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The effect of deproteinization temperature on chitosan extraction from shrimp shell in Sabah

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ABSTRACT Chitosan is a straight amino-polysaccharide made after the alkaline deacetylation of biopolymer chitin. N-acetyl-D-glucosamine molecules are converted into D-glucosamine components, which contain free amino groups with an advantageous ionic charge. Chitosan and its derivatives have been used in a wide variety of applications such as food industry, biomedicine, cosmetics and agriculture. There are three stages to extract chitosan from shrimp shell waste such as deproteinization, demineralization and deacetylation. This present study aims to examine the influence of different temperatures and using 60% NaOH during the deproteinization process while 5% of HCl during demineralization and 60% NaOH during deacetylation. The temperature being studied during deproteination was 30 °C – 80 °C. The results show that the percentage of chitosan yield was higher when using low temperature at the first stage. The yield obtained in this study ranged from 6.90 – 9.50%, while degree of deacetylation ranged from 85.28 – 85.34%.

KEYWORDS: Chitosan; Deproteinization Temperature; Chitosan Yield; Degree of deacetylation (DDA)
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Original Article

INTRODUCTION

Seafood is one of the favourite foods among citizen in Malaysia especially in Sabah such as shrimp, crab and fish. Shrimps are rich in nutrients, possesses a unique scent, and tastes amazing. The flesh of prawns is primarily consumed as food. The skin, head, and tail of shrimp are among the portions that are rarely eaten and frequently end up as shrimp trash. When the demand of these crustaceans is getting higher, the waste also increase which is harmful and toxic to the people and environment (Setiati *et al.*, 2021) On the other hand, the high demand could encourage the growth of fishing sector and increase employment for villagers (Chik *et al.*, 2023).

Chitin which a polysaccharide composed of N-acetyl-D-glucosamine components, is the second most prevalent biopolymer on Earth after cellulose. It is mostly found in bugs, fungus, algae, and yeasts, as well as in crustacean shells (Al-Hoqani *et al.*, 2020). Most research using traditional method to extract chitosan from marine wastes such as deproteinization, demineralization and deacetylation. The product is called chitin after treated with alkaline solution during deproteinization and using acid solution during demineralization. When chitin undergo deacetylation, it will become chitosan due to the process of removal of acetyl groups from chitin and substitution of reactive amino groups ($-NH_2$) as shown in the Figure 1 in next page (Pakizeh *et al.*, 2021; Ewais *et al.*, 2023).

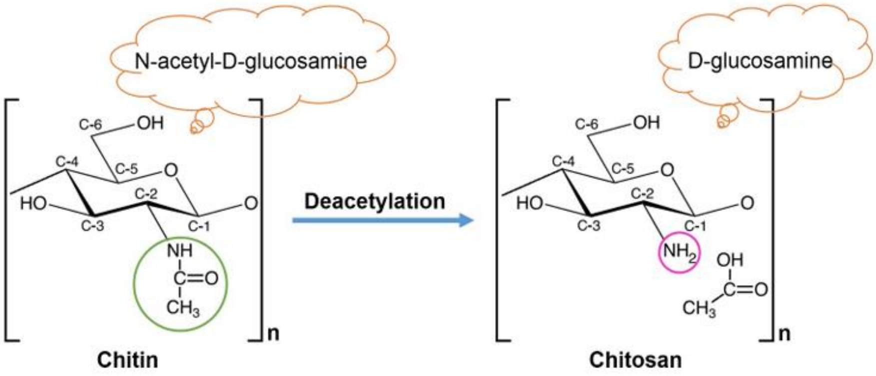

Figure 1. Chemical structure of chitin and chitosan (Pakizeh *et al.,* 2021)

Ewais *et al.* (2023) stated that chitosan is transformed into a cationic form by the free amino groups, which may have advantageous antiviral or antibacterial properties similar to those of cationic proteins. Chitosan's positive ionic charge enables it to aim for the bacterial negative cytoplasmic membrane and chemically attach to lipids and bile acids. Jasim (2021) also mentioned that organic functional polymers such as chitosan have received a lot of scientific interest and have been utilised as an environmentally hazardous substitute for artificial polymers. Bioactive polymers like chitosan and its derivatives have drawn a lot of interest from a variety of industries, including the food sector, medical technology, beauty products, and agriculture (Hisham *et al.,* 2021). Therefore, the aim of this study is to extract chitosan from shrimp shell waste to substitute the hazardous chemicals which is harmful for the ecosystem. Besides, the focus of this research is to determine the effect of temperature during the deproteinization process by calculating the percentage of chitosan yield and degree of deacetylation.

METHODOLOGY

Sample Collection and Preparation

The shrimp shell waste utilised in this study was collected from Kiang Huat Seagull Trading Frozen Sdn Bhd which located in Putatan, Sabah. The species of the shrimp was identified as *vannamei* species. The collected shell was subsequently cleaned and dried in an oven for 4 hours at 85ºC. Then, dried sample was grind using mortar and pestle into powder form (Hisham *et al.,* 2021).

Production of Chitin by Chemical Method

Deproteinization (DP)

This experiment was carried out on a laboratory scale using six (6) of 250 mL beakers. A total of 5g grounded shrimp shell waste was added to 60% of NaOH using the ratio of 1:16 (w/v) for each beaker. The solution was heated at various temperatures such as 30 ºC, 40 ºC, 50 ºC, 60 ºC, 70 ºC and 80 ºC while stirred for 1 hour using magnetic stirrer. Then, all samples were washed and filtered until reached pH 7. All samples were dried in oven overnight at 80ºC (Aldila *et al.,* 2020).

Demineralization (DM)

All the dried samples were then added into 5% HCl while stirring for 1 hour using 1:10 (w/v) at room temperature. Then, all samples were washed and filtered again until neutral pH to remove excess acid. All samples were dried overnight in oven at 80 ºC. After drying, the samples were called chitin (Tamzi *et al.,* 2020).

Chitosan Extraction

Deacetylation (DA)

The chitin was then treated again with 60% NaOH while stirring for 1 hour at 30 ºC using 1:15 (w/v). After that, all the samples were washed and filtered again with distilled water until neutral pH. The samples were dried overnight in the oven at 80 ºC. The samples were called chitosan after drying (Ahing & Wid, 2016; Aldila *et al.*, 2020).

Analysis of chitosan yield

Chik *et al.*, (2023) mentioned that the yield for chitosan was determined by comparing the dry weight of the powder to the wet weight of the dry shrimp shell. The following (Equation 1) is an example of how equation is written given as:

$$\text{Chitosan extraction yield, \%} = \frac{\text{Dry weight of the chitosan powder}}{\text{Wet weight of the dry shrimp shell}} \quad (1)$$

Characterization of Chitosan

Degree of Deacetylation (DDA)

The wavelengths of chitosan were measured using a Fourier Transform Infrared Spectroscopy (FTIR) instrument (Thermo Nicolet Nexus 670 spectrometer, USA) at a frequency of 4000-400cm^{-1} at 4cm^{-1} resolution. The degree of deacetylation (DDA) of chitosan was determined by using the baseline technique. The equation for the baseline was found as follows.

$$\text{Degree of deacetylation} = 100 - \frac{A1655}{A3450} \times \frac{100}{1.33} \quad (2)$$

where the absorbances at 1655 cm^{-1} of the amide-I band, which indicates the N-acetyl group content were denoted as A_{1655}. The absorbances at 3450 cm^{-1} of the hydroxyl band serve as an internal benchmark for determining disc thickness. The factor 1.33 is the ratio of A_{1655}/A_{3450} for completely N-acetylated chitosan (Selvaraj *et al.*, 2023).

RESULT AND DISCUSSION

Chitosan Product

This research effectively extracted chitosan from wet shrimp shell waste by using traditional method. The physical appearance of chitosan obtained at different temperatures through observation. The chitosan at lower temperatures from 30 – 50 ºC were slightly brownish while chitosan at higher temperature from 60 – 80 ºC were white in colour as shown in Figure 1.

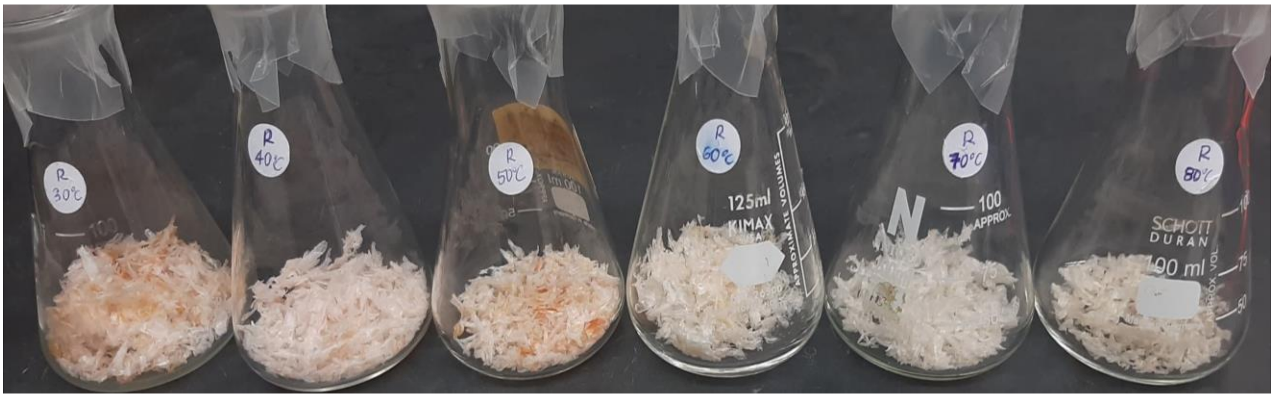

Figure 2. Chitosan at different temperatures (30 ºC, 40 ºC, 50 ºC, 60 ºC, 70 ºC, 80 ºC)

The samples showed the shape of crystalline flakes and had no smell. The properties of the chitosan generated in this investigation were equal to the chitosan produced from past studies which located at India, Turkey and Indonesia (Allwin *et al.*, 2015; Kucukgulmez *et al.*, 2011; Mulyani *et al.*, 2019). In addition, this study also successfully yielded 6.90 to 9.50 chitosan with an average of 8.27 % from wet shrimp shell waste. Based on Table 1, the higher chitosan yield was 9.50 % which is at the lower temperature, 30 ºC while the lower extracted chitosan was only 6.90 % at the higher temperature, 90 ºC. When using low temperature during the first process called deproteinization, the higher the percentage of chitosan yield. Previous study from Kucukgulmez *et al.* (2011) also mentioned the extraction of chitosan decreasing when the temperature is increasing. At temperature of 20 ºC, the amount of chitosan yield was 56.52 % while only 26.73 % chitosan extracted at 40 ºC.

Table 1. Result of yield and degree of deacetylation (DDA) percentage at various temperatures

Temperature (ºC)	Result	
	Yield (%)	DDA (%)
30	9.50	85.28
40	8.12	85.30
50	8.45	85.34
60	8.44	85.29
70	8.19	85.31
80	6.90	85.29

Aldila *et al.*, (2020) also studied that the temperature during deproteinization was one among the most important variables influencing deacetylation degree (DD) of chitosan. The most common method for removing these proteins was to use NaOH solutions in different concentrations over an extended period of time at high temperatures. Nevertheless, treatment at high temperatures give harmful effect on the chitosan DD. At the deproteinization temperature at 30 °C, the maximum chitosan DD was obtained. It then progressively drops as the temperature rises until the final temperature of 90 °C at the same NaOH concentration.

Meanwhile, one of the most crucial factors that affects the quality of chitosan is the degree of deacetylation (DDA). The DDA increases with the purity of the chitosan. It is also often mentioned as a crucial element in assessing the biological activity, polymeric and physicochemical properties, and biomedical uses of chitosan (Hosney *et al.*, 2022). Jadhav & Diwan (2018) mentioned that the degree of deacetylation was influenced by temperature and NaOH concentration. This statement supported by Aldila *et al.*, (2020) by saying that at 30 °C during deproteinization and 60 % NaOH concentration, the maximum chitosan DD of 88.89% was recorded.

The percentage of DDA was calculated by using the wavenumber from the FTIR spectra. Table 1 indicates that the percentage of DDA for each sample was in the range 85.28 – 85.34 % which was slightly different. The higher DDA was 85.34 % at 50 ºC while lower DDA was 85.28 % at 30 ºC using 60 % NaOH which parallel with previous study that mentioned DDA was affected by the temperature and NaOH concentration. The bar graph for both results of yield and DDA were plotted as shown in Figure 3.

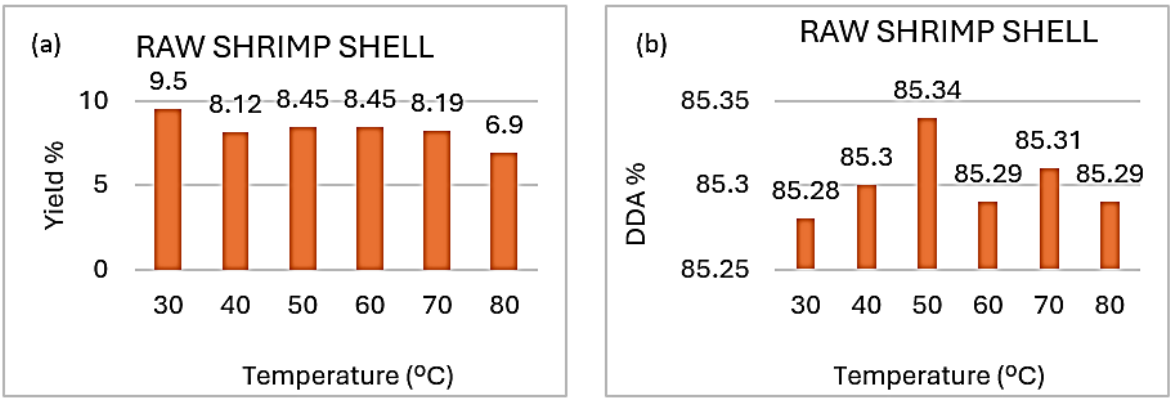

Figure 3. The effect temperature on raw shrimp shell for (a) Yield %, (b) degree of deacetylation (DDA) %

Mulyani *et al*., (2019) mentioned that FTIR spectra of chitosan showed C=O stretching on the bond ($NHCOCH_3$) (1660.71 cm^{-1}), CO stretching (1026.13 cm^{-1}), OH stretching (3448.72 cm^{-1}), NH bending ($R\text{-}NH_2$) (1564.27 cm^{-1}) and CH stretching (2887.44 cm^{-1}). Kucukgulmez *et al*., (2011) also stated that FTIR spectroscopy was applied to identify the chitosan's structure. The peak seen about 1555 cm^{-1} belongs to the N-H bending of secondary amide II bands. The amide I band, which usually appears around 1655 cm^{-1}, is not visible, though. Additional bands seen in the 1380–1460 cm^{-1} range correspond to the methyl groups' symmetric and asymmetric bending vibrations (Hassan *et al*., 2022).

Besides, Figure 4 shows the reading for one sample of chitosan obtained from shrimp shell waste in Sabah at 30 ºC. The wavenumber at 3260.51 cm^{-1} indicates the presence hydroxyl group, 1622cm^{-1} represent the C=O stretching, and 1550.89 cm^{-1} represent amine group. These indicates the confirmation of chitosan chemical structure.

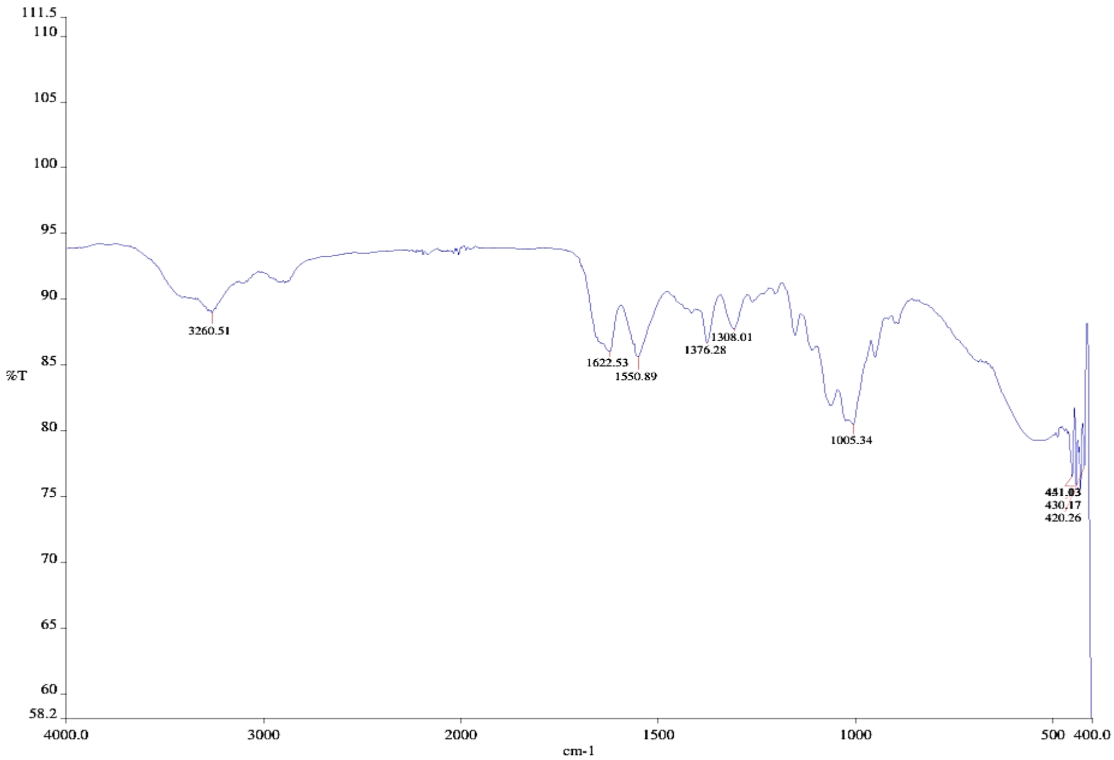

Figure 4. FTIR spectra of chitosan at 30 ºC deproteinization

CONCLUSION

According to the findings from this study, the average yield percentage obtained was 8.27%, while the average of DDA was 85.30%. When the temperature during deproteinization was lower, the yield was higher at 9.50%. The effect of temperature on the DDA was slightly different in the range of 85.28 – 85.34 %. The higher value of DDA was 85.34% which was at 50 ºC. At low temperature as 30 ºC, the DDA was slightly lower which was 85.28%. Therefore, the effect of temperature was not significant for DDA. The yield percentage indicates that shell waste in Sabah has the potential to produce a high-quality chitosan that can be used for many applications including agriculture, wastewater treatment, food sector and cosmetics.

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