

Degree of Deacetylation of Chitosan Extracted from White Snapper (*Lates* sp.) Scales Waste

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Abstract—Chitosan is natural biopolymer which can be obtained from natural resources even from wastes such fish scales. Indonesia is one of countries that having high fish production. White snapper (*Lates* sp.) is seawater fish that has an economical value since it is preferred by many consumers. New challenge to this condition is how to manage the waste from fish processing into useable materials. This study was aimed to extract chitosan from white snapper (*Lates* sp.) scales and to determine the degree of deacetylation of the chitosan based on the treatment differences in temperature, heating duration, and the NaOH concentration. Chitosan from white snapper scales were extracted through three stages, deprotenization, demineralization and deacetylation. Different treatment on deacetylation stages based on variation of NaOH concentration, temperature and duration of heating. Degree of deacetylation were measured using FTIR spectrophotometry based on infrared light absorbance. Result showed that chitosan produced were fulfilled minimum requirement for good quality of chitosan. Highest deacetylation degree was 84.05 %, resulted from treatment number 15 with NaOH concentration of 80%, temperature of 120°C and duration of heating for 4 hours.

Index Terms—chitosan, degree of deacetylation, fish scale, white snapper, FTIR

I. INTRODUCTION

Chitosan is a natural product which is a derivative of the polysaccharide chitin. Chitosan has the chemical name Poly D-glucosamine (beta (1-4) 2-amino-2-deoxy-D-glucose). Chitosan can be used in various fields of biochemistry, medical or pharmaceutical, food and nutrition, agriculture, microbiology, paper industries, textile membrane or film, cosmetics, and wastewater treatment.

Waste from seafood processing for example crustaceans shell are abundantly found and are the most common source of chitosan[1]. Chitosan also can be isolated from freshwater fishes such as carp (*Cyprinus carpio* L.) scales. In addition, Uawonggul *et al.* (2002) [2] and Weeraphan (2011) [3], also managed to extract chitin and chitosan from fish scales of tilapia (*Tilapia nilotica*). Kumaria & Rath (2014) [4] stated that chitin and chitosan can be extracted from fish scales of *Labeo rohita*, too. There are other potential uses of fish scales according to Ikoma *et al.* (2003) [5], which is as an inorganic

absorbent materials. Other than that, scales can also be used not only in separation technology but also in catalysis and biomedical applications.

Further research is needed to determine the method and characteristic of chitosan extracted from seawater fish scales, for example white snapper. Local name of white snapper is Barramundi. Barramundi (*Lates* sp) has a body covered with scales. The scales derived from the skin layer called the dermis.

Degree of deacetylation is a quality parameter that indicates an acetyl group which can be removed from the chitosan. The higher the degree of deacetylation of chitosan, the lower the acetyl group contained in the chitosan (Knoor, 1983) [6]. Degree of deacetylation is also determined as a percentage that indicates an acetyl missing or replaced with amines. Analysis Fourier-Transform Infra-Red (FTIR) is used to determine the functional groups present in chitosan. In addition, this method can be used also to determine the degree of deacetylation. The working principle of FTIR is infrared radiation will pass through the sample and then the radiation will be absorbed. The ones that are not absorbed will be forwarded passing the transmittances resulting in a spectrum of absorption in the infrared region therefore cause the vibration of the bond in the molecules. This study was aimed to extract chitosan from white snapper fish (*Lates* sp.) scales and to determine the degree of deacetylation of the chitosan based on the treatment differences in temperature, heating duration, and the NaOH concentration.

II. METHODOLOGY

A. Preparation of Fish Scale

15 kg of fish scales were collected from Bening Food Companies at Parung, Bogor. Fish scales then washed using running water to remove dirt and other odd particles. After that, samples were air-dried. Dry fish scales were stored in room temperature before the extraction process started.

B. Extraction of Chitosan from Fish Scale

Chitosan were extracted based on [3]. The extraction consists of three stages, deproteinization, demineralization, and deacetylation. Deproteinization was done using a solution of 4.2% NaOH and the ratio between the fish scales with a solution of NaOH was 1: 6.

The process of making 4.2% NaOH solution was by dissolving 504 grams solid NaOH using distilled water up to 1000 ml volume in the beaker glass. The solution then was added by 11 L of distilled water. 2 kg of fish scales were added to 2 L of 4.2% NaOH solution, mixed, then heated using hotplate with a constant temperature of 60 °C, and stirred using spatula periodically for 6 hours. Deproteinization residue was filtered and neutralized using distilled water. The residue that has reached a pH of 7 was dried using an oven with a temperature of 60 °C for 12 hours. The dried residue was then prepared for demineralization process.

Demineralization was carried out using 2 N 4.2% HCl solution and the ratio between deproteinization residue with a solution of HCl was 1: 6. Demineralization began by adding the 4.2% HCl solution in a pyrex glass containing deproteinization residue. The solution is then stirred periodically in room temperature for 5 hours. Demineralization residue then filtered and neutralized using distilled water until its pH reached 7. The residue was dried using an oven with a temperature of 60 °C for 12 hours. Final product of demineralization is chitin.

Samples resulted from demineralization process were tested using complete random design with variation on NaOH concentration, temperature, and duration of heating.

Table I showed parameters used which were: NaOH concentrations (60%, 70%, 80%), temperature (110, 120, 130°C) and duration of heating (4, 6 hours). The steps of deacetylation of white snapper fish scales divided into 18 treatments (number 1-18).

C. Measurement of Degree of Deacetylation on Chitosan

Degree of deacetylation was characterized using FTIR spectrophotometry with computation by providing infrared light on a sample of chitosan and then the infrared absorbance was recorded. Hydroxyl groups calculated at a wavelength of 3450 cm⁻¹, whereas the amide group is at a wavelength of 1655 cm⁻¹. Deacetylation degree of chitosan can be calculated using this following equation as in (1) [7]:

$$\% DD = 100 - \left[\frac{A_{1655}}{A_{3450}} \times \frac{100}{1.33} \right] \quad (1)$$

Chitosan A₁₆₅₅ = absorbance at a wavelength of 1655 cm⁻¹
Chitosan A₃₄₅₀ = absorbance at a wavelength of 3450 cm⁻¹

TABLE I. DEACETYLATION TREATMENT

| Treatment number | Parameter | | |
|------------------|-----------|------------------|--------------|
| | NaOH (%) | Temperature (°C) | Time (hours) |
| 1 | 60 | 110 | 4 |
| 2 | 60 | 110 | 6 |
| 3 | 60 | 120 | 4 |
| 4 | 60 | 120 | 6 |
| 5 | 60 | 130 | 4 |
| 6 | 60 | 130 | 6 |
| 7 | 70 | 110 | 4 |
| 8 | 70 | 110 | 6 |
| 9 | 70 | 120 | 4 |
| 10 | 70 | 120 | 6 |
| 11 | 70 | 130 | 4 |
| 12 | 70 | 130 | 6 |
| 13 | 80 | 110 | 4 |
| 14 | 80 | 110 | 6 |
| 15 | 80 | 120 | 4 |
| 16 | 80 | 120 | 6 |
| 17 | 80 | 130 | 4 |
| 18 | 80 | 130 | 6 |

III. RESULT AND DISCUSSION

Chitosan is produced through three processes, which are deproteinization, demineralization and deacetylation. Deproteinization is protein removal process using alkaline solution, to produce deproteinization residue. Meanwhile, the demineralization process is removal of the metal content of deproteinization residue, generating residual demineralized form of chitin. Chitosan is produced from chitin by a deacetylation process. Deacetylation is the removal of acetyl groups (-COCH₃) on chitin and turn it into an amine group (NH₂) using an alkaline solution. Degree of deacetylation on chitosan extracted from white snapper fish scales is showed at Table II.

TABLE II. DEGREE OF DEACETYLATION ON CHITOSAN EXTRACTED FROM WHITE SNAPPER FISH SCALE

| Treatment number | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
|------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| DD (%) | 78,35 | 80,51 | 77,09 | 79,25 | 81,72 | 82,02 | 80,19 | 80,59 | 82,30 | 81,45 | 75,00 | 79,79 | 77,49 | 79,22 | 84,05 | 76,09 | 77,41 | 75,58 |

Formation of chitosan from chitin occurs through an amide hydrolysis reaction using a base. Chitin plays role as an amides and NaOH as a base. The process begins with an additional reaction, namely the inclusion of the -OH group into the group NHCOCH₃. Then chitosan produced through the elimination of cluster CH₃COO- [8]. The degree of deacetylation of the chitosan obtained from white snapper fish scales ranged from 75.00% to 84.05%. Degree of deacetylation was calculated based on FTIR using equation Dosmzy & Robert (1984). The highest degree of deacetylation was 84.05% which was

obtained from treatment number 15 (NaOH concentration of 80%, temperature of 120°C, duration of heating for 4 hours), while the treatment number 11 (NaOH concentration of 70%, temperature of 130°C, duration of heating for 4 hours) showed lowest degree of deacetylation which was 75.00%. The quality standard of chitosan according to SNI 7949: 2013 is when the degree of deacetylation of chitosan reaches a minimum value of 75%. It showed that chitosan resulted from white snapper fish scales fulfilled the standard.

Fig. 1 showed the FTIR graph which performed chitosan with higher degree of deacetylation by calculating the distance between two absorbance values

which were 1655 and 3450 wavelength. The distances then were measured based on there the highest and lowest point.

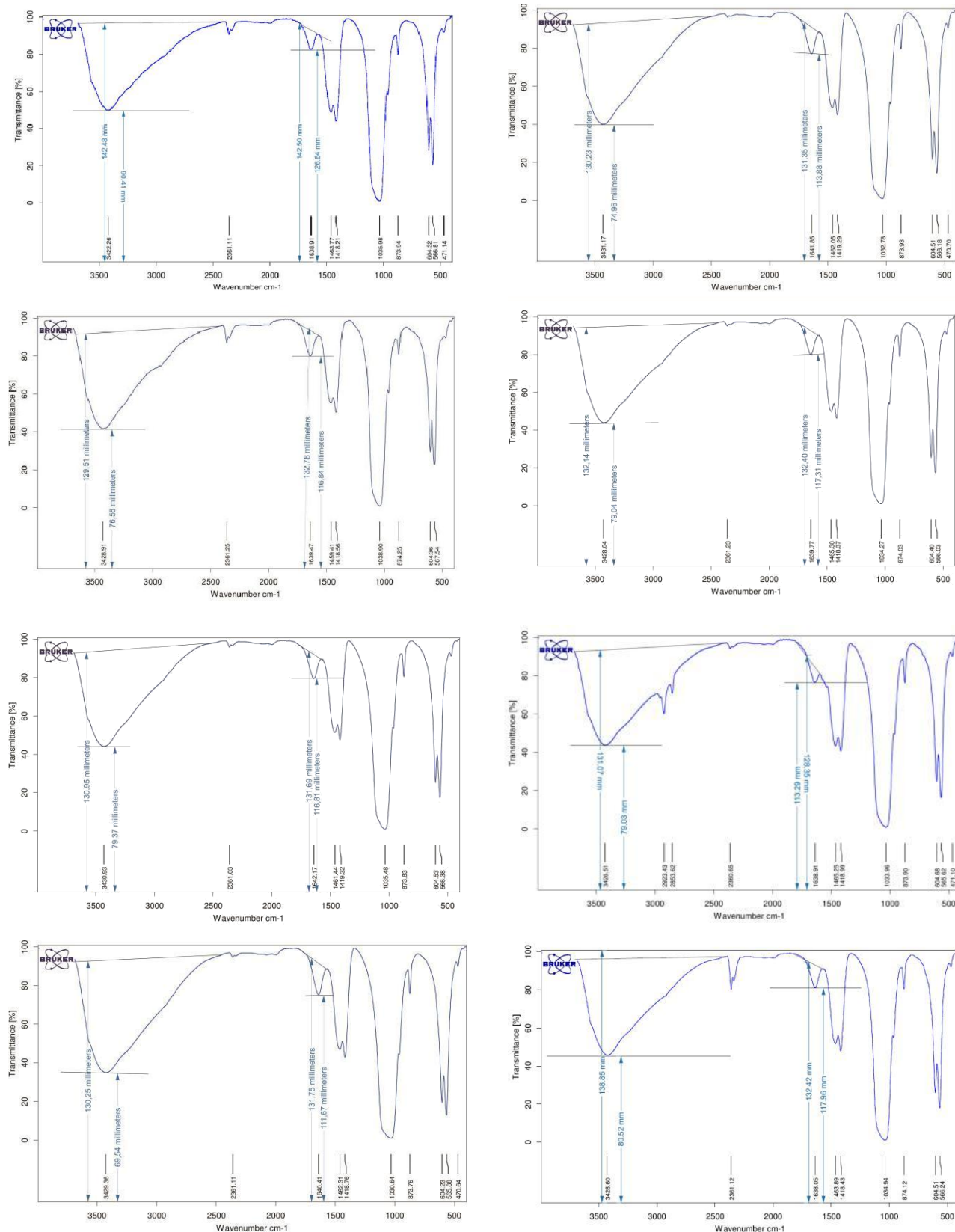


Figure 1. FTIR graph of treatment showing chitosan with DD higher than 80% (Treatment 2, 5, 6, 7, 8, 9, 10, 15)

The amount of percent degree of deacetylation can be used to determine the quality of chitosan. Base line method proposed by [7] used to calculate the percentage of the degree of deacetylation of the chitosan scales white snapper. Chitosan is said to have been deacetylated perfectly if DD > 90% [9].

IV. CONCLUSION

Deacetylation degree of 84,05% was resulted from treatment number 15 with temperature of 120 °C, NaOH concentration of 80%, and duration of heating for 4 hours.

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REFERENCES

- [1] S. G. Zaku, S. A. Emmanuel, O. C. Aguzue, and S. A. Thomas, "Extraction and characterization of chitin, a functional biopolymer obtained from scales of common carp fish (*Cyprinus carpio* L.): A lesser known source," *African Journal of Food Science*, vol. 5, no. 8, pp. 478-483, 2011.
- [2] N. Uwonggul, S. Kongsri, and S. Chantai, "Study on dye-binding interactions of chitosan obtained from fish scale of tilapia (*Tilapia nilotica*)," *International Journal of Pure and Applied Chemistry*, vol. 6, no. 2, pp. 129-147, 2011.
- [3] W. Weeraphan, "Chitosan extraction from fish scale of tilapia (*Tilapia nilotica*) for edible film production," thesis, Chiang Mai University, Thailand, 2011.
- [4] S. Kumaria and P. K. Rathb, "Extraction and characterization of chitin and chitosan from (*Labeo rohita*) fish scales," *Procedia Materials Science*, vol. 6, pp. 482 – 489, 2014.
- [5] T. Ikoma, K. Hisatoshi, T. Junzo, W. Dominic, and M. Stephen, "Microstructure, mechanical and biomimetic properties of fish scales from pagrus major," *Journal of Structural Biology*, vol. 142, pp. 327–333, 2003.
- [6] D. Knorr, "Dye binding properties of chitin and chitosan," *Journal of Food Science*, vol. 48, pp. 36–37, 1983.
- [7] J. G. Domszy and G. A. F. Robert, "Evaluation of infrared spectroscopic technique for analyzing chitosan," *Journal of Macromolecular: Science- Pure Applied Chemistry*, vol. 8, pp. 1671-1677, 1984.
- [8] F. W. Mahatmanti, "Study adsorben logam seng (II) dan timbal (II) pada kitosan dan kitosan sulfat dari kulit udang windu (*Penaus monodon*)," thesis, UGM, Yogyakarta, 2001.

- [9] B. Srijanto, "Kajian pengembangan teknologi proses produksi kitin dan kitosan secara kimiawi," in *Proc. Seminar Nasional Teknik Kimia Indonesia*, 2003, vol. 1, pp. F01-1 –F01-5.



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