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Effectiveness Study of Chitosan from Pearl Oyster Shells (*Pinctada maxima* sp.) as Antibacterial in Bone Scaffold Application

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Abstract: Chitosan functional material is a promising material in manufacturing bone scaffolds, as it has antibacterial properties, low toxicity, biodegradability, biocompatibility with human tissues, and facilitates regenerative processes in wound healing. Graft placement is prone to bacterial invasion, and alternatives are made scaffolds with antibacterial activity. This study aims to identify changes in functional groups in each isolation process and identify the effect of chitosan concentration (5%, 10%, 20%, 40%, and 80%) on the activity of *Straphylococcus aureus* and *Escherichia coli* bacteria. Chitosan isolation methods are demineralization, deproteination, decolorization, and deacetylation by microwave irradiation. Analysis of chitosan functional groups using FTIR, while antibacterial activity test using diffusion method. Isolation of chitosan from pearl oyster shells (*Pinctada maxima* sp.) obtained a degree of deacetylation of chitosan of 95.37%. Pearl oyster shell powder identified typical peaks of calcium carbonate ($CaCO_3$). The demineralized powder sample had calcium carbonate (CO_3^{2-}) peaks that disappeared. Furthermore, the deproteinated powder sample produced peaks with amide groups (C=O dan N-H) of reduced protein. Decolorized powder samples did not show drastic changes in the bands of the deproteinated powder spectra, but the spectra could show cleaner and clearer peaks without any interference from pigments. The last, deacetylated powder sample showed a decrease in peak intensity in the 1650 cm^{-1} (C=O amide). The analysis of the ability of chitosan to inhibit the growth of E. Coli and S. Aureus bacteria was effective at a minimum chitosan concentration of 20%. In comparison, antibacterial activity in S. aureus is better than in E. coli. Therefore, chitosan from this shell can be used as an antibacterial, but it is necessary to optimize chitosan manufacturing techniques to obtain better antibacterial efficacy.

Keywords: Biomaterials; Degree of Deacetylation; Functional Groups

Introduction

Bone structure loss due to periodontal disease or bone trauma can be addressed with graft placement. Graft placement is susceptible to bacterial invasion so an alternative is to make a scaffold with antibacterial activity. Antibacterials are substances that can inhibit the growth of bacteria and kill bacteria that cause infection. *Staphylococcus aureus* and *Escherichia coli* are Gram-positive and Gram-negative bacteria that can cause disease in the body. One of the biopolymer compounds that can be utilized as an antibacterial is the chitosan compound.

The activity of chitosan as an antibacterial is influenced by various intrinsic and extrinsic factors including PH, concentration, chitosan source, type of bacteria, degree of polymerization, degree of deacetylation, and others (Ardean et al., 2021). The source of raw materials for the production of chitosan affects its antimicrobial activity against gram-positive and negative bacteria, as well as fungi. Various raw materials for chitosan sources have been tested for antibacterial activity including crab shells, silkworms, shrimp shells, cuttlefish, squid cartilage, book clam shells, and green mussel shells. The results of the antibacterial activity test show that chitosan isolated from these animals can be used as an antibacterial from various types of bacteria such as *Staphylococcus aureus, Bacillus thuringiensis, Burkholderia pseudomallei, Mycobacterium tuberculosis, Escherichia coli, Vibrio chlores, Pseudomonas aureginosa, and Staphylococcus epidermidis* (Ningsih et al., 2022). The antibacterial action of chitosan will always depend on the intrinsic parameters and working conditions, especially the PH of the environment and the subtract where it is done. The deacetylation degree (DD) of chitosan is one of the intrinsic parameters that determine the characteristics of chitosan.

Isolation of chitosan from pearl oyster shells by deacetylation, demineralization, and deacetylation processes. The isolation results obtained a degree of deacetylation (DD) of up to 80.53% (Handayani et al., 2022). The method of isolating chitosan from pearl oyster shells was also carried out using the gradual deacetylation technique, where in this technique deacetylation was carried out with three additions of 60% NaOH. The technique obtained a degree of deacetylation of 57.50% (Nurlaili et al., 2022). In addition to producing chitosan, Pearl clam shell powder can also be synthesized into nano chitosan with DD reaching 88.63% (Alawiyah et al., 2024). Research conducted by Nurmaulida et al., (2023) succeeded in isolating chitosan with a high DD of more than 80%, but only produced a yield of 7.06%. The resulting yield is so small that innovation is needed in the chitosan isolation process to improve the characteristics of the chitosan produced. The isolation process utilizing microwave irradiation with high power produces purity of up to 89.75%, low crystallinity, and low viscosity (Kurniawidi et al., 2024). Another study showed that microwave deacetylation of chitin resulted in a degree of deacetylation of 83.4% and a molecular weight of 222.185 Da (Destrianingtyas et al., 2024).

Several studies above indicate that the isolation of chitosan by microwave irradiation is very advantageous compared to conventional methods as previously done. Furthermore, antibacterial test research on chitosan from Pearl clam shells has also never been done. The source of chitosan, chitosan isolation method, and DD of chitosan will affect its ability as an antibacterial. Therefore, in this study, chitosan production was carried out by microwave method and then analyzed the antibacterial activity of chitosan against *Straphylococcus aureus* and *Escherichia coli*. The results of this study will play an important role in functional development as a bone scaffold-forming material.

Materials and Method

Materials

The study utilized pearl oyster shell waste (Pinctada maxima sp.) as the main material. Other materials used for isolation include distilled water, NaOH pro analyst (Merck, Germany), and HCl 1 M pro analyst (Mallinckrodt, USA). The tools used in the chitosan preparation and isolation process are grinder (SY-150 Pulp Grinder Yamamoto, Indonesia), Milling (FGD-Z100 Fomac, Indonesia), magnetic stirrer and hot plate (IKA-CMAG HS7, Indonesia), oven (Mito, Indonesia), and microwave (Sharp, Indonesia).

Method

The pearl oyster shell powder preparation process involves cleaning and drying the clam shells. The shells are reduced in size using a grinder and milling to form clam shell powder (Sample A). Furthermore, the powder was isolated through several processes, namely demineralization, deproteination, decolorization, and deacetylation (Destrianingtyas et al., 2024). This isolation method modifies the method of Kurniawidi et al, 2023, namely adding the decolorization process and the demineralization process as the first process. Demineralization begins by dissolving 80 grams of clam shell

powder in 1 M HCl solution with a ratio of 1:15 (m/v) using a magnetic stirrer for 3 hours at room temperature. The precipitate obtained was rinsed until neutral and oven at 80 ºC (Sample B). Furthermore, deproteination by dissolving the demineralized powder into NaOH in a ratio of 1:10 (m/v) and homogenized. The resulting precipitate was rinsed until neutral and dried to form chitin powder (Sample C). The chitin powder was dissolved in 0.5% NaOCl in a ratio of 1:10 (m/v) and homogenized at 75 ºC for 1 hour. This process is referred to as the decolorization process which produces chitin powder with brighter white pigments (Sample D). Furthermore, the final process of isolation is deacetylation. Chitin powder was dissolved in 60% NaOH solution then homogenized for 1 hour and given microwave radiation for 15 minutes. The precipitate from the radiation was rinsed until neutral and oven-dried (Sample E).

Five powder samples from each isolation process were analyzed for functional groups using FTIR. From the results of the functional group analysis, the changes in functional groups that occur in each process will be identified. In addition, the vibration and degree of deacetylation of the powder were identified through this analysis. The degree of deacetylation of chitosan can be calculated by comparing the absorbance value between the wavelength $\lambda\,1655\ \mathrm{cm}^{-1}$ (A_{1655}) with the absorbance at wavelength $\lambda\,3450\ \mathrm{cm}^{-1}$ (A_{3450}) (Fatima, 2020):

$$DD\ (\%) = \left[100 - \left(\frac{A_{1655}}{A_{3450}} \times \frac{100}{1{,}33}\right)\right] \qquad (1)$$

Chitosan isolated from clam shells was tested for antibacterial activity using a modified diffusion method of Magani et. Al (2020). A petri dish equipped with 10 mL of NA media as the first layer was left to harden. Next, 10 mL of NA media was poured in which there were test bacteria as the second layer. Then the media was allowed to stand in laminar air flow for two hours so that the media hardened and the bacteria diffused. The blocker was removed so that wells with a diameter of 6 mm were obtained. Pouring antibacterial chitosan and comparative control into each well as much as 200 µL after that put into an incubator at 37 ºC for 3x24 hours. The incubation results were observed for a clear zone or zone of inhibition every 24 hours. Calculation of the chitosan antibacterial inhibition zone is measured based on the radius (r_p) and the inhibitor in the form of a clear area around the test well. The radius measurement is done by measuring the distance from the edge of the test well to the circular boundary of the inhibition zone (accuracy 0.05 mm), on several sides of the test well, then averaged (Muhardi, 2002). Calculation of the inhibition zone area followed the procedure of Adam et al., (2014).

$$D = \frac{d_1 + d_2}{2} \qquad (2)$$

D is the antibacterial diameter of chitosan, d1 the vertical diameter of chitosan antibacterial, and d2 the horizontal diameter of chitosan antibacterial (Magani et al., 2020).

Result and Discussion

Pearl oyster shell powder has been successfully extracted into chitosan through the isolation method. The chitosan isolation process starts with demineralization, deproteination, decolorization, and deacetylation. Each process produces powders with physical characteristics that are not too much different (Figure 1).

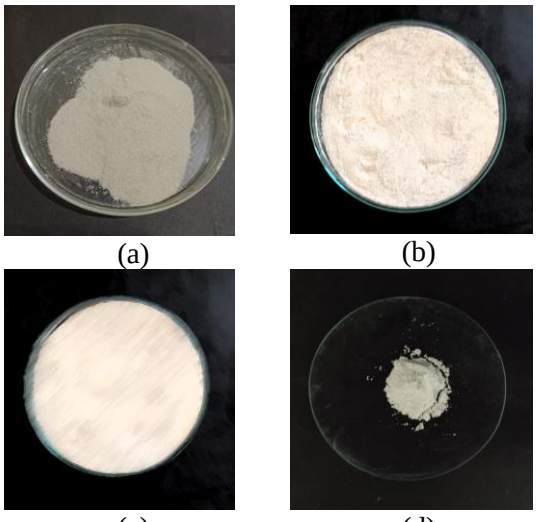

Figure 1. Powder from the isolation process at each stage (a) demineralization (b) deproteination (c) decolorization, and (d) deacetylation.

The physical changes in the powder occur due to the removal of non-chitosan components during the isolation process. Isolation of clam shell powder removes minerals, proteins, color

pigments, and acetyl groups (Abolude, 2016). Once these components are removed, the color of the powder tends to be lighter and is white or light yellowish depending on the final purity of the chitosan. Usually, the purity of chitosan is related to its degree of deacetylation (DD). The %DD value of chitosan in sample E based on the IR analysis results showed a value of 95.37% which indicates that its purity has met the medical material standards.

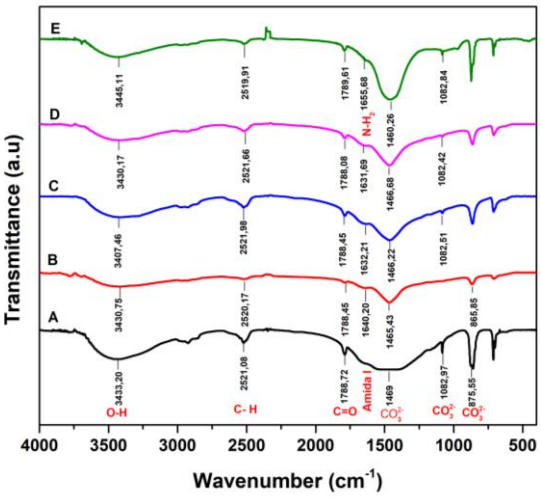

Figure 2. IR analysis of sample a (pearl oyster shell powder), sample B (demineralization), sample C (Deproteination), sample D (decolorization), and sample E (deacetylation)

IR spectrum analysis (Figure 2) shows the waveform shift in each isolation process. Interpretation of the spectrum of each stage shows the characteristic features of each material. Sample a (pearl oyster shell powder) identified the characteristic peaks of the main components of the shell, such as calcium carbonate ($CaCO_3$). An absorption band around 1400-1500 cm-1 indicates the presence of asymmetric vibrations of the carbonate group (CO_3^{2-}) (Capelo-Avilés et al., 2024). Then in the area of 875 cm^{-1} visible vibration thread of the group CO_3^{2-} indicates the presence of calcium carbonate. Protein or chitin groups may also appear in certain areas but are not dominant. Sample B (demineralized) has calcium carbonate-related peaks (CO_3^{2-}) reduced or disappeared, mainly peaks in the vicinity of 1400-1500 cm^{-1} and 875 cm^{-1}. The demineralization process is aimed at removing minerals such as calcium carbonate so that the peaks associated with the vibration of CO_3^{2-} become significantly reduced or disappear completely. The remaining bands indicate the presence of organic compounds such as chitin. Furthermore, sample C (deproteination) produces peaks with amide groups (C=O and N-H) of the reduced protein. The amide I and amide II vibrations of the protein usually appear around 1650-1550 cm^{-1} (Sadat & Joye, 2020). After deproteination, the peak intensity in this area decreases, signaling protein removal. The dominant vibrations on this spectrum are most likely from chitin. The D (decolorized) sample does not show a drastic change in the C band of the spectrum, but the spectrum can show cleaner and clearer peaks without any interference from the pigment. Finally, the sample E (deacetylation) there is a decrease in peak intensity in the region of 1650 cm^{-1} (C=O amide I), and there is a shift in the wavenumber around 1655.58 cm^{-1} which indicates the vibrational stretching of the N-H bond of chitosan the emergence of a new peak around 1590 cm^{-1} which indicates the vibrational N-H of chitosan (Kurniawidi et al., 2024). The reduction in amide intensity indicates the success of the deacetylation process, which converts chitin to chitosan by breaking off the acetyl group. Peaks in the 3400 cm^{-1} area also become wider due to the increased content of hydroxyl groups (-OH) associated with chitosan.

Table 1. The results of the analysis of antibacterial test E.Coli and S.Aureus

Chitosan Concentration (%)	Ability To Inhibit Bacteria	
	E. Coli	S. Aureus
10	-	-
20	+	+
30	+	+
40	+	+
50	+	+
60	+	+
70	+	+
80	+	+

From the data presented (Table 1) the ability of chitosan to inhibit the growth of E. Coli bacteria and S. Aureus bacteria is effective at a minimum chitosan concentration of 20%. This shows that chitosan from pearl shells has the potential as an antibacterial of gram-negative (E.Coli) and gram-positive (S. Aureus). The antibacterial properties of chitosan arise because

it has an amino group (-NH_2) that is positively charged at acidic to neutral pH (F). The positive charge can interact with the negatively charged cell wall of bacteria (especially in bacteria E. coli). The interaction of positive and negative charges disrupts the bacterial cell wall resulting in the death of the bacteria.

Table 2. Analysis of the inhibitory zone of bacteria E.Coli and S.Aureus against chitosan

Type of bacteria	Negative Control (mm)	Positive Control (mm)	Inhibition Zone (mm)
E. Coli	0.7	0.9	1.0
S. Aureus	0.7	0.9	1.3

Bacterial activity in chitosan was analyzed based on positive and negative control in the experiment (Table 2). Antibacterial activity in S.Aureus is better than E.Coli. Although the inhibitory zone is increased compared to the control, the inhibitory effect of chitosan on both bacteria is still relatively small, which may indicate that chitosan concentrations or conditions can be adjusted to increase their effectiveness.

Conclusion

Isolation of chitosan from pearl oyster shells (Pinctada maxima sp) obtained a degree of deacetylation of chitosan 95.37%. The functional group analysis of each isolation process shows the changes typical of each process. Pearl shell powder identified the typical peaks of calcium carbonate ($CaCO_3$). Demineralized powder samples have calcium carbonate (CO_3^{2-}) related peaks reduced or disappeared; subsequently, deproteinated powder samples produce peaks with amide Groups (C=O and N-H) of the reduced protein. Decolorized powder samples do not show drastic changes in the spectral band of the deproteinated powder, but the spectrum can show cleaner and clearer peaks without any interference from the pigment. In the last, deacetylated powder sample there was a decrease in peak intensity in the area of 1650 cm^{-1} (C=O amide). The ability of chitosan to inhibit the growth of E.Coli and S. Aureus bacteria Aureus is effective at a minimum chitosan concentration of 20%. The antibacterial properties of chitosan arise because it has an amino group (-NH_2) that is positively charged at acidic to neutral pH. Antibacterial activity in S. aureus is better than E.Coli.

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Referensi

Abolude, I. O. (2016). Modification of Emulsion Paint Using Chitosan-Grafted- Acrylic Acid [AHMADU BELLO UNIVERSITY]. In *AHMADU BELLO UNIVERSITY*. https://doi.org/10.13140/RG.2.2.30482.63686

Adam, A. A., Posangi, J., Tumewu, E., & Tallei, T. E. (2014). Aktivitas Antibakteri Ekstrak Kasar Tunikata Polycarpa Aurata Terhadap Streptococcus Mutans. In *Dentire Journal* (Vol. 3, Issue 2).

Akbarzadeh, I., Keramati, M., Azadi, A., Afzali, E., Shahbazi, R., chiani, M., Norouzian, D., & Bakhshandeh, H. (2021). Optimization, physicochemical characterization, and antimicrobial activity of a novel simvastatin nano-niosomal gel against E. coli and S. aureus. *Chemistry and Physics of Lipids*, *234*(October 2020), 105019. https://doi.org/10.1016/j.chemphyslip.2020.105019

Alawiyah, G., Septiani, N., Rahayu, S., Kurniawidi, D. W., Ardianto, T., Budianto, A., Alaa, S., & Syamsuddin. (2024). *Synthesis of nanochitosan from oyster pearl shell (Pinctada maxima) as renewable energy candidate*. *7*(2), 526–533. https://doi.org/10.29303/aca.v7i2.205

Amor, I. Ben, Hemmami, H., Laouini, S. E., Abdelaziz, A. G., & Barhoum, A. (2024). Influence of chitosan source and degree of deacetylation on antibacterial activity and adsorption of AZO dye from water. *Biomass Conversion and Biorefinery*, *14*(14), 16245–16255.

https://doi.org/10.1007/s13399-023-03741-9

Ardean, C., Davidescu, C. M., & Neme, N. S. (2021). Ardean2021.Pdf. *Factors Influencing the Antibacterial Activity of Chitosan and Chitosan Modified by Functionalization*, *22*(14), 7449.

Capelo-Avilés, S., Tomazini de Oliveira, R., Gallo Stampino, I. I., Gispert-Guirado, F., Casals-Terré, A., Giancola, S., & Galán-Mascarós, J. R. (2024). A thorough assessment of mineral carbonation of steel slag and refractory waste. *Journal of CO2 Utilization*, *82*(January). https://doi.org/10.1016/j.jcou.2024.102770

Destrianingtyas, A. S., Rahayu, S., Illahi, R. R., & Kurniawidi, D. W. (2024). Isolasi Kitosan dari Cangkang Kerang Mutiara (Pinctada Maxima) Menggunakan Deasetilasi dengan Gelombang Mikro. *Kappa Journal*, *8*(2), 262–269.

Dubey, A., Vahabi, H., & Kumaravel, V. (2023). Antimicrobial and Biodegradable 3D Printed Scaffolds for Orthopedic Infections. *ACS Biomaterials Science and Engineering*, *9*(7), 4020–4044. https://doi.org/10.1021/acsbiomaterials.3c00115

Fatima, B. (2020). Quantitative Analysis by IR: Determination of Chitin/Chitosan DD. In M. Khan, G. M. do Nascimento, & M. El-Azazy (Eds.), *Modern Spectroscopic Techniques and Applications*. IntechOpen. https://doi.org/10.5772/intechopen.89708

Gomes, L. C., Faria, S. I., Valcarcel, J., Vázquez, J. A., Cerqueira, M. A., Pastrana, L., Bourbon, A. I., & Mergulhão, F. J. (2021). The effect of molecular weight on the antimicrobial activity of chitosan from Loligo opalescens for food packaging applications. *Marine Drugs*, *19*(7). https://doi.org/10.3390/md19070384

Gonçalves, C., Ferreira, N., & Lourenço, L. (2021). Production of low molecular weight chitosan and chitooligosaccharides (COS): A review. *Polymers*, *13*(15), 1–23. https://doi.org/10.3390/polym13152466

Ha, S., Lee, J. W., Choi, S. H., Kim, S. H., Kim, K., & Kim, Y. (2019). Calcination characteristics of oyster shells and their comparison with limestone from the perspective of waste recycling. *Journal of Material Cycles and Waste Management*, *21*(5), 1075–1084. https://doi.org/10.1007/s10163-019-00860-2

Handayani, D., Alaa, S., Kurniawidi, D. W., & Rahayu, S. (2022). Pengolahan Limbah Cangkang Kerang Mutiara (Pinctada Maxima) Sebagai Adsorben Logam Berat Fe. *Jurnal Pertambangan Dan Ligkungan*, *3*(2), 10–15.

Khrunyk, Y., Lach, S., Petrenko, I., & Ehrlich, H. (2020). Progress in Modern Marine Biomaterials Research. *Marine Drugs*, *18*(12), 1–47. https://doi.org/10.3390/md18120589

Kurniawidi, D. W., Alawiyah, G., Rahayu, S., Masruroh, Wirawan, R., Destrianingtyas, A. S., Septiani, N., Ardianto, T., & Illahi, R. R. (2024). Modification of Chitosan Isolation Method from Pearl Oyster Shell (Pinctada maxima sp) as A Source of Natural Polymer Modification of Chitosan Isolation Method from Pearl Oyster Shell (Pinctada maxima sp) as A Source of Natural Polymer. *Journal of Physics: Conference Series 2866 (2024) 012015 IOP*, 1–9. https://doi.org/10.1088/1742-6596/2866/1/012015

Lu, J., Wang, Z., Zhang, H., Xu, W., Zhang, C., Yang, Y., Zheng, X., & Xu, J. (2022). Bone Graft Materials for Alveolar Bone Defects in Orthodontic Tooth Movement. *Tissue Engineering - Part B: Reviews*, *28*(1), 35–51. https://doi.org/10.1089/ten.teb.2020.0212

Magani, A. K., Tallei, T. E., & Kolondam, B. J. (2020). Antibacterial Test of Chitosan Nanoparticles against Staphylococcus aureus and Escherichia coli. *Jurnal Bios Logos*, *10*(1), 7–13.

Muhardi. (2002). *Isolasi dan Karateristik Komponen Antibakteri dan Biji Atung (Parinarium glaberrium Hassak)*. IPB University.

Ningsih, S. N. R., Tania, E., Azizah, N. N., Lutfiah, S. L., & Gunarti, N. S. (2022). Aktivitas Antibakteri Kitosan Dari Berbagai Jenis Bahan Baku Hewani : Review Journal. *Jurnal Buana Farma*, *2*(4), 25–30. https://doi.org/10.36805/jbf.v2i4.576

Nurlaili, Alaa, S., & Rahayu, S. (2022). Modifikasi Teknk Isolasi Biopolimer Kitosan dari Cangkang Kerang Mutiara (Pinctada maxima) Sebagai Adsorben Zat Warna Metilen Blue. *ORBITA. Jurnal Hasil Kajian, Inovasi, Dan Aplikasi Pendidikan Fisika*, *8*(2), 268–273.

Nurmaulida, S. E., Alawiyah, G., Rahayu, S., Taufik S, A., Hidayatullah, K., Kurniawidi, D. W., & Ali, M. (2023). FABRICATION OF CHITOSAN BIOPOLYMER FROM PEARL OYSTER SHELLS (Pinctada maxima) FOR MEDICAL APPLICATIONS. *Indonesian Physical Review*, *6*(2), 240–249. https://doi.org/10.29303/ipr.v6i2.227

Pei, J., Wang, Y., Zou, X., Ruan, H., Tang, C., Liao, J., Si, G., & Sun, P. (2021). Extraction, Purification, Bioactivities and Application of Matrix Proteins From Pearl Powder and Nacre Powder: A Review. *Frontiers in Bioengineering and Biotechnology*, *9*(April), 1–12. https://doi.org/10.3389/fbioe.2021.649665

Sadat, A., & Joye, I. J. (2020). Peak fitting applied to fourier transform infrared and raman spectroscopic analysis of proteins. *Applied Sciences (Switzerland)*, *10*(17). https://doi.org/10.3390/app10175918

Weißpflog, J., Vehlow, D., Müller, M., Kohn, B., Scheler, U., Boye, S., & Schwarz, S. (2021). Characterization of chitosan with different degree of deacetylation and equal viscosity in dissolved and solid state – Insights by various complimentary methods. *International Journal of Biological Macromolecules*, *171*, 242–261. https://doi.org/10.1016/j.ijbiomac.2021.01.010

Yarnpakdee, S., Senphan, T., & Karnjanapratum, S. (2025). Structural characterization and antibacterial activity of pearl oyster (Pinctada maxima) shell as affected by calcination temperature. *Journal of Agriculture and Food Research*, *19*(November 2024), 101551. https://doi.org/10.1016/j.jafr.2024.101551