Synthesis of water-soluble chitosan from squid pens waste as raw material for capsule shell: temperature deacetylation and reaction time

Malinda Syifa Yusharani\textsuperscript{1}, Stenley\textsuperscript{1}, Harmami\textsuperscript{1}, Ita Ulfin\textsuperscript{1}, Yatim Lailun Ni’mah\textsuperscript{1}\textsuperscript{*}

\textsuperscript{1} Department of Chemistry, Faculty of Sciences, Institute Technology Sepuluh Nopember Surabaya 60111, Indonesia
\textsuperscript{*} Corresponding author: yatimnikmah@gmail.com

Abstract. Water-Soluble Chitosan (WSC) was synthesized from squid pens waste by optimization temperature and time of deacetylation process from chitin to become chitosan. Chitin isolated from squid pens waste had been recovered using demineralization and deproteinization methods. HCl 7\% was used for demineralization process and NaOH 10\% at 60\°C was used for deproteinization process. Deacetylation process has been made by NaOH 50\% solution for 10 hours on temperature variation 60, 70, 80, 90 and 100\°C. Afterwards for time variation on 2, 4, 6, 8 and 10 hour using the optimized temperature. WSC has been synthesized by shortening polymer chain of chitosan using $\text{H}_2\text{O}_2$ 30\%. The synthesis result was then characterized by FTIR. The result of squid chitin yield showed at 33.9\%. The optimum temperature and time of chitosan deacetylation occurred at 90\°C for 8 hours as indicated by the value degree of deacetylation (DD) in equal to 83.94\% and 82.22\%. The percentage of WSC yield at an optimized temperature (90\°C) was 27.59\% and at optimized time (8 hours) was 23.16\%. WSC solubility test has been done in water and HCl 0.1 N. In addition, optimum chitosan products were insoluble on water and HCl 0.1 N.

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

1. Introduction

Currently the pharmaceutical industry was having many problems, one of them was the capsule shell. The capsule shell was a solid shell that functions to wrap the drugs because it had an unpleasant taste like bitter and smelly [1]. The capsule shell was generally made from gelatine as the base material because it had a flexible, easy to form, odourless and long lasting properties [2]. The animals most commonly used as sources of gelatine were cows and pigs. Capsule shells made from pork gelatine have a much cheaper selling price than capsule shells from cow gelatine. This difference in the selling price of raw material for gelatine was the reason why many drug manufacturers prefer using shells of pig gelatine capsules rather than shells of cow gelatine capsules [1]. The weakness of the capsule shell from pig gelatine was that some people cannot consume it because of the belief that was held, while the capsule shell that comes from cow gelatine made people worried about the issue of mad cow disease. Various studies have been carried out to find alternatives for pork and cattle gelatine including fish gelatine [3], hydroxy propyl methyl cellulose [4], starch [5], alginate [6], and chitosan [7].

Chitosan was a biopolymer that composed of poly (2-deoxy-2-acetylamino-2-glucose) and poly (2-deoxy-2-aminoalcohol) which form bonds (1,4) β-glycosidic. This compound was a derivative of chitin which has partial or total N-deacetylation in alkaline conditions [8]. Chitin was the second largest
biopolymer after cellulose [9] and can be obtained in marine animal waste types of crustaceans and mollusks [10] e.g. shrimp shells, crab shells, and squid pens. Chitosan has biodegradable properties that are often used in the pharmaceutical and biomedical industries [11]. Several previous studies that have used chitosan and its derivatives as the basic ingredients of capsule shell are shrimp chitosan [12], chitosan/polyacrylate acid [13], chitosan/hyaluronic acid [14] and chitosan/sodium cellulose sulphate [15].

Based on Chandumpai et al. [16], chitosan from squid pens and shrimp shells have been compared. Chitosan from squid pen powders has been more weight than shrimp shell, which is about 25-30% from dry weight and 15-20% for shrimp shell [17]. The results from Rahayu and Purnavita [18] 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 for this study.

Chitosan was insoluble in water, but soluble in acetic acid. Low solubility in water made limited use of chitosan. One factor to increase solubility was by increasing the deacetylation degree. The degree of deacetylation was the value of the removal of acetyl groups in chitin acetamide groups. The properties of chitosan were related to 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 make chitosan become more reactive and increase solubility in water [19].

The degree of deacetylation was influenced by several factors, such as the concentration of strong bases, temperature and the duration of the deacetylation process [20]. Some method on several research used different temperature settings on deacetylation reaction. For example, Du et al. [21] used 60°C for 8 hours, Huang et al. [22] used 80°C for 2 hours with ultrasonic rays, Tolaimate et al. [23] used 80°C with conditions on N₂ gas and Abdelmalek et al. [24] used 120°C for 4 hour. In this study optimization of temperature and deacetylation time was done to get chitosan 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. For chitosan that has a high molecular weight will have lower solubility compared to chitosan which has a low molecular weight, so we will try to make chitosan with a lower molecular weight without changing the chemical structure [25].

2. Experimental

2.1. Materials

The starting materials squid pens waste (was collected from Icha Deles stall Surabaya and squid rice stall in Atom market Surabaya), aqua demineralization, HCl (Merck, 37%), NaOH (Merck, 99%), absolute ethanol (Merck, 100%), ethanol (SAP chemicals, 96%), glacial acetic acid (Merck, 100%), and H₂O₂ (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 h. The residue was filtered and neutralized. Afterward, the residue was saturated in NaOH 10% for 24 h at 60°C. The residue was washed with 96% ethanol and dried at 50°C for 8 hours [21]. Squid pens chitin was characterized by Fourier-Transform Infrared (FTIR) spectrophotometer (Shimadzu FTIR-8400S). Yield percentages of chitin were calculated using equations (1):

\[
\% \text{Chitin} = \frac{\text{weight of chitin}}{\text{weight of squid pens powders}} \times 100\%
\]  

(1)

2.2.2. Synthesis Chitosan and Water-Soluble Chitosan (WSC). In this research chitosan was synthesized using temperature and time optimization reactions deacetylation. Chitin was soaked in 50% NaOH (1:10) for 10 h at 60, 70, 80, 90 and 100°C temperature variations and for time optimization we used
variations 2, 4, 6, 8, and 10 h at the optimum temperature on the previous process. The residue was filtered and neutralized with hot aqua demineralization then dried. Chitosan with temperature variations labelled as C60, C70, C80, C90, and C100. However, chitosan with time variations labelled as C2, C4, C6, C8, and C10.

Chitosan was dissolved in acetic acid 2% then added H₂O₂ 30% and reacted for 4 h. During this reaction, the viscosity of solution decreased which indicates water-soluble chitosan has been formed. After that, NaOH 10% was used to adjust the solution into neutral. The residue was removed by filtration, while twofold volumes of absolute ethanol were added to the filtrate. After that, the mixture was incubated at ambient condition until formed white gel then dried in 50°C. The yield percentages of chitosan and water-soluble chitosan were calculated using equation (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 degree of deacetylation of squid pens chitosan was measured by baseline method spectra FTIR of chitosan. Value of degree deacetylation was calculated using equations (4). \(A_{1655}\) was the absorbance at 1655 cm\(^{-1}\) of the amide band as a measure of the N-acetyl group content and \(A_{3450}\) was of the hydroxyl band. The factor ‘1.33’ denotes the value of the ratio of \(A_{1655}/A_{3450}\) for fully N-acetylated chitosan [26].

\[
\text{DD} = 100 \times \left[ \frac{A_{1655}}{A_{3450}} \right] x \frac{100}{1.33} \quad (4)
\]

2.2.4. Solubility Test
The solubility test of chitosan and WSC was done in neutral (water; pH=7) and acid (HCl 0.1 N; pH=1) medium at 40°C [27]. Chitosan and water-soluble chitosan was weighed 0.1 g, respectively. Then, dissolved in 50 mL solution while stirring for 4 h. Afterward, the residue was filtered and 40 mL filtrate was dried at 50°C. After that weighed the mass of dried filtrate. The solubility of chitosan and water-soluble chitosan was determined by equation (5).

\[
\text{Solubility (mg/mL)} = \frac{\text{weight of WSC (mg)}}{40 \text{ mL}} \times 100\% \quad (5)
\]

3. Result and Discussions
3.1. Chitin Squid Pens and Optimization of chitosan
In this research, chitin and chitosan synthesis was based on research method [21] which has been modified in the deacetylation process. Squid pens chitin has been purified by demineralization, deproteination and depigmentation reaction. Demineralization used HCl 7% solution at room temperature to remove minerals contained calcium carbonate (CaCO\(_3\)). Foam (CO\(_2\)) appeared when the solution added, this process was shown by equation (6) [28].

\[
2 \text{HCl} + \text{CaCO}_3 \rightarrow \text{CaCl}_2 + \text{H}_2\text{O} + \text{CO}_2 \quad (6)
\]

The residue was neutralized because acids (HCl) can be entangled and diffused in crystal [29]. Afterward, deproteination process using NaOH 10% solution to remove protein content in squid pens. In this process we used high temperature to break off the bonds between protein and chitin because they bounded by covalent bonds which requires a large amount of energy to break off the bond. Ion Na\(^+\) will attached to amino acid and re-formed of Na-protein [30] and can be precipitated become yellow residues. Deproteination reaction was shown on Fig. 1. The residue washed by ethanol to remove impurities then
dried. The average percentage of chitin from squid pens from three replication was 33.90\% in was in accordance with the results of previous research [31]. The result of chitin production shown in table 1.

![Figure 1. Deproteination reaction by NaOH [29].](image1)

**Table 1. Percentage chitin yield squid pens.**

| Replication | mass of squid pens (g) | mass of chitin (g) | Chitin yield(\%) |
|-------------|------------------------|--------------------|------------------|
| 1           | 50.02                  | 16.34              | 32.67            |
| 2           | 50.00                  | 17.69              | 35.38            |
| 3           | 50.03                  | 16.84              | 33.66            |
| Average     |                        |                    | 33.90            |

Chitosan has been made by deacetylation reaction which optimization of temperature and time reaction. The deacetylation reaction removed an acetyl group from an acetamide group in chitin into an amine group (partial/total) by strong alkali solution. Strong alkali solution would break off the bond between carbon and nitrogen to form amine group (–NH₂). Deacetylation reaction was shown on Fig. 2. The colour of chitosan synthesis was pale to tanned according to the variations of temperature and time reaction. The higher temperature and time reaction used, the chitosan will more tanned. The temperature and time of optimum deacetylation were observed from degree of deacetylation based on the FTIR spectra.

![Figure 2. Deacetylation chitin to chitosan [32].](image2)
Table 2. Percentage of chitosan yield temperature and time variations.

| variation temperature chitosan | result mass of chitosan (g) | chitosan yield (%) |
|---------------------------------|------------------------------|--------------------|
| C60                             | 7.73                         | 26.15              |
| C70                             | 7.84                         | 26.55              |
| C80                             | 8.00                         | 27.12              |
| C90                             | 8.40                         | 28.45              |
| C100                            | 7.14                         | 24.20              |

| variation reaction time chitosan | result mass of chitosan (g) | chitosan yield (%) |
|----------------------------------|------------------------------|--------------------|
| C2                               | 7.40                         | 25.09              |
| C4                               | 7.94                         | 26.92              |
| C6                               | 7.95                         | 26.95              |
| C8                               | 8.16                         | 27.66              |
| C10                              | 7.34                         | 24.98              |

The percent yield of chitosan from temperature and time optimization deacetylation was shown in table 2. The most yield of chitosan for temperature variations occurred at 90°C was 28.45% and for time variations showed at 8h was 27.66%. This result was appropriate that deacetylation was affected by several factors such as temperature and time reaction [23].

3.2. Water-Soluble Chitosan (WSC)
Water-soluble chitosan has been synthesized by shortening the polymer chain of chitosan by H2O2 called depolymerization. The hydroperoxide anion on H2O2 was very unstable and easily decomposed into high reactive hydroxyl radical (HO•). The hydroxyl radical was a very powerful oxidant. The main action of HO• would break down the glycosidic bond on chitosan polymers [33]. This process would shortening the polymer chain of chitosan and make the molecular weight of chitosan will be decreased. The result WSC from squid pens was yellowish to tanned after dried and it was hygroscopic. The percent yield of water-soluble chitosan was shown in table 3. The highest yield of WSC for temperature variations chitosan happened at 90°C was 27.59% and for time variations chitosan showed at 10h was 26.71%.

Table 3. Percentage of water-soluble chitosan yield.

| variation temperature chitosan | result mass of WSC (g) | WSC yield (%) |
|---------------------------------|-------------------------|---------------|
| C60                             | 1.78                    | 23.28         |
| C70                             | 1.87                    | 24.83         |
| C80                             | 1.66                    | 22.51         |
| C90                             | 1.94                    | 27.59         |
| C100                            | 1.22                    | 14.76         |

| variation reaction time chitosan | result mass of WSC (g) | WSC yield (%) |
|----------------------------------|-------------------------|---------------|
| C2                               | 0.87                    | 11.45         |
| C4                               | 1.48                    | 19.48         |
| C6                               | 1.55                    | 20.40         |
| C8                               | 1.76                    | 23.16         |
| C10                              | 2.03                    | 26.71         |

3.3. FTIR Characterization
The FTIR spectra of chitin, chitosan temperature and time optimization from squid pens were shown in Fig. 3 respectively. Both FTIR spectra of chitin and chitosan have identic characteristic with the literature [34]. In FTIR spectra of chitin, there were peaks at 3466.20 cm⁻¹ (O–H stretching vibration); 2916.47 cm⁻¹ (C–H sp³ vibration); 1649.19 cm⁻¹ (C=O amide); 1546.96 cm⁻¹ (–NH amide bending vibration); 1383.01 cm⁻¹ (C–N amide stretching vibration); 1030.02 cm⁻¹ (C–O–C stretching vibration) and 848.71 cm⁻¹ (glycosidic bond β-1.4). In FTIR spectra of chitosan, there were peaks at 3525.99 cm⁻¹ (O–H overlap with N–H stretching vibration); 2877.89 cm⁻¹ (C–H sp³ vibration); 1637.62 cm⁻¹ (C=O amide); 1537.32 cm⁻¹ (–NH₂ amine); 1269.20 cm⁻¹ (C–N amine stretching vibration); and 1153.47 cm⁻¹ (C–O–C stretching vibration).
Figure 3. (a) FTIR chitin and chitosan; (b) chitosan temperature variations; (c) chitosan time variations.

The FTIR spectra of water-soluble chitosan were shown in Fig. 4 and have an identical characteristic with the literature [33]. In this 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). In C–O–C absorption band (1070-1080 cm$^{-1}$) from chitin and chitosan there was almost different but the absorption decreased in WSC because the depolymerization made the C–O–C bond break off become –OH bond.
3.4. Degree of Deacetylation

Determination degree of deacetylation chitosan was carried out by baseline method on chitosan FTIR spectra [26]. The principle of determining the degree of deacetylation was the absorbance of amides in acetyl groups and hydroxyl groups at wavelength of 1655 cm\(^{-1}\) and 3450 cm\(^{-1}\). Representation absorbance of sample C90 was shown in Fig. 5. The equation of \(A_{1665}\) and \(A_{3450}\) shown in Equation (12) and (13). and total equation of degree deacetylation was shown in equation (4). The results of the DD calculations of each chitosan sample and WSC sample were given in table 4.

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

\[
(A_{3450})\text{ hydroxyl} = \log\left(\frac{AC}{AB}\right) \quad (13)
\]
Table 4. Degree of deacetylation (DD) of chitosan and water-soluble chitosan (WSC).

| Variations temperature chitosan | DD (%) | Variation reaction time chitosan | 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 temperature chitosan | DD (%) | Variation reaction time chitosan | 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 result were 83.94% on chitosan with optimized temperature at 90°C and 82.22 % for chitosan with deacetylation process for 8 hours. Furthermore, for water-soluble chitosan the highest degree of deacetylation were 73.67% and 75.14 for WSC at 90°C variations and WSC at 8 hours’ variations. The result means that the acetyl groups was released 82.22% from total acetyl groups on chitin. The higher degree of deacetylation. the better quality of chitosan. In this research. all chitosan has been synthesized already have met the standard of chitosan (DD >60%). respectively.

3.5. Solubility
In this study. solubility tests were carried out to determine the effect of deacetylation time and deacetylation temperature on water-soluble chitosan. The solubility test process was carried out in water and acid (0.1 M HCl) at 40°C. The solubility value was obtained through equation 5. Comparison graph between solubility with variation of deacetylation time and deacetylation temperature was shown in Fig. 6.

Figure 6. Comparison graph between WSC solubility and variation of (a) deacetylation time. (b) deacetylation temperature.
The solubility of WSC on acid and neutral medium follows the trend of the degree of deacetylation. Solubility values in the variation of deacetylation time increased from a variation of 2 hours to 8 hours and decreased in variation of 10 hours. The highest solubility was in the variation of deacetylation time 8 hours with the solubility value was 2.8325 mg/mL in acidic media and 0.8125 mg/mL in neutral media. Roughly, solubility values in the variation of deacetylation temperature increased from a variation of 60°C to 90°C and decreased in variation of 100°C. The highest solubility was in the variation of deacetylation temperature 90°C with the solubility value is 2.5075 mg/mL in acidic media and 0.9900 mg/mL in neutral media. Based on result, the highest solubility of WSC at the deacetylation time of 8 hours and temperature on 90°C.

Solubility test was also conducted to compare chitosan and WSC on acidic media and neutral media. Chitosan and WSC were used from one variation to see the difference in solubility values. Comparison of chitosan and WSC solubility was shown in table 5.

| 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 results of chitosan solubility in acidic media have a higher value than WSC, but not significantly different. WSC solubility on water medium had a much higher value than chitosan. Based on result, that the depolymerization reaction made chitosan has a higher solubility.

4. Conclusion
Water-soluble chitosan (WSC) from squid pens waste has been synthesized by optimization temperature and time of deacetylation process. The modification of deacetylation process gave the optimized temperature at 90°C with degree of deacetylation 83.94% and optimized reaction time for 8 hours with degree deacetylation 82.22%. The higher degree of deacetylation will decreased the molecular weight of chitosan as a water-soluble chitosan. The percentage of WSC yield for chitosan optimized temperature and time reaction were 27.59% and 23.16%. Solubility test has been done to prove solubility of WSC in acid medium and neutral medium.

Acknowledgments
The financial supports from the Institute for research and community services (LPPM, Lembaga Penelitian and Pengabdian Masyarakat) ITS (1438/PKS/ITS/2018) and the facilities supports from Chemistry Department. Faculty of Natural Sciences. Institut Teknologi Sepuluh Nopember Surabaya (ITS).

References
[1] Gadri A and Priani S E 2012 Stabilitas Kadar dan Laju Disolusi Ketoprofen Dalam Sediaan Kapsul Gelatin dan HPMC-Karagenan Prosiding SNAPP: Sains, Teknologi 3 1 87-94
[2] Ansel H C 2008 Introduction to Pharmaceutical Dosage Forms: Lea & Febiger
[3] Karim A and Bhat R 2009 Fish gelatin: properties, challenges, and prospects as an alternative to
mammalian gelatins *Food Hydrocoll.* 23 3 563-76

[4] Dagadiye R, Kajale A, Mahajan V and Joshi M 2012 Advancement in manufacturing of non-gelatin capsule shell—a review *Int. J. Adv. Pharm. Res* 3 1178-87

[5] Zema L, Loreti G, Melocchi A, Maroni A and Gazzaniga A 2012 Injection molding and its application to drug delivery *J. Control. Release* 159 3 324-31

[6] Pudjiastuti P, Al Rizqi Dharma Fauzi M and Darmokoesoemo H 2017 Drug Delivery Hard Shell Capsules from Seaweed Extracts *J. Chem. Technol. Metall.* 52 6 1140-4

[7] Wu Q-X, Lin D-Q and Yao S-J 2014 Design of chitosan and its water soluble derivatives-based drug carriers with polyelectrolyte complexes *Mar. Drugs* 12 12 6236-53

[8] Shavandi A, Bekhit A A, Bekhit A E-D A, Sun Z and Ali M A 2015 Preparation and characterisation of irradiated crab chitosan and New Zealand Arrow squid pen chitosan *Mater. Chem. Phys.* 167 295-302

[9] Huang J, Cheng Z-H, Xie H-H, Gong J-Y, Lou J, Ge Q, Wang Y-J, Wu Y-F, Liu S-W and Sun P-L 2014 Effect of quaternization degree on physiochemical and biological activities of chitosan from squid pens *Int. J. Biol. Macromol.* 70 545-50

[10] Reys L L, Silva S S, Pircacco R P, Marques A P, Mano J F, Silva T H and Reis R L 2017 Influence of freezing temperature and deacetylation degree on the performance of freeze-dried chitosan scaffolds towards cartilage tissue engineering *Eur. Polym. J.* 95 232-40

[11] Vázquez J A, Noriega D, Ramos P, Valcarcel J, Novoa-Carballal R, Pastrana L, Reis R L and Pérez-Martin R I 2017 Optimization of high purity chitin and chitosan production from Illex argentinus pens by a combination of enzymatic and chemical processes *Carbohydr. Polym.* 174 262-72

[12] Alami R and Permatasari L 2016 Industry Pharmaceuticals: Chitosan as an Alternative Replacement Gelatin Capsules on Shell *J. Med. Bioeng.* 5 1 67-71

[13] Silva C L, Pereira J C, Ramalho A, Pais A A and Sousa J J 2008 Films based on chitosan polyelectrolyte complexes for skin drug delivery: development and characterization *J. Memb. Sci.* 320 1-2 268-79

[14] Feng Q, Zeng G, Yang P, Wang C and Cai J 2005 Self-assembly and characterization of polyelectrolyte complex films of hyaluronic acid/chitosan *Colloids Surf. A Physicochem. Eng. Asp.* 257 85-8

[15] Ianiro A, Giosia M D, Fermani S, Samori C, Barbalinardo M, Valle F, Pellegrini G, Biscarini F, Zerbetto F and Calvaresi M 2014 Customizing properties of β-chitin in squid pen (gladius) by chemical treatments *Mar. Drugs* 12 12 5979-92

[16] Chandumpai A, Singhpibulporn N, Faroongsarng D and Sornprasit P 2004 Preparation and physico-chemical characterization of chitin and chitosan from the pens of the squid species, Loligo lessoniana and Loligo formasoma *Carbohydr. Polym.* 58 4 467-74

[17] Youn D K, No H K and Prinyawiwatkul W 2013 Preparation and characteristics of squid pen β-chitin prepared under optimal deproteinisation and demineralisation condition *Int. J. Food Sci. Technol.* 48 3 571-7

[18] Rahayu L and Purnavita S 2007 Optimasi pembuatan kitosan dari kitin limbah cangkang rajungan (Portunus pelagicus) untuk adsorben ion logam merkuri *Reaktor* 11 1 45-9

[19] Younes I and Rinaudo M 2015 Chitin and chitosan preparation from marine sources. Structure, properties and applications *Mar. Drugs* 13 3 1133-74

[20] Kumirska J, Czerwicka M, Kaczynski Z, Bychowska A, Brzozowski K, Thöming J and Stepnowski P 2010 Application of spectroscopic methods for structural analysis of chitin and chitosan *Mar. Drugs* 8 5 1567-636

[21] Du Y, Zhao Y, Dai S and Yang B 2009 Preparation of water-soluble chitosan from shrimp shell and its antibacterial activity *Innov. Food Sci. Emerg. Technol.* 10 1 103-7

[22] Huang J, Zhao D, Hu S, Mao J and Mei L 2012 Biochemical activities of low molecular weight chitosans derived from squid pens *Carbohydr. Polym.* 87 3 2231-6

[23] Tolaimate A, Desbrières J, Rhazi M and Alaguí A 2003 Contribution to the preparation of chitins
and chitosans with controlled physico-chemical properties Polymer 44 26 7939-52

[24] Abdelmalek B E, Sila A, Haddar A, Bougatet A and Ayadi M A 2017 β-Chitin and chitosan from squid gladius: Biological activities of chitosan and its application as clarifying agent for apple juice Int. J. Biol. Macromol. 104 953-62

[25] Roncal T, Oviedo A, de Armentia I L, Fernández L and Villarán M C 2007 High yield production of monomer-free chitosan oligosaccharides by pepsin catalyzed hydrolysis of a high deacetylation degree chitosan Carbohydr. Res. 342 18 2750-6

[26] Bagheri-Khouljenjani S, Taghizadeh S and Mirzadeh H 2009 An investigation on the short-term biodegradability of chitosan with various molecular weights and degrees of deacetylation Carbohydr. Polym. 78 4 773-8

[27] da Silva S B, Krollicka M, van den Broek L A, Frissen A E and Boeriu C G 2018 Water-soluble chitosan derivatives and pH-responsive hydrogels by selective C-6 oxidation mediated by TEMPO-laccase redox system Carbohydr. Polym. 186 299-309

[28] Zuber M, Zia K M and Barikani M 2013 Advances in natural polymers: Springer) pp 55-119

[29] Afriani Y, Fadli A, Maulana S and Karina I 2016 Sintesis, Kinetika Reaksi dan Aplikasi Kitin dari Cangkang Udang: Review Seminar Nasional Teknik Kimia Teknologi Petro dan Oleokimia 2016 184-96

[30] Dompeipen E J, Kaimudin M and Dewa R P 2016 Isolasi Kitin Dan Kitosan Dari Limbah Kulit Udang Majalah Biam 12 1 32-9

[31] Kurita K, Tomita K, Tada T, Ishii S, Nishimura S I and Shimoda K 1993 Squid chitin as a potential alternative chitin source: deacetylation behavior and characteristic properties J. Polym. Sci. A Polym. Chem. 31 2 485-91

[32] Champagne L M 2008 The synthesis of water soluble N-acyl chitosan derivatives for characterization as antibacterial agents Department of Chemistry Xavier University of Louisiana New Orleans, Louisiana

[33] Tian F, Liu Y, Hu K and Zhao B 2003 The depolymerization mechanism of chitosan by hydrogen peroxide J. Mater. Sci. 38 23 4709-12

[34] Kumari S, Rath P, Kumar A S H and Tiwari T 2015 Extraction and characterization of chitin and chitosan from fishery waste by chemical method Environ. Technol. Innov. 3 77-85