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Synthesis Chitosan from Squid Pens Waste

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Abstract. Squid is one of the fisheries commodities that is quite important and ranks in the third position after fish and shrimp. Still, many people don't know that these animals have bones that usually discarded when processing squid meat. Squid pens used as a source of medicinal ingredients, one of which is chitosan. Chitin was obtained by deproteination process with 3.5% (w/v) NaOH, and demineralization with 1 M HCl. Chitosan was obtained from the chitin deacetylation process using a variation of 50% (w/v) NaOH and 60% (w/v) NaOH. In this research, chitosan only produced use method 60% acetylation degree. Chitosan compounds obtained from the deacetylation process, with 60% NaOH were analyzed using FTIR spectrophotometers, received functional groups: C-H, O-H, N-H, and NH_2.

Keywords : chitin, chitosan, deacetylation degree, squid pens

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Introduction

Squid (*Loligo sp*) is one of the most marine products in the world. In Indonesia, people process sea mollusks as a variety of dishes. Squid is a type of cephalopods known in the world of trade besides squid, fish, and also octopus [1]. But there are still not many people who know that these animals have bones in the so-called pen, which usually discarded when processing squid meat. Squid pen used as a source of medicinal ingredients, one of which is chitin [2].

Some sources of chitin that have been tested regarding the isolation of chitin and chitosan from several sources, namely snail shells with the degree of deacetylation obtained by 74.78-77.99% [3], chitosan shrimp shell with a degree of deacetylation 79.57% [4] and sea crab shells with a degree of deacetylation of 82 % [5].

The process of making chitosan from chitin is carried out with several processes, namely deproteinization, demineralization, and deacetylation. Based on the description above, researchers are interested in conducting research. Making chitosan from chitin squid pen waste, using variations NaOH 50% and 60% with 2 variations in the deacetylation process to get the optimal degree of deacetylation.

Methods

This research was carried out in July-August 2019 in the Stikes Harapan Bunda Jambi Research Laboratory, and the FTIR test will be conducted at Padang State University (UNP). The sample studied and determined was a squid pen obtained from the results of the waste Seafood restaurant in the City of Jambi. 1 kg squid pen washed with water until clean, dried in direct sunlight, mashed using grinding and sieved with a 60 Mesh sieve.

Deproteinization. 500 grams of squid pen powder were added with a 3.5% NaOH (Merck) solution in a ratio of 1:20 (w/v) then heated and stirred with a hot magnetic stirrer for 1 hour to a temperature of 80 ° C and cooled then filtered and neutralized with distilled water to neutral pH [6]. The solids obtained are roasted at a temperature of 80 ℃ to dry.

Demineralization. Squid pen powder from deproteinization was added with 1 M HCl (Merck) at a ratio of 1:20 (w/v) and a stirrer for 30 minutes at 40 ℃, then cooled. After chilling filtered and washed with distilled water. Then dried in an oven at 80 ℃ until a constant weight [7].

Analysis Qualitative of Chitin. Chitin identification test carried out by reacting with a solution of I2 in KI (Merck) will produce a violet color [8].

Chitosan Synthesis. Chitosan synthesis was carried out through the chitin deacetylation process using the Knorr method by adding 50% NaOH and 60% NaOH at a ratio of 1: 20 (w / v) and stirred at 90 – 100 ℃ for 2 hours. After chilling filtered and solids washed with distilled water. The solid is then dried in an oven at 80 ℃ until the weight is constant. Chitosan was identified using the FTIR spectrophotometer.

Chitosan Characterization. Chitosan characterization carried out included organoleptic, determination of the degree of deacetylation, yield transformation of chitin to chitosan, water content, and solubility of chitosan.

1. Degree of deacetylation

The degree of deacetylation states the percentage of the acetyl group that has been removed from chitin. The FTIR spectrum was used to determine the degree of chitosan deacetylation. The frequency used is around the wave number 4000-400 cm^{-1} [9].

2. Yield

The transformation of chitin to chitosan is calculated based on the percentage of the weight of chitosan produced against the chitin weight obtained using the following formula :

$$\text{Yield} = \frac{\text{Weight of Chitosan}}{\textit{Weight of Chitin}} \text{ x } 100\% \quad \textbf{(1)}$$

3. Solubility

Chitosan solubility is a standard parameter for chitosan quality assessment. Chitosan is dissolved in acetic acid at a concentration of 10% in a ratio of 1: 100 (g/ml).

4. Drying Shrinkage

Porcelain dishes were weighed then 0.5 g of the sample were added followed by heating in an oven at 100 ℃ for approximately 1-2 hours. Then it is cooled in a desiccator for about 30 minutes and weighed. Repeat the experiment to get a constant weight. Dry shrinkage calculations can use the formula 2:

$$\% \text{ Drying shrinkage} = \frac{a-b}{c} \text{ x } 100\% \quad (2)$$

Explanation :
a: weight of porcelain + sample wet (g)
b: the weight of porcelain + sample dry (g)
c: the weight of sample wet (g)

5. Ash content

Ash content was carried out by Kumari et al. [10] method. Ash content was determined using 0.5 grams of chitosan placed in a porcelain cup of known empty weight and then weighed. After that, the samples are incubated in a furnace with a temperature of 500 ℃ for 30 – 45 minutes. After that, it is put into the desiccator to be cooled to room temperature then weighed. To calculate the ash content following formula :

$$\text{Ash content} = \frac{B2-B1}{BS} \text{ x } 100\% \quad (3)$$

Explanation :
Bs = weight of the sample (g)
B1 = weight of porcelain (g)
B2 = weight of (sample + porcelain) after heated up (g)

Chitosan Characterization. The characterization test results obtained chitosan in powder form, slightly white color, and odorless. The chitosan drying shrinkage results obtained from deacetylation dehydration with 50% NaOH by 10% while with 60% NaOH with 8%. Chitosan solubility test results showed that chitosan was soluble in 10% acetic acid. The results of ash content test with 50% NaOH deacetylation were 12% while the results of 60% NaOH deacetylation ash content were 10%. The results of the degree of deacetylation of chitosan obtained from the variation of 50% non-chitosan NaOH while the degree of deacetylation with the variation of 60% NaOH obtained results of 73%. This result showed in Table 1, Figure 1, and 2.

Discussion. In the process of making chitin from squid pen waste the sample is cleaned, dried and then smoothed sing grinding and sieved with 60 mesh sieves to obtain white powder. Sifted sample results are used to obtain chitin by deproteinization process using 3.5% NaOH which aims to separate or release protein bonds contained in squid pens dissolved in bases so that proteins that are covalently bound to chitin functional groups will separate, then the result of deproteinization is in the form of white powder [11].

Table 1. Wavenumber chitosan FTIR result

No.	Wavenumber (cm^{-1})		Wave Number Chitosan [11]	Functional groups [11]	Functional group chitosan produced
	NaOH 50 %	NaOH 60%			
1	1375,40 (medium)	1373,89 (medium)	1470 – 1430	CH_2 and CH_3	C-H
2	1636,89 (strong)	1644,41 (strong)	1650 – 1560	N-H	N-H
3	-	2260,61 (medium)	2700 – 2250	NH_2	NH_2
4	3269,00 (weight)	3348,21 (weight)	3800 – 2700	O-H and N-H	O-H

Result and Discussion

Result

Chitin Identification. The results of the chitin qualitative test showed that the positive squid pen contained chitin after the chitin qualitative test with I_2 in KI obtained an orange-red color.

Chitin and Chitosan Yield. The result of chitin from squid pens was 29.970%, while the non-chitosan yield from chitin with 50% NaOH deacetylation was 87.54%, and the results of chitosan from chitin with 60% NaOH deacetylation obtained results of 88.15%.

This demineralization process aims to eliminate the mineral content found in squid pens. The mineral content of the squid pen is $CaCO_3$, the mineral contained in the squid pen is more easily separated than protein because the mineral is only physically bound. In the demineralization process, he results of deproteinization are reacted again with hydrochloric acid (HCl) with the reaction :

$$CaCO_{3(s)} + 2HCl_{(aq)} \text{—> } CaCl_{2(aq)} + H_2O_{(l)} + CO_{2(g)} \quad (4)$$

The process that occurs at the demineralization stage is the minerals contained in the squid pen waste react with HCl so that the mineral separation

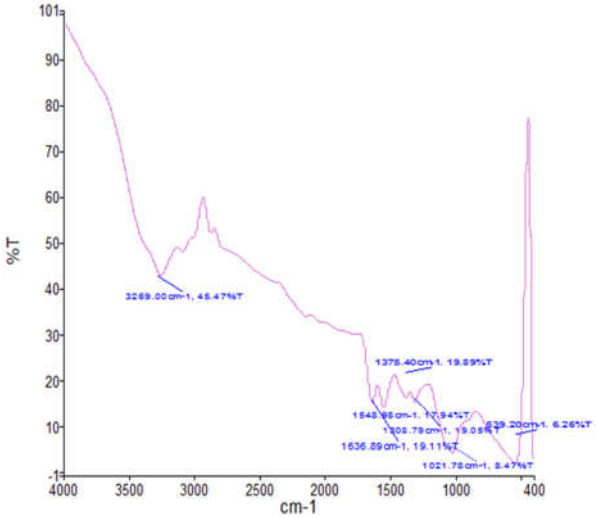

Figure 1. FTIR of chitosan produced by NaOH 50%

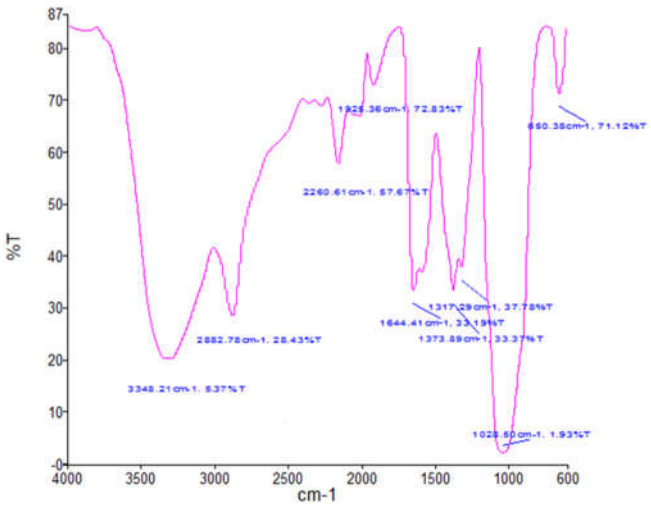

Figure 2. FTIR of chitosan produced by NaOH 60%

from the squid pen waste will occur. The mineral separation process is demonstrated by the presence of CO_2 gas in the form of air bubbles when the HCl solution is added to the sample so that the addition is done slowly so that the sample does not overflow [12].

The ketone identification test that produces an orange-red color indicates that the positive sample contains chitin, as was the study conducted by Artiningsih et al. [13] which says that the results of chitin identification are orange-red.

The yield of chitin is the weight of chitin produced after passing through the deproteinization process, and demineralization in this research yielded a chitin yield of 29,970% while in research conducted by Wahyuni et al. [14] chitin yield results obtained by 34.63% of other studies with different yields obtained yields of 16% according Dompeipen et al. [15] while we get another different yield from [16] yield of 89.71%. This difference in yield is thought to be due to the length of time of deproteinization and demineralization. The longer the process will cause more and more minerals and proteins to be eliminated, causing the weight of chitin produced to be smaller.

The chitin process becomes chitosan through the process of deacetylation. The deacetylation process is the process of removing acetyl groups (-$COCH_3$) from chitin by using an alkaline solution to turn into a group (NH_2). The deacetylation process uses sodium hydroxide (NaOH) with variations in the concentration of 50% NaOH and 60% NaOH at temperatures of 90 – 100 ℃ for 2 hours. The use of high alkaline solutions with high temperatures during the deacetylation process affects the degree of deacetylation produced. This research was conducted by varying NaOH 50% and NaOH 60% to determine the degree of deacetylation [12].

Chitosan yield was obtained from chitin deacetylation (removal of acetyl groups). The yield of chitosan -based on the weight of chitosan produced divided by the weight of chitin obtained in the study produced a yield with a variation of 50% NaOH of 87.53%, the results of chitin deacetylation using 60% NaOH produced chitosan yield of 88.14%, and the research conducted by Dompeipen et al. [15] Chitosan obtained 63% and stated that there was a relationship between molecular weight and yield. The chitosan yield decreases with increasing concentration of sodium hydroxide solution and temperature.

Chitosan characterization test was obtained to determine the quality of chitosan produced. Characterization included calculation of yield, drying shrinkage test, ash content test, solubility test, and degree of deacetylation.

Dry shrinkage and ash content are parameters used as chitosan quality standards. High drying shrinkage causes chitosan freshness and shelf life to be shorter. Ash content indicates the success rate of demineralization so that low ash levels indicate the purity of chitosan [17].

Chitosan characterization showed that chitosan has a low drying loss at 50% NaOH deacetylation variation obtained by 10% while with 60% NaOH at 8% in the chitosan specification which states that chitosan drying losses <10%. The air and storage area affect chitosan drying loss because chitosan is hygroscopic.

Shrinkage drying can affect the water content of chitosan, which affects the growth of microorganisms [15]. While ash content obtained from varying NaOH 50% by 12% and ash content obtained from variations of 60% NaOH by 10%, while ash content obtained from research results from Agustina et al. [12] of 0.17% in the chitosan specifications stated that the ash content <2%.

The solubility of chitosan in acetic acid is one of the main parameters in the chitosan quality assessment standard. According to Rochima et al. [18] and Chattopadhyay and Inamdar [19], the higher the chitosan solubility in acetic acid 10% shows the better the quality of chitosan produced. The resultant chitosan has perfect solubility in 10% acetic acid.

The results of this study indicate that the solubility of chitosan in 10% acetic acid can dissolve completely. The solubility of chitosan in acetic acid solution is influenced by the temperature and the length of immersion in the NaOH solution.

Chitosan analysis was carried out using the FTIR spectrophotometer to determine the functional groups contained in the research sample used. From the results of tests using FTIR, the experiment with 50% NaOH variation showed that the sample did not become chitosan perfectly, this is because the results of the FTIR spectrophotometer showed that no -NH functional group was found in the sample, where the absorption wavenumber of the -NH function number ranged between 1650 – 1560 cm^{-1}. In the variation of 60% NaOH with absorbance of amide group wavenumbers 1644.41 cm^{-1} with absorbance values of hydroxyl group wavenumbers OH^- 3348.21 cm^{-1} [20] by 73%, whereas research has been done by Li et al. [21] with squid pens samples obtained by deacetylation degrees of 70.42% and [22] chitosan shell skin with a degree of deacetylation 79.57% and sea crab shells with a degree of deacetylation of 40.90% [23].

From the research that has been done, the chitosan results with the best degree of deacetylation 73% of the variation of 60% NaOH, this is in accordance with research conducted by Wahyuni, et al. [14] and Murniati and Mudassir [23] which states the degree of deacetylation > 60% is called chitosan.

Conclusion

Based on the results of research that has been done, it can be concluded that Chitosan produced from deacetylation with 60% NaOH is the optimal degree of deacetylation of 73%, whereas 50% NaOH does not produce chitosan.

Daftar Pustaka

[1] Agusandi, A., Supriadi, A. & Lestari, S. D. Pengaruh Penambahan Tinta Cumi-Cumi (*Loligo sp*) Terhadap Kualitas Nutrisi dan Penerimaan Sensoris Mi Basah. *Fishtech*. **2**. 22–37. (2013). DOI: 10.36706/fishtech.v2i1.1100.

[2] Singh, S. K. Solubility of Lignin and Chitin in Ionic Liquids and Their Biomedical Applications. *Inter Jour of Bio Macro*. **132**. 265–277. (2019). DOI: 10.1016/j.ijbiomac.2019.03.182.

[3] Kusumaningsih, T. Masykur, A. & Arief, U. Pembuatan Kitosan dari Kitin Cangkang Bekicot (*Achatina fulica*) Synthesis of chitosan from chitin of escargot (*Achatina fulica*). *J. Biofarmasi*. **2**. 64–68. (2004). DOI: 10.13057/biofar/f020204.

[4] Sularsih, S., Yuliati, A. & Dian, C. P. Degrees of Chitosan Deacetylation from White Shrimp Shell Waste as Dental Biomaterials. *Dent. J. (Maj Kedokt. Gigi)*. **45.** 17-21. (2012). DOI: 10.20473/j.djmkg.v45.i1.p17-21.

[5] Ocloo, F., Adu-Gyamfi, A., Quarcoo, E. A., Serfor-Armah, Y., Asare, D. K. & Owulah, C. Effect of Gamma Irradiation on Antibacterial Properties of Sea Crab Shell Chitosan. *Eur J of Nutr & Food Saf.* **2**. 69-78. (2012).

[6] Mariod, A. A. Extraction, Purification, and Modification of Natural Polymers in Natural Polymers. *Springer.* 63-91. (2015). DOI: 10.1007/978-3-319-26414-1_3.

[7] Omogbai, B. A. & Ikenebomeh, M. J. Subchronic Toxicity Study of a Characterized Food Grade Chitosan from Crab (*Callinectes sapidus*). *NISEB J*. **16**. 34-44. (2016).

[8] Dhandapani, B. & Lyer, R. G. Biosynthesis,

Characterization and Application of Chitin Nanoparticle with (*Cassia auriculata*). *Am. J. Biomed. Life Sci*. **6**. 96-102. (2018). DOI: 10.11648/j.ajbls.20180605.11.

[9] Fang, Y., Zhang, R., Duan, B., Liu, M., Lu, A. & Zhang, L. Recyclable Universal Solvents for Chitin to Chitosan with Various Degrees of Acetylation and Construction of Robust Hydrogels. *ACS Sustain. Chem. Eng*. **5**. 2725 –2733. (2017). DOI: 10.1021/acssuschemeng.6b03055.

[10] Kumari, S., Rath, P., Kumar, A. S. H. & Tiwari, T. N. Extraction and Characterization of Chitin and Chitosan from Fishery Waste by Chemical Method. *Environ. Technol*. **3**. 77–85. (2015). DOI: 10.1016/j.eti.2015.01.002.

[11] Branca, C., D'Angelo, G., Crupi, C., Khouzami, K., Rifici, S., Ruello, G. & Wanderlingh, U. Role of The OH and NH Vibrational Groups in Polysaccharide-nanocomposite Interactions: A FTIR-ATR Study on Chitosan and Chitosan/Clay Films. *Polymer*. **99**. 614–622. (2016). DOI: 10.1016/j.polymer.2016.07.086.

[12] Agustina, S., Swantara, M. D. & Suartha, I. N. Isolasi Kitin, Karakterisasi, dan Sintesis Kitosan dari Kulit Udang. *J. Kim*. **9**. 271–278, 2015.

[13] Artiningsih, A. Noor, A. & Natsir, H. Usaha Biokonversi Khitin Asal Kepiting Rajungan Menjadi Khitosan. *Repos Unhas*. **4**. 1-10. (2003).

[14] Wahyuni, W., Ridhay, A. & Nurakhirawati, N. Pengaruh Waktu Proses Deasetilasi Kitin dari Cangkang Bekicot (*Achatina fulica*) Terhadap Derajat Deasetilasi. *Kovalen*. **2**. 1-7. (2016).

[15] Dompeipen, E. J., Kaimudin, M. & Dewa, R. P. Isolasi Kitin Dan Kitosan Dari Limbah Kulit Udang. *Majalah Biam*. **12**. 32-38. (2016).

[16] Sukma, S., Lusiana, S. E., Masruri, M. & Suratmo, S. Kitosan dari Rajungan Lokal *Portunus Pelagicus* Asal Probolinggo. *Kim Stud J*. **2**. 506–512. (2014).

[17] Herdyastuti, N., Raharjo, T. J., Mudasir, M. & Matsjeh, S. Chitinase and Chitinolytic Microorganism: Isolation, Characterization and Potential. *Indo. J. Chem*. **9**. 37–47. (2009).

[18] Rochima, E., Suhartono, M., Syah, D. & Sugiono, S. Derajat Deasietilase Hasil Reaksi Enzimatis Kitin Deasetilasi Isolat Bacillus Papandayan K29-14. *Repository IPB*. (2004).

[19] Chattopadhyay, D. P. & Inamdar, M. S. Aqueous Behaviour of Chitosan. *Int. J. Polym. Sci*. **2010**. 1-7. (2010). DOI: 10.1155/2010/939536.

[20] Dimzon, I. K. D. & Knepper, T. P. Degree of Deacetylation of Chitosan by Infrared Spectroscopy and Partial Least Squares. *Int. J. Biol. Macromol*. **72**. 939–945. (2015). DOI: 10.1016/j.ijbiomac.2014.09.050.

[21] Li, H., Du, Y. & Xu, Y. Adsorption and Complexation of Chitosan Wet-end Additives in Papermaking Systems. *J. Appl. Polym. Sci*. **91**. 2642–2648. (2004). DOI: 10.1002/app.13444.

[22] Hossain, M. S. & Iqbal, A. Production and Characterization of Chitosan from Shrimp Waste. *J. Bangladesh Agri. Univ*. **12**. 153–160. (2014). DOI: 10.3329/jbau.v12i1.21405.

[23] Murniati, D. & Mudasir, M. Isolasi Kitin dari Cangkang Kepiting Laut (*Portunus pelagicus Linn.*) serta Pemanfaatannya untuk Adsorpsi Fe dengan Pengompleks 1,10-fenantrolin. *J. Kim. Val*. **3**. 15–22. (2013). DOI: 10.15408/jkv.v3i1.499.