Comparison of extraction sequence on yield and physico-chemical characteristic of chitosan from shrimp shell waste

W William¹ and N Wid¹,²
¹Faculty of Science and Natural Resources, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia.
²Water Research Unit, Faculty of Science and Natural Resources, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia.

Email: newati@ums.edu.my

Abstract. The focus of this study was to compare the yield and characteristics of chitosan produced from different sequences of treatment for deproteination, demineralization and deacetylation. In Process 1, deproteination occurred in the first step followed by demineralization and deacetylation. While Process 2 was started with demineralization step and followed by deproteination and deacetylation. The results show that the percentage yield of chitosan was slightly higher when Process 2 was adopted with 22.22 %. However, the process produced chitosan with lower degree of deacetylation and solubility with high ash content, which were 76.47 %, 37.79 % and 2.67 %, respectively. While, when Process 1 was carried out, the degree of deacetylation of chitosan, solubility and ash content were improved to 91.26 %, 100 % and 0.38 %, respectively, with lower yield of 19.01 %. Therefore, this study suggests the extraction process should be performed by carrying out deproteination step before demineralization and deacetylation (Process 1) to produce a good quality of chitosan.

1. Introduction
Shrimp is one of the most important fisheries worldwide. Throughout the years, the world of fisheries and aquaculture production volume has increased rapidly due to the rising demand in the international market. Subsequently, shrimp aquaculture is expanding rapidly and has become one of the most important sectors in Malaysia. With the growing production of shrimp aquaculture every year, it has become one of the major industries that contributes to the economic growth of Malaysia [1]. In Sabah, shrimp processing is increasing vastly with the growing of cultured shrimp production at the local area. Seafood processing especially shellfish is usually involved with the removal of head and shell. Constant repetition of this process without any improper waste management will cause the accumulation of solid waste and bringing negative impacts towards the environment as well as the human health. Shrimp shell are made of components such as protein and chitin. Chitin is an amino polysaccharides built a long polymer consisting of N-acetyl glucosamine units connected by β-1,4-glycoside bonds that can be found at the outer surface of the crustacean. Separation of chitin from shrimp shell involved in the chemical processing steps such as deproteination and demineralization by using strong acid and base to remove protein and calcium carbonate respectively. Chitosan is the deacetylated derivative of chitin. It is a natural polymer composed of β-(1 →4)-linked D-Glucosamine, randomly distributed.

Chitosan has proven to be beneficial for industrial application, as it possesses characteristics such as biodegradable, ability to chelate metal, sorption properties and more. However, usage of chitosan is still...
limited for industrial application due to the difficulties in maintaining the quality of chitosan. For industrial application, high quality of chitosan is required. Therefore, several factors such as concentration of chemical, heating temperature and process sequence should be taken into consideration during the extraction process. Extraction of chitin and chitosan involved deproteination, demineralization and deacetylation process. In this study, different serial of process sequence was conducted with the aim to investigate the yield and characteristic of chitosan, which includes the ash content, degree of deacetylation and its solubility in acetic acid.

2. Material and method

2.1. Sample collection and preparation
Shrimp shell wastes were collected from a local market, near Kota Kinabalu City. The samples then washed with tap water to remove insoluble material on the shell and followed by drying it under the sun for 8 hours. The samples were blended and then stored in a closed container prior to use.

2.2. Different Series Extraction of Chitosan
Chitosan was prepared by two different treatments in which deproteination, demineralization and deacetylation were applied in either sequence.

2.2.1. Process 1 (P1): Deproteination preceded demineralization. A 20 g of Shrimp shell was deproteinated with 2.0 M of NaOH at ratio 1:16 (w/v) at room temperature for 48 hours, then demineralized with 1.0 M of HCl at ratio 1:16 (w/v) for 24 hours at room temperature [2]. Chitin obtained from the process were dried in an oven at 60 °C for 4 hours [3]. Deacetylation of chitin with 48 % of NaOH was carried out at temperature 70 °C for 48 hours and followed by similar drying process.

2.2.2. Process 2 (P2): Demineralization preceded deproteination. A 20 g of shrimp shell was demineralized with 1.0 M of HCl at ratio 1:16 (w/v), then followed by deproteination with 2.0 M NaOH at room temperature at ratio 1:16 (w/v). Dried chitin was then deacetylated using 48 % of NaOH at temperature 70 °C for 48 hours and dried in an oven at 60 °C for 4 hours.

2.3. Chitosan Yield
Chitosan yield (%) was calculated as dry weight of chitosan flakes relative to the wet weight of shrimp shell Equation 1 [2].

\[
\text{Chitosan Extraction Yield (\%)} = \frac{\text{Extracted Chitosan (g)}}{\text{Shrimp Shell Waste (g)}} \times 100\% \\
\text{(Equation 1)}
\]

2.4. Characterization of chitosan

2.4.1. Ash content. 1 g of chitosan was heated in a furnace at 600 °C for 6 h [4]. The crucibles then allowed to cool in the furnace to < 200 °C and then placed into a desiccator. The mass of crucible and ash content was weighed and calculated using Equation 2.

\[
\text{Ash(\%)} = \frac{\text{Residue (g)}}{\text{Sample (g)}} \times 100\% \\
\text{(Equation 2)}
\]

2.4.2. Degree of deacetylation. The chitosan samples were characterized by using Fourier Transform Infrared (FTIR) spectrophotometer in the range of 400 to 4000 cm⁻¹ and repeated for three replicates. The DDA of chitosan were determined by using baseline purposed by Brugnerotto et al., (2001) at
ratio $A_{1320}/A_{1420}$. The band produced have been reported to have narrower experimental error. Thus, Equation 3 was used to calculate the DDA of chitosan [5]:

$$DA(\%) = \left( \frac{A_{1320}}{A_{1420}} \right) - 0.3822 \quad (\text{Equation 3})$$

$$DDA(\%) = 100 - \% DA$$

Where,

- DDA = Degree of deacetylation (\%)
- DA = degree of acetylation (\%)
- $A_{1320}$ = Peak area for band 1320 cm$^{-1}$
- $A_{1420}$ = Peak area for band 1420 cm$^{-1}$

2.4.3. Solubility in Acetic Acid. A 1 g of chitosan was dissolved in 100 mL of 1% acetic acid solution and placed on an orbital shaker at 200 rpm for 24 h [6]. The chitosan acidic solution was then filtered using vacuum pump. The percentage of the solubility of chitosan in the acetic acid was calculated using Equation 4.

$$\text{Insoluble} (\%) = \frac{\text{Insoluble (g)} - \text{Initial weight of filter paper (g)}}{\text{Sample Weight (g)}} \times 100\% \quad (\text{Equation 4})$$

$$\text{Solubility} (\%) = 100 - \% \text{ insoluble}$$

3. Results and Discussion

3.1. Chitosan yield

P1 has produced an average of 19.01\% of chitosan from wet weight of shrimp shell waste. Whereas, P2 yielded 22.22\% of chitosan. When extraction was started with deproteination (P1), less chitosan was produced and this is due to the native structure of chitin (protein) that lost during the deproteination process. The process which resulting to more hydrolysis and losses of solid material, eventually decrease the weight of chitosan. However, when first extraction was started with demineralization (P2), more chitosan was produced. A higher yield of chitosan (22.22\%) is caused by the presence of native chain that still stays intact with the chitosan. Therefore, there is less hydrolysis of the backbone and higher yield of chitosan [6] [7].

3.2. Physical characteristics of chitosan

The results also found that chitosan from both processes showed distinctive physical appearances. The physical appearance of chitosan produced from both process was carried out through observation. Chitosan produced from P1 was whiter compared to the chitosan from P2, which has pinkish pigments. The presence of this pigments might due to the presence of coloured matter and some remaining of protein material bounded on the solid matrix caused by the incomplete deproteination in P2. Figure 1(a) and Figure 1(b) show the chitosan produced from P1 and P2, respectively.
3.3. Ash content
Effectiveness of demineralization can be determined through the percentage of ash content in the chitosan. The ash content in chitosan is an important parameter as the ash residual may affect the solubility and thus contribute to lower viscosity or other important characteristic of the final products [8]. Nouri et al., (2016) reported that good quality of chitosan should possess less than 1 % of ash content. For this study, analysis of ash showed that chitosan from P1 has lower ash content compared to chitosan produced by P2, which was 0.24 % and 2.67 %, respectively. The significant difference of ash percentage shows that, the demineralization process in P1 was more effective than in P2. This is because when demineralization was carried out before deproteination, the protein layer will act as the adhering layer and prevent the hydrolysis of chitin from occurring and also further depolymerisation [6]. Consequently, produced chitosan with higher mineral content. Meanwhile, when deproteination occurred in the first step the protein layer was removed during the deproteination process, leaving the exposed mineral layers. Accordingly, allowed the mineral to be easily removed during demineralization treatment and produced chitosan with low ash content.

3.4. Degree of Deacetylation (DDA)
The efficiency of acetyl group removal can be measured through DDA. DDA of chitosan provides major influences on the physical, chemical and biological properties of chitosan, such as acid base and electrostatic characteristic, biodegradability, self-aggregation, sorption properties and the ability to chelate metal ions [9]. For current study, comparison between the FTIR spectra of the synthesized chitosan samples from each respective processes were made with standard chitosan sample.

FTIR spectrum of chitosan from P1 showed peaks at 3355 cm\(^{-1}\) that indicated the stretching vibration of (–OH) group, (-NH\(_2\)) group of amines and hydrogen bonding which comparable to spectrum peak of standard chitosan, 3420 cm\(^{-1}\). (C=O) in NHCOCH\(_3\) group and amide II band (N-H bending) were observed to occur at band 1647 cm\(^{-1}\) and 1567 cm\(^{-1}\) respectively. Whereas in standard chitosan, these functional groups were observed at band 1654 cm\(^{-1}\) and 1580 cm\(^{-1}\). Amide III band (C-N stretching) were shown at 1313 cm\(^{-1}\) and 1320 cm\(^{-1}\) for standard chitosan. For (–CH) stretch with vibration of OH and CH in the ring, peaks were observed at 2881 cm\(^{-1}\) and 1421 cm\(^{-1}\). 1374 cm\(^{-1}\) showed the peak for (–CH) group of NHCOCH\(_3\) (amide bond), 1027 cm\(^{-1}\) shows the C-O stretching in acetamide. The peaks showed that the P1 has produced chitosan as it provided similar peaks as the standard chitosan. Meanwhile, chitosan produced from P2 also showed similar peaks as the standard chitosan and chitosan produced from P1. However, for P2 chitosan extra peak can be observed at 1739.89 cm\(^{-1}\) in which indicates the presence of acetyl group (C=O) in chitosan. When demineralization occurred first, conversion of chitin into chitosan was not as effective as when deproteination occurred first due to the presence of coloured matter and protein materials. Accordingly, it leads to the insufficient reaction to
convert acetyl group into amino group and thus explained the remaining acetyl group in P2 chitosan. The FTIR spectrum of chitosan samples are as illustrated in Figure 2.

![FTIR spectrum](image)

**Figure 2.** FTIR spectrum of (a) standard chitosan [11], (b) chitosan from P1 and (c) chitosan from P2.

### 3.5. Solubility

Chitosan is insoluble in water, alkaline solution or most common organic solvents but it is soluble to some extent in dilute aqueous solutions [2]. Solubility of chitosan are usually affected by the ash content and DDA. Solubility proportionally increased with increasing DDA. Chitosan with high DDA contained large amount of amino group (-NH) which would allow it to be easily protonated in aqueous acid solution with pKa smaller than 6.2 making chitosan soluble as shown in Equation 5 [10].

\[
\text{-NH}_2 + H_2O \leftrightarrow \text{NH}_3^+ + OH^- 
\]

Chemical reaction for amine group in water molecule  

Equation 5

On the contrary, when chitosan contained large amount of ash, it is unlikely becoming soluble in acetic acid. The ineffectiveness of demineralization may cause some minerals impurities remained bounded with chitosan and therefore affect the solubility of chitosan. In this study, chitosan produced from P1 was found completely soluble in acetic acid, while P2 produced chitosan with 37.79 % of solubility. As mentioned earlier, the P2 chitosan has lower DDA and higher ash content compared to the chitosan from P1. As result, it is less soluble compared to chitosan extracted from P1. Besides that, the coloured matter and protein residuals also other reasons for the less soluble of P2 chitosan. Table 1 shows the percentage yield and physico-chemical characteristics of chitosan from P1 and P2.
Table 1. Yield and physico-chemical characteristic of chitosan from P1 and P2.

| Physico-chemical properties | Process Sequence |
|-----------------------------|------------------|
|                             | P1               | P2               |
| Yield (%)                   | 19.01 ± 0.55     | 22.22 ± 0.56     |
| Colour                      | White            | Pinkish          |
| Ash content (%)             | 0.24 ± 0.14      | 2.67 ± 0.44      |
| Solubility (%)              | 100              | 37.79            |
| DDA (%)                     | 91.26 ± 2.75     | 76.46 ± 0.83     |

1 Each physico-chemical parameters were conducted with at least 3 replicates.

4. Conclusion
Chitosan is known for its potential for industrial application. However, maintaining the quality of chitosan has always been the reason for limited industrial application. Generally, extraction of chitosan involves three main stages which are deproteination, demineralization and deacetylation. Apart from known factors such as concentration of chemical, heating duration and temperature, process sequence for extraction of chitosan also can affect the quality of chitosan. In current study, better quality of chitosan was extracted when the extraction was started with deproteination (P1). When the process was started with demineralization (P2), higher yield of chitosan was produced, but the quality in terms of colour, ash content, solubility and the DDA were lower compared to P1. Consequently, extraction of chitosan should be carried out using P1 to obtain a better quality of chitosan.

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