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The role of base types and concentration in the deacetylation process of manufacturing chitosan from green mussel shells (*Perna viridis*)

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Abstract

In the food industry, chitosan is useful as a preservative, edible film, heavy metal binder, and so on. Chitosan is produced by removing the acetyl groups in chitin using a strong base. Green mussel (*Perna viridus*) shells, which are waste from the fishing industry, have the potential to be utilized as raw materials for chitosan production because they are estimated to contain about 15-35% chitin. The deacetylation process was conducted using alkali at high temperatures, leading to the release of the acetyl group (CH_3CHO-) from the chitin molecule. The choice of a strong base over an acid for deacetylation, particularly in the context of converting chitin to chitosan, is rooted in the mechanism of the reaction and the desired product properties. The purpose of this study was to determine the influence of the type and concentration of bases in the deacetylation process on the characteristics of chitosan produced from green mussel shells. The experimental design used was a completely randomized factorial design with two factors. The first factor was the strong base (NaOH, KOH, and a mixture of NaOH and KOH) used, while the second factor was the base concentration (40%, 50%, and 60%). The data obtained were analyzed using analysis of variance (ANOVA) and Duncan multiple range test (DMRT). The results revealed that the type and concentration of the base affected the yield, deacetylation degree, water content, ash content, nitrogen content, and solubility. The best treatment in this study was a KOH treatment with a concentration of 60%, resulting in a deacetylation degree of 78.95%, a yield of 3.20%, a moisture of 2.035, an ash content of 0.26%, a nitrogen content of 6.38%, and a solubility of 99.19%. Identification of chitosan functional groups was conducted on chitosan from the best treatment using FT-IR Spectrophotometer. From the results of FT-IR spectra analysis, the functional groups present in the sample were –OH, C-H, and N-H, which were the main functional groups of chitosan.

1. Introduction

Chitosan is a chitin derivative formed through the isolation of the exoskeleton of shrimp, clams, or crabs (Kadak *et al.*, 2023). In the food industry, chitosan is useful as a preservative, edible film, heavy metal binder, and so on. Chitin production typically involves three stages: demineralization, deproteination, and depigmentation. Several deacetylation methods include alkaline hydrolysis, acid hydrolysis, enzymatic deacetylation and chemical methods. In contrast, chitosan is obtained by deacetylating chitin with a high-concentration alkaline solution. The choice of a strong base over an acid for deacetylation, particularly in the context of converting chitin to chitosan, is rooted in the mechanism of the reaction and the desired product properties. One significant source of chitin is green mussel shells, commonly found in coastal waters, with substantial cultivation occurring on the north coast of Java, especially in Gresik Regency. The green mussel caught in 2019, per hectare, could reach 200-300 tons of whole mussels (Ummah, 2019). This substantial production directly contributes to the volume of shellfish waste generated. In Gresik Regency alone, green mussel shell waste can amount to 25 kg per day for a single family. This considerable quantity of green mussel shell waste presents an opportunity for utilization as a source material for chitosan production.

Green mussel shells consist of various components, including calcium carbonate ($CaCO_3$), calcium phosphate ($CaPO_4$), calcium bicarbonate ($Ca(HCO_3)_2$),

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tricalcium silicate (Ca_3S), and active calcium. Additionally, they possess a chitin content of approximately 20-30% (Pratama *et al.*, 2023). Chitin itself is comprised of 2000-3000 units of N-acetyl D-Glucosamine monomers, interconnected through 1,4-glycosidic bonds (Aranaz *et al.*, 2021). It is worth noting that chitin naturally lacks complete acetylation, typically exhibiting a degree of deacetylation of less than 10%. The utilization of chitin is constrained by its insolubility and the challenge of separating it from other associated materials, particularly proteins. Therefore, for practical use, chitin must undergo a conversion process to become chitosan. Chitosan is characterized by its easy biodegradability and acts as a cationic polyelectrolyte due to the presence of amine functional groups. In addition to the amine group, chitosan also features primary and secondary hydroxyl groups. These functional groups contribute to the high chemical reactivity of chitosan. Chitosan production involves the reduction of acetyl groups ($-COCH_3$) in chitin to amine groups ($-NH_2$) through heating in a strong alkali solution with a high concentration (Younes and Rinaudo, 2015). This process is commonly referred to as deacetylation. The choice of a strong base over an acid for deacetylation, particularly in the context of converting chitin to chitosan, is rooted in the mechanism of the reaction and the desired product properties. The extent to which the acetyl group is removed from the acetamide group of chitin is termed the degree of deacetylation (DD). The degree of deacetylation indicates the number of acetyl groups lost during the deacetylation process, and a higher degree of deacetylation reflects the purity of the produced chitosan (Aranaz *et al.*, 2021). Various factors influence the degree of deacetylation in chitosan production, including the choice of basic materials and process conditions such as the type of strong base solution, concentration, temperature, and duration of the process.

The commonly used strong alkaline solution in the chitosan extraction process is NaOH. However, in a previous study conducted by Khairi *et al.* (2019), it was observed that the degree of deacetylation of chitosan in green mussels reached 38.91% with 45% NaOH immersion. Consequently, there is a need for efforts to enhance the degree of deacetylation. Another strong base solution that can be utilized in the deacetylation process, aside from NaOH, is KOH. Setiati *et al.* (2021), in their examination of the synthesis of chitin into chitosan polymer from shrimp shells, asserted that the deacetylated chitin (DD) is higher when using KOH solution compared to NaOH solution. The degree of deacetylation in chitin is influenced by several factors, including alkali strength, alkali concentration, reaction time, and temperature. In a study conducted by Takarina *et al.* (2017), the degree of deacetylation (DD) of chitosan synthesized from red snapper ranged from 79.32% to 90.83%. The lowest DD, 79.32%, was observed in the deacetylation process with the addition of 60% NaOH, boiled at a temperature of 110°C for 4 hours. On the other hand, the highest DD, 90.83%, was found in the deacetylation process with the addition of 80% NaOH, boiled at a temperature of 110°C for 4 hours. Interestingly, the boiling temperature and duration did not exhibit a significant effect on the DD. Based on this background, our research aimed to investigate the impact of using NaOH, KOH, and a combination of NaOH and KOH with varying concentrations on the characteristics of the chitosan derived from green mussel shells.

2. Materials and methods

2.1 Materials

The raw material utilized in this research was sourced from green mussel shells obtained from Gresik Regency, Indonesia. The material for analysis (NaOH ≥98% anhydrous pellet), HCl (37%, emprove® essential), KOH (≥98% anhydrous pellet), CH_3COOH (≥99%, glacial, ReagentPlus®), and Kjeldahl tablets) were obtained from Merck KGaA, Darmstadt, Germany, along with distilled water. The instruments utilized in this research comprised a cabinet dryer, digital scales, thermometer, 40-mesh sieve, and blender. Analytical equipment such as analytical balances, furnaces, ovens, glassware, magnetic stirrers, Kjeldahl apparatus, and Fourier Transform Infrared (FTIR) spectrophotometer Shimadzu were also used.

2.2 Chitosan production

Chitosan from green mussel shells was extracted by the method of Takarina *et al.* (2017) with some modifications. The green mussel shells underwent a cleaning process with water and a brush to eliminate any dirt or residual flesh. Subsequently, they were dried in a cabinet dryer. The dried shells were then further reduced in size through pounding and the use of a blender to increase the surface area. The resulting material was sifted using a 40-mesh sieve. The chitosan processing comprised three stages: deproteinization, demineralization, and deacetylation. In the deproteinization stage, green mussel shell powder was heated in a 1 N NaOH solution with a ratio of 1:6 (w/v) at 80°C for 1 hr. The precipitate was then filtered and washed with distilled water until neutral. Moving on to the demineralization stage, the dry sediment was treated with 1.5 N HCl in a ratio of 1:12 (w/v) and stirred for 30 minutes at room temperature. The resulting precipitate was filtered, washed with distilled water until neutral,

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and then dried at 80°C for 1 hr. The dried precipitate obtained from the demineralization stage was termed chitin. In the deacetylation stage, this chitin was immersed in a base solution at a ratio of 1:10 (w/v) corresponding to the treatment (NaOH, KOH, and mixture) with concentrations of 40%, 50%, and 60%. The soaking process took place for 2 hr at a temperature of 100°C with continuous stirring. After cooling, the mixture underwent filtration using filter paper, followed by washing with distilled water until neutral. Subsequently, the resulting residue was dried using an oven at a temperature of 80°C for 1 hr. The product of this deacetylation process was referred to as chitosan. The obtained dry chitosan underwent analysis to determine its characteristics.

2.3 Characterization of chitosan

Characterization of chitosan includes yield, moisture, ash content, nitrogen content, solubility, and degree of deacetylation. Weight measurements of the raw material and chitosan acquired after treatment were compared in order to calculate the chitosan yield (Ahing and Newati, 2016). The moisture was determined after drying samples at 103°C for 4 hrs whereas ash content was determined by heating at 550°C for 4 hrs (Kadak *et al.,* 2023). Nitrogen was calculated based on the Kjeldahl method (Takarina *et al.,* 2017). Solubility was calculated based on 1 g of chitosan dissolved in 100 mL of 1% acetic acid solution and placed on an orbital shaker at 200 rpm for 24 hrs, the chitosan acidic solution was then filtered using a vacuum pump, then the difference between insolubility with the initial weight being the solubility (Wiliam and Wid, 2019). The degree of deacetylation was measured based on the potentiometric titration method, 10 mL from 0.30 M HCl (v/v) was used to dissolve 250.0 mg of chitosan. After being diluted to 50.0 mL by using ultrapure water, the solution was titrated with 0.10 M NaOH (w/v). Consumed NaOH (w/v) solution volume that corresponds to the number of amine groups in chitosan was calculated according to the difference between two inflection points of acid-base titration (Kadak *et al.,* 2023). Fourier transform infrared spectroscopy (FTIR) was employed to ascertain the vibration frequencies of the functional groups. The spectra were measured using an FTIR spectrometer within the wave number range of 400-4000 cm^{-1}. Approximately 0.1 g of the dry polymer was meticulously mixed with KBr, pressed into a pellet, and the FTIR spectrum was subsequently recorded (Setiati *et al.,* 2021).

2.4 Data analysis

The experimental design employed was a completely randomized factorial design with two factors, each repeated three times. The data obtained were subjected to Analysis of Variance (ANOVA) for statistical analysis. Post hoc comparisons between treatments were conducted using the Duncan multiple range test (DMRT) with a significance level set at 5%, and the analysis was carried out using Minitab Ver. 17 software.

3. Results and discussion

The analysis of chitosan from green mussel shells in this study encompassed the assessment of yield, moisture, ash content, nitrogen content, solubility, degree of deacetylation, and the functional group analysis of chitosan using FT-IR (Fourier transform infrared spectrophotometer) for the optimal treatment. The characteristics of chitosan derived from green mussel shells are presented in Table 1.

3.1 Yield of chitosan

This study has produced about range of 3.14-4.44% of chitosan from wet weight of green mussel shell waste. Previous research by Ahing and Newati (2016) showed that the yield of chitosan derived from shrimp shells was 3.10 to 5.2%. While the research by Sumaila *et al.* (2020) produced a yield of 15.4% dry weight. The deacetylation process was the process of removing acetyl

Table 1. Characteristic of chitosan from green mussel shells.

Treatment	Yield (%)	Moisture (%)	Ash content (%)	Total nitrogen (%)	Degree of deacetylation (%)	Solubility (%)
			Type of base			
NaOH	3.73±1.92^{c}	2.06±0.21^{a}	0.22±0.74^{b}	1.06±0.72^{b}	78.44±1.28^{b}	98.82±1.12^{b}
KOH	3.78±1.98^{c}	2.10±0.13^{a}	0.22±0.73^{b}	1.05±0.52^{b}	78.54±1.30^{b}	98.81±1.13^{b}
Mixed	3.70±1.90^{c}	2.03±0.2^{a}	0.23±0.77^{b}	1.07±0.89^{b}	78.33±1.44^{b}	98.84±1.08^{b}
			Concentration (%)			
40	4.44±0.16^{d}	2.01±0.11^{a}	0.18±0.05^{a}	1.1 ±0.38^{c}	77.99±0.36^{a}	98.47±0.11^{a}
50	3.58±0.15^{b}	2.04±0.15^{a}	0.23±0.07^{b}	1.05±0.14^{b}	78.41±0.21^{b}	98.80±0.03^{b}
60	3.18±0.07^{a}	2.13±0.06^{b}	0.27±0.02^{c}	1.02±0.06^{a}	78.88±0.21^{b}	99.21±0.05^{c}

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different (P<0.05).

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groups from chitin using a strong alkaline solution to convert them into amine groups. Removal of the acetyl group causes the chains to break in the chitin polymer, causing the yield weight to become smaller. The type of base treatment did not show a significant effect (p≥0.05). Pellis *et al.* (2022) assert that an increase in the number of acetyl groups bound by the strong base solution during the deacetylation process leads to a decrease in yield. However, this results in chitosan of increasingly pure quality, as more acetyl groups are released from the chitin polymer. Regarding the treatment with different base concentrations, the findings indicate that higher concentrations lead to lower yields (William and Wid, 2019).

3.2 Moisture

Based on Table 1, the type of base treatment did not show a significant effect (p≥0.05), but the base concentration had a significant effect (p≤0.05) on moisture content. The moisture content obtained was ranging from 2.01 to 2.13%. Almost the same results were obtained in research conducted by Kadak *et al.* (2023) which produced chitosan with a moisture content of 1.03 to 3.32%. Several studies produced greater water content, as reported by Ahing and Newati (2016), chitosan moisture content was between 6.02 to 8.01% with different base concentration treatments. A high base concentration causes more acetyl groups to be eliminated so that more amine groups are formed, and the resulting hydrogen bonds with water molecules contribute to increased moisture content in the chitosan (Pellis *et al.*, 2022). The study performed by Szymanska and Winnicka (2015) suggested that the moisture content of chitosan must be low which ranges from 6-10% so that it has a greater capability to form hydrogen bonding.

3.3 Ash content

Based on Table 1, the type of base treatment did not exhibit a significant effect (p≥0.05), but the base concentration had a significant effect (p≤0.05) on the ash content value of chitosan derived from green mussel shells. Ash content is one of the important parameters that determine the quality of chitosan. The lower the ash content then the higher the chitosan quality. In this study, the ash content produced was between 0.18 to 0.27%. Research conducted by Wiliam and Wid (2019) produced chitosan with an ash content of 0.24 to 2.67%. Ash content can be influenced by the chitosan washing process during neutralization. A high NaOH concentration requires a longer neutralization time than a low NaOH concentration, as a result, the remaining minerals can increase the ash content (Younes and Rinaudo, 2015). Nouri *et al.* (2016) reported that good quality chitosan should possess less than 1% of ash content.

3.4 Total nitrogen

Based on Table 1, the type of base treatment did not show a significant effect (p≥0.05), but the base concentration had a significant effect (p≤0.05) on the total nitrogen content of chitosan derived from green mussel shells. In this research, chitosan was produced with a total nitrogen of 1.02 to 1.10%. Meanwhile, research conducted by Takarina *et al.* (2017) produced total nitrogen of 0.0136 to 0.0278%. The high concentration of NaOH in the deacetylation process causes more acetyl groups to be eliminated, so that more free amine groups are available, and therefore, the nitrogen content in chitosan will also be higher. However, in this study there is a contradiction, this may be due to the presence of degradation of the chitosan polymer (Kanani *et al.,* 2023).

3.5 Degree of deacetylation

Based on Table 1, the type of base treatment did not exhibit a significant effect (p≥0.05), but the base concentration showed a significant effect (p≤0.05) on the degree of deacetylation in chitosan derived from green mussel shells. The degree of deacetylation produced in this study was between 77.99 to 78.88%. This result is slightly lower than that produced by Takarina *et al* (2017) which produced a degree of deacetylation of 79.32%. The degree of deacetylation refers to the distribution of amino groups along the polymer chain. The degree of deacetylation is another factor that determines the physicochemical properties of chitosan, its activity, and its application. Increasing the base concentration tends to increase the degree of deacetylation because stronger bases or higher concentrations are more efficient at removing acetyl groups. In the food industry, chitosan with a deacetylation degree of more than 75% is very effective as an antimicrobial agent, fortification agent and clarification agent in beverage processing (Thambiliyagodage *et al.*, 2023)

3.6 Solubility

Based on Table 1, the type of base treatment did not show a significant effect (p≥0.05), but the base concentration had a significant effect (p≤0.05) on the solubility value of chitosan. In this study, the chitosan produced had a solubility between 98.47 and 99.2%. Previous research showed that the solubility of chitosan was 97.92% (Ahing and Newati, 2016) and 100% (Wiliam and Wid, 2019). The solubility of chitosan is usually affected by the ash content and degree of deacetylation. Solubility proportionally increased with increasing degree of deacetylation. Chitosan with a high

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degree of deacetylation contained a large amount of amino group (-NH) which would allow it to be easily protonated in an aqueous acid solution. On the contrary, when chitosan contains a large amount of ash, it is unlikely to become soluble in acetic acid. High base concentrations cause many acetyl groups to be eliminated, resulting in chitosan with a high degree of deacetylation, so the solubility also increases.

The deacetylation process in strong bases and heat causes the loss of acetyl groups in chitin, resulting in positively charged chitosan that can dissolve in organic acetic acid (Alemu *et al.,* 2023)

3.7 Chitosan functional group analysis

Based on Figure 1, the infrared spectrum of chitosan in the research reveals absorption bands in the wave number range of 3645.9 to 3481.29 cm^{-1}, indicating the presence of bending vibrations of OH and NH groups. This observation aligns with the findings of research conducted by Abdulsahib *et al.* (2015). Additionally, an absorption band at 2927.48 cm^{-1} indicates absorption from the -CH group, while the absorption at 2801.50 cm^{-1} suggests the presence of the C-H group of the alkane, specifically indicating the stretching vibration of the CH_2 group. A narrow band below 3000 cm-1, showing aliphatic compounds. For example, the absorption band for long-chain linear aliphatic compounds is identified at 2935 and 2860 cm-1 as noted by Nandiyanto *et al.* (2019). Furthermore, the absorption band at 1657.30 cm^{-1} is attributed to the N-H group, and as reported by Negrea *et al.* (2015), the typical absorption of chitosan is seen at 1666.30 cm^{-1}, indicating N-H stretching vibrations of amides.

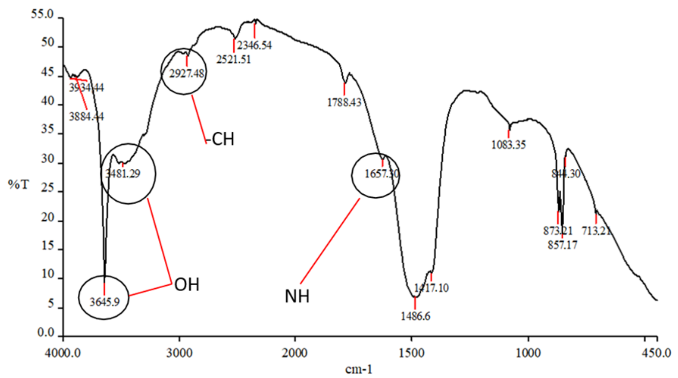

Figure 1. Spectrum results of green mussel shell chitosan.

4. Conclusion

Chitosan extracted from green mussel shells is not statistically influenced by the type of base but is significantly influenced by the base concentration. A higher base concentration increases the water content, ash content, degree of deacetylation and solubility, but reduces the total nitrogen and yield of chitosan produced.

The results of the FT-IR spectra analysis reveal that the functional groups present in the sample are –OH, C-H, and N-H, which are the main functional groups of chitosan.

Conflict of interest

The authors declare no conflict of interest.

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