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Synthesis of Water-Soluble Chitosan From Squid Pens Waste for Capsule Shell Materials

Malinda Syifa Yusharani, Stenley, Harmami, Ita Ulfin, Suprapto Suprapto and Yatim Lailun Ni'mah*

Department of Chemistry, Faculty of Sciences, Institut Teknologi Sepuluh Nopember, 60111, Indonesia.
*Corresponding Author: Yatim Lailun Ni'mah. Email: yatimnikmah@gmail.com.

Abstract: Water-Soluble Chitosan (WSC) has been sucessfuly synthesized from squid pens waste. The synthesis of chitosan from chitin was carried out by optimization of deacetylation temperature and time. Chitin was obtained from squid pens waste by demineralization and deproteinization process. HCl 7% was used for demineralization and NaOH 10% at 60°C was applied for deproteinization process. Deacetylation reaction was carried out at varied temperatures i.e., 60°C, 70°C, 80°C, 90°C and 100°C in NaOH 50% solution for 10 hours. Deacetylation reaction time were varied for 2 hours, 4 hours, 6 hours, 8 hours, and 10 hours. The crude chitosan obtained then reacted with H_2O_2 30% to depolymerize. The synthesis product obtained then characterized by FTIR. The result of squid chitin yield was 33.9%. The optimum temperature and time of chitosan deacetylation process were 90°C for 8 hours as indicated by the value of deacetylation degree (DD) that equal to 83.94% at optimum temperature and 82.22% at optimum reaction time. The percentage of WSC yield at optimum temperature (90°C) and optimum time (8 hours) were 27.59% and 23.16%, respectively. WSC solubility test was done in water and HCl 0,1N. The solubility of 2.8325 mg/mL and 0.8125 mg/mL were obtained in acid medium and water medium, respectively.

Keywords: Chitosan; deacetylation; squid pens; temperature; time; water-soluble chitosan

1 Introduction

The capsule shell was a solid shell that has functions to wrap the drug because of its bitter taste and unpleasant smell [1]. The capsule shell was commonly made from gelatin as base material because its elasticity, easy to mold, odorless and durability properties [2]. Gelatin for capsule shell usually produced from animal gelatin, such as from cows or pigs gelatin. Capsule shells made from pork gelatin was cheaper than capsule shells from cow gelatin. This was one of the reasons why many drug manufacturers prefer pig gelatin shell capsules than cow gelatin [1]. Because of religious reason, muslim refuse to consume capsule shell that produced from pig gelatin, while some people worried about mad cow disease contamination from capsule shell produced from cow gelatin. Various studies have been carried out to find alternatives for pork and cattle gelatin i.e., fish gelatin [3], hydroxy propyl methyl cellulose [4], starch [5], alginate [6], and chitosan [7].

Chitosan was a biopolymer that composed of poly (2-deoxy-2-acetylamine-2-glucose) and poly (2-deoxy-2-aminoglucose) which form bonds (1,4) β-glycosidic. This compound was a derivative of chitin which has partial or total N-deacetylation in alkaline conditions [9]. Chitin was the second largest biopolymer after cellulose [10] and can be obtained in marine animal waste types of crustaceans and mollusks [11] e.g., shrimp shells, crab shells, and squid pens. Chitosan has biodegradable properties that are often used in the pharmaceutical and biomedical industries [12]. Several previous studies that have used chitosan and its derivatives as the basic ingredients of capsule shell are shrimp chitosan [13], chitosan/polyacrylate acid [14], chitosan / hyaluronic acid [15] and chitosan/sodium cellulose sulfate [16].

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Chandumpai's (2004) [17] has compared chitosan content between squid pens and shrimp shells, where the percentage of chitosan produced from squid pen powder was about 25-30% of its dry weight, while chitosan produced from shrimp shell powder was at about 15-20% of its dry weight [18]. The research by Rahayu [19] found that chitosan produced from crab shell powder was about 20-30% of its dry weight. Squid pen has a high yield of chitosan so that it will be used as a source of chitosan in this study.

Chitosan was insoluble water, but soluble in acetic acid. Its low solubility in water made the application of chitosan limited. One factor to increase chitosan solubility was by increasing deacetylation degree. The degree of deacetylation was the value of acetyl groups removal in chitin acetamide groups. The chemical properties of chitosan were influenced by the presence of amine functional groups, primary hydroxy groups and secondary hydroxy. This functional group causes chitosan to become more reactive than chitin so that the removal of acetyl groups will made chitosan become more reactive and increase its solubility in water [20].

The degree of deacetylation was determined by several factors, i.e. the concentration of strong bases, temperature and the duration of the deacetylation process [21]. Many studies have been carried out to optimize the deacetylation process. Deacetylation temperature of 60°C for 8 hours [21], a temperature of 80°C for 2 hours with ultrasonic rays [22], a temperature of 80°C with N_2 atmospher [23] and a temperature of 120°C for 4 hours [24] have been studied. In this study, the optimization of temperature and deacetylation time was done to get the chitosan product with optimum deacetylation degree.

In addition to the degree of deacetylation, converting chitosan to water-soluble chitosan (WSC) through the degradation process will also increase the solubility of chitosan. Chitosan that has a high molecular weight has low solubility compared to chitosan with low molecular weight. So, it was an important study to obtain chitosan with lower molecular weight without changing its chemical structure [25].

2 Experimental

2.1 Materials

The starting materials squid pens waste (collected from local market in Surabaya, Indonesia), aqua demineralization, HCl (Merck, 37%), NaOH (Merck, 99%), absolute ethanol (Merck, 100%), ethanol (SAP chemicals, 96%), glacial acetic acid (Merck, 100%), and H_2O_2 (SAP chemicals).

2.2 Methods

2.2.1 Purification of Chitin

Squid pens waste was cleaned and dried for 5 hours at 60°C then grounded. Squid pens powders (50 g) was soaked in HCl 7% at room temperature for 24 hours then the residue was filtered and neutralized. Afterward, the residue that obtained was saturated in NaOH 10% for 24 hours at 60°C. The residue was washed with ethanol 96% and dried at 50°C for 8 hours [22]. Squid pens chitin that obtained was characterized by Fourier-Transform Infrared (FTIR) spectrophotometer (Shimadzu FTIR-8400S). Yield percentages of chitin were calculated using Eq. (1):

$$\% \text{ Chitin} = \frac{\text{weight of chitin}}{\text{weight of squid pens powders}} \text{ x } 100\% \quad (1)$$

2.2.2 Synthesis Chitosan and Water-Soluble Chitosan (WSC)

Chitosan was synthesized by deacetylation of chitin by temperature and reaction time optimization. Chitin was soaked in NaOH 50% (1:10) for 10 hours at 60°C, 70°C, 80°C, 90°C and 100°C. Reaction time was optimized for 2 hours, 4 hours, 6 hours, 8 hours, and 10 hours at the optimum temperature. The residue was filtered and neutralized with hot aqua demineralization then dried. Chitosan obtained by temperature variations was labeled as C60, C70, C80, C90, and C100. However chitosan with time variations labeled as C2, C4, C6, C8, and C10.

Chitosan was dissolved in acetic acid 2% then H_2O_2 30% was added and reacted for 4 hours. After reaction, NaOH 10% was used to neutralize the solution. The residue was removed by filtration, while twofold volumes of absolute ethanol were added to the filtrate. The crystal of water-soluble chitosan was produced after incubation at ambient condition overnight then dried in 50°C. The yield percentages of chitosan and water-soluble chitosan were calculated using Eqs. (2) and (3).

$$\% \text{ Chitosan} = \frac{\text{weight of chitosan}}{\text{weight of sample}} \times \% \text{ chitin} \quad (2)$$

$$\% \text{ WSC} = \frac{\text{weight of WSC}}{\text{weight of sample}} \times \% \text{ chitosan} \quad (3)$$

2.2.3 The degree of Deacetylation (DD)

The deacetylation degree of squid pens chitosan was calculated by baseline method from chitosan FTIR spectra. The sample preparation for FTIR measurements was carried out by adding sample to KBr pellets at ratio of 1:9 (sample:KBr). The mixture then pressed to form thin film disc. The disc then measured by FTIR spectrophotometer and FTIR at wavenumbers between 400-4000 cm^{-1}. Deacetylation degree was calculated by baseline methods from chitin, chitosan and water soluble-chitosan FTIR spectra.

Degree of deacetylation was calculated using Eq. (4). A_{1655} was the absorbance at 1655 cm^{-1} from amide band of N-acetyl group and A_{3450} was peak of hydroxyl band. The factor '1,33' denotes the ratio of A_{1655}/A_{3450} for fully N-acetylated chitosan [27].

$$\text{DD} = 100 - \left[\left(\frac{A_{1655}}{A_{3450}}\right) x \ \frac{100}{1{,}33}\right] \quad (4)$$

2.2.4 Solubility Test

The solubility test of chitosan and WSC was carried out in neutral (water) and acid (HCl 0,1N) medium at 40°C [28]. Chitosan and water-soluble chitosan of 0,1 g each were dissolved in 50 mL solution and stirred for 4 h. Afterward, the residue was filtered and 40 ml of filtrate that obtained was dried at 50°C. The solubility of chitosan and water-soluble chitosan was determined by Eq. (5):

$$\text{Solubility (mg/mL)} = \frac{\text{weight of dissolved WSC (mg)}}{\text{40 mL}} \quad (5)$$

3 Result and Discussions

3.1 Chitin Squid Pens and Optimization of Chitosan

In this research, chitin and chitosan synthesis based on research method by Du [22] with some modification. Squid pens chitin was made by demineralization, deproteination and depigmentation reaction. Demineralization was carried out using HCl 7% at room temperature to remove minerals contains such as calcium carbonate ($CaCO_3$). Foam (CO_2) was observed when acid was added, this process was described in Eq. (6) [29]:

$$2\ HCl + CaCO_3 \rightarrow CaCl_2 + H_2O + CO_2 \quad (6)$$

The residue obtained then neutralized to remove acids that may be entangled and diffused in crystal lattice or in association with free amino acids and protein residues [30]. Afterward, deproteination process was carried out using NaOH 10% to remove protein in squid pens. In this process, high temperature was applied to break the bonds and to form Na-Proteins where the Na^+ ions were attached to the point re-formed of protein [31]. Precipitation was observed and the color become yellowish. Deproteination reaction was expalined in Fig. 1. The residue obtained was washed using ethanol to remove impurities then dried. The average percentage of chitin that obtained from squid pens in three replication measurement was 33.90% that is in accordance to the results of previous research [32]. The result was shown in Tab. 1.

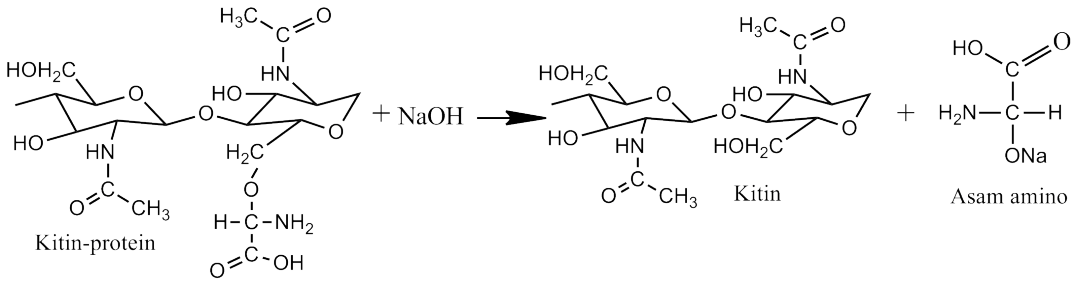


Figure 1: Deproteination reaction by NaOH [30]

Table 1: Percentage chitin yield squid pens

Variation	Mass of squid pens (g)	Mass of chitin (g)	Rendement chitin (%)
A	50.02	16.34	32.67
B	50.00	17.69	35.38
C	50.03	16.84	33.66
		Average	33.90

Chitosan has been successfully synthesized by optimization of temperature and reaction time during deacetylation reaction. The deacetylation reaction was conducted using strong alkali solution to convert acetyl from acetamide group in chitin into an amine group (partial/total) that called chitosan . Strong alkali solution break the bond between carbon and nitrogen to form amine group ($-NH_2$). Deacetylation reaction was shown on Fig. 2. In this research, the temperature for reaction time optimization was 10 hours. The optimum deacetylation temperature and reaction time was determined from degree of deacetylation based on the FTIR spectra.

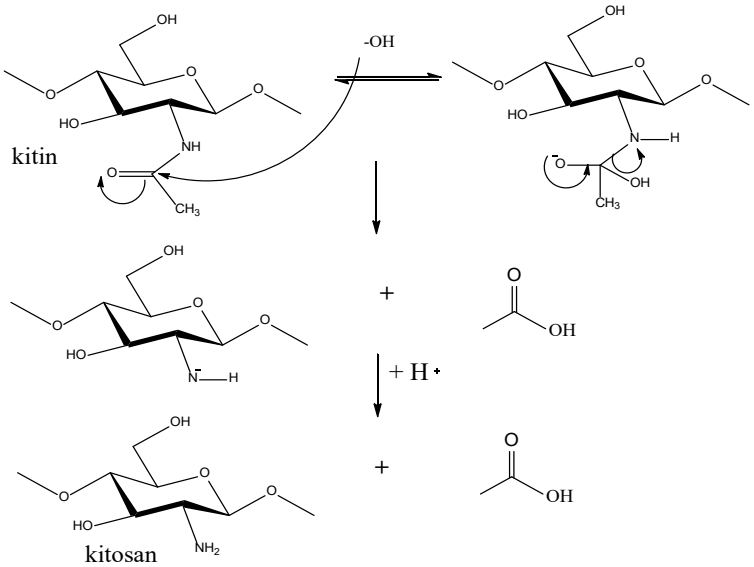


Figure 2: Deacetylation chitin to chitosan [32]

Table 2: Percentage of chitosan yield temperature and time variations

Variation	Mass of chitosan (g)	Rendement chitosan (%)	Variation	Mass of chitosan (g)	Rendement chitosan (%)
C60	7.73	26.15	C2	7.40	25.09
C70	7.84	26.55	C4	7.94	26.92
C80	8.00	27.12	C6	7.95	26.95
C90	8.40	28.45	C8	8.16	27.66
C100	7.14	24.20	C10	7.34	24.98

The percent yield of chitosan deacetylation at varied temperature and reaction time was shown in Tab. 2. The highest yield of chitosan deacetylation for temperature optimization was 28.45% obtained at 90°C. The optimum reaction time was 27.66% that obtained for 8 hours reaction. This result was in accordance with other research conclusion that deacetylation reaction was affected by several factors such as temperature and reaction time [24]. Chitosan that obtained was pale to tanned.

3.2 Water-Soluble Chitosan (WSC)

Water-soluble chitosan has been successfully synthesized in this research [22]. WSC was obtained by partial decomposition of chitosan polymer chain by H_2O_2, that called depolymerization. WSC from squid pens was hygroscopic yellowish powders. Depolymerization reaction of chitosan with H_2O_2, was shown in Eqs. (7) and (8), and the total reaction was shown in Eq. (9).

$$R–NH_2 + H^+ = R–NH_3^+ \quad (7)$$

$$H_2O_2 = H^+ + HOO^- \quad (8)$$

$$H_2O_2 + R–NH_2 + H^+ = R–NH_3^+ + HOO^- + H^+ \quad (9)$$

$$HOO^- \rightarrow OH^- + O^{\bullet} \quad (10)$$

$$H_2O_2 + HOO^- \rightarrow HO\bullet + O_2\bullet^- + H_2O \quad (11)$$

The hydroperoxide anion was very unstable and easily decomposed into high reactive hydroxyl radical ($HO^{\bullet}$). The hydroxyl radical was a powerful oxidant. The main action of $HO^{\bullet}$ was break down the glycosidic bond on chitosan polymers [34]. The percent yield of water-soluble chitosan obtained was shown in Tab. 2. The highest yield of WSC from temperature variations was 27,59% obtained at 90°C. The optimum WSC from reaction time variations was 26,71% obtained from deacetylation reaction for 10 hours. Water-soluble chitosan from squid pens was tanned when dried.

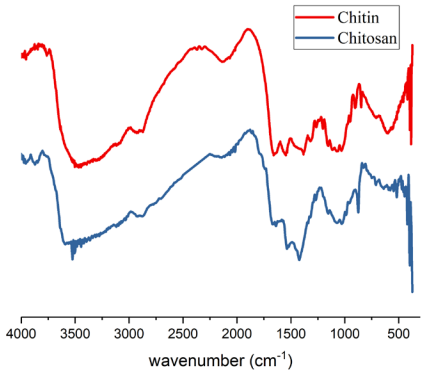
Table 3: Percentage of water-soluble chitosan yield

Variation	Mass of WSC (g)	Rendement WSC (%)	Variation	Mass of WSC (g)	Rendement WSC (%)
C60	1.78	23.28	C2	0.87	11.45
C70	1.87	24.83	C4	1.48	19.48
C80	1.66	22.51	C6	1.55	20.40
C90	1.94	27.59	C8	1.76	23.16
C100	1.22	14.76	C10	2.03	26.71

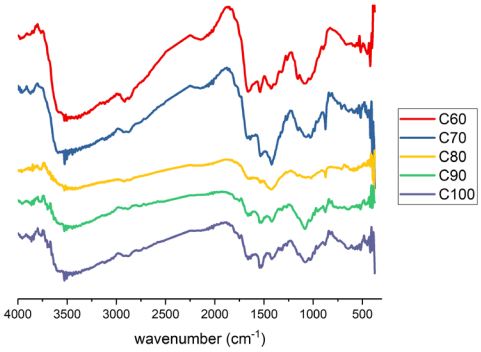
3.3 FTIR Characterization

The FTIR spectra of chitin and chitosan was shown in Fig. 3. There were peaks at 3466,20 cm^{-1} (O-H streching vibration); 2916,47 cm^{-1} (C-H sp^3 vibration); 1649,19 cm^{-1} (C=O amide); 1546,96 cm^{-1} (-NH

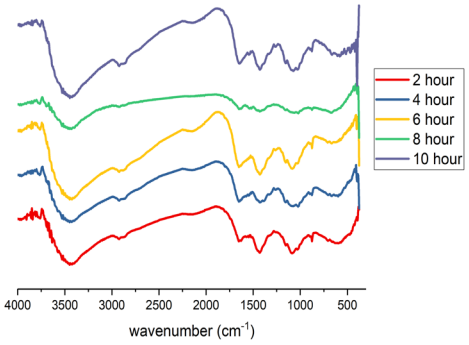
amide bending vibration); 1383,01 cm^{-1} (C-N amide streching vibration); 1030,02 cm^{-1} (C-O-C streching vibration) and 848,71 cm^{-1} (glicosidic bond β-1,4) for chitin FTIR spectra. In FTIR spectra of chitosan, there were peaks at 3525,99 cm^{-1} (O-H overlap with N–H streching vibration); 2877,89 cm^{-1} (C-H sp^3 vibration); 1637,62 cm^{-1} (C=O amide); 1537,32 cm^{-1} (-NH_2 amine); 1269,20 cm^{-1} (C-N amine streching vibration); and 1153,47 cm^{-1} (-C-O-C streching vibration) [34].



(a)



(b)



(c)

Figure 3: (a) FTIR chitin and chitosan at varied (b) temperature (c) time variations

The FTIR spectra of water-soluble chitosan was shown in Fig. 4 and has identical characteristic with that from literature [33]. In the spectra there were peaks at 3446,91 (O-H overlap with N-H stretching vibration); 1649,19 cm^{-1} (C=O amide); 1550,82 cm^{-1} (-NH_2 amine); 1074,39 cm^{-1} (O-H stretching vibration); 1024,24 cm^{-1} (-C-O-C stretching vibration) and 927,24 cm^{-1} (glycosidic bond β-1,4). WSC was the break down result of chitosan polymer chain so the spectra was not significantly different from that of chitosan. FTIR spectrum of WSC and chitosan at varied temperature and reaction time were shown in Fig. 5.

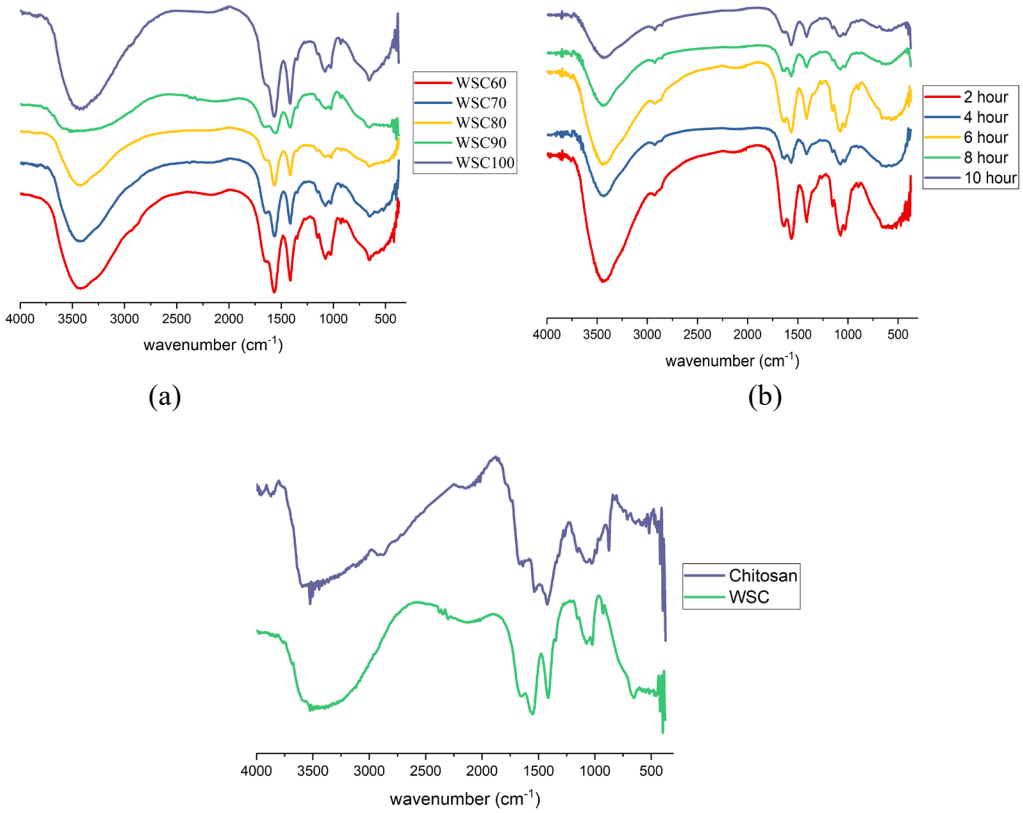


Figure 4: (a) FTIR spectra of WSC (C60-C100); (b) WSC (C2-C10); (c) chitosan and WSC

3.4 Degree of Deacetylation

Chitosan was synthesized by deacetylation reaction of chitin. During this process, the acetyl group convert the acetamide group into amine group. The percentage of acetyl groups removal during the deacetylation process was referred as degree of deacetylation. Degree of deacetylation was determined by baseline method on chitosan FTIR spectra [26]. The degree of deacetylation was calculated from the absorbance of amides in acetyl groups and hydroxyl groups at wavelength of 1655 cm^{-1} and 3450 cm^{-1}. Absorbance representation of C90 sample was shown in Fig. 5. The equation for the calculation were shown in Eqs. (12) and (13), and equation of total deacetylation degree was shown in Eq. (4). The DD calculations result of chitosan and WSC samples were shown in Tab. 4.

$$(A_{1655}) \text{ amide} = \log\left(\frac{DF}{DE}\right) \quad (12)$$

$$(A_{3450}) \text{ hydroxyl} = \log\left(\frac{AC}{AB}\right) \quad (13)$$

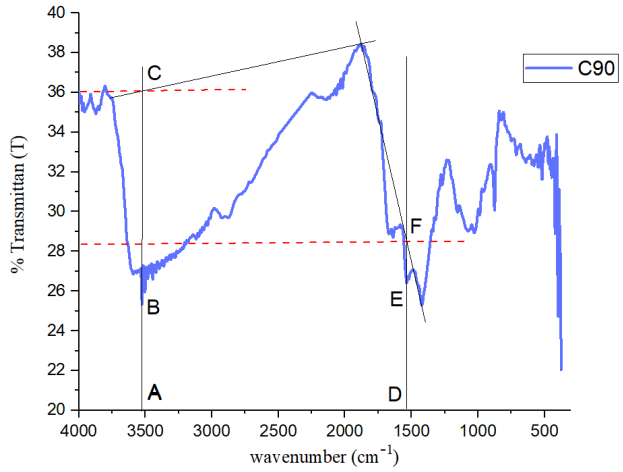


Figure 5: Baseline method for degree deacetylation

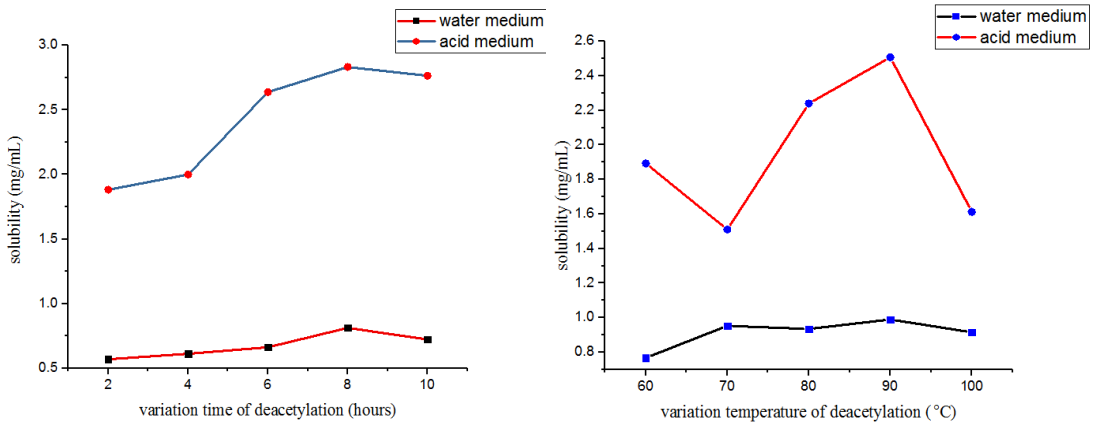
Table 4: Degree of deacetylation (DD) of chitosan and water-soluble chitosan (WSC)

Variations	DD (%)	Variations	DD (%)
C60	63.67	C2	66.56
C70	72.39	C4	70.34
C80	74.06	C6	72.34
C90	83.94	C8	82.22
C100	62.38	C10	71.49
Variations	**DD (%)**	**Variations**	**DD (%)**
WSC60	69.27	WSC2	71.32
WSC70	71.21	WSC4	74.31
WSC80	73.42	WSC6	71.34
WSC90	73.67	WSC8	75.14
WSC100	68.05	WSC10	74.15

The highest degree of deacetylation was 83.94% (C90) and 82.22% (C8). Futhermore, the highest degree of deacetylation of 73.67% and 75.14 was obtained from WSC90 and WSC8. The results show that the acetyl groups removal was about 82.22% from total acetyl groups on chitin. The higher degree of deacetylation, the better quality of chitosan. In this research, all chitosan that have been synthesized have met the standard of chitosan (DD > 60%).

3.5 Solubility

Solubility test was carried out to determine the effect of deacetylation time, deacetylation temperature, and depolymerization reaction to the obtained product solubility. The solubility test was carried out in water and acid (0.1 M HCl) at 40°C. The solubility value was calculated using Eq. (5). The solubility at varied deacetylation time and temperature was shown in Fig. 6.



(a) (b)

Figure 6: The solubility product at varied (a) deacetylation time (b) deacetylation temperature

Based on Fig. 5, the solubility of WSC on acid and water medium in accordance with the degree of deacetylation. Solubility at varied deacetylation time increased from 2 hours to 8 hours and decreased for 10 hours reaction. The highest solubility was obtained by 8 hours deacetylation time with the solubility value of 2.8325 mg/mL in acidic media and 0.8125 mg/mL in neutral media. Roughly, solubility values at varied deacetylation temperature increased from product that treated at 60°C to 90°C and decreased when treated at 100°C. The highest solubility was obtained by deacetylation at temperature of 90°C with the solubility value 2.5075 mg/mL in acidic media and 0.9900 mg/mL in neutral media. Based on the result, the degree of deacetylation affects the solubility of chitosan. The increase in deacetylation degree will increase the solubility of chitosan. This was in accordance with previous study [19].

Solubility test was also conducted to compare chitosan and WSC on acidic media and neutral media. Chitosan and WSC were used from the optimum product to see the difference in their solubility values. The solubility of optimum chitosan and WSC product was shown in Tab. 5.

Table 5: Comparison of chitosan and WSC solubility

Sample	Solubility (mg/mL)	Sample	Solubility (mg/mL)
C90 Acid medium	3.2350	C8 Acid medium	3.0308
WSC90 Acid medium	2.5075	WSC8 Acid medium	2.8325
C90 Water medium	0.1300	C8 Water medium	0.1667
WSC90 Water medium	0.9900	WSC8 Water medium	0.8125

The solubility of chitosan in acidic media was higher than WSC, but the different was not significant. This was because chitosan was a compound that dissolves in low pH. WSC solubility on neutral media was 5-7 times higher than chitosan. This was because WSC had a lower molecular weight than chitosan so that the solubility become higher. The result proved that the depolymerization reaction produce chitosan that has higher solubility than chitosan without depolymerization reaction.

4 Conclusion

Water-soluble chitosan from squid pens waste has been successfully synthesized by optimization of deacetylation temperature and time. The chitosan optimum deacetylation temperature was 90°C with 83.94% DD. The optimum reaction time was 8 hours with 82.22% DD. The WSC yield percentage at optimum temperature (90°C) was 27.59% and 23,16% at optimum reaction time. The solubility of WSC at optimum temperature was 2.5075 mg/mL in acid medium and 0.9900 mg/mL in water medium. Furthermore, WSC that produced at optimum reaction time has solubility of 2.8325 mg/mL and 0.8125 mg/mL in acid and water medium, respectively.

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