International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(3): 255-260

Received: 14-03-2018
Accepted: 18-04-2018

Indri Hermiyati
Department of Rubber and Plastic Processing Technology, ATK, Yogyakarta, Indonesia

Iswahyuni
Department of Rubber and Plastic Processing Technology, ATK, Yogyakarta, Indonesia

Swatika Juhana
Department of Leather Processing Technology Politeknik ATK, Yogyakarta, Indonesia

Correspondence
Swatika Juhana
Department of Leather Processing Technology Politeknik ATK, Yogyakarta, Indonesia

Synthesis of chitosan from Stary Tigger Fish Skin (*Abalistes stellaris*)

Indri Hermiyati, Iswahyuni and Swatika Juhana

Abstract
Synthesis of chitosan from stary tigger fish skin was carried out in three stages: deproteination using 10% NaOH solution, demineralization using 0.63 M HCl solution, and deacetylation using NaOH solution with variations in 60%, 50%, and 40% NaOH concentrations. Characterization results show that chitosan is best obtained from deacetylation process with 60% NaOH. The degree of deacetylation obtained 74% has met the DD standard of at least 70% chitosan. Ash level 88.05%; 6% water content; 2.29% protein content.

Keywords: Chitosan, stary tigger fish skin, deacetylation degree

Introduction
The use of polymers has developed in all aspects of life, thus continuing to encourage the desire to find a type of polymer that is focused on natural resources that are cheap and easily available. The polymer is a biopolymer which is a polymer that is easily degraded and environmentally friendly. Solid waste of crustaceans (shells, heads, feet and scales) is one of the problems that need to be overcome in the seafood processing industry, including stary tigger fish.

Stary tigger fish that have hard skin cause consumers to only use their meat, so that fish skin is usually only disposed of as scattered waste on the beach side of Tasik Agung, Rembang Regency, Indonesia. The waste disrupts the beauty around the beach and the amount of waste wasted is approximately 25-50 kg per day, so that the estimated number of chicken skins per month is 750-1500 kg.

Chitin was first discovered in 1811 by Henry Braconot by extracting certain types of fungi using water, alcohol and dilute alkalis that produce insoluble deposits called fungieu. The extraction results as a pure form of cellulose although the presence of nitrogen is unknown. Then in 1823 Odie found the same component in the insect ectodermal (cuticle) and named it chitin from the Greek word "chiton" which means cover or cover.

Chitin is composed of units of acetyl glucosamine which bind to each other, with (1-4) beta bonds. Chemically chitin is a polysaccharide group polymer composed of units of β- (1-4) 2-acetamide-2-deoxy-D-glucose, which can be digested by mammals (Suhardi, 1992) [8]. Chitin is a formless solid (amorphous), insoluble in water, dilute organic acids, dilute and concentrated alkalis, alcohol, has a physical form in the form of crystals which are white to light yellow, tasteless, odorless with the molecular formula $[C_6H_{13}O_5N]^{-}_n$. The molecular weight of chitin is very large depending on the length of the chain. From the molecular formula above, the molecular weight is $[203,19]_n$. Chitin has no melting point, and has a nitrogen content of less than 7% (Suhardi, 1992) [8].

Chitin is obtained from stary tigger fish skin waste through deproteinization and demineralization processes. Deprotenation is the process of removing protein, which is extracted with NaOH solution. In principle the deproteinization process is to separate or release bonds between protein and chitin. This process is generally carried out with treatment using NaOH solution at relatively high temperatures for a relatively long time. With this treatment protein will be released and form soluble Na-proteinate (Suhardi, 1992) [8].

Demineralization is a stage in the removal of minerals which serves to separate chitin from inorganic salts by adding dilute HCl at room temperature, so that the existing minerals will react with HCl (Suhardi, 1992) [8]. The reactions that occur are:

$CaCO_{3\,(s)} + 2\,HCl_{\,(s)} \longrightarrow CaCl_{2\,(l)} + H_2O_{(g)} + CO_{2\,(g)}$
$Ca_3(PO_4)_{2\,(s)} + 6\,HCl_{(l\,)} \rightarrow 3CaCl_{2(l)} + 2H_3PO_{4(l)}$

Treatment with dilute hydrochloric acid at room temperature is more effective and produces chitin with a lower residual mineral content, although it can cause breaking of the chain or the release of its acetyl groups.

Chitosan is chitin which is removed by acetyl group, so this material is a polymer of glucosamine which is also called β-1,4-2, amino-2-dioxy-D-glucose (Muzarelli, 1985 in Suhardi, 1992) [5, 8]. Chitosan is white, non-toxic, odorless, easily biodegradable, slightly soluble in HCl, HNO_3 and H_3PO_4. The free amino groups they contain make this polymer polycationic so it is not soluble in water and dissolves in strong bases. Chitosan is also insoluble in organic solvents such as alcohol, acetone, dimethylformamide, dimethylsulfoxide, but is easily soluble in organic acids such as citric acid and high concentrated formic acid (0.2% -100%) in water (Sugiyo, 2001) [7]. Besides that chitosan easily interacts with organic substances such as proteins, so it is relatively more widely used in various applied industries and the health industry.

Chitosan has the same chemical structure as chitin, consisting of long molecular chains and large molecular weight. The difference between chitin and chitosan is that in each ring of chitin molecules there is an acetyl group (CH3-CO-) on the second carbon atom, whereas in chitosan there is an amine group (NH2-). Chitosan contains more than 7% nitrogen (Muzarelli, 1977 in Jumadi, 2006) [3].

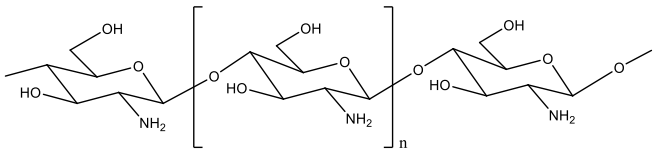


Fig 1: Structure chitosan

Chitosan can be produced from chitin by deacetylation process by reacting using high concentration alkali with relatively long time and high temperature, by removing acetyl group (-$COCH_3$) so that the molecule can dissolve in acid solution. This process is called deacetylation, which releases acetyl groups so that chitosan has the characteristics of a cation. In general, the deacetylation degree of chitosan is around 60% and around 90-100% for chitosan which has full deacetylation, this price depends on the chitin raw material used and the process carried out (Suhardi, 1992) [8].

According to Oh, *et al.*, 2001 in Rochima, 2007 [6] that the degree of deacetylation was influenced by alkaline solution concentration, temperature, reaction time, chitin pre treatment, particle size and chitin concentration.

Table 1: Chitosan Quality Parameters

Quality Parameters	Standard value of chitosan
Particle size	Pellets
Ash content (%b/b)	<1.0
Water content(%b/b)	<10.0
Deacetylation degree (%)	> 70.0 for technical types
Solubility in an acidic atmosphere	> 95.0 for pharmacic types
Color	Only at pH <6

Source: Bastaman, 1989 in Jumadi, 2006 [3]

One of the quality parameters of chitosan is the degree of deacetylation. The degree of deacetylation is a parameter of chitosan quality which shows the percentage of acetyl groups that can be removed from chitin yield. The higher the deacetylation degree of chitosan, the lower the acetyl chitosan group so that the interaction between ions and hydrogen bonds becomes stronger. The more amine groups in chitosan also cause chitosan to become more reactive.

Experimental

Materials

Sodium Hydroxide (NaOH) pa, Chloride Acid (HCl) pa, stary tigger fish skin, aquades, pH indicator paper, pure chitosan.

Isolation of Chitin

Stary tigger fish skin are cleaned by washing in running water, then dried under the sun. The dried of stary tigger fish skin are mashed to a size of 100 mesh. Chitin isolation was carried out through deproteinization stage by refluxing 50 grams of stary tigger fish skin powder with 500 ml of 4% (b/v) NaOH solution, at 65ºC for 2 hours, then refluxed with distilled water until the pH of the neutral washing water and filtered results dried in oven.

After the deproteination process continued with the demineralization process, the residue plus 0.5M HCl with a ratio of 1:15 was stirred at room temperature for 3 hours. Then the mixture is filtered and washed with aquades until the pH is washed neutral. The washing residue is then ovened, and then called chitin.

Synthesis Chitosan

Making chitosan is carried out through a deacetylation process. 50 grams of chitin were refluxed with 3 different treatments, namely 60%, 50% and 40% (b/v) NaOH with a ratio of 1:10 at 70 ºC for 1 hour. The reflux product is cooled and filtered to a neutral washing pH. The residue is then ovenered and chitosan is obtained which will then be characterized.

Characterization of chitosan

Chitin and chitosan obtained by characterization include water content, ash content, nitrogen content and deacetylation degree.

a) Water Content (AOAC, 1999)

One gram of chitosan was put in a porcelain cup then oven at 105ºC for 3 hours, then cooled in a desiccator and weighed. Water content is calculated as follows:

$$\text{kadar air (\%)} = \frac{X - Y}{X}\, x\, 100\%$$

with
X = weight of chitin or initial chitosan (gram)
Y = weight of chitin or final chitosan (gram)

b) Ash content (AOAC 1999)

As much as 1 gram of dried chitosan is put into a cup, then burned on an electric stove until it is no longer smoky and then put in the furnace for 5 hours at 650ºC. The cup is cooled in a desiccator then weighed. Ash content is calculated as follows:

$$\text{kadar abu (\%)} = \frac{\text{berat abu}}{\text{berat sampel}}\, x\, 100\%$$

c) Protein content (AOAC 1995)

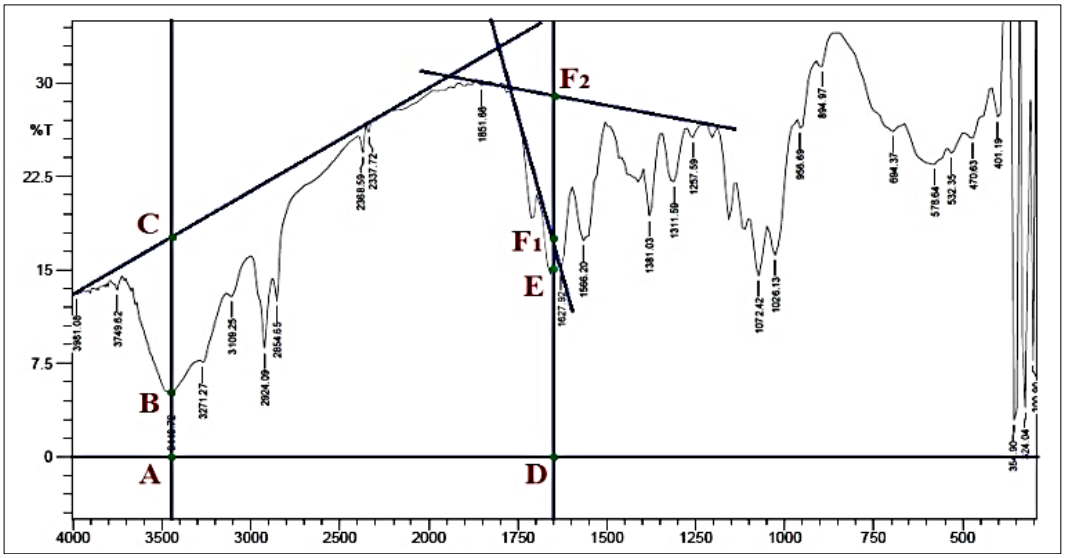
Protein content analysis consists of three stages: destruction, distillation and titration. A total of 0.25 grams of chitin or

chitosan were added to 100 mL of Kjeldahl flask and added with one kjeltab and 3 mL of concentrated H_2SO_4. The mixture is degenerated at 410 ^oC for 1 hour until the clear solution is then cooled. 50 mL of distilled water and 20 ml of 40% NaOH were added to the Kjeldah flask and distilled at 100^oC. The results of the distillation were collected in an Erlenmeyer 125 flask and added a mixture of 2 mL boric acid (H_3BO_3) 2% and 2 drops of bronchosol green-methyl red indicator. The distillation process is stopped when the volume of distillate reaches 40 mL and is bluish green. The distillate is then titrated with 0.1N HCl until it forms a pink color. Protein levels are calculated by the following formula:

$$\text{kadar N (\%)} = \frac{(\text{mL HCl} - \text{mL blangko}) \text{ x N HCl x } 14{,}007}{\text{mg sampel}} \text{ x } 100\%$$

d) The degree of deacetylation

The degree of deacetylation is determined by infrared spectrophotometry methods. The deacetylation degree of chitin and chitosan can be calculated based on the comparison of the absorbance of amide groups (about 1655 cm^{-1}) with hydroxyl groups (about 3450 cm^{-1}). The absorbance ratio was calculated after first determined the baseline in each spectra so as to produce two different baselines in the absorption area of 1655 cm^{-1} namely baseline A and B (Khan *et al*., 2002) [4]. Baseline determination techniques are determined as follows:



The formula for determining baseline A according to Domzy and Robert in Hung *et al*., (2002) [2] is as follows:

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

The area of absorbance of amine groups and hydroxyl groups can be presented further by the mathematical equations proposed by Sabnis and Block in Hung *et al*., (2002) [2] namely:

$$\log\left(\frac{DF_1}{DE}\right) = A_{1655}$$

$$\log\left(\frac{DF_2}{DE}\right) = A_{1655}$$

$$\log\left(\frac{AC}{AB}\right) = A_{3450}$$

DF1 (used for baseline A) or DF2 (used for baseline B), DE, AC and B show the absorbance band of the functional group as the wavelength region.

Results and Discussion

The results of chitosan rendement testing stated the efficiency of chitosan isolation process from stary tigger fish skin. The randement calculation was obtained from the weighing of chitosan results compared to the weight of processed stary tigger fish skin powder in Table 2.

Table 2: Results of Making Chitosan

No	Process	Initial Weight (gram)	Final Weight (gram)	Rendemen (%)
1	Chitosan 1 (deasetilation with NaOH 60%)	20	3.00	15
2	Chitosan 2 (deasetilation with NaOH 50%)	30	16.05	53,5
3	Chitosan 3 (deasetilation with NaOH 40%)	54	16.24	30,0

To find out the quality of chitosan produced, it is necessary to do several tests including moisture content, ash content, and protein content. The test results are listed in table 3.

Table 3: Results of characterization of water content, ash content and chitosan protein content of stary tigger fish skin

No	Procces	Water Content (%)	Ash Content (%)	Protein Content (%)
1	Chitosan 1	6,02	88,50	2,29
2	Chitosan 2	3.43	87,67	1,07
3	Chitosan 3	5,05	91,80	1,08

The results of the calculation of water content and protein content of all chitosan are below 10%. This shows that chitosan has met chitosan standards. The test results of ash content for the three chitosan are still very high which shows the mineral content of stary tigger fish skin is very large, according to the characteristics of hard scales, according to Talumepa, Anggun CN *et al* said that, the mineral component shows the indicator of the hardness of the minerals the harder the mineral texture of the material.

Characterization of chitosan produced was carried out by FTIR to determine the formation of NH2 groups in chitosan and to calculate the deacetylation degree of chitosan. Characterization results with FTIR in Figures 2.3, and 4.

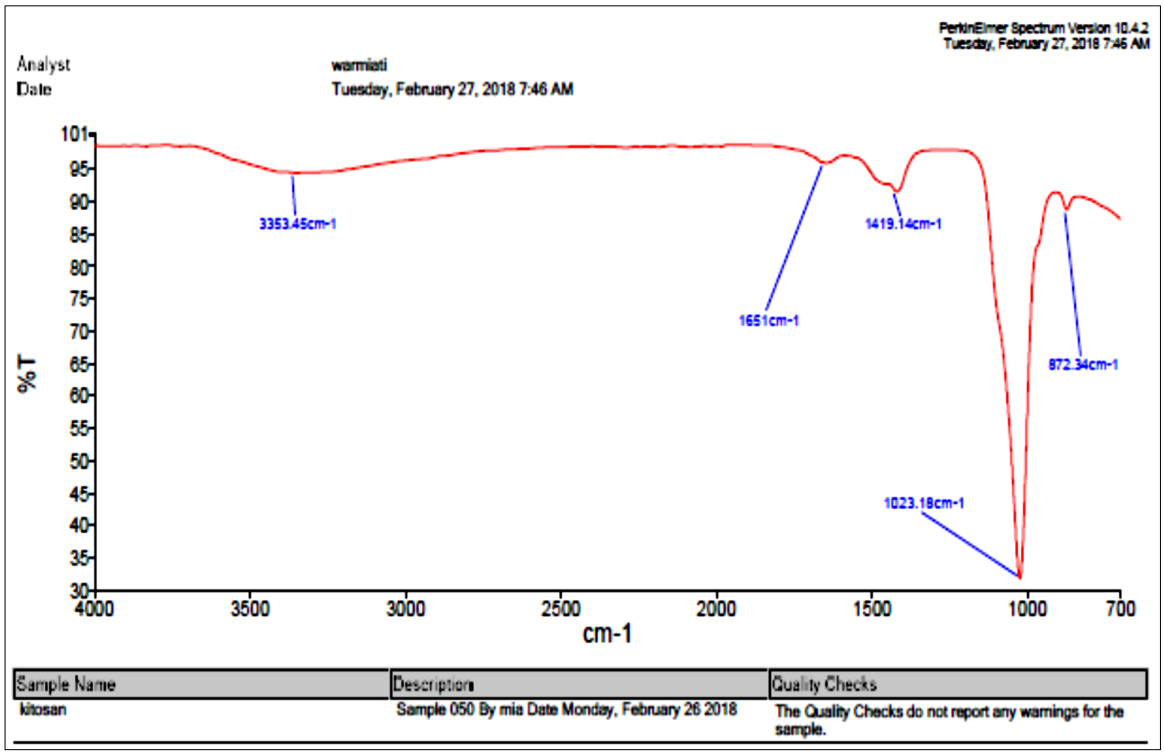


Fig 2: Chitosan FTIR Spectra with 60% NaOH

Figure 2 shows the chitosan FTIR spectra from the deacetylation process with 60% NaOH. The spectra results show that there is an -OH group of chitosan groups shown in wave number 1419 cm-1, -CH group at wave number 1651 cm-1, group -NH at wave number 3353.45 cm-1. The calculation of deacetylation degree for this chitosan is 74.25%.

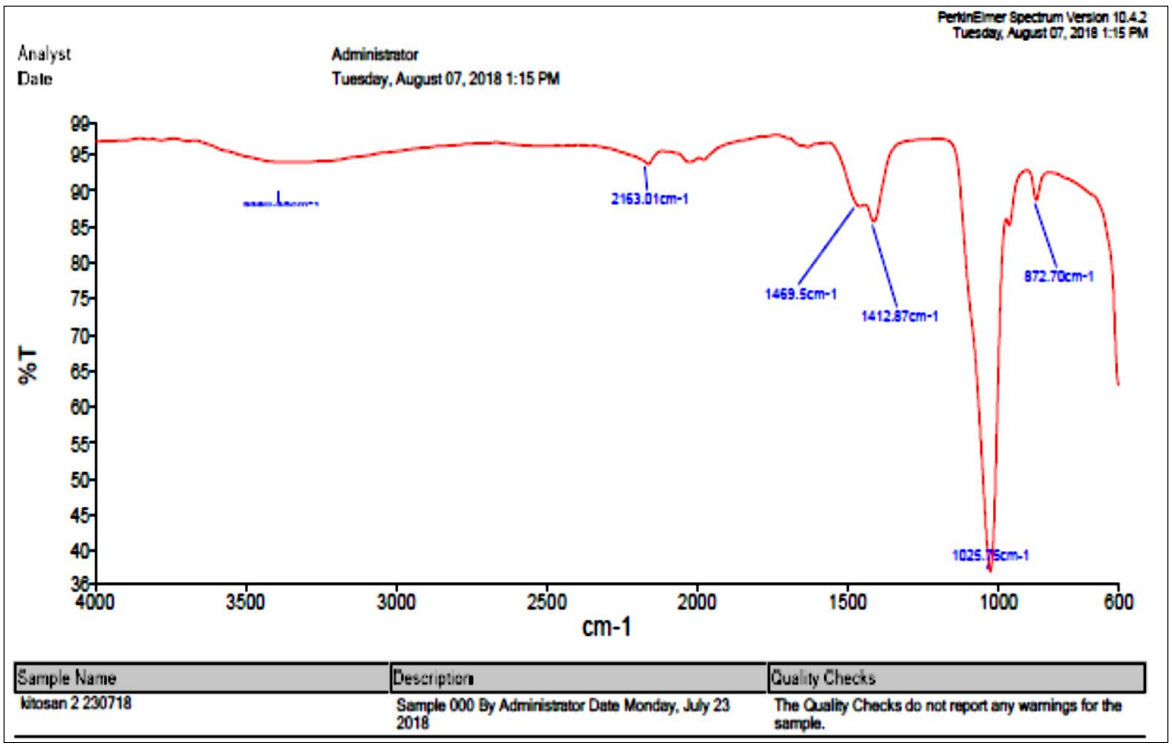


Fig 3: Chitosan FTIR Spectra with 50% NaOH

Figure 3 shows the chitosan FTIR spectra from the deacetylation process with 50% NaOH. The spectra results show there is an -OH group of chitosan groups which are shown in wave numbers 1412.87 cm-1, -CH groups at wave numbers 1469 cm-1, –CO groups at wave numbers 1025.75 cm-1, CH-alkene groups at wave number 872.70. The calculation of deacetylation degree for this chitosan is 47.58%.

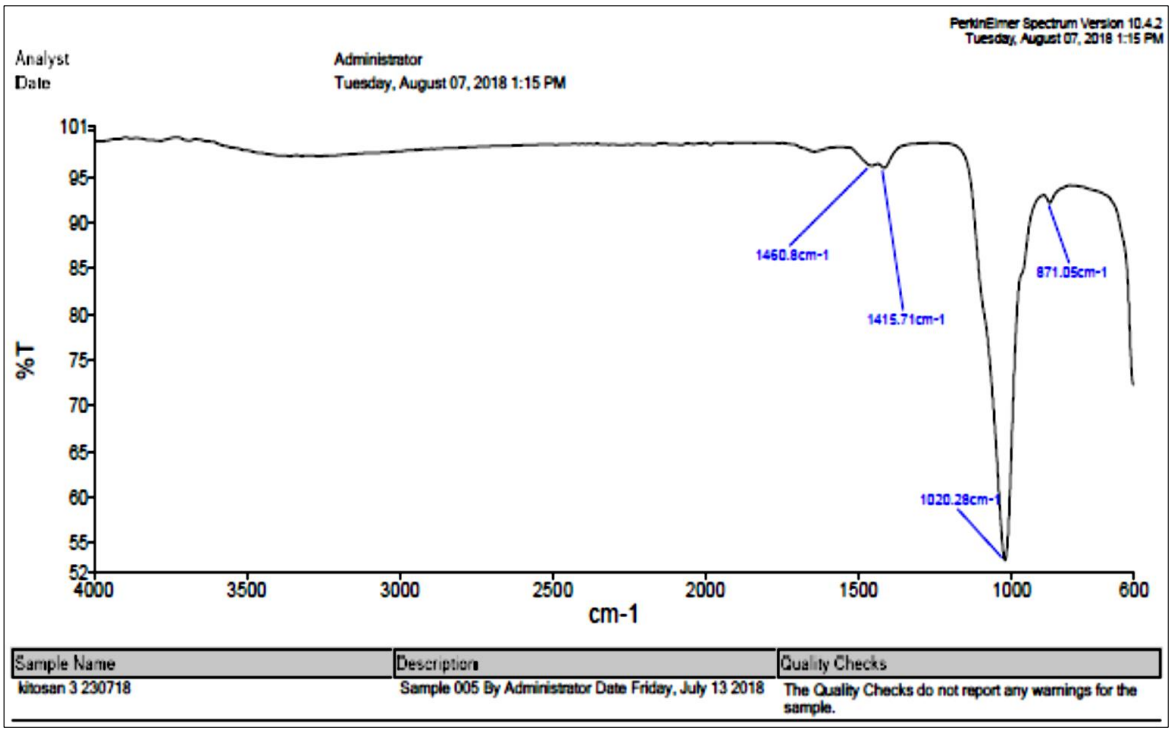


Fig 4: Chitosan FTIR spectra with 40% NaOH

Figure 4 shows the chitosan FTIR spectra from the deacetylation process with 40% NaOH. Spectra results show that there is an -OH group of chitosan groups shown in wave numbers 1415.71 cm-1, -CH groups at wave numbers 1460.8 cm-1, –CO groups at wave numbers 1020.28 cm-1, groups -CH alkene at wave number 871.05. The calculation of deacetylation degree for this chitosan is 40.19%.

The calculation results of Deacetylation Degrees calculated based on the formula from Domzy and Robert in Hung *et al*., (2002) [2] are listed in table 4.

Table 4: Calculation Results of Deacetylation

No	Process	Deacetylation Degree (%)
1	Chitosan 1	74
2	Chitosan 2	47,58
3	Chitosan 3	40,19

The deacetylation degree (DD) value of chitosan 1 is 74%, chitosan 2 is 47.58%, chitosan 3 is 40.19. This value shows that chitosan that meets the standard is chitosan 1, the minimum quality parameter of chitosan DD is 70%. This is because the smaller the NaOH concentration in the deacetylation degree has not been able to remove the acetyl group in chitin.

Conclusion

1. Synthesis of chitosan from stary tigger fish skin was carried out in three stages: deproteination with 10% NaOH, demineralization with 0.63 M HCl, and deacetylation using NaOH with variations in 60%, 50%, and 40% NaOH concentrations.
2. The results of characterization with FTIR showed that chitosan was best obtained from the deacetylation process with 60% NaOH with 74% deacetylation obtained with a good DD chitosan standard of at least 70% DD. DD on deacetylation with 50% NaOH was 47.58 and DD with 40% NaOH was 40.19.
3. The results of chemical testing of the quality of chitosan with 60% NaOH solution obtained moisture content is 6.02%; ash content of 88.50%; 2.29% protein.

Acknowledgement

The authors acknowledge the kindness and co-operation of the inforsmal and local administrators in the study area, and supported by the Politeknik ATK Yogyakarta, Indonesia.

References

1. (AOAC) Association of Official Analytical Chemist. Official Methods of Analysis (18Edn). The Association of Official Analytical Chemist. Inc. Mayland. USA 2005.
2. Hung S, Peh KK, Khan TA. Reporting Degree of Deacetyllation Value of Chitosan: The Influence of Analytical Methods, J. Pharm. Pharmaceut. Sci 2002;5(3):202-212.
3. Jumadi. Karakteristik Adsorpsi Cr (III) dengan Adsorben Kitin dan Humat Kitin Dalam Sistem Fluidized Bed. *Tesis*. Jurusan Kimia FMIPA UGM. Yogyakarta 2006.
4. Khan SH, Bhatti BM. Effect of feeding cooked hatchery waste on the performance of broilers. Pakistan Vet. J 2002;22(1):27-30.
5. Muzarelli RAA. Chitin In GO Aspinall, Polysaccharides. (Vol 3). Academic Press. New York 1985.
6. Rochima E. Karakteristik Kitin dan Kitosan Asal Limbah Rajungan Cirebon, Jawa Barat. Universitas Padjajaran. Bandung 2007.
7. Sugiyo. Studi Pemanfaatan Kepiting Hijau (*Scylla serrata*) Sebagai Adsorben Untuk Ion Logam Cobalt (III)

dan Nikel (II) Dalam Medium Air. Tesis. Jurusan Kimia FMIPA UNY. Yogyakarta 2001.
8. Suhardi. Buku Monograf Khitin dan Khitosan. Pusat Antar Universitas, Pangan dan Gizi Universitas Gadjah Mada. Yogyakarta 1992.
9. Talumepa, Anggun CN, Suptijah Pipih, Wullur Stenly, Rumengan, Inneke FM. Kandungan Kimia Dari Sisik Beberapa Jenis Ikan Laut. Jurnal LPPM Bidang sains dan Teknologi 2016;3:1.