattern is acquired within the angular range of 10° to 90° (2θ), with an increment of 0.02° and a scanning rate of 1° per minute. In addition, the surface morphology of chitosan was analyzed by the utilization of a scanning electron microscope (SEM) (JSM-6510, JEOL, Japan) operating at an accelerating voltage of 15 kV [20,22]. The obtained scanning electron microscopy (SEM) images will be utilized to calculate the particle size of chitosan with the assistance of ImageJ and Origin software. The Fourier Transform Infrared (FTIR) technique is employed for the evaluation of inorganic, organic, and polymeric substances. Variations in the characteristic absorption band patterns are indications of modifications in the material's composition. The identification of chitosan specimens in this investigation was performed using a Perkin Elmer Spotlight 400 Frontier FT-IR Spectrophotometer equipped with a recording area ranging from 4,000 to 400 cm^{-1}. The degree of deacetylation of chitosan (DD, %) will be calculated using the FTIR test data using a calculating approach offered in previous studies [4].

A deacetylation degree ranging from 55% to 70% is considered to be indicative of a low deacetylated degree of chitosan, resulting in its near-full insolubility in water. The deacetylation degree of chitosan typically ranges from 70% to 85%, representing the intermediate level of deacetylation. At this degree, chitosan exhibits partial solubility in water. In conclusion, a deacetylation degree of 85-95% is considered to be very high for chitosan, as it exhibits favorable solubility in water. On the other hand, achieving a deacetylation degree of 95-100% is referred to as an ultrahigh deacetylation degree of chitosan, which poses considerable challenges. Concurrently, the enhancement of chitosan's water solubility can be achieved through the degradation-induced reduction of its molecular weight [23].

3. Results and Discussion

Figure 2 presents a comparison of the X-ray diffraction (XRD) test results obtained from crab shell powder, deacetylation products, and depolymerization products. The presence of calcite and aragonite crystalline phases has been observed in crab shell powder. Based on the JCPDS card number 05-0586, the crystalline calcite phase observed in crab shell powder is often represented by the following values: 2θ angles of 29.404, 39.399, and 43.143. The aragonite crystal phase is observed for 2θ values of 26.312, 31.176, and 33.180, as shown by the JCPDS Card No. 05-0453. The present investigation reveals that the composition of crab shell powder primarily consists of calcite crystals, accompanied by a small proportion of aragonite crystals.

The presence of chitosan after deacetylation and depolymerization was identified based on JCPDS number 39-1894. The presence of chitosan in Figure 2 is indicated by the presence of peaks at 2θ: 15.18, 20.3, 21.2, 23.9, and 29.9.

In this study, the crystallinity index is calculated as the ratio between the area of the crystal contribution and the total area under the XRD peaks using Origin software using the formula from previous studies [4].

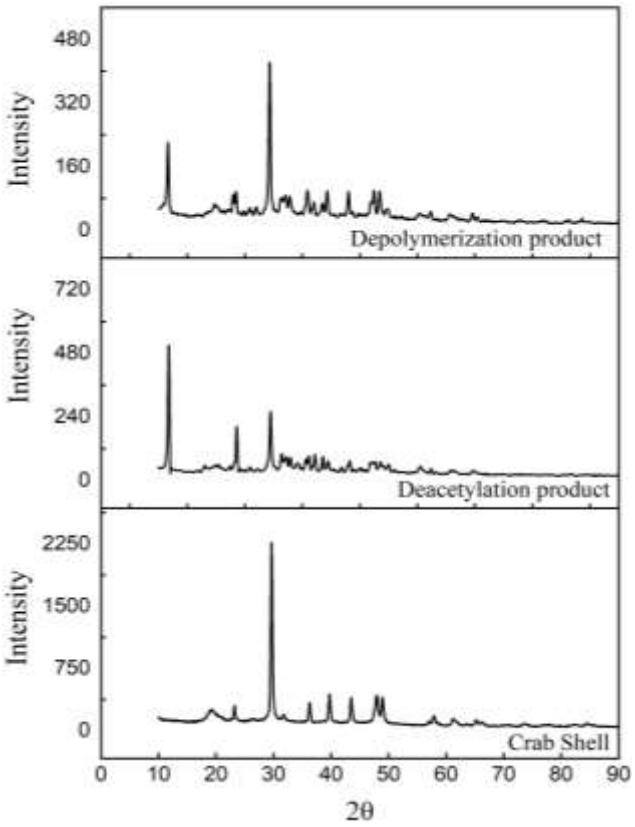


Fig. 2. XRD diffractogram on crab shell, deacetylated chitosan product, and depolymerized chitosan product

The crystallinity index comparison obtained in this study is shown in Figure 3. The crystallinity index (CI) is a numerical measure used to assess the degree of crystallinity in a material. It is determined by calculating the ratio of crystalline peaks to the overall number of peaks, which includes both crystalline and amorphous areas. The concept of crystallinity relates to the level of organization and dimensions of crystals within a given crystalline material. A higher value of the crystallinity index (CI) corresponds to an increased presence of well-defined crystalline peaks within a given material [24].

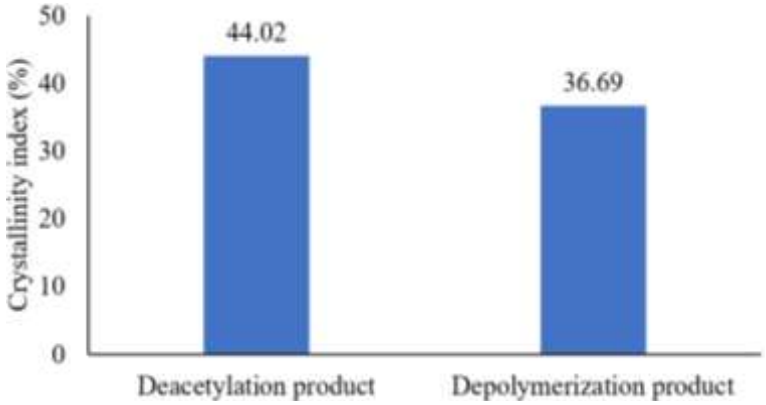


Fig. 3. Effect of depolymerization treatment on crystallinity index

The findings of this study demonstrated that the depolymerization treatment led to a reduction in the crystallinity index of chitosan. The depolymerization treatment process involves the fragmentation of the extended chitosan chains, leading to the formation of smaller fragments and subsequently causing a reduction in the overall crystallinity of the material. Furthermore, the depolymerization process causes an increase in entropy and disrupts the regular organization of chitosan molecules, resulting in a reduction in crystalline structure.

Furthermore, the depolymerization process of chitosan has the potential to enhance molecular mobility. As a result of this phenomenon, the utilization of low molecular weight chitosan (LMWC) leads to the generation of a comparatively less ordered or amorphous structure, thus resulting in a reduction in the crystallinity index. Another study investigated the impact of the depolymerization process on chain orientation, which can disrupt the formation of crystalline areas and consequently lead to a reduction in the crystallinity index [25-28].

Figure 4 shows the graphical comparison of FTIR test results of chitosan produced from deacetylation and depolymerisation processes. The -OH, -CH stretching, -NH2 cutting, -CH3, and -C-O-C- functional groups in chitosan produced from deacetylation process were shown at wavelengths (cm^{-1}) of 3451, 2891, 1642, 1419, and 1151, respectively. Whereas in chitosan produced from the depolymerisation process, the functional groups -OH, -CH stretching, -NH2 cutting, -CH3, and -C-O-C- were shown at wavelengths (cm^{-1}) of 3435.22, 2897, 1631, 1420.03, and 1040.05, respectively. The graph generated from the FTIR test results was used to determine the degree of deacetylation of chitosan (DD, %) using an equation based on previous research.

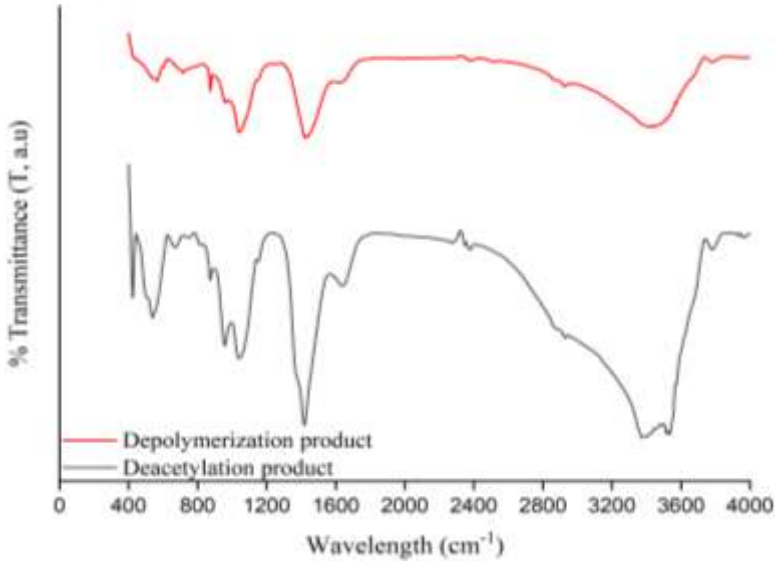


Fig. 4. FTIR spectra of chitosan produced from deacetylation and depolymerization process

The effect of the depolymerization process on the degree of deacetylation of chitosan (DD, %) is shown in Figure 5. The results of this study indicate that the depolymerization process causes an increase in the degree of deacetylation of chitosan (DD, %). Chitosan produced from the deacetylation process has a degree of deacetylation of chitosan (DD, %) of 81%. While the chitosan produced from the depolymerization process produced a degree of deacetylation of chitosan (DD, %) of 91%. The depolymerization process frequently leads to a reduction in the molecular weight of chitosan. The observed effect can be attributed to the ability to catalyze the hydrolysis of the glycosidic linkages present in the chitosan polymer chain. The abundance of fragmented chitosan

chains results in the generation of depolymerized chitosan, characterized by a progressively reduced molecular weight.

A decrease in the molecular weight of chitosan has the potential to result in an elevation of the degree of deacetylation. Furthermore, the depolymerization process typically leads to the liberation of an increasing number of acetyl groups and free amide active groups (-NH_2), thereby causing an elevation in the degree of deacetylation [14,17].

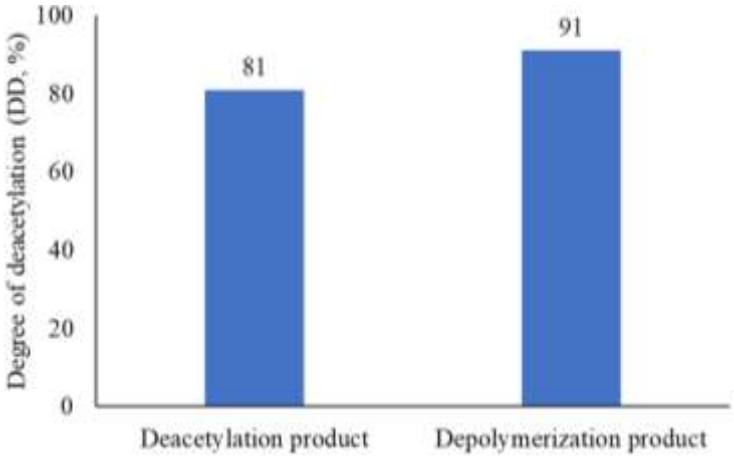
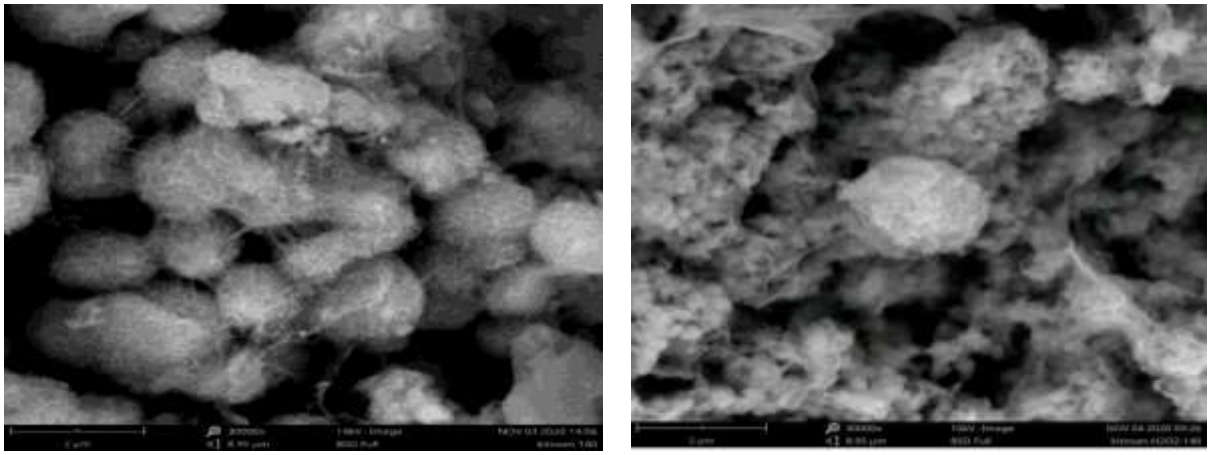


Fig. 5. Effect of depolymerization treatment on degree of deacetylation of chitosan (DD, %)

The results of this study indicate that the deacetylated chitosan product falls into the category of chitosan with a medium degree of deacetylation. This is because the resulting deacetylation degree of 81% is in the range of 70% to 85%. Meanwhile, the depolymerized chitosan product belongs to chitosan with a high degree of deacetylation. This is because the degree of deacetylation in the depolymerized chitosan is in the range of 85-95% and shows good solubility in water [23]. The degree of deacetylation (DD, %) is a crucial parameter that significantly influences the characteristics of chitosan. An increase in the degree of deacetylation (DD, %) is associated with a decrease in molecular weight, enhanced antimicrobial abilities, higher water solubility, and improved mechanical properties. In addition, an increase in the degree of deacetylation (DD, %) indicates a better purity of the chitosan produced [29-31]. Chitosan with a high degree of deacetylation can be applied in various fields, including biodegradable packaging, the food industry, biomedical applications, environmental applications, and industrial applications. In the field of biomedical applications, chitosan with a high degree of deacetylation can be used in drug delivery systems, wound dressings, orthopedic implants, and tissue engineering [31,32].

SEM images of the chitosan produced from the deacetylation and depolymerization process are shown in Figure 6. The morphology of chitosan derived from crab shells exhibited a layered structure characterized by flakes, which displayed a porous structure and contained fibres. Scanning electron microscopy (SEM) images were used to calculate the particle size of chitosan with the help of ImageJ and Origin software. The effect of the depolymerization process on the particle size of chitosan is shown in Figure 7.



(a) (b)

Fig. 6. SEM images of chitosan produced from deacetylation and depolymerization process

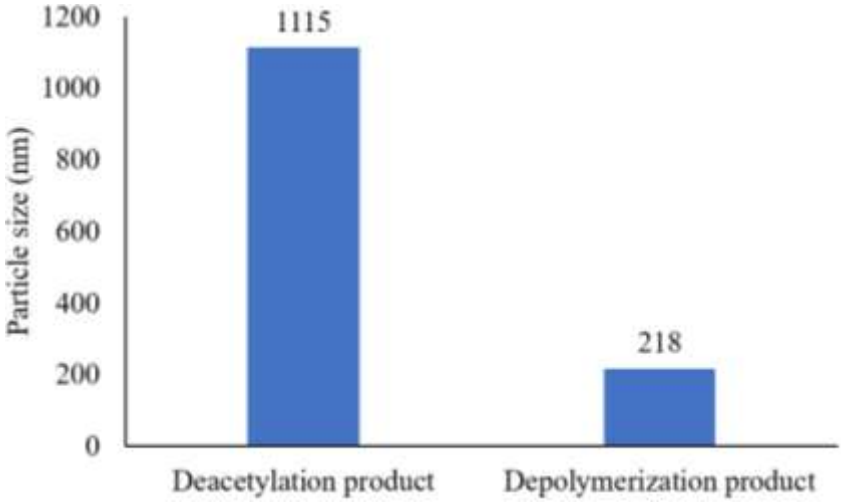


Fig. 7. Effect of depolymerization treatment on particle size of chitosan

The findings of this investigation revealed that the depolymerization procedure resulted in a reduction in the particle size of the obtained chitosan. This phenomenon occurs due to the depolymerization process, which is employed to cleave the extended chitosan chains into shorter chains, or oligomers. As a consequence, the chitosan's molecular weight decreases, which may result in smaller chitosan particles [31,33,34]. This is due to the fact that smaller molecules can organize themselves more closely and tightly, resulting in a reduction in particle size [35]. The characteristics of chitosan nanoparticles in medical applications are significantly influenced by the particle size. Reducing the particle size can result in the encapsulation of a greater concentration of therapeutic substances, leading to enhanced drug stability and improved bioavailability [36]. The surface area-to-volume ratio of chitosan particles increases as their size decreases. The increase of surface area facilitates enhanced interaction with other substances, such as drugs or pollutants, hence resulting in enhanced efficacy across diverse applications [37,38]. Shameli *et al.,* [39] and Yusefi *et al.,* [40] found that the smaller the size of chitosan particles (nano-sized), the larger the surface area created. Furthermore, nanosized chitosan offers benefits in terms of biocompatibility and biodegradability. This is a reason for the widespread application of nanosized chitosan in various industries, including food packaging, cosmetics, and biomedicine.

4. Conclusions

This study aimed to transform crab shells into chitosan, a versatile biopolymer that has promise for various applications in the medical domain. Additionally, the study sought to evaluate the impact of depolymerization on the characteristics of chitosan obtained from crab shells. The findings from this investigation indicate that the application of depolymerization treatment results in a decrease in the crystallinity index of chitosan. This may be due to the cleavage of longer chitosan chains during the depolymerization process, which consequently results in smaller fragments and ultimately leads to an overall decrease in chitosan crystallinity. Furthermore, the depolymerization process results in an increase in the degree of deacetylation of chitosan, expressed as a percentage (DD, %).

The chitosan derived from the deacetylation process showed a deacetylation degree of 81%, while the depolymerized chitosan product showed a deacetylation degree of 91%. The chitosan derived from the depolymerization process showed a fairly high degree of deacetylation, being in the range of 85-95%. An increase in the degree of deacetylation (DD%) results in lower molecular weight, greater antibacterial ability, higher water solubility, improved mechanical properties, and better chitosan purity. The findings of this investigation revealed that the depolymerization procedure resulted in a reduction in the particle size of the obtained chitosan. The particle sizes of chitosan produced from the deacetylation and depolymerization processes were 1115 nm and 218 nm, respectively. Reducing the particle size can result in the encapsulation of a greater concentration of therapeutic substances, leading to enhanced drug stability and improved bioavailability.

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References

[1] Azizah, Dewiantika, Nurwanti Fatnah, and Mutiara Cahyani. "Literature Study on Small Crab Shell (Portunus pelagicus) Waste Management in Mertasinga Village to Produce Valuable Economic Product." In *Proceedings of the 2nd International Conference on Education, ICE 2019*, 27-28 September 2019, Universitas Muhammadiyah Purworejo, Indonesia. 2020.

[2] Dliyauddin, Moh, Tri Ardyati, and Suharjono Suharjono. "Evaluation of Proteolytic and Chitinolytic Activities of Indigenous Bacillus Species from Crab Shell Waste." *The Journal of Experimental Life Science* 10, no. 1 (2020): 1-5. https://doi.org/10.21776/ub.jels.2019.010.01.01

[3] Lian, Federico, Enrico De Conto, Vincenzo Del Grippo, Sabine M. Harrison, John Fagan, James G. Lyng, and Nigel P. Brunton. "High-Pressure processing for the production of added-value claw meat from edible crab (Cancer pagurus)." *Foods* 10, no. 5 (2021): 955. https://doi.org/10.3390/foods10050955

[4] Ismail, Rifky, Deni Fajar Fitriyana, Athanasius Priharyoto Bayuseno, Jamari Jamari, Putut Yoga Pradiptya, Rilo Chandra Muhamadin, Fariz Wisda Nugraha, Rusiyanto Rusiyanto, Andri Setiyawan et al. "Investigating the effect of deacetylation temperature on the characterization of chitosan from crab shells as a candidate for organic nanofluids." *Journal of Advanced Research in Fluid Mechanics and Thermal Sciences* 103, no. 2 (2023): 55-67. https://doi.org/10.37934/arfmts.103.2.5567

[5] Khoiriah, Siti. "Kupas Tuntas - Volume Ekspor Daging Rajungan Asal Lampung 1.019 Ton Senilai Rp 418 Miliar." *Kupastuntas*. February 21, 2023.

[6] Fachry, Mardiana E., and Alpiani Alpiani. "Literature review: Economic value of utilization of crab shell waste (Case study of PT. Toba Surimi Industri in Tanjungpinang City, Riau Island Province)." *Akuatikisle: Jurnal Akuakultur, Pesisir dan Pulau-Pulau Kecil* 5, no. 2 (2021): 49-52. https://doi.org/10.29239/j.akuatikisle.5.2.49-52

[7] Nerdy, N., P. Lestari, D. Simorangkir, V. Aulianshah, F. Yusuf, and T. K. Bakri. "Comparison of chitosan from crab shell waste and shrimp shell waste as natural adsorbent against heavy metals and dyes." *International Journal of Applied Pharmaceutics* 14, no. 2 (2022): 181-185. https://doi.org/10.22159/ijap.2022v14i2.43560

[8] Pardi, Hilfi, Dina Fitriyah, Friska Septiani Silitonga, Tri Widya Edelwis, Annisa Fitri, and Martin Agustina Sari. "Optimization of Pb Adsorption from Seawater from Former Bauxite Mines Using Crab Shell Waste." In *IOP Conference Series: Earth and Environmental Science*, vol. 1148, no. 1, p. 012005. IOP Publishing, 2023. https://doi.org/10.1088/1755-1315/1148/1/012005

[9] Zivanovic, S., R. H. Davis, and D. A. Golden. "Chitosan as an antimicrobial in food products." *Handbook of Natural Antimicrobials for Food Safety and Quality* 20, no. 1 (2015): 153-181. https://doi.org/10.1016/B978-1-78242-034-7.00008-6

[10] Umoren, Saviour A., Moses M. Solomon, and Viswanathan S. Saji. *Polymeric materials in corrosion inhibition: fundamentals and applications*. Elsevier, 2022. https://doi.org/10.1016/B978-0-12-823854-7.00026-6

[11] Kim, Yevgeniy, Zharylkasyn Zharkinbekov, Kamila Raziyeva, Laura Tabyldiyeva, Kamila Berikova, Dias Zhumagul, Kamila Temirkhanova, and Arman Saparov. "Chitosan-based biomaterials for tissue regeneration." *Pharmaceutics* 15, no. 3 (2023): 807. https://doi.org/10.3390/pharmaceutics15030807

[12] Sultankulov, Bolat, Dmitriy Berillo, Karina Sultankulova, Tursonjan Tokay, and Arman Saparov. "Progress in the development of chitosan-based biomaterials for tissue engineering and regenerative medicine." *Biomolecules* 9, no. 9 (2019): 470. https://doi.org/10.3390/biom9090470

[13] Foster, Leslie John Ray, Sonia Ho, James Hook, Monica Basuki, and Helder Marcal. "Chitosan as a biomaterial: Influence of degree of deacetylation on its physiochemical, material and biological properties." *PloS One* 10, no. 8 (2015): e0135153. https://doi.org/10.1371/journal.pone.0135153

[14] Mathaba, Machodi, and Michael Olawale Daramola. "Effect of chitosan's degree of deacetylation on the performance of PES membrane infused with chitosan during AMD treatment." *Membranes* 10, no. 3 (2020): 52. https://doi.org/10.3390/membranes10030052

[15] de Souza, Eber Euripedes, Adão Lincon B. Montel, Robson D. S. Barbosa, Ilsamar Mendes Soares, Miguel Daniel Noseda, Raimundo Wagner de Souza Aguiar, Tarso da Costa Alvim, and Sergio Donizeti Ascêncio. "Obtaining Hexoses from Chitosan through Depolymerization with Nitrous Acid." *Current Organic Synthesis* 19, no. 7 (2022): 767-771. https://doi.org/10.2174/1570179419666220127145745

[16] Alves, Helton J., Mônica Vieceli, Cássio Alves, Graciela IB Muñiz, Cristiano LP de Oliveira, Michael Feroldi, and Mabel K. Arantes. "Chitosan depolymerization and nanochitosan production using a single physical procedure." *Journal of Polymers and the Environment* 26 (2018): 3913-3923. https://doi.org/10.1007/s10924-018-1267-7

[17] Ridhay, A., and A. H. Laenggeng. "Depolymerization of chitosan from snail (Pilla ampullaceae) field shell using α-amylase." In *Journal of Physics: Conference Series*, vol. 1242, no. 1, p. 012005. IOP Publishing, 2019. https://doi.org/10.1088/1742-6596/1242/1/012005

[18] Aguila-Almanza, Eva, René Salgado-Delgado, Zully Vargas-Galarza, Edgar García-Hernández, and Heriberto Hernández-Cocoletzi. "Enzymatic depolimerization of chitosan for the preparation of functional membranes." *Journal of Chemistry* 2019, no. 1 (2019): 5416297. https://doi.org/10.1155/2019/5416297

[19] Fitriyana, D. F., F. W. Nugraha, M. B. Laroybafih, R. Ismail, A. P. Bayuseno, R. C. Muhamadin, M. B. Ramadan, A. RA Qudus, and J. P. Siregar. "The effect of hydroxyapatite concentration on the mechanical properties and degradation rate of biocomposite for biomedical applications." In *IOP Conference Series: Earth and Environmental Science*, vol. 969, no. 1, p. 012045. IOP Publishing, 2022. https://doi.org/10.1088/1755-1315/969/1/012045

[20] Fitriyana, Deni Fajar, Agustinus Purna Irawan, Aldias Bahatmaka, Rifky Ismail, Athanasius Priharyoto Bayuseno, Rilo Chandra Muhamadin, Tezara Cionita, Januar Parlaungan Siregar, Emilianus Jehadus, Gregorius Dimas Baskara, Dwinta Laksmidewi, and Natalino Fonseca Da Silva Guterres. "The Effect of Temperature on the Hydrothermal Synthetis of Carbonated Apetite from Calcium Carbonate Obtained from Green Mussels Shells." *ARPN Journal of Engineering and Applied Sciences* 18, no. 11 (2023): 1215-1224.

[21] Ismail, Rifky, Muhammad Bagus Laroybafih, Deni Fajar Fitriyana, Sri Nugroho, Yanuar Iman Santoso, Ahmad Jazilussurur Hakim, Mohammad Syahreza Al Mulqi, and Athanasius Priharyoto Bayuseno. "The effect of hydrothermal holding time on the characterization of hydroxyapatite synthesized from green mussel shells." *Journal of Advanced Research in Fluid Mechanics and Thermal Sciences* 80, no. 1 (2021): 84-93. https://doi.org/10.37934/arfmts.80.1.8493

[22] Fitriyana, D. F., Hazwani Suhaimi, Sulardjaka, R. Noferi, and Wahyu Caesarendra. "Synthesis of Na-P Zeolite from geothermal sludge." In *NAC 2019: Proceedings of the 2nd International Conference on Nanomaterials and Advanced Composites*, pp. 51-59. Springer Singapore, 2020. https://doi.org/10.1007/978-981-15-2294-9_5

[23] Lv, S. H. "High-performance superplasticizer based on chitosan." In *Biopolymers and Biotech Admixtures for Eco-Efficient Construction Materials*, pp. 131-150. Woodhead Publishing, 2016. https://doi.org/10.1016/B978-0-08-100214-8.00007-5

[24] Sa, Yue, Yaru Guo, Xiaowei Feng, Man Wang, Ping Li, Yixue Gao, Xu Yang, and Tao Jiang. "Are different crystallinity-index-calculating methods of hydroxyapatite efficient and consistent?." *New Journal of Chemistry* 41, no. 13 (2017): 5723-5731. https://doi.org/10.1039/C7NJ00803A

[25] Podgorbunskikh, Ekaterina, Timofei Kuskov, Denis Rychkov, Oleg Lomovskii, and Aleksey Bychkov. "Mechanical amorphization of chitosan with different molecular weights." *Polymers* 14, no. 20 (2022): 4438. https://doi.org/10.3390/polym14204438
[26] Bentley, Flor Estefany, Renaud Passieux, Laurent David, and Anayancy Osorio-Madrazo. "Pure Chitosan Biomedical Textile Fibers from Mixtures of Low-and High-Molecular Weight Bidisperse Polymer Solutions: Processing and Understanding of Microstructure-Mechanical Properties' Relationship." *International Journal of Molecular Sciences* 23, no. 9 (2022): 4767. https://doi.org/10.3390/ijms23094767
[27] Suryani, Suryani, Anis Yohana Chaerunisaa, I. Made Joni, Ruslin Ruslin, La Ode Ahmad Nur Ramadhan, Yoga Windhu Wardhana, and Sitti Hadijah Sabarwati. "Production of low molecular weight chitosan using a combination of weak acid and ultrasonication methods." *Polymers* 14, no. 16 (2022): 3417. https://doi.org/10.3390/polym14163417
[28] Nguyen, Hien Thi-Thanh, Tinh Ngoc Tran, Anh Cam Ha, and Phu Dai Huynh. "Impact of deacetylation degree on properties of chitosan for formation of electrosprayed nanoparticles." *Journal of Nanotechnology* 2022, no. 1 (2022): 2288892. https://doi.org/10.1155/2022/2288892
[29] Kadak, Ali Eslem, Aygül Küçükgülmez, and Mehmet Çelik. "Preparation and characterization of crayfish (Astacus leptodactylus) chitosan with different deacetylation degrees." *Iranian Journal of Biotechnology* 21, no. 2 (2023): e3253.
[30] Gîjiu, Cristiana Luminița, Raluca Isopescu, Daniel Dinculescu, Maria Memecică, Manuela-Rossemary Apetroaei, Mirela Anton, Verginica Schröder, and Ileana Rău. "Crabs marine waste-A valuable source of chitosan: Tuning chitosan properties by chitin extraction optimization." *Polymers* 14, no. 21 (2022): 4492. https://doi.org/10.3390/polym14214492
[31] Alsharabasy, Amir M. "Semi-synthesis of chitosan with high molecular weight and enhanced deacetylation degree." *Polymer Sciences* 2, no. 2 (2016): 11.
[32] Mametapo, Maria, Arijjal Fadhli Aulia, and Nabila Qurrata A'yun. "Chitosan application as edible packaging raw material." *Asian Journal of Fisheries and Aquatic Research* 12, no. 5 (2021): 44-54. https://doi.org/10.9734/ajfar/2021/v12i530247
[33] Al-Jbour, Nawzat D., M. D. H. Beg, Jolius Gimbun, and A. K. M. Moshiul Alam. "Preparation and characterization of low molecular weight chitosan with different degrees of deacetylation by the acid hydrolysis method." *International Journal of Applied Pharmaceutics* 13, no. 2 (2021): 153-164. https://doi.org/10.22159/ijap.2021v13i2.32229
[34] Thanakkasaranee, Sarinthip, Kittisak Jantanasakulwong, Yuthana Phimolsiripol, Noppol Leksawasdi, Phisit Seesuriyachan, Thanongsak Chaiyaso, Pensak Jantrawut et al. "High substitution synthesis of carboxymethyl chitosan for properties improvement of carboxymethyl chitosan films depending on particle sizes." *Molecules* 26, no. 19 (2021): 6013. https://doi.org/10.3390/molecules26196013
[35] Marianti, A., Y. U. Anggraito, and W. Christijanti. "Effective gamma irradiation dose on viscosity and molecular weight reduction of chitosan." In *Journal of Physics: Conference Series*, vol. 1567, no. 4, p. 042096. IOP Publishing, 2020. https://doi.org/10.1088/1742-6596/1567/4/042096
[36] Warsito, Mega Ferdina, and Fitri Agustiani. "A review on factors affecting chitosan nanoparticles formation." In *IOP Conference Series: Materials Science and Engineering*, vol. 1011, no. 1, p. 012027. IOP Publishing, 2021. https://doi.org/10.1088/1757-899X/1011/1/012027
[37] Sethi, Aisha, Mahmood Ahmad, Tayyaba Huma, and Waqas Ahmad. "Pharmacokinetic variables of medium molecular weight cross linked chitosan nanoparticles to enhance the bioavailability of 5-fluorouracil and reduce the acute oral toxicity." *Drug Delivery* 28, no. 1 (2021): 1569-1584. https://doi.org/10.1080/10717544.2021.1944398
[38] Zhang, Yaling, Xianling Wu, Bin Yan, Meilan Xu, Zewei Lin, and Su Xu. "Optimization of preparation process of chitosan-nano-selenium." In *Journal of Physics: Conference Series*, vol. 2021, no. 1, p. 012038. IOP Publishing, 2021. https://doi.org/10.1088/1742-6596/2021/1/012038
[39] Shameli, Kamyar, Syamim Arsyad Saiful, and Mostafa Yusefi. "Cross-linked Chitosan-Based Hydrogels Nanocomposites for Treatment of Disease." *Journal of Research in Nanoscience and Nanotechnology* 5, no. 1 (2022): 65-97. https://doi.org/10.37934/jrnn.5.1.6597
[40] Yusefi, Mostafa, Pooneh Kia, Siti Nur Amalina Mohamad Sukri, Roshafima Rasit Ali, and Kamyar Shameli. "Synthesis and properties of chitosan nanoparticles crosslinked with tripolyphosphate." *Journal of Research in Nanoscience and Nanotechnology* 3, no. 1 (2021): 46-52. https://doi.org/10.37934/jrnn.3.1.4652