Effect of deacetylation conditions on physicochemical properties of chitosan derived from shrimp shell and squid pen

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Abstract. Chitosan is a biopolymer derived from chitin. The physicochemical properties of chitosan end products resulted from many factors such as chitin source of origin, deacetylation conditions such as alkali concentration, ratios of chitin to alkali, temperature and extent of the reaction. The objective of this study was to investigate the effects of deacetylation time length and alkali treatments on physicochemical properties of chitosan products prepared from shrimp shell, and squid pen. The result demonstrated that the % yield of chitosan products were in the range of 62.23-90.83% for α form and 71.30-98.07% for β form. The molecular weight (MW) of chitosan products were in a range of 342.98-1603.48 kDa for α form, and 127.11-908.18 kDa for β form. The % deacetylation degree (%DD) of chitosan products derived from shrimp shell were in a range of 85.36-91.39%. In addition, the result showed that %DD of chitosan products was significantly negative correlated with MW of chitosan products.

1. Introduction
Chitin (β-1,4-N-acetylglucosamine), the second most abundant natural polymer on earth next to cellulose, exist in different polymorphic form varying in packing and polarities of adjacent chitosan chains in successive sheets. The α-form has antiparallel chains, the most abundant and present in chitin samples isolated from crabs and shrimp shells, while the β-form has parallel chains found in chitin samples isolated from squid pens [1]. Chitosan is a derived compound from chitin. α-Chitosan has been commercially manufactured from shrimp and crab shells and has been widely applied in various fields, whereas β-chitosan has been commonly obtained from squid pens and has a limited number of studies and applications, primarily due to its limited availability [2]. Chitosan is obtained from deacetylation of chitin. The deacetylation process requires a strong alkali solvent, such as NaOH to remove the acetyl group from...
chitin to an amine group [3]. Deacetylation time length and alkali treatment are ones among those factors affecting the physicochemical characteristics of chitosan. The objective of this study was to investigate the effects of deacetylation time length and alkali treatments on physicochemical properties of chitosan products prepared from shrimp shell, and squid pen.

2. Material and method
2.1 Materials
Dried shrimp shell and squid pen were obtained from local food processing plant (Samutsakhon, Thailand). N-Acetyl-d-glucosamine was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Acetic acid and sodium hydroxide (NaOH) were obtained from VWR (Radnor, PA, USA). Sodium chloride (NaCl) was purchased from Ajax Finechem (North Ryde, NSW, Australia). All the chemicals were of analytical grade.

2.2 Preparation of chitin
α-Chitin and β-chitin were prepared from dried shrimp shell and squid pen respectively according to the method of Jampafuang et al. [1]. Dried shrimp shell was demineralized with 1 M HCl for 2 h at 60°C and then deproteinized with 2 M NaOH for 2 h at 60°C, and oven dried at 60°C. Dried squid pen was deproteinized with 2 M NaOH for 2 h at 60°C, and oven dried at 60°C.

2.3 Preparation of chitosan.
α-Chitosan and β-chitosan were prepared from α-chitin and β-chitin respectively. α-Chitin products were divided into 2 sets. Firstly, α-Chitin products were deacetylated with 50 % NaOH (w/w) at 126°C for 2, 4, 6, 8, and 10 hours without alkali solution refreshed resulting in 5 α-chitosan products with different %DD and MW combinations, that is, SSC1, SSC2, SSC3, SSC4, and SSC5. Secondly, α-Chitin products were deacetylated with 50 % NaOH (w/w) at 126°C for 2, 4, 6, 8, and 10 hours with alkali solution refreshed every 2 hours resulting in 5 α-chitosan products with different %DD and MW combinations, that is, SSC6, SSC7, SSC8, SSC9, and SSC10. In the same manner, β-Chitin products were divided into 2 sets. Firstly, β-Chitin products were deacetylated with 35 % NaOH (w/w) at 120°C for 2, 4, 6, 8, and 10 hours without alkali solution refreshed resulting in 5 β-chitosan products with different %DD and MW combinations, that is, SPC1, SPC2, SPC3, SPC4, and SPC5. Secondly, β-Chitin products were deacetylated with 35 % NaOH (w/w) at 120°C for 2, 4, 6, 8, and 10 hours with alkali solution refreshed every 2 hours resulting in 5 β-chitosan products with different %DD and MW combinations, that is, SPC6, SPC7, SPC8, SPC9, and SPC10. All samples were washed with water until the pH is 7 after the deacetylation process, and oven dried at 60°C.

2.4 Characterization of chitosan
2.4.1 Determination of %yield.
%Yield was determined by the amount of chitosan products obtained divided by the total amount of initial chitin products, and expressed as a percentage.

2.4.2. Determination of molecular weight (MW)
The MW of chitosan samples were done according to the method of Jung and Zhao [4]. An approximately 0.076 grams of chitosan was dispersed and homogenized in 10 ml of acetic acid 1 M for 12 hours. Chitosan samples were dissolved in 0.1 M acetic acid and 0.2 M NaCl solvent system. An Ubbelohde viscometer (Cannon Instrument Company, State College, PA, USA) was used to measure the efflux time at 25°C. Intrinsic viscosity (η) was obtained from linear plots of reduced viscosity (ηsp/C) against
concentration (C, g/ml), extrapolating to zero concentration. The MW was assessed using the Mark-Houwink relationship given in equation (1):

\[ \eta = K(MW)^a \]  

(1)

where: \( K = 1.8 \times 10^{-3} \)
\( a = 0.93 \).

2.4.3. Determination of % deacetylation degree (%DD).
The % DD of chitosan samples were determined by the first derivative UV spectrophotometry method of Kiang et al. [5]. Firstly, UV visible absorbance spectra of 0.01, 0.02 and 0.03 M acetic acid solutions were obtained from 190–250 nm scanning using a UV-visible spectrophotometer (UV-1700, Shimadzu, Kyoto, Japan). The zero-crossing point (ZCP) was obtained from the intersection of the first derivative absorbance spectra. The standard curve was plotted using 0.005 to 0.035 mg/ml N-acetyl-d-glucosamine in 0.01 M acetic acid. The height, H, was measured from the ZCP to the first derivative spectra of the standard solutions. The % DD of chitosan was obtained by scanning absorbance spectra of 0.1 mg/mL chitosan in 0.01 M acetic and the concentration of N-acetyl-d-glucosamine was determined and the % DD was then calculated.

3. Results and discussion

3.1. Yield
The % yields of α-chitosan and β-chitosan products are illustrated in Figure 1, and Figure 2 respectively. As shown in Figure 1, the % yield of α-chitosan derived from deacetylation of α-chitin for 2, 4, 6, 8, and 10 hours without alkali solution refreshed (SSC1, SSC2, SSC3, SSC4, and SSC5) were in a range of 68.50–90.83%. The % yield of α-chitosan derived from deacetylation of α-chitin for 2, 4, 6, 8, and 10 hours with alkali solution refreshed every 2 hours (SSC6, SSC7, SSC8, SSC9 and SSC10) were in a range of 62.23–79.23%. As shown in Figure 2, the % yield of β-chitosan derived from deacetylation of α-chitin for 2, 4, 6, 8, and 10 hours without alkali solution refreshed (SPC1, SPC2, SPC3, SPC4, and SPC5) were in a range of 71.30–90.53%. The % yield of β-chitosan derived from deacetylation of β-chitin for 2, 4, 6, 8, and 10 hours with alkali solution refreshed every 2 hours (SPC6, SPC7, SPC8, SSC9 and SPC10) were in a range of 70.33–84.20%. As shown in Figure 1, and Figure 2 the % yield of chitosan samples showed no significant difference among all treatments studied.

![Figure 1. %yield of α-chitosan derived from deacetylation of α-chitin for 2, 4, 6, 8, and 10 hours without alkali solution refreshed (SSC1, SSC2, SSC3, SSC4, and SSC5), and with alkali solution refreshed every 2 hours (SSC6, SSC7, SSC8, SSC9 and SSC10).]
Figure 2. %yield of β-chitosan derived from deacetylation of β-chitin for 2, 4, 6, 8, and 10 hours without alkali solution refreshed (SPC1, SPC2, SPC3, SPC4, and SPC5), and with alkali solution refreshed every 2 hours (SPC7, SPC8, SPC9 and SPC10).

3.2. Molecular weight
The MW of α-chitosan and β-chitosan products are illustrated in Figure 3, and Figure 4 respectively. As shown in Figure 3, the MW of α-chitosan derived from deacetylation of α-chitin for 2, 4, 6, 8, and 10 hours without alkali solution refreshed (SSC1, SSC2, SSC3, SSC4, and SSC5) were in a range of 342.98-1140.51 kDa. The MW of α-chitosan derived from deacetylation of α-chitin for 2, 4, 6, 8, and 10 hours with alkali solution refreshed every 2 hours (SSC6, SSC7, SSC8, SSC9 and SSC10) were in a range of 456.81-1603.48 kDa similar to the previous reports [1,6]. As shown in Figure 4, the MW of β-chitosan derived from deacetylation of α-chitin for 2, 4, 6, 8, and 10 hours without alkali solution refreshed (SPC1, SPC2, SPC3, SPC4, and SPC5) were in a range of 150.17-908.18 kDa. The MW of β-chitosan derived from deacetylation of β-chitin for 2, 4, 6, 8, and 10 hours with alkali solution refreshed every 2 hours (SPC6, SPC7, SPC8, SPC9 and SPC10) were in a range of 127.11-660.34 kDa similar to the previous reports [1,6]. As shown in Figure 3, and Figure 4, the longer deacetylation time length, the shorter the MW of chitosan samples obtained.

Figure 3. Molecular weight of α-chitosan derived from deacetylation of α-chitin for 2, 4, 6, 8, and 10 hours without alkali solution refreshed (SSC1, SSC2, SSC3, SSC4, and SSC5), and with alkali solution refreshed every 2 hours (SSC6, SSC7, SSC8, SSC9 and SSC10).
3.3. Deacetylation degree of chitosan

The %DD of α-chitosan is illustrated in Figure 5. As shown in Figure 5, the % DD of α-chitosan derived from deacetylation of α-chitin for 2, 4, 6, 8, and 10 hours without alkali solution refreshed (SSC1, SSC2, SSC3, SSC4, and SSC5) were in a range of 85.36–90.39%. The % DD of α-chitosan derived from deacetylation of α-chitin for 2, 4, 6, 8, and 10 hours with alkali solution refreshed every 2 hours (SSC6, SSC7, SSC8, SSC9 and SSC10) were in a range of 85.46–91.39% similar to the previous reports [1,6]. As shown in Figure 5, the longer deacetylation time length, the higher the % DD of chitosan samples obtained.

4. Conclusions

It could be concluded that the deacetylation time length and alkali treatments showed similar effects on physicochemical characteristics of chitosan products either prepared from shrimp shell or prepared from squid pen. This study suggested that the longer deacetylation time length, the shorter the MW, and higher

![Figure 4](Image)

**Figure 4.** Molecular weight of β-chitosan derived from deacetylation of β-chitin for 2, 4, 6, 8, and 10 hours without alkali solution refreshed (SPC1, SPC2, SPC3, SPC4, and SPC5), and with alkali solution refreshed every 2 hours (SPC6, SPC7, SPC8, SPC9 and SPC10).

![Figure 5](Image)

**Figure 5.** %Deacetylation degree of α-chitosan derived from deacetylation of α-chitin for 2, 4, 6, 8, and 10 hours without alkali solution refreshed (SSC1, SSC2, SSC3, SSC4, and SSC5), and with alkali solution refreshed every 2 hours (SSC6, SSC7, SSC8, SSC9 and SSC10).
% DD of chitosan samples obtained and % DD was significantly negative correlated with the MW of chitosan products.

5. References

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