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Effect Of Deacetylation Temperature On The Degree Of Deacetylation Of Chitosan Extracted From Panulirus Penicillatus *Shells*

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Abstract: Chitosan is a valuable biopolymer derived from chitin, whose quality is strongly influenced by its degree of deacetylation (DD). Deacetylation temperature is one of the key parameters affecting the conversion of chitin to chitosan. This study aimed to investigate the effect of deacetylation temperature on the degree of deacetylation of chitosan extracted from *Panulirus penicillatus* shells. Chitosan was produced through demineralization, deproteinization, and deacetylation processes, with deacetylation temperatures varied at 80°C, 90°C, and 100°C. The DD of the resulting chitosan was determined using Fourier Transform Infrared (FTIR) spectroscopy and calculated based on the absorbance ratio of characteristic functional groups. Statistical analysis was performed to evaluate the significance of temperature variation on DD values. The results indicated that deacetylation temperature significantly affected the degree of deacetylation of chitosan, with higher temperatures generally leading to higher DD values within the studied range. These findings demonstrate that temperature control during the deacetylation process plays a crucial role in improving chitosan quality and provides important insights for optimizing chitosan production from lobster shell waste.

Keywords: Chitosan, Deacetylation temperature, Degree of deacetylation, *Panulirus penicillatus*, Shell waste.

INTRODUCTION

Chitosan is a biodegradable, biocompatible, and non-toxic biopolymer derived from chitin, which is abundantly found in the exoskeletons of crustaceans such as shrimp, crabs, and lobsters[1]. Due to its unique physicochemical properties, chitosan has been widely applied in various fields, including food preservation, pharmaceuticals, agriculture, water treatment, and biomedical applications [2,3]. Indonesia, as a maritime country, produces a significant amount of crustacean shell waste, which has considerable potential to be utilized as a sustainable raw material for chitosan production [4].

The quality and functionality of chitosan are strongly influenced by its degree of deacetylation (DD), which represents the proportion of deacetylated amino groups in the polymer chain[5]. A higher DD generally enhances chitosan solubility, chemical reactivity, and biological performance, making DD one of the most important parameters in determining chitosan suitability for various applications [6]. Therefore, controlling the deacetylation process is essential to obtain chitosan with desired characteristics.

Among the factors affecting the deacetylation process, temperature plays a critical role in the conversion of chitin to chitosan. Increasing deacetylation temperature can accelerate the removal of acetyl groups and promote the formation of free amine groups[7]. However, excessively high temperatures may cause polymer chain degradation, which can negatively affect chitosan

quality and molecular integrity[8]. Several studies have reported that deacetylation temperature significantly influences the DD of chitosan, although the optimal temperature range may vary depending on the chitin source and processing conditions[9,10].

Spiny lobster (*Panulirus penicillatus*) shells represent an underutilized marine waste with high chitin content[11]. However, studies focusing on chitosan extraction from this specific biological source remain limited. In particular, systematic investigations on the effect of deacetylation temperature on the degree of deacetylation of chitosan derived from *P. penicillatus* shells are still scarce[12]. Understanding this relationship is important for optimizing processing parameters and enhancing the value of lobster shell waste.

Therefore, this study aimed to investigate the effect of deacetylation temperature on the degree of deacetylation of chitosan extracted from *Panulirus penicillatus* shells. The results of this study are expected to provide scientific insight into temperature optimization during chitosan production and support the sustainable utilization of marine shell waste.

MATERIALS AND METHODS

1. Materials

Spiny lobster (*Panulirus penicillatus*) shells were obtained from local fishery processing waste in West Sumatra, Indonesia. The shells were washed thoroughly with tap water to remove adhering impurities, dried at room temperature, and ground into smaller particles. Analytical-grade chemicals were used throughout the experiments, including hydrochloric acid (HCl), sodium hydroxide (NaOH), and distilled water.

2. Chitin Extraction

Chitin was extracted from lobster shells through demineralization and deproteinization processes. Demineralization was carried out by treating the shell powder with HCl solution under constant stirring to remove calcium carbonate, followed by washing with distilled water until neutral pH was achieved. Deproteinization was then performed using NaOH solution to remove residual proteins. The resulting chitin was washed repeatedly with distilled water and dried for further processing.

3. Chitosan Preparation by Deacetylation

Chitosan was produced by deacetylating the extracted chitin using concentrated NaOH solution. The deacetylation process was conducted at three different temperatures: 80°C, 90°C, and 100°C. Each temperature treatment was performed in triplicate. After completion of the reaction, the samples were washed with distilled water until neutral pH and dried to obtain chitosan powder.

4. Determination of Degree of Deacetylation

The degree of deacetylation (DD) of chitosan was determined using Fourier Transform Infrared (FTIR) spectroscopy. FTIR spectra were recorded in the range of 4000–400 cm^{-1}. The DD was calculated based on the absorbance ratio of characteristic peaks corresponding to the amide and hydroxyl groups, following a commonly accepted calculation method. The DD values obtained from each treatment were expressed as mean ± standard deviation.

5. Statistical Analysis

Statistical analysis was performed to evaluate the effect of deacetylation temperature on the degree of deacetylation of chitosan. The DD data were analyzed using one-way analysis of variance (ANOVA) at a 95% confidence level. Differences among treatment means were considered statistically significant when $p < 0.05$.

RESULTS AND DISCUSSION

Chitosan Extraction from ***Panulirus penicillatus*** Shells

Chitosan was successfully extracted from *Panulirus penicillatus* shells through sequential demineralization, deproteinization, and deacetylation processes. The extracted material exhibited typical physical characteristics of chitosan, including a pale color,

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fibrous appearance, and hygroscopic behavior. These features are commonly reported for chitosan derived from crustacean shell waste and indicate successful conversion from chitin to chitosan[13,14].The results of chitosan extraction at different deacetylation temperatures are shown in Figure 1.

(a) (b) (c)

Figure 1. Chitosan derived from rock lobster waste at different deacetylation temperatures: (a) 80 °C; (b) 90 °C; (c) 100 °C.

Based on the figure above, the produced chitosan meets the standard, exhibiting a light cream to white color and a powdery texture. Relatively high deacetylation temperatures and the use of concentrated NaOH resulted in a darker, slightly brownish coloration of the chitosan. The demineralization step effectively removed inorganic components, primarily calcium carbonate, which is a major constituent of lobster shells. Efficient demineralization is essential to improve alkali penetration during subsequent processing stages and to prevent interference with deacetylation efficiency[15,16]. Similar results have been reported for chitosan extraction from shrimp and crab shells using acid-based demineralization methods[17].

Deproteinization using alkaline treatment further eliminated residual proteins bound to the chitin matrix. Protein removal is a critical step to improve the purity of the extracted polymer, as residual proteins may affect the structural and functional properties of the resulting chitosan [18,19]. Previous studies have demonstrated that alkaline deproteinization effectively disrupts protein–polysaccharide interactions, facilitating protein solubilization and removal [20].

Following chitin isolation, deacetylation was performed to convert chitin into chitosan. The formation of chitosan was qualitatively confirmed by structural analysis, which revealed characteristic functional groups of chitosan. These results demonstrate that lobster shell waste can serve as a viable raw material for chitosan production, although further optimization of processing conditions is required to improve product quality.

Effect of Deacetylation Temperature on the Degree of Deacetylation

The degree of deacetylation (DD) is a key parameter in chitosan characterization, as it reflects the proportion of acetyl groups removed from the chitin structure during the deacetylation process. DD strongly influences the physicochemical properties of chitosan, including solubility, surface charge, and intermolecular interactions, and is closely associated with its biological activity [1].

In this study, the DD of chitosan produced at different deacetylation temperatures (80°C, 90°C, and 100°C) was evaluated using Fourier Transform Infrared (FTIR) spectroscopy. Quantitative determination of DD was limited due to the dominant presence of the amide I absorption band around 1655 cm^{-1} compared to the –OH/–NH stretching band /near 3450 cm^{-1} in all samples. This spectral characteristic indicates that acetyl groups were still prevalent in the polymer structure, suggesting that the deacetylation process was not fully effective under the studied conditions. [21,22].

In this study, the DD of chitosan extracted from *Panulirus penicillatus* shells was evaluated using Fourier Transform Infrared (FTIR) spectroscopy. Qualitative evaluation was based on the relative intensity of the amide I absorption band at approximately

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1655 cm^{-1}, corresponding to the C=O stretching vibration of residual acetyl groups, and the broad –OH/–NH stretching band around 3450 cm^{-1}. The ratio of corrected peak areas (A1655/A3450) was used as a relative indicator of DD, where lower ratios indicate a reduced presence of acetyl groups and thus a higher degree of deacetylation [23].Therefore, DD evaluation was conducted using a qualitative and semi-quantitative approach based on the ratio of corrected FTIR peak areas (A_{1655}/A_{3450}), as presented in Table 1.

Tabel 1. Ratio of corrected FTIR peak areas

Suhu Deasetilasi	Corr. Area 1655 cm^{-1}	Corr. Area 3450 cm^{-1}	Rasio A1655/A3450
80 °C	13,215	9,013	1,47
90 °C	5,074	1,830	2,77
100 °C	90,526	6,440	14,06

As shown in Table 1, the A1655/A3450 ratio for chitosan deacetylated at 80°C was 1.47, increasing to 2.77 at 90°C, and rising markedly to 14.06 at 100°C. The dominance of the amide I band across all temperature treatments indicates that acetyl groups were not effectively removed under the applied deacetylation conditions. These results suggest that increasing the deacetylation temperature within the investigated range did not result in a proportional enhancement of DD.

Chemically, the deacetylation process is influenced not only by temperature but also by alkali concentration, reaction time, and solid–liquid ratio[24]. Although higher temperatures may accelerate reaction kinetics, excessive thermal conditions can induce degradation of the chitosan polymer chain through cleavage of β-(1→4) glycosidic bonds, potentially altering FTIR peak intensities without significantly improving acetyl group removal[25,26]. The persistence of strong amide I absorption in this study indicates that the applied temperatures were insufficient to achieve effective deacetylation, resulting in chitosan with a relatively low DD or partially deacetylated chitin.

Quantitative DD values calculated using the Domzy method yielded DD values of ≤75% for all temperature treatments. Quantitative determination of DD using FTIR is highly dependent on spectral processing procedures, including baseline correction and peak area integration, which can introduce uncertainty when amide absorption remains dominant[27]. This methodological limitation likely contributed to the relatively low and closely distributed DD values observed across the different deacetylation temperatures.

Statistical analysis supported these observations. Normality testing indicated that the DD data were not normally distributed ($p < 0.05$); therefore, the Kruskal–Wallis test was applied. The results revealed no statistically significant differences in DD among deacetylation temperatures of 80°C, 90°C, and 100°C ($p > 0.05$). The absence of a significant temperature effect suggests that temperature alone, under the experimental conditions employed, was not a determining factor in enhancing the degree of deacetylation.

Overall, the results demonstrate that variations in deacetylation temperature within the studied range did not significantly affect the DD of chitosan derived from *P. penicillatus* shells. The relatively low DD obtained highlights the necessity of further optimization of deacetylation parameters, such as alkali concentration and reaction duration, to improve acetyl group removal and obtain chitosan with enhanced functional properties[28–30].

CONCLUSION

Chitosan was successfully extracted from *Panulirus penicillatus* shells, and the effect of deacetylation temperature on the degree of deacetylation (DD) was evaluated using FTIR analysis. Increasing the deacetylation temperature from 80°C to 100°C did not significantly affect DD ($p > 0.05$), indicating incomplete deacetylation under the studied conditions. These results suggest that temperature alone was insufficient to improve deacetylation efficiency, and optimization of other process parameters is required.

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