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EVALUATION OF CHITOSAN QUALITY FROM SHRIMP, CRAB, AND BLUE SWIMMING CRAB WASTE: YIELD, WATER CONTENT, AND DEGREE OF DEACETYLATION

EVALUASI KUALITAS KITOSAN DARI LIMBAH UDANG, KEPITING, DAN RAJUNGAN: RENDEMEN, KADAR AIR, DAN DERAJAT DEASETILASI

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ABSTRACT

Mud crab, blue swimming crab, and vannamei shrimp carapace are crustacean wastes that have not been optimally utilized in Bangka Island and have the potential to pollute the environment. Converting the biomass from this waste into chitosan supports the principles of a circular economy. This study evaluates the quality of chitosan produced from crustacean waste based on yield, moisture content, and degree of deacetylation (DD), and compares two FTIR-based methods for estimating DD using the spectral band ratios A1320/A1420 and A1655/A3450. Carapace waste (100 g) was processed through demineralization (1.5 M HCl), deproteinization (3.5% NaOH), and deacetylation (60% NaOH) with two replications per species. The chitosan yields were 4.0% ± 0.5 (mud crab), 8.7% ± 0.5 (blue swimming crab), and 12.4% ± 0.9 (shrimp), respectively. The water content was still within the limits of SNI 7949:2013 (<12%), namely 6.4% ± 2.0, 10.7% ± 2.7, and 6.3% ± 0.6. Based on the A1320/A1420 ratio, the DD values were 86.8% ± 0.4, 84.4% ± 0.1, and 95.3% ± 2.5, respectively, all exceeding the minimum standard of 75%. In contrast, the A1655/A3450 method produces much lower DD values (<75%). These findings indicate that local crustacean shell waste has strong potential as a source of high-quality chitosan. FTIR is a practical method for DD estimation, but it still needs further validation, especially with standard methods such as ^{1}H-NMR.

Keywords: chitosan, crustacean, shell waste, valorization

ABSTRAK

Cangkang kepiting, rajungan, dan udang vannamei merupakan limbah krustasea yang belum dimanfaatkan secara optimal di Pulau Bangka dan berpotensi mencemari lingkungan. Konversi biomassa dari limbah ini menjadi kitosan mendukung prinsip ekonomi sirkular. Penelitian ini mengevaluasi kualitas kitosan yang dihasilkan dari limbah krustasea berdasarkan rendemen, kadar air, dan derajat deasetilasi (DD), serta membandingkan dua metode berbasis FTIR untuk estimasi DD menggunakan rasio pita spektral A1320/A1420 dan A1655/A3450. Limbah cangkang (masing-masing 100 g) diproses melalui tahap demineralisasi (HCl 1,5 M), deproteinasi (NaOH 3,5%), dan deasetilasi (NaOH 60%) dengan dua ulangan per jenis. Hasil rendemen kitosan berturut-turut adalah 4,0% ± 0,5 (kepiting), 8,7% ± 0,5 (udang), dan 12,4% ± 0,9 (rajungan). Kadar air masih berada dalam batas SNI 7949:2013 (<12%), yaitu 6,4% ± 2,0, 10,7% ± 2,7, dan 6,3% ± 0,6. Berdasarkan rasio A1320/A1420, nilai DD masing-masing adalah 86,8% ± 0,4, 84,4% ± 0,1, dan 95,3% ± 2,5, seluruhnya melampaui standar minimum 75%. Sebaliknya, metode A1655/A3450 menghasilkan nilai DD jauh lebih rendah (<75%). Temuan ini menunjukkan bahwa limbah cangkang krustasea lokal memiliki potensi kuat sebagai sumber kitosan berkualitas tinggi. FTIR merupakan metode praktis untuk estimasi DD, namun tetap perlu divalidasi lebih lanjut, terutama dengan metode standar seperti ^{1}H-NMR.

Kata kunci: kitosan, krustase, limbah cangkang, valorisasi

INTRODUCTION

Blue swimming crabs (*Portunus pelagicus*) and mud crabs (*Scylla serrata*) are leading capture fisheries commodities, while whiteleg shrimp (*Litopenaeus vannamei*) is a favorite in aquaculture on Bangka Island, Bangka Belitung Islands Province (Mayu *et al*. 2021; Bidayani and Valen 2023). The abundance of these resources generates large amounts of crustacean carapace waste. The molting phase of whiteleg shrimp, the stripping of crab meat with a carapace composition of 50-60%, and the high preference for crab consumption contribute to the accumulation of waste that can to pollute the environment (Amalia *et al*. 2021). The utilization of blue swimming crab carapace by the people of Bangka Island is limited to fish feed (Supratman and Umroh 2016), organic fertilizer (Kurniawan *et al*. 2017), and shrimp carapace as animal feed. Waste valorization can be done through waste-to-product, converting waste into chitin and chitosan biomaterial products and supporting the circular economy (Muthu *et al*. 2021; Cooney *et al*. 2023).

The deacetylation of chitin produces chitosan, a polysaccharide derived from chitin undergoes deproteinization and demineralization. Manufacturers typically produce commercial chitosan by partially deacetylating chitin, resulting in D-glucosamine and N-acetylglucosamine. During this reaction, the acetamido group converts into an amino group (Maliki *et al*. 2022). Chitosan is found in various types of crustaceans with varying compositions and is abundant after cellulose. One characteristic of chitosan, the degree of deacetylation (DD), directly affects the quality of chitosan and its effectiveness in adsorption, covalent bond formation, encapsulation, and other applications. High-quality chitosan is usually required for industrial-scale applications (William and Wid 2019).

Hosney *et al*. (2022) stated that chitosan synthesis using different raw materials and different demineralization, deproteinization, and deacetylation treatments will produce different chitosan qualities. Sarofa *et al*. (2025) also reported that the concentration of the base solution used significantly affects the chitosan extraction process from *Perna viridis*, influencing the physical and chemical properties of the resulting chitosan. The extracted chitosan is widely used in industry and medical applications such as drug delivery and tissue engineering because it has biodegradable, biocompatible, and antimicrobial properties (Ghezelsofloo and Dehghani 2024). Ihsan *et al*. (2025) extracted chitosan from *Panulirus homarus* and demonstrated its potential as a feed binder and antifungal agent against *Fusarium* sp. in aquaculture.

Based on those references, researchers are interested in extracting chitosan from abundant local resources on Bangka Island, which are often considered waste, in the form of mud crab, blue swimming crab, and vannamei shrimp carapace. In addition, this study also aims to analyze the yield percentage, water content, and degree of deacetylation (DD) of chitosan produced using FTIR (Fourier Transform Infrared Spectroscopy) instruments and compare the calculation of DD values with different wave numbers. The urgency of this study lies in the need to manage crustacean waste sustainably through a valorization approach to become valuable products such as chitosan, in addition to the importance of the accuracy of analytical methods for determining the quality of chitosan, especially the degree of deacetylation (DD), which plays a crucial role in various functional applications of chitosan. Although there are several studies on the extraction of chitosan from crustacean waste, there has not been a study that simultaneously evaluates the potential of several types of local crustacean waste on Bangka Island as raw materials for chitosan. In addition, studies on the comparison methods for determining the degree of deacetylation quantitatively using FTIR wave numbers are still minimal. This study provides a practical contribution to validating chitosan quality and improving the efficiency of local chitosan production in a competitive and applicable manner. The results offer a scientific foundation for producing high-quality local chitosan and guide the development of more sustainable and value-added crustacean waste management strategies.

METHODS

Research sample collection

This study used a laboratory experimental approach to extract and evaluate the quality of chitosan from three types of local crustacean waste conducted from July to September 2024. Crustacean waste samples were collected purposively

from three different locations. Vannamei shrimp carapace was a molted product from a shrimp pond in Belinyu District, Bangka Regency. In contrast, blue swimming crab carapaces were obtained from a crab peeling MSME (Micro, Small, and Medium Enterprises) in Toboali District, South Bangka Regency, and crab shells were obtained from seafood restaurants around Pangkalpinang City. Each sample was taken as much as 2 kg, then dried, ground, and homogenized (Reshad *et al.* 2021), after which the extraction process into chitosan was carried out with two replications.

Chitin and chitosan extraction

The extraction of shrimp, crab, and swimming crab carapace was carried out through a series of chemical reactions described by Reshad *et al.* (2021). Mineral content, such as $CaCO_3$ and $Ca_3(PO_4)_2$, in shrimp, swimming crab, and crab carapace samples can be removed through demineralization. Demineralization was carried out with 1.5 M HCl at 80°C for 2 hours (1:10 ratio, w/v), for each 100 g sample, followed by washing to neutral pH and drying. The deproteination and deacetylation processes were carried out using 3.5% and 60% NaOH, respectively, under similar conditions. The effectiveness of HCl was 10% higher than that of H_2SO_4 in dissolving mineral content in carapace samples. The addition of NaOH was done because the protein contained in the sample is soluble in alkali. The 60% NaOH concentration was chosen based on previous literature (Hosney *et al.* 2022), which showed an optimal deacetylation rate under these conditions. Calculating the percentage yield, water content, and analyzing the degree of deacetylation were carried out to analyze the quality of the chitosan produced.

Data analysis methods

Analysis of material functional groups using FTIR and DD calculations

The FTIR (Fourier Transform Infrared Spectroscopy) spectrum was recorded in the range of 400-4,000 cm^{-1} using KBr tablets (ratio 100:1). FTIR spectra of chitosan samples were initially recorded in transmittance mode. Before further analysis, the spectral data were converted to absorbance using the equation A = log(1/T). Baseline correction and peak height measurements were performed using OriginLab software to calculate the degree of deacetylation (DD) (Satrohamidjoyo 2018). Two band ratios were used for DD estimation: A1320/A1420, following the method of Brugnerotto *et al.* (2001), and A1655/A3450, as described by Duarte *et al.* (2002).

The formulas are,

$$1.\ DA[\%] = \frac{A1655}{A3450} \times 100/1.33$$

(Duarte *et al.* 2002)

$$2.\ DA[\%] = \frac{A1320}{A1420} - 0.3822/0.03133$$

(Brugnerotto *et al.* 2002)

$$3.\ DD[\%] = 100 - DA$$

Calculation of yield percentage

Chitosan yield is the percentage of chitosan obtained from the chitosan extraction process from raw materials, such as blue swimming crab, crabs, and shrimp carapace. This yield is calculated by comparing the dry weight of the chitosan produced to the initial dry weight of the raw materials used, usually expressed as a percentage. The yield indicates how efficient the chitosan production process is. The higher the yield, the more effective the extraction process (Andrade *et al.* 2012).

Water content analysis method

Water content was determined using a calibrated moisture analyzer. A 0.5-gram chitosan sample was heated at 120°C for 10 minutes, and the device automatically displayed the water percentage after a few minutes. Considering that the FTIR absorption band around 3,450 cm^{-1} (associated with OH and NH stretching vibrations) is highly sensitive to residual moisture, all samples were dried before FTIR analysis to minimize potential interference in the calculation of the degree of deacetylation (DD), particularly when using the A1655/A3450 band ratio.

Data analysis methods

The results of the yield, water content, and degree of deacetylation (DD) tests were analyzed descriptively and quantitatively to present the characteristics of chitosan produced from each type of crustacean

waste. The analysis was conducted on three types of raw materials with two replications (n = 2) and presented as average values and standard deviations as basic statistical parameters.

RESULTS AND DISCUSSION

Chitosan is a polysaccharide that can be extracted from the crustacean carapace. Based on the research results presented in Table 1, the chitosan yield from crustacean waste shows significant variations. The highest yield value was obtained from swimming crab at 12.4% ± 0.9, followed by shrimp (8.7% ± 0.5) and crab (4.0% ± 0.5). This difference indicates that the type of species affects the amount of chitosan that can be produced from its exoskeleton waste. This value is in line with Bolat *et al.* (2010), when chitosan was extracted from 100 g of freshwater crab carapace, *Potamon potamios*, was obtained 4.65% chitosan. This is thought to be due to a reduction in sample mass, which can occur due to removing proteins (deproteination) and minerals such as $CaCO_3$ (demineralization) or deacetylation. The result of the study is in line with Maliki *et al.* (2022), which states that the skin/carapace components of different biomaterials have different percentages of chitin and contain mineral elements such as $CaCO_3$ and protein, so not all carapace/skins can be converted into chitosan. Interestingly, the results of the study obtained are not the same as the results of the study by Gbenebor *et al.* (2016), which stated that the calcium carbonate content in the exoskeleton of crab carapace (crab and swimming crab) is higher than that of shrimp. High calcium carbonate content is generally associated with a denser and thicker shell structure, which should reduce the yield of chitin and chitosan because organic fractions, such as chitin, are less abundant. However, high mineral content is not the only determining factor in low yield. The effectiveness of the processing process, especially in the neutralization and washing stages, also has a significant influence. Low chitosan yield can also be caused by the loss of sample mass during the washing process or pH neutralization after demineralization, deproteinization, and deacetylation. Mass loss during these processes can significantly impact the amount of chitosan produced. Guo *et al.* (2013) stated that the neutralization process of residual alkali solution (NaOH) with CO_2 was more effective in reducing chitosan loss than regular water washing, which can cause losses of up to more than half.

In addition to yield, this study also analyzed the water content of the chitosan samples. Based on Table 1, the results of chitosan extraction from 100 g of carapace waste show that most of the water content is below the maximum threshold set by SNI No. 7949:2013, which is 12%. This water content can be affected by the drying process, drying time, and storage location (Szymańska and Winnicka 2015). Chitosan is more hygroscopic than chitin because its polymer structure has amine, N-acetyl, and hydroxyl groups, hydrogen bonds with water to form. Therefore, the absorbed water is free moisture and chemically bound to these functional groups.

In addition to the yield and water content previously described, the degree of deacetylation (DD) is a key parameter that significantly determines the quality and function of chitosan. The degree of deacetylation reflects the proportion of acetyl groups ($–COCH_3$) successfully removed from the chitin structure during the deacetylation process and directly affects the functional properties of chitosan, including its ability to bind metal ions and other chemical reactivities (Djaenudin *et al.* 2019). One critical factor affecting the DD value is the NaOH concentration, as strongly alkaline conditions are required to break the acetyl groups from the chitin polymer chain. Hosney *et al.* (2022) state that a minimum concentration of 40% NaOH is required to initiate an effective and significant deacetylation process.

Table 1. Yield quality (%) and water content (%) of chitosan from crustacean waste (n=2).

Parameter	Standard SNI (7949:2013)	Mud Crab	Shrimp	Blue Swimming Crab
Chitin yield (%)	-	31.5 ± 6.9	25.5 ± 7.8	14.4 ± 1.9
Chitosan yield (%)	-	4.0 ± 0.5	8.7 ± 0.5	12.4 ± 0.9
Water content (%)	<12%	6.4 ± 2.0	10.7 ± 2.7	6.3 ± 0.6

Confirmation of successful deacetylation was carried out through FTIR spectrum analysis shown in Figure 1, in the range of 400-4,000 cm^{-1} range. Chitosan extracted from different samples showed consistent spectral characteristics, namely the presence of typical absorption bands of amine (–NH), hydroxyl (–OH), and carbonyl (C=O) groups. Furthermore, the identification of these absorption bands became the basis for calculating DD using two quantitative FTIR-based approaches: the A1655/A3450 ratio, which compares the absorption intensity of the amide I group with the hydroxyl/amine group, and the A1320/A1420 ratio, which measures the absorption intensity of the amide III group relative to the C–H band as a reference.

The DD calculation results in Table 2 show significant variation between calculation methods, even for the same sample. For example, the chitosan sample from crab shells showed a DD value of 95.3% ± 2.5 using the A1320/A1420 method, but only 39.91% ± 0.88 when calculated using the A1655/A3450 method. This value is far below the chitosan quality standard according to SNI 7949:2013, which requires a minimum deacetylation degree of 75%. A similar pattern was also observed in chitosan samples from crab carapace and shrimp skin.

These results are not much different from Brugnerotto *et al.* (2001), who stated that the use of the A1320/A1420 ratio produced good DD, with an r value of 0.99, and provided results that were not much different when compared to the results of analysis using ^{1}H-NMR and 13C-NMR instruments. Conversely, it was found that A1655/A3450 gave poor values. Czechowska-Biskup *et al.* (2012) also stated that the A1320/A1420 ratio was unaffected by humidity, meaning the data obtained were more stable and reliable in determining DD, regardless of environmental variations. Abdou *et al.* (2008) and Ahing and Wid (2016) also used the A1320/A1420 ratio and obtained DD results >70%. However, a different opinion was expressed by Amitaye *et al.* (2024), who found that the A1320/A1420 ratio produced unreliable values when compared with the ^{1}H-NMR method, which is considered a standard reference. On the other hand, if using the A1655/A3450 ratio, the DD value will approach the standard value of the ^{1}H-NMR results.

Based on these results, although FTIR offers a faster and more practical approach, its limitations in terms of quantitative reproducibility and sensitivity to environmental conditions remain a significant challenge. Therefore, FTIR results are not absolute, so to determine the DD value, it needs to be correlated with other, more accurate methods (Brugnerotto *et al.* 2001). The ^{1}H-NMR method remains the standard reference in determining DD because it provides precise results and is free from operator bias, although it requires more time and cost. Therefore, cross-validation with standard methods such as ^{1}H-NMR is still necessary to ensure the accuracy and consistency of the results.

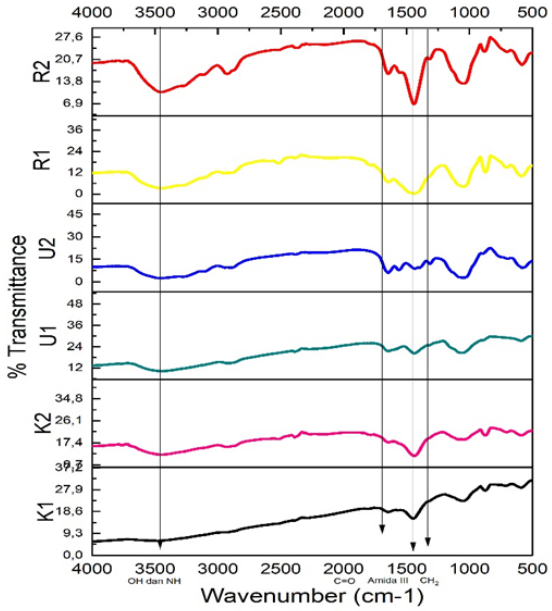

Figure 1. FTIR spectrum of chitosan from crab (K), shrimp (U), and blue swimming crab (R) carapace.

Table 2. Degree of deacetylation of chitosan with different formulas.

Sample	$DA[\%] = \frac{A1320}{A1420} - 0.3822/0.03133$		$DA[\%] = \frac{A1655}{A3450} \times 100/1.33$		Standard SNI (7949:2013)
	DA (%)	DD (%)	DA (%)	DD (%)	
Mud crab	13.2 ± 0.4	86.8 ± 0.4	54.72 ± 12.11	45.29 ± 12.11	
Shrimp	15.6 ± 0.1	84.4 ± 0.1	54.09 ± 4.90	45.92 ± 4.90	DD>75%
Blue swimming crab	4.7 ± 2.5	95.3 ± 2.5	60.09 ± 0.88	39.91 ± 0.88	

CONCLUSION

Based on the research results, the blue swimming crab, mud crabs, and shrimp carapace, as local resources of Bangka Island, have the potential to be extracted into valuable chitosan. Chitosan from crab carapace showed the highest yield of 12.4% ± 0.9 and the lowest water content of 6.3% ± 0.6 according to the SNI 7949:2013 standard (<12%). Two FTIR methods showed significant variations, where the A1320/A1420 ratio produced a DD>75%, while the A1655/A3450 method produced a DD below the SNI 7949:2013 standard. Both methods can be used for DD analysis, but a comparison with standard methods such as ^{1}H-NMR and further validation is needed to obtain more precise and universally comparable results. These findings support the potential of utilizing crustacean waste as a source of local chitosan with added value. They are relevant in the development of the biomaterial industry and the implementation of a circular economy.

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