Optimization of Chitosan Production from Mangrove Crab Shell Waste Using Response Surface Methodology (RSM)

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ABSTRACT

This study optimized the extraction of chitin into chitosan from mangrove crab shells using the Response Surface Methodology (RSM) approach. Three independent factors were used: NaOH concentration (A), deacetylation temperature (B), and deacetylation time (C) at five levels (-α, -1, 0, +1, α). The NaOH concentration ranged from 43.2% to 78.8%, the deacetylation temperature ranged from 83.2°C to 116.8°C, and the deacetylation time ranged from 0.4 hours to 3.6 hours. The dependent variables were yield (Y1), degree of deacetylation (Y2), intrinsic viscosity (Y3), and molecular weight (Y4). The results of chitin deacetylation to chitosan optimization using the Design Expert program with the RSM approach led to 42 optimization formulas. The formula with the highest desirability value of 0.927 was obtained using the following extraction conditions: 50% NaOH concentration, 110°C deacetylation temperature, and 1 hour deacetylation time. Chitosan obtained under these extraction conditions yielded 33.61%, a deacetylation degree of 79.2%, an intrinsic viscosity of 34.96 ml/g, and a molecular weight of 9.25 kDa. The deacetylation of the chitosan met the quality standards from Protan Laboratory, as deacetylation was ≥70%.

Keywords: Chitosan; degree of deacetylation; mangrove crab shells

ABSTRAK

Penelitian ini mengoptimasi ekstraksi kitin menjadi kitosan dari cangkang kepiting bakau menggunakan pendekatan *Response Surface Methodology* (RSM). Tiga faktor independen digunakan: konsentrasi NaOH (A), suhu deasetilasi (B), dan waktu deasetilasi (C) pada lima taraf (-α, -1, 0, +1, α). Konsentrasi NaOH berkisar antara 43,2% hingga 78,8%, suhu deasetilasi berkisar antara 83,2°C hingga 116,8°C, dan waktu deasetilasi berkisar antara 0,4 jam hingga 3,6 jam. Variabel dependen adalah rendemen (Y1), derajat deasetilasi (Y2), viskositas intrinsik (Y3), dan berat molekul (Y4). Hasil optimasi deasetilasi kitin menjadi kitosan menggunakan program Design Expert dengan pendekatan RSM menghasilkan 42 formula optimasi. Formula dengan nilai desirabilitas tertinggi sebesar 0,927 diperoleh dengan menggunakan kondisi ekstraksi sebagai berikut: konsentrasi NaOH 50%, suhu deasetilasi 110°C, dan waktu deasetilasi 1 jam. Kitosan yang diperoleh pada kondisi ekstraksi tersebut menghasilkan rendemen 33,61%, derajat deasetilasi 79,2%, viskositas intrinsik 34,96 ml/g, dan berat molekul 9,25 kDa. Deasetilasi kitosan memenuhi standar mutu dari Laboratorium Protan, yaitu deasetilasi ≥70%.

Kata kunci: Kitosan; derajat deasetilasi; cangkang kepiting bakau

INTRODUCTION

In Lamjabat Village, Meraxa District, Banda Aceh, soft crabs (soka) are cultivated. These mangrove crabs, also known as soft-shell crabs, undergo a moulting phase and are increasingly popular due to their distinct flavour, high protein content, and low carbohydrate levels (Waiho et al., 2021). However, the shells produced during farming are discarded in landfills without economic use. Crustacean shells contain valuable components like calcium carbonate, protein, and chitin (Rahman et al., 2018). Converting crab shell waste into chitosan offers a promising solution by providing economic value and reducing waste volume (Tan et al., 2021). Utilizing these waste shells could reduce landfill waste and create valuable products, thus promoting economic growth and new business opportunities (Rahman et al., 2018).

Chitosan is a naturally occurring polymer derived from chitin, a biopolymer found in the exoskeletons of crustaceans such as crabs, shrimp, and lobsters, through deacetylation. As a linear polymer, chitosan contains primary amino groups, which enable chemical modifications to enhance its solubility and biological activity. Furthermore, chitosan exhibits antimicrobial, antioxidant, and anti-inflammatory properties, demonstrating potential in wound healing and cancer therapy. In the food industry, it functions as a natural preservative and contributes to developing biodegradable packaging. In environmental applications, chitosan is an adsorbent for removing pollutants, including heavy metals, from water (Aranaz et al., 2021).

The conversion of chitin into chitosan involves three primary steps: demineralization, which entails the removal of calcium carbonate and calcium phosphate; deproteinization, which involves the removal of proteins; and deacetylation, which refers to the removal of acetyl groups (–COCH3) from chitin. The degree of deacetylation (DD) refers to the percentage of acetyl groups (–COCH3) removed from chitin during deacetylation to produce chitosan. DD indicates the extent to which acetyl groups have been eliminated, thereby influencing the properties of the polymer. The higher the DA, the more acetyl groups have been removed, and the polymer is more likely to be classified as chitosan (Pellis et al., 2022). According to the research conducted by Czechowska-Biskup et al. (2012), a degree of deacetylation (DD) ranging from 70% to 95% is generally considered to represent an optimal range for chitosan, as it ensures the material exhibits favourable physicochemical properties that are suitable for a wide range of applications.

The deacetylation process is influenced by three primary factors: NaOH concentration, reaction temperature, and reaction time(Tolaimate et al., 2003). Despite the extensive research on chitosan, the deacetylation process frequently produces inconsistent results. Response Surface Methodology (RSM) enhances the process and achieves optimal outcomes. RSM is a collection of mathematical and statistical techniques used for modelling and analyzing situations where multiple factors influence a response variable to identify the optimal conditions (Anwar et al., 2015).

EXPERIMENTAL SECTION

Materials and Tools

The raw material used was mangrove crab shells, which were obtained from Lamjabat Village, Meraxa District, Banda Aceh. The chemicals used included sodium hydroxide (NaOH), hydrochloric acid (HCl), distilled water (aquadest), acetic acid, sodium hypochlorite, and acetone. The tools used in this study included 80-mesh sieves, a pestle and mortar, beakers, Erlenmeyer flasks, basins, drop pipettes, tissue paper, washcloths, filter paper, a desiccator, a hot plate, a magnetic stirrer, a thermometer, an analytical balance, volumetric flasks, measuring cups, funnels, spatulas, Petri dishes, and a Soxhlet extractor. The analytical instruments used were a Fourier Transform Infrared Spectrometer (FTIR) (Agilent Technologies), an Ostwald viscometer (BrandTech), and a furnace (Thermo Fisher Scientific).

Sample Preparation

1. Production of Crab Shell Powder

The crab shell powder preparation followed the method by Baron et al. (2017). The shells were washed with running water, air-dried, and then oven-dried at 70°C for 6 hours. Afterward, they were crushed with a pestle and mortar, blended, and sifted through an 80-mesh sieve.

2. Deproteination

The deproteinization process followed the method described by Patria (2013). Crab shell powder was mixed with 3.5% NaOH at 1:5 (w/v). The mixture was stirred while heated and maintained at 90°C for 1 hour. After cooling, the mixture was filtered to obtain the solid fraction. The solid was washed with distilled water until the pH reached neutral and dried at 60°C for 4 hours.

3. Demineralization

The demineralization process followed the method described by Patria (2013). The deproteinized crab shell powder was mixed with

2N HCl at a ratio of 1:5 (w/v) and heated at 90°C for 1 hour. The solution was then filtered to collect the solid material. The solid was washed with distilled water until the pH reached neutral and dried at 60°C for 4 hours. The resulting product was chitin.

4. Depigmentation

The depigmentation process followed the procedure described by Patria (2013). The chitin obtained from demineralization was extracted with acetone at a 1:5 (w/v) ratio for 4 hours using a Soxhlet extractor. The residue was then bleached with 0.38% sodium hypochlorite (NaOCl) for 5 minutes at room temperature. Afterwards, the residue was washed with distilled water until the pH became neutral and dried in an oven at 60°C for 4 hours.

5. Deacetylation of Chitin into Chitosan

The deacetylation process followed the method Patria (2013) described, with several modifications. Chitin powder was mixed with NaOH solution (43.2%–78.8%) at a ratio of 1:10 (w/v) and heated at varying temperatures from 83°C to 116°C (B) for 0.4 to 3.6 hours (C). The solids obtained were washed with distilled water until the pH reached neutral and then dried at 60°C for 24 hours. The product formed was chitosan.

Analysis Procedure

Yield

The procedure for determining chitosan yield follows the method established by Apriyantono et al. (1989). The yield is calculated by comparing chitosan's mass to chitin's mass. The calculation is expressed as:

Yield = $\frac{\text{Mass of chitosan}}{\text{Mass of Chitin}} x100\%$

Degree of Deacetylation Analysis

The analytical procedure for determining the degree of deacetylation (DD) follows the method established by Ahmad Khan et al. (2002), A one-gram chitosan sample is mixed with KBr in a 1:3 ratio, followed by homogenization. The resulting pellet is then placed into a Fourier-transform infrared (FTIR) spectrometer for spectral analysis. The degree of deacetylation is quantified by analyzing the Absorbance at specific wavelengths. The degree of deacetylation is then calculated using the formula:

Degree of Deacetylation (DD) = $\left[\frac{A1655}{A3450} x \frac{1}{1.33}\right] x100\%$

Where :

A1655 = Absorbance at the wavenumber 1655 cm^{-1} for the absorption of the amide/acetamide group (CH_3CONH)

A3450 = Absorbance at the wavenumber 3450 cm^{-1} for the absorption of the hydroxyl group (OH^-)

Intrinsic Viscosity Analysis

Viscosity Analysis follows the procedure established by J.-K. Hwang et al. (1997). The Viscosity is measured using an Oswald viscometer. The specific Viscosity is first calculated to determine the intrinsic Viscosity. The specific Viscosity is determined by comparing the flow rate of a given solution to that of its solvent. The procedure is as follows: First, prepare chitosan solutions with concentrations of 0.2%, 0.4%, 0.6%, 0.8%, and 1% in a 1% acetic acid solvent. The solutions are then placed in the Oswald viscometer, and the flow rate for each concentration is recorded. Second, 1% acetic acid solvent is used as a blank and introduced into the Oswald viscometer, and its flow rate is recorded. The specific viscosity value is determined by calculating both the specific Viscosity and the reduction viscosity. Using the Least method, the intrinsic viscosity is computed by extrapolating data from the graph relating reduction viscosity to various chitosan concentrations. The specific viscosity and reduction viscosity are calculated using the following formulas:

$\eta_{sp} = \frac{t-t0}{t0}$, $\eta_{red} = \frac{\eta sp}{c}$,

Where :

η_{sp} = Specific Viscosity, t = time required for the sample solution to flow (second)

η_{red} = Reduction Viscosity t0 = time required for the acetic acid solvent to flow (second)

C = Chitosan concentration

Molecular Weight Analysis

The molecular weight of chitosan is determined following the procedure outlined by J.-K. Hwang et al. (1997). The molecular weight of chitosan is calculated based on intrinsic Viscosity. Chitosan solutions are prepared in varying concentrations of 0.2%, 0.4%, 0.6%, 0.8%, and 1% in a 1% acetic acid solvent. The solutions are then placed in the Oswald viscometer. The obtained data are plotted on a (ηsp) / c graph. The molecular weight is determined using the Mark-Houwink equation:

$\eta = KM^{\alpha}$

Where:

η = Intrinsic Viscosity

K = Solvent constant for acetic acid (0.0474)

α = Mark-Houwink constant

M = Molecular weight

Experimental Design using RSM

This study applied Response Surface Methodology (RSM) to optimize the deacetylation process of chitin into chitosan. Three independent variables were selected: NaOH concentration (A), deacetylation temperature (B), and deacetylation time (C), each evaluated at five levels (−α, −1, 0, +1, α) based on a Central Composite Design (CCD) as described in Table 1. The NaOH concentration ranged from 43.2% to 78.8%, the temperature from 83°C to 116°C, and the heating time from 0.4 to 3.6 hours. The responses (dependent variables) measured were yield (Y1), degree of deacetylation (Y2), intrinsic viscosity (Y3), and molecular weight (Y4).

Table 1. The independent variables and their corresponding levels in the three-factor, five-level central composite rotatable design

Variable independent	Symbol	Code Level				
		-α	-1	0	+1	+α
NaOH concentration (%)	A	43.2	50	60	70	76.8
Deacetylation temperature (℃)	B	83	90	100	110	116
Deacetylation time (hours)	C	0.4	1	2	3	3.6

RESULTS AND DISCUSSION

Response Analysis for the Optimization of Chitin Deacetylation into Chitosan

The response variables selected for optimizing the deacetylation of chitin to chitosan were yield, degree of deacetylation, intrinsic viscosity, and molecular weight. Each response was evaluated based on the coefficient of determination (R^2), with the model exhibiting the highest R^2 value being chosen as the optimal response model. The model analysis, including the evaluation of the lack of fit, is presented in Table 2. A lack-of-fit value that is not statistically significant is desirable, as it indicates a strong correlation between the experimental data and the predicted model (Mourabet et al., 2017). The lack-of-fit values for all responses in

Table 2 indicate no significant effect, confirming that the response data for chitin deacetylation optimization to chitosan is predicted by the model. Consequently, the response variables selected for optimization were those with statistically significant models ($p \leq 0.05$).

Table 2. Model Analysis

Response	Model	p- Value (p<0.05)		Lack of fit (p<0.05)		R^2
Yield	Linier	0.0031	Significant	0.0617	tn	0.5693
Degree of Deacetylation	Linier	< 0.0001	Significant	0.3143	tn	0.8737
Intrinsic viscosity	2FI (Two Factor Interaction)	0.0024	Significant	0.1523	tn	0.7494
Molecular Weight	2FI	0.0035	Significant	0.0848	tn	0.7339

Chitosan Yield Response

The chitosan yield in this study ranged from 14.74% to 40.53%, with an average of 31.05%. ANOVA results showed that NaOH concentration and deacetylation temperature significantly affected yield ($P \leq 0.01$). The 3D response surface plot (**Error! Reference source not found.**) illustrates that increasing NaOH concentration (%) and deacetylation temperature (°C) tend to reduce chitosan yield due to their interactive effects. Conversely, lower NaOH concentrations and deacetylation temperatures correlate with higher yields. These

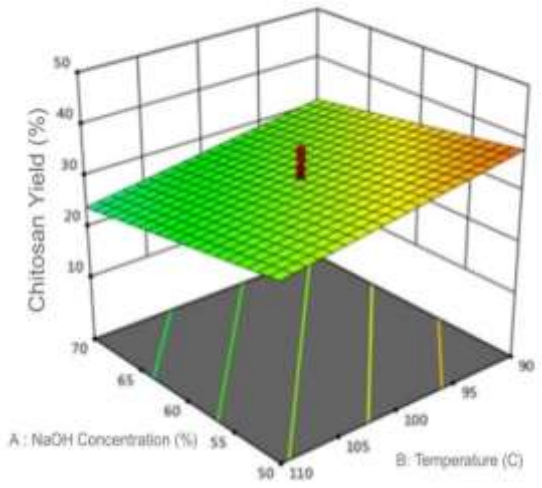


Figure 1. The effect of NaOH concentration (%) and deacetylation temperature (°C) on chitosan yield

findings are consistent with Mathaba & Daramola (2020), who reported that increasing NaOH volume and deacetylation time decrease chitosan yield. This phenomenon occurs because higher NaOH solvent volume enhances solvent-solid contact, promoting more extensive removal of acetyl groups ($–COCH_3$) from chitin. Additionally, prolonged deacetylation time increases the duration of contact between chitin and the NaOH solution, leading to greater diffusion of NaOH molecules into the chitin structure. This process depolymerises a portion of the chitosan, thereby reducing yield. Then, Pellis et al. (2022) state that yield tends to decrease as the number of acetyl groups bound by the strong base solution increases during the deacetylation process.

According to Hasriani et al. (2024), temperature can enhance the deacetylation reaction rate; however, if the temperature exceeds optimal levels, it may lead to the excessive release of acetylation chains from chitin. This results in the formation of fine chitosan particles, which subsequently dissolve in the NaOH solution during the deacetylation process. As a result, this dissolution contributes to a reduction in the overall chitosan mass.

Degree of Deacetylation Response

The degree of deacetylation is a key quality parameter in chitosan, representing the percentage of acetyl groups removed from chitin to produce chitosan. A higher degree of deacetylation indicates a lower acetyl group content in chitosan. As the acetyl group decreases, the interactions between ions and hydrogen bonds strengthen (Mathaba & Daramola, 2020).). A desirable degree of deacetylation for chitosan is ≥70% (Protan Lab, 1987). Based on the Czechowska-Biskup et al. (2012) study, a degree of deacetylation (DD) ranging from 70% to 95% is typically regarded as the optimal range for chitosan.

The degree of deacetylation (DD) can be determined through FTIR (Fourier Transform Infrared) spectroscopy, a method that quantifies the absorption of infrared radiation by atoms undergoing vibrational motions. These vibrations occur within the infrared region at specific frequencies, typically between 400 and 4000 cm^{-1}. The degree of chitosan deacetylation is calculated by comparing the absorbance values of the absorption bands at wavelengths of 1655 cm^{-1} and 3450 cm^{-1}. The absorption band at 1655 cm^{-1} corresponds to the carbonyl stretch of the N-acetyl group, while the absorption band at 3450 cm^{-1} corresponds to the stretch of the NH2 group. Absorbance (A) is expressed by equation (1), while the DD value is derived using equation (2) (Gargioni et al., 2006).

$$A = \text{Log } \frac{Po}{P} \quad \text{.....(1)}$$

Where:
Po = % transmittance of the baseline (maximum absorption)
P = % transmittance at maximum absorption

$$\%DD = 1 - \left[\frac{A1655}{A3450} x \frac{1}{1,33}\right] x\ 100\% \quad \text{.....(2)}$$

Where:
A1655 = Chitosan absorbance at a wavelength of 1655 cm^{-1}
A3450 = Chitosan absorbance at a wavelength of 3450 cm^{-1}

In this study, the degree of deacetylation (DD) ranged from 76.6% to 98.6%, with a mean value of 86.69%. ANOVA analysis indicated that both NaOH concentration and deacetylation temperature had a highly significant effect on DD ($P \leq 0.01$). As shown in Figure 2, increasing NaOH concentration and deacetylation temperature enhanced the degree of deacetylation. These results align with findings by Mathaba & Daramola (2020), who reported that DD increased from 33.9% to 73.05% when NaOH concentration was elevated from 20% to 40% at a constant temperature of 80°C. A similar upward trend in DD was observed with temperature increments to 100°C and 120°C at the same NaOH concentration. This behaviour can be attributed to the higher alkalinity of the NaOH solution, which facilitates more effective removal of acetyl groups, thus exposing additional amine groups and resulting in increased DD.

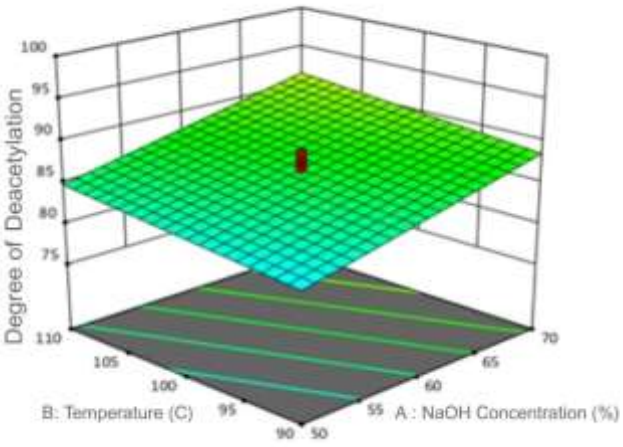


Figure 2. The effect of NaOH concentration (%) and deacetylation temperature (°C) on degree of deacetylation

Intrinsic Viscosity Response

Intrinsic viscosity is essential in chitosan quality analysis to determine its molecular weight. Intrinsic viscosity plays a crucial role in characterizing chitosan, as it reflects the polymer's chain length and molecular conformation in solution, which are directly related to its molecular weight. It is also important for predicting rheological properties, which affect the processing behaviour, film-forming ability, and biological activity of chitosan in various applications such as pharmaceuticals food packaging, and biomedical materials (Rinaudo, 2006).

The intrinsic viscosity in this study ranged from 10.98 mL/g to 1320.30 mL/g, with an average value of 478.27 mL/g. The ANOVA results showed that NaOH concentration and the interaction between NaOH concentration and deacetylation temperature had a highly significant effect ($P \leq 0.01$) on the intrinsic viscosity response. In contrast, the individual effects of deacetylation temperature and time and their interaction were not statistically significant ($P > 0.05$). A three-dimensional graph of the intrinsic viscosity response, as shown in Figure 3, was generated using Response Surface Methodology (RSM). An ideal intrinsic viscosity for chitosan depends on its intended application. For example, low to medium-viscosity chitosan (50–800 mL/g) is generally preferred for drug delivery systems and wound healing materials due to its good solubility and bioactivity. Meanwhile, higher viscosity chitosan (>800 mL/g) is typically used in film formation and water treatment, where higher molecular weight enhances mechanical strength and adsorption properties (Aranaz et al., 2021).

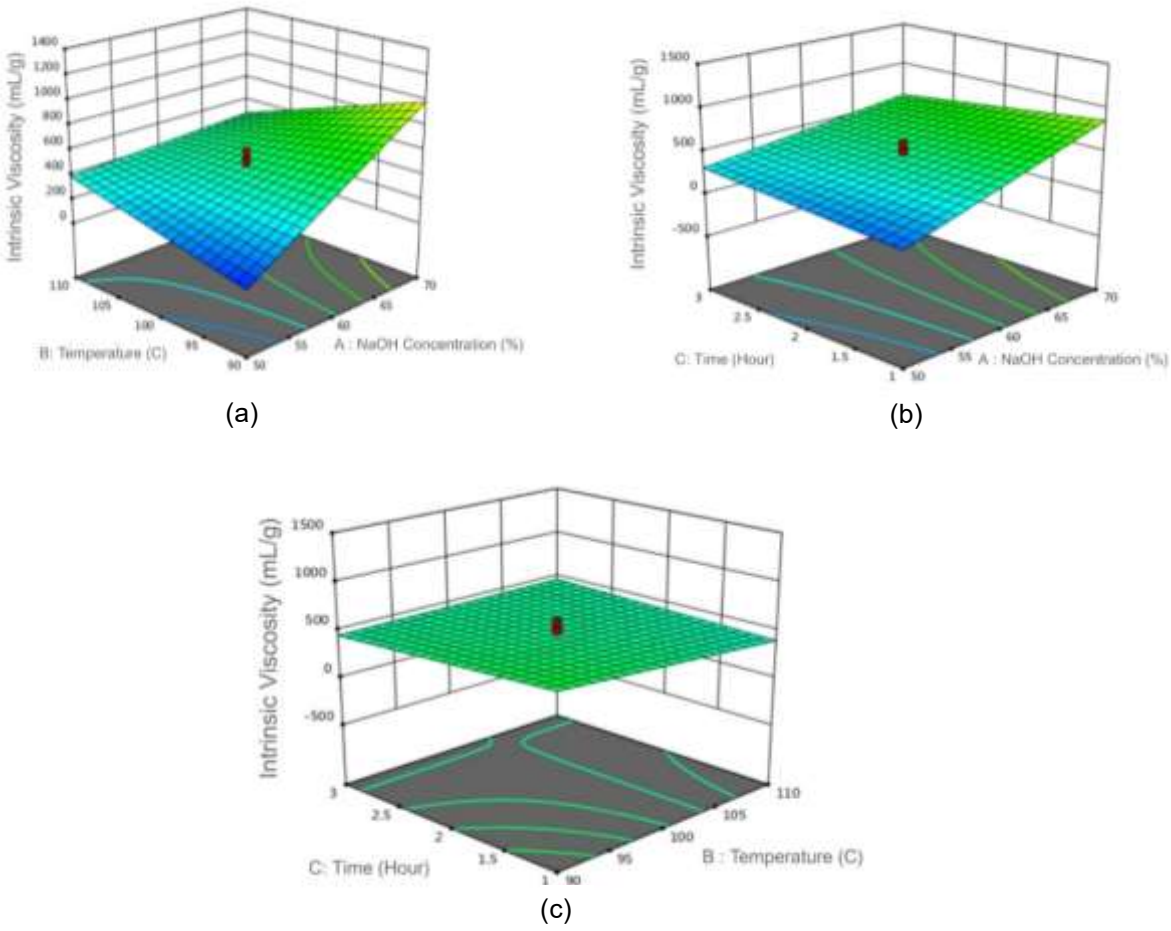


Figure 3. Three-dimensional graph of the effect of chitosan intrinsic viscosity due to (a) the effect of NaOH concentration (%) and deacetylation temperature (° C), (b) the effect of NaOH concentration (%) and deacetylation time (hours), and (c) time effect and deacetylation temperature (° C)

The three-dimensional response surface (Figure 3a) shows high NaOH concentration and low deacetylation temperature, resulting in high intrinsic viscosity. Low NaOH concentration and high temperature lead to low viscosity. This trend is consistent with findings from several studies, which report that increasing NaOH concentration improves the deacetylation process by providing more hydroxide ions (OH^-) to break acetyl

groups from chitin, leading to higher molecular weight chitosan and, consequently, higher intrinsic viscosity. Conversely, higher temperatures tend to increase the rate of deacetylation but may reduce molecular weight if the process is too aggressive, leading to lower viscosity (Lyalina et al., 2017).

According to Figure 3b, high NaOH concentration combined with longer deacetylation time increases viscosity, while lower NaOH concentration and shorter time reduce it. This observation is supported by research indicating that prolonged deacetylation allows for more complete removal of acetyl groups, thus increasing the degree of deacetylation and enhancing viscosity. The higher the concentration of NaOH, the more hydroxide ions are available, which accelerates the reaction, leading to a more pronounced effect on intrinsic viscosity (Kadak et al., 2023)

According to Figure 3c, deacetylation temperature and time have minimal effect on intrinsic viscosity. Temperature and time do not significantly influence the degree of deacetylation when NaOH concentration is controlled within the experimental conditions. Similar observations have been made in studies where, beyond a certain threshold, temperature and time were found to have little additional impact on viscosity as the reaction reached a saturation point (Dinculescu et al., 2024).

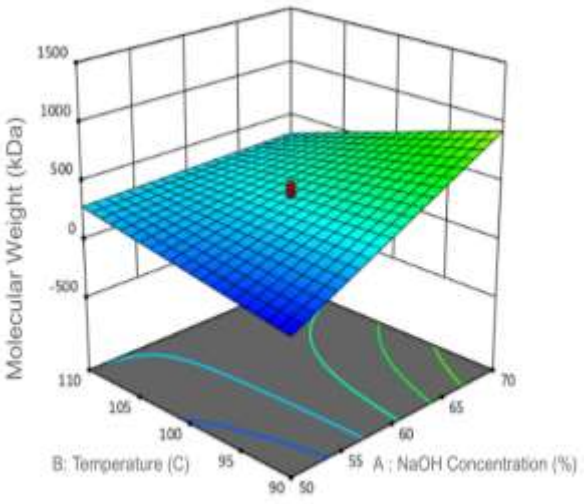
Molecular Weight Response

Chitosan molecular weight is a critical parameter for assessing the quality of chitosan, as it determines its suitability for various applications. Lower molecular weight chitosan is often preferred as an antibacterial, antitumor, and antioxidant agent. Chitosan with a medium molecular weight (30 kDa) exhibits higher anti-cholesterol activity compared to high molecular weight chitosan (250 kDa) (Brzezinski et al., 2004). In this study, the molecular weight ranged from 1.90 kDa to 1405.08 kDa, with an average molecular weight of 383.37 kDa. The ANOVA results indicated that NaOH concentration and the interaction between NaOH and deacetylation temperature had a highly significant effect ($P < 0.01$) on the molecular weight response. However, deacetylation temperature, deacetylation time, and their interaction did not significantly affect the molecular weight response. Figure 4 presents a three-dimensional graph illustrating the deacetylation degree response test results using Response Surface Methodology (RSM).

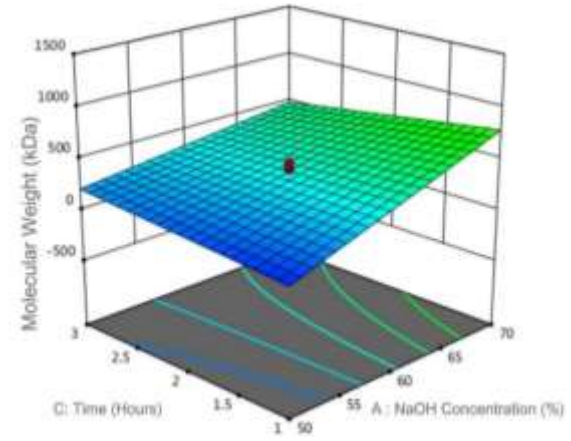
The graph in Figure 4a shows that the chitosan molecular weight is lower when NaOH concentration is high, and the deacetylation temperature is high. This result aligns with Hwang et al. (2002) used Response Surface Methodology (RSM) to control the molecular weight (MW) and degree of deacetylation (DD) of chitosan in a chemical process. The results showed that the interaction between temperature, NaOH concentration, and reaction time significantly influences the MW of chitosan. Increasing the temperature, NaOH concentration, and reaction time led to a significant decrease in the MW of chitosan, with the MW decreasing from 1,100 kDa to 100 kDa under these conditions. This finding aligns with research indicating that excessively high temperatures can cause polymer backbone degradation, thereby reducing molecular weight (Hwang et al., 2002).

(b)

The graph in Figure 4b reveals that a higher NaOH concentration combined with longer deacetylation times results in lower molecular weights. This outcome supports the notion that NaOH concentration directly affects the efficiency of the deacetylation process. EL Knidri et al. (2019) reported that extending the deacetylation duration substantially reduces molecular weight. Specifically, their research reported a decrease from 360 KDa to 300 KDa as the deacetylation time increased. This reduction can be attributed to the progressive removal of acetyl groups from the chitin structure during the extended deacetylation process, leading to a decrease in the molecular weight of the resulting chitosan. Figure 4c shows that the interaction between deacetylation time (hours) and temperature (°C) minimally impacts the chitosan molecular weight. This suggests that, after a certain threshold, variations in either temperature or time do not significantly affect the molecular weight.



(a)



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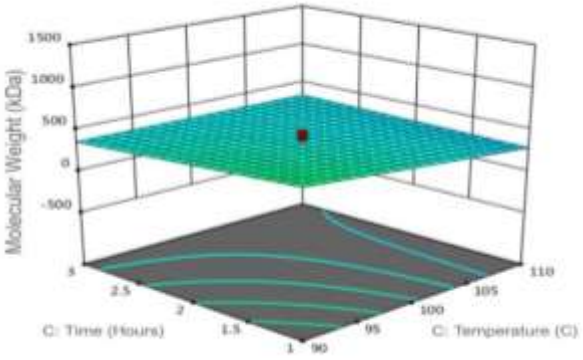

(c)

Figure 4. (a) The effect of NaOH concentration (%) and deacetylation temperature (°C) on molecular weight; (b) The effect of NaOH concentration (%) and deacetylation time (hours) on molecular weight; and (c) The effect of deacetylation time (hours) and temperature (°C) on molecular weight.

Optimization of the Chitin Deacetylation Process into Chitosan

The objective of the optimization process is to minimize the required effort while maximizing the desired outcomes. The responses, including yield, deacetylation degree, intrinsic viscosity, and molecular weight, demonstrated a significant impact on the model, with no significant lack of fit, thus ensuring that all responses were appropriate for optimization. Table 2 outlines the components being optimized, their respective targets, the minimum and maximum limits, and the level of importance assigned to each component during the optimization process.

The concentration of NaOH is optimized within the 50-70% range, with a minimum target of 50% and an importance level of 3(+++). Given the environmental concerns associated with NaOH, its application should be minimized without compromising its efficacy in enhancing chitosan quality. Additionally, the temperature is optimized with a maximum target of 110°C to ensure effective deacetylation. In contrast, the time is optimized with a minimum target, as extended deacetylation times may result in chitosan depolymerization. The temperature and time of deacetylation significantly influence the degree of deacetylation, (b) which has an importance level of 3(+++).

Table 3. Optimized response components

Response Component	Target	Lower Limit	Upper Limit	Significance
NaOH Concentration (%)	Minimum	50	70	3(+++)
Deacetylation Temperature (°C)	Maximum	90	110	3(+++)
Deacetylation time (hours)	Minimum	1	3	3(+++)
Yield (%)	Maximum	14.74	40.53	5(+++++)
Degree of Deacetylation (%)	Maximum	76.6	98.6	5(+++++)
Intrinsic Viscosity (ml / g)	*In range*	10.97	1320.30	3(+++)
Molecular Weight (kDa)	*In range*	1.899	1450.08	3(+++)

Furthermore, the yield response component is optimized with an importance level of 5 (+++++), reflecting its substantial impact on overall chitosan production. Achieving optimal yield also directly influences the economic value and profitability of the chitosan production process. Moreover, the degree of deacetylation is optimized with a maximum target and an importance level of 5 (+++++), as it is the primary quality parameter for chitosan. According to Zahiruddin et al. (2008), an increase in the degree of deacetylation results in a reduction of acetyl groups present in chitosan, leading to stronger ionic and hydrogen bond interactions. Consequently, removing acetyl groups from chitosan forms positively charged chitosan, capable of binding to negatively charged compounds such as proteins and polysaccharide anions, forming neutral ions (Suhartono, 1989).

In addition, the intrinsic viscosity response component is optimized within an

appropriate range due to the relatively high average intrinsic viscosity produced. Intrinsic viscosity is strongly correlated with molecular weight and the degree of deacetylation. As noted by Dinculescu et al. (2024), as the temperature and NaOH concentration increase, more acetyl groups are dissolved, leading to increased deacetylation and a corresponding rise in viscosity, which thickens the chitosan. This further supports the idea that a higher degree of deacetylation correlates with increased viscosity.

Furthermore, the molecular weight response component is optimized with a minimum target. Emmawati et al. (2007) indicate that chitosan with low molecular weight exhibits greater potential as an antibacterial, antioxidant, and antifungal agent, offering enhanced benefits in agricultural food processing applications. Low molecular weight chitosan easily permeates Gram-negative bacteria (E. coli) cells, where its positive charge binds to the bacterial surface, disrupting the cell membrane and enhancing permeability. Additionally, chitosan stabilizes the cell membrane, is electronegative, and induces flocculation, impairing bacterial physiological activities and ultimately leading to bacterial cell death (Akdaşçi et al., 2025). All response components in this study align with the generated model, as evidenced by a non-significant lack of fit value.

During the optimization of chitin deacetylation, 42 different formulation solutions were developed and ranked based on their desirability (Table 3). According to Montgomery (2001), the formulation with the highest desirability score represents the optimal condition. The optimal parameters were determined to be a NaOH concentration of 50%, a deacetylation temperature of 110°C, and a reaction time of 1 hour, yielding a desirability value of 0.927. This formulation is projected to produce chitosan with a yield of 33.42%, a degree of deacetylation of 80.30%, an intrinsic viscosity of 128.65 ml/g, and a molecular weight of 51.30 kDa. Experimental validation of this formula resulted in chitosan with a yield of 33.61%, a degree of deacetylation of 79.2%, an intrinsic viscosity of 34.96 ml/g, and a molecular weight of 9.25 kDa. The produced chitosan contained moisture and ash contents of 3.73% and 1.94%, respectively, which comply with the quality standards set by Protan Laboratory (1987), stipulating moisture ≤10%, ash ≤2%, and degree of deacetylation ≥70%. These results demonstrate that the optimized conditions successfully produce high-quality chitosan suitable for intended applications.

Table 4. Optimization formula for chitin deacetylation to chitosan from mangrove crab shell waste based on desirability value.

No	NaOH Concentration (%)	Deacetylation Temperature (°C)	Deacetylation time (hours)	Yield (%)	Degree of Deacetylation (%)	Intrinsic Viscosity (ml / g)	Molecular Weight (kDa)	*Desirability*
1	50.00	110.00	1.00	33,42	80.30	128.65	51.30	0.927
2	50.00	109.91	1.00	33.46	80.29	128.65	51.16	0.927
3	50.02	109.82	1.00	33.48	80.29	128.39	51.26	0.926
4	50.00	109.72	1.00	33.52	80.27	127.85	50.86	0.926
5	70.00	110.00	1.00	33.40	80.35	130.49	52.90	0.925
6	50.12	110.00	1.00	33.38	80.34	130.23	52.77	0.925
7	50.14	110.00	1.00	33.37	80.35	130.48	53.00	0.925
8	50.00	109.60	1.00	33.57	80.25	127.49	50.66	0.925
9	50.00	109.48	1.00	33.62	80.22	127.04	50.41	0.924
10	50.00	110.00	1.02	33.36	80.40	132.82	54.94	0.924
11	50.00	109.30	1.00	33.68	80.20	126.60	50.17	0.923
12	50.26	110.00	1.00	33.33	80.39	132.08	54.48	0.923
13	50.00	109.19	1.00	33.71	80.18	126.29	49.99	0.923
14	50.00	110.00	1.03	33.32	80.47	135.79	57.51	0.921
15	50.14	109.24	1.00	33.64	80.24	128.27	51.76	0.921
16	50.01	108.89	1.00	33.81	80.14	125.56	49.63	0.921
17	50.48	110.00	1.00	33.26	80.46	134.74	56.94	0.920
18	50.00	110.00	1.05	33.28	80.55	138.94	60.25	0.918
19	50.60	108.37	1.00	33.21	80.51	136.58	58.64	0.918
20	50.00	108.37	1.00	34.00	80.06	123.90	48.65	0.918
21	50.00	108.28	1.00	34.04	80.05	123.66	48.52	0.917
22	50.69	110.00	1.00	33.18	80.54	137.71	59.69	0.916
23	50.00	107.57	1.00	34.29	79.94	121.59	47.36	0.913
24	50.00	110.00	1.09	33.17	80.74	146.61	66.91	0.912
25	50.00	107.36	1.00	34.37	79.91	120.97	47.02	0.911
26	50.93	109.69	1.00	33.21	80.57	139.92	62.05	0.911

No	NaOH Concentration (%)	Deacetylation Temperature (°C)	Deacetylation time (hours)	Yield (%)	Degree of Deacetylation (%)	Intrinsic Viscosity (ml / g)	Molecular Weight (kDa)	*Desirability*
27	50.00	106.60	1.00	34.64	79.80	118.75	45.77	0.906
28	50.00	105.56	1.00	35.02	79.64	115.72	44.08	0.898
29	50.00	110.00	1.18	32.93	81.14	163.47	81.57	0.898
30	50.00	105.40	1.00	35.08	79.62	115.24	43.81	0.896
31	50.00	110.00	1.24	32.78	81.40	174.29	90.96	0.888
32	52.30	109.28	1.00	32.89	80.97	156.63	77.93	0.887
33	51.31	106.67	1.00	34.17	80.25	136.11	61.74	0.887
34	53.17	110.00	1.00	32.34	81.37	170.15	89.67	0.877
35	50.00	110.00	1.37	32.44	81.99	198.55	112.05	0.867
36	50.00	101.54	1.02	36.47	79.04	104.02	37.54	0.859
37	50.00	100.76	1.00	36.75	78.93	101.74	36.26	0.849
38	55.99	110.00	1.00	31.39	82.32	206.95	123.69	0.829
39	50.00	99.05	1.00	37.37	78.67	96.76	33.48	0.826
40	57.03	110.00	1.00	31.03	82.67	220.47	136.19	0.810
41	50.00	110.00	1.75	31.46	83.69	268.94	173.20	0.800
42	50.00	110.00	1.78	31.38	83.84	274.91	178.39	0.787

Conclusion

The optimization of the deacetylation process of chitin into chitosan using the Design Expert 11 program with the Response Surface Methodology approach resulted in 42 optimized formulas. The formula with the highest desirability value of 0.927 corresponds to the extraction conditions of 50% NaOH concentration, a deacetylation temperature of 110°C, and a deacetylation time of 1 hour, with predicted values of 33.42% yield, 80.30% deacetylation degree, 128.65 ml/g intrinsic viscosity, and 51.30 kDa molecular weight. These predicted results align with the chitosan produced in this study, which showed a yield of 33.61%, a deacetylation degree of 79.2%, an intrinsic viscosity of 34.96 ml/g, and a molecular weight of 9.25 kDa. The degree of deacetylation of the produced chitosan meets the quality standards set by Protan Laboratory, with a result of ≥ 70%.

REFERENCES

Ahmad Khan, T., Khiang Peh, K., & Seng Ch, H. (2002). Reporting degree of deacetylation values of chitosan: the influence of analytical methods. *J Pharm Pharmaceut Sci*, *5*(3), 205–212.

Akdaşçi, E., Duman, H., Eker, F., Bechelany, M., & Karav, S. (2025). Chitosan and Its Nanoparticles: A Multifaceted Approach to Antibacterial Applications. *Nanomaterials 2025*, *15*(2), 126.

Anwar, K., Said, M., Afizal, M., & Amin, M. (2015). Overview of the Response Surface Methodology (RSM) in Extraction Processes. *Journal of Applied Science & Process Engineering*, *2*(1).

Apriyantono, A., Fardiaz, D., Puspitasari, N. L., Sedarnawati, & Budiyanto, S. (1989). *Analisis Pangan*. IPB Press.

Aranaz, I., Alcántara, A. R., Civera, M. C., Arias, C., Elorza, B., Caballero, A. H., & Acosta, N. (2021). Chitosan: An Overview of Its Properties and Applications. *Polymers*, *13*(19), 3256.

Baron, R. D., Pérez, L. L., Salcedo, J. M., Córdoba, L. P., & Sobral, P. J. do A. (2017). Production and characterization of films based on blends of chitosan from blue crab (Callinectes sapidus) waste and pectin from Orange (Citrus sinensis Osbeck) peel. *International Journal of Biological Macromolecules*, *98*, 676–683.

Brzezinski, R., LeHoux, J.-G., & Kelly, A. (2004). Clinical studies on the innocuousness of chitosan and its short-chain derivative generated by enzymatic hydrolysis. *Asia Pacific Journal of Clinical Nutrition*, *13*, 13–96.

Czechowska-Biskup, R., Rokita, B., Ulański, P., Rosiak, J. M., Jarosińska, D., Rokita, B., Czechowska-Biskup, R., Jarosińska, D., Rokita, B., Ulański, P., & Rosiak, J. M. (2012). Determination of Degree of Chitosan. *Progress on Chemistry and Application of Chitin and Its Derivatives*, *17*, 5–20.

Dinculescu, D. D., Apetroaei, M. R., Gîjiu, C. L., Anton, M., Enache, L., Schröder, V., Isopescu, R., & Rău, I. (2024). Simultaneous Optimization of Deacetylation Degree and Molar Mass of Chitosan from Shrimp Waste. *Polymers 2024, Vol. 16, Page 170*, *16*(2), 170.

EL Knidri, H., Dahmani, J., Addaou, A., Laajeb, A., & Lahsini, A. (2019). Rapid and efficient extraction of chitin and chitosan for scale-up production: Effect of process parameters on deacetylation degree and molecular weight. *International Journal of Biological Macromolecules*, *139*, 1092–1102.

Emmawati, A., Jenie, B. S. L., & Fawzya, Y. N. (2007). Kombinasi Perendaman dalam Natrium Hidroksida dan Aplikasi kitin deasetilase terhadap Kitin Kulit Udang untuk Menghasilkan Kitosan dengan Berat molekul rendah. *Jurnal Teknologi Pertanian*, *3*(1), 12–18.

Gargioni, K., Correa De Mello, P., De Cássia Bernusso, L., Nogueira, R., Pitombo, M., & Polakiewicz, B. (2006). Synthesis and Physicochemical Characterization of Chemically Modified Chitosan by Succinic Anhydride. *Brazilian Archives of Biology and Technology*, *49*, 665–668.

Hasriani, Olii, A., & Najib, A. (2024). The Effect of Temperature Variations on the Deacetylation Process of Chitosan Characteristics from Mud Crab (Scylla serrata) Shell Waste. *Universal Journal of Pharmaceutical Research*.

Hwang, J.-K., Hong, S.-P., & Kim, C.-T. (1997). *Effect of Molecular Weight and NaCl Concentration on Dilute Solution Properties of Chitosan*. *2*(1), 1–5.

Hwang, K. T., Jung, S. T., Lee, G. D., Chinnan, M. S., Park, Y. S., & Park, H. J. (2002). Controlling molecular weight and degree of deacetylation of chitosan by response surface methodology. *Journal of Agricultural and Food Chemistry*, *50*(7), 1876–1882.

Kadak, A. E., Küçükgülmez, A., & Çelik, M. (2023). Preparation and Characterization of Crayfish (Astacus leptodactylus) Chitosan with Different Deacetylation Degrees. *Iranian Journal of Biotechnology*, *21*(2), 87–94.

Lyalina, T., Zubareva, A., Lopatin, S., Zubov, V., Sizova, S., & Svirshchevskaya, E. (2017). Correlation Analysis of Chitosan Physicochemical Parameters Determined by Different Methods. *Organic & Medicinal Chemistry International Journal*, *1*(3), 1–9.

Mathaba, M., & Daramola, M. O. (2020). Effect of Chitosan's Degree of Deacetylation on the Performance of PES Membrane Infused with Chitosan during AMD Treatment. *Membranes*, *10*(3), 52.

Mourabet, M., El Rhilassi, A., El Boujaady, H., Bennani-Ziatni, M., & Taitai, A. (2017). Use of response surface methodology for optimization of fluoride adsorption in an aqueous solution by Brushite. *Arabian Journal of Chemistry*, *10*, 3292–3302.

Patria, A. (2013). Production and characterization of Chitosan from shrimp shells waste. *International Journal of the Bioflux Society*, *6*(4), 339–344.

Pellis, A., Guebitz, G. M., & Nyanhongo, G. S. (2022). Chitosan: Sources, Processing and Modification Techniques. *Gels*, *8*(7), 393.

Rahman, M., Haque, S. M., Wahab, A., & Egna, H. (2018). Soft-Shell Crab Production in Coastal Bangladesh: Prospects, Challenges, and Sustainability. *World Aquaculture*, *1*(2), 43–47.

Rinaudo, M. (2006). Chitin and chitosan: Properties and applications. *Progress in Polymer Science*, *31*(7), 603–632.

Suhartono, M. T. (1989). *Enzim dan Bioteknologi*. Pusat antar Universitas Bioteknologi IPB.

Tan, H. W., Lim, Z. Y. J., Muhamad, N. A., & Liew, F. F. (2021). Potential Economic Value of Chitin and Its Derivatives as Major Biomaterials of Seafood Waste, with Particular Reference to Southeast Asia. *Journal of Renewable Materials*, *10*(4), 909–938.

Tolaimate, A., Desbrieres, J., Rhazi, M., & Alagui, A. (2003). Contribution to the preparation of chitins and chitosans with controlled physico-chemical properties. *Polymer*, *44*(26), 7939–7952.

Waiho, K., Ikhwanuddin, M., Baylon, J. C., Jalilah, M., Rukminasari, N., Fujaya, Y., & Fazhan, H. (2021). Moult induction methods in soft-shell crab production. In *Aquaculture Research*, 52(9), 4026–4042.

Zahiruddin, W., Ariesta, A., & Salamah, E. (2008). Karakteristik Mutu dan Kelarutan Kitosan dari Ampas Silase Kepala Udang Windu (Panaeus monodon). *Buletin Teknologi Hasil Perikanan*, *XI*(2), 140–151.