THE EFFECT OF TIME DEACETYLATION TO CHARACTERIZE CHITOSAN FROM WASTE SHRIMP

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ABSTRACT
This research aims to look at the effect of time deacetylation of the chitosan characterization of the results of the utilization of waste shrimp. The method was used in this research is an experimental method to the treatment of different deacetylation time. Observation results include moisture, ash, fat and protein are shown in figures and tables. The content of chitosan in the shells of 40.35% to the deacetylation for 3 hours, 37.27% to deacetylation for 4 hours and 33.15% to the deacetylation for 5 hours. The characterization results obtained are water content of 75.10% for shrimp waste, 15.26% to chitosan with deacetylation for 3 hours, 10.15% to deacetylation for 4 hours and 9.66% for the deacetylation for 5 hours, ash content of 5.02% for shrimp waste, 3.18% of chitosan by deacetylation for 3 hours, 1.14% to deacetylation for 4 hours and 0.10% to deacetylation for 5 hours. At 2.47% fat content for shrimp waste, 2.02% for chitosan with deacetylation for 3 hours, 1.71% for deacetylation for 4 hours and 0.29% to deacetylation for 5 hours. The protein content of 14.85% for shrimp waste, 3.34% for chitosan with deacetylation for 3 hours, 1.93% for deacetylation for 4 hours and 0.69% for the deacetylation for 5 hours. The characterization results that meet the protant laboratory standard Inc. chitosan is ash, fat and protein, while the water content does not meet the standards.

Degree of deacetylation of 83.25% on deacetylation for 5 hours. Chitosan typical uptake seen in the results of chemical tests FTIR with wavenumbers 1,666.50 cm⁻¹.

The effect of time deacetylation of the chitosan are very significant characterization in accordance with the test results analysis of variance.

Keywords: Characterization; Chitin; Chitosan; Process; Shrimp waste
1. INTRODUCTION

The shrimp is a non-oil export commodities reliable and high economic value. Indonesian shrimp are generally exported in frozen form has been thrown head, tail and skin. Until now the waste is not processed and utilized. New utilization used as animal feed, causing environmental pollution in particular odor and poor environmental aesthetics (Kurniasih and Kartika, 2011).

The potential shrimp waste reached 56,200 tons per year (Direktorat Jenderal Perikanan Tangkap, 2009). Based on data from the Minister of Maritime Affairs and Fisheries, Indonesia's shrimp exports reached 240-250 tons or 27.29% of the 881,413 tons of fishery exports. Total shrimp export value reached US$ 1.576 billion. In 2014, Indonesia became the largest shrimp producer and exporter of the world (Ministry of Marine Affairs and Fisheries, 2011). The larger the shrimp production, the greater the waste from the shrimp production.

Shrimp waste can be obtained from the shrimp processing industry. The shrimp waste generally consists of the heads, tails and shrimp peel into small little besides shrimp meat. Weight shrimp waste 30-40% by weight of shrimp. Thus, the number of parts discarded from shrimp processing business is quite high. However, the waste that is easily obtainable and available in such large quantities, has not been used optimally. Commodities shrimp in Moluccas with the production value in 2012 amounted to 8,091.4 tons, mostly exported in the form of frozen shrimp and about 80% in the form of a headless (headless) or without bark (peeled) (Maluku in Figures, 2013). The shrimp exports in 2013 amounted to 2,881 tons of total shrimp exports are expected to head and shrimp shell waste reaches 1 ton. In line with the development of the shrimp export value of shrimp shell waste and the head of this will be greater in number so that its utilization should be improved. As evidence, it was reported that Wahana Lestari Investama Private Company, every day can produce 5 tons of waste shrimp waste is usually at IDR 5,000 per 50 kg, and even many wasted. Waste freshly made shrimp in their utilization for the purposes of flour to feed (Puri, 2015). Shrimp shell waste that so far only used for animal feed, can be processed to manufacture chitin which can be further processed to produce chitosan.
Shrimp waste containing protein, chitin and calcium carbonate. The content of chitin in shrimp waste reached 42-57%, the crab waste reached 50-60%, squid and mussels for 40% and 14-35%. Because the shrimp raw materials are easier to obtain the synthesis of chitin and chitosan more use of waste shrimp (Yurnaliza 2002).

Chitin is an N-acetyl-D-glucosamine with β-1,4 bond that is widely available in nature, especially in crustacean shells (Figure 1). The content of shrimp shell chitin can achieve 40-60% dry weight of his body. Chitin obtained from various sources has the same structure, except its binding with proteins and calcium carbonate which is two (2) other components of the shells (Helda and Dodi, 2014). Chitin is very difficult to dissolve in water and some organic solvents. Low reactivity and highly hydrophobic chemicals, causing the use of chitin relatively less developed compared to chitosan and its derivatives (Kaban, 2009). When the deacetylation of chitin experienced either chemically or enzymatically will produce chitosan. Chitin, chitosan and its derivatives is a biopolymer that has great potential to be developed in Indonesia given the broad usefulness ranging from the fields of medicine, food industry, pharmaceuticals, cosmetics, agriculture and others. That is because chitosan and its derivatives have special properties in terms of biocompatibility, biodegradability, biology activity, non-toxic, which is not cause allergies and ability to form fibers and films (Wibowo et al, 2005).

Chitosan is a natural biopolymer with the second largest abundance of cellulose, a product of deacetylation of chitin either through chemical reaction or enzymatic reaction. These compounds can be found in the shells of shrimp, crab, clams, insects, annelids and some cell walls of fungi and algae. Chitosan is composed of units of N-acetyl glucosamine and N glucosamine (Figure 2). Results modified chitosan produce nature and specific benefits, that is the nature of the bioactive, biocompatible, chelating, anti-bacterial and can biodegraded with reactive groups amino atom C-2 and the hydroxyl groups of atoms C-3 and C-6. Seeing hydrophilic properties, chemical reactivity, the ability to form films
and good mechanical properties, the chitosan is a good material for use in various fields of application (Kaban, 2009). For the application, chitosan have a role as a preservative and stabilizer color fishery food products, as flocculants and help the process of reverse osmosis in water treatment, additives for agrochemical products and seed preservation (Muzzarelli et al, 1997).

Chitin and chitosan difference lies in the comparison group of amines (NH₂) with an acetyl group (-OCH₃) called the degree of deacetylation. According of Thate (2004) in his dissertation, noted that chitosan has a degree of deacetylation of more than 70%, whereas the degree of deacetylation of chitin is less than 70%. According to the description, can be synthesized chitin into chitosan by replacing the acetyl group into the amine group (deacetylation process).

The stage transformation deacetylation chitin into chitosan to provide treatment with highly concentrated alkali. Deacetylation reaction was to decide the acetyl group (-HCOCH₃) attached to the nitrogen in the compound chitin structure to increase the percentage of the amine group (-NH₂) on chitosan. Deacetylation reaction was shown in Figure 3.

Chitosan is produced commercially on a large scale in various parts of the world, including Japan, North America, Poland, Italy, Russia, Norway, and India (Zargar et al, 2015). Chitosan was triggered many requests that be unique for biological characteristics such as biodegradability, biocompatibility and non-toxic, thus enabling applications in various fields (Sheng et al., 2011). Although, chitosan very abundant in nature, but the low utilization of the new chitosan developed in the last two decades (Zargar et al, 2015). Chitosan is now widely used in food, pharmaceutical, medical, textile, agriculture and other industries, for example the purification of waste. In recent years, chitosan attracted much attention because it shows antimicrobial activity against fungi, bacteria, and
viruses. Commercial applications of chitosan commercial activities include its use as a food preservative, anti-infective drugs and microbial-free textiles.

Nowadays the country that uses a lot of chitin and chitosan products are Japan, about 700 tons/year and the United States about 500 tons/year. Because the benefits of chitin and chitosan as well as the availability of raw materials shrimp shells galore, even a waste that can pollute the environment and damage the aesthetics of the environment, and these compounds need to get the most attention, especially in terms of utilization efforts, research processes and products, as well as counter measures pollution.

![Figure 3. Reaction Mechanism of Deacetylation (Transformation Chitin).](image)

Chitosan has many benefits in the areas of industry, such as food preservatives are harmless (non-toxic) substitute for formalin (Goosen, 1997). Chitosan in Indonesia is the result of importing from India, Korea, and Japan. Indonesia as the country's shrimp providers should be able to treat waste generated shrimp into chitosan because it is cheap and relatively easy manufactured (Pratiwi et al., 2008 in Dompeipen, 2015). Levels of
chitin in shrimp weight around to 60-70% and when processed into chitosan yielding 15-20% (Marganof, 2003).

Lately, chitosan widely used in a variety of industries with industrial waste reason seafood is so big and need to be processed into something useful than that because the properties of chitosan that is non-toxic and biodegradable (Suhardi, 1992). Application of chitosan in various areas determined by the characterization, which includes the intrinsic nature of the degree of deacetylation, solubility, viscosity, and molecular weight. Type the source of the chitin (raw materials) to determine the characteristics of chitosan and derivatives produced. Physical and chemical structure of chitin and chitosan are very varied, among other things depending on the position of the chain of N-acetyl glucosamine, degree of deacetylation and crosslinking structural components with other components such as protein and glucose (Svitil et al., 1997). In addition to the raw materials, the quality or characteristics of chitosan is also determined by the production process (Kaban, 2009). In addition, chemical modification of chitosan continue to be developed more actively to explore new product applications. This effort will make chitosan into a multi-use material that can be applied to the field of pharmaceutical, health, biotechnology, food, sewage treatment, cosmetics, agro-industry, textile industry, and other industries (Uragami and Tokura, 2012). The application of chitosan in various areas is supported by the quality of chitosan can be seen from its intrinsic properties, namely its purity, molecular mass, and degree of deacetylation. Generally chitosan has a degree of deacetylation of 75-100%.

The rapid interest in exploring chitosan, chitosan has proved that prospects so promising. Economically, the chitosan form of glucosamine polymer or monomer form, is cheaper than its derivatives, such as carboxymethyl chitosan citrate. Shrimp shell price IDR 5,000 for 50 kg, while the price of chitosan IDR 150,000 for 250 g (CV. Chimulti, 2016). Therefore, chitosan and its derivatives has enough potential to be developed in Indonesia, given the availability of waste as raw material is abundant (Puri, 2015), low production cost and environment friendly (Hasri, 2007). The aim of this research is to find the effect of time deacetylation of the chitosan characterization of the results of the utilization of waste shrimp.
2. RESEARCH METHOD

2.1. Materials and tools

The main material used is waste shrimp shells. The chemical material used is sodium hydroxide, hydrochloric acid, acetone and distilled water. The equipment used in this study include knives, basins, scales, a set of glasses, a stirrer, a thermometer, oven, vacuum pump, burettes, coarse filter paper or fabric filter, hot plate and water bath.

2.2. Method

The method used in this study is the experimental method. The process of making the chitosan in accordance with Sukardjo and Mawarni (2011) with slightly modified, namely at the stage of deacetylation with a different time, i.e. D3, D4, and D5 are degree of deacetylation treated for 3, 4, and 5 hours, respectively. In addition, the commercial chitosan (KK), shrimp waste (KU), and Protan Laboratory Standar Inc (S) were also used in this research.

2.3. Parameter test

In this study was observed objectively covers yield, moisture, ash, fat, protein and degree of deacetylation. Moisture, ash, fat and protein were analyzed according to SNI 01-2891-1992, the deacetylation degree in chitosan treatment appear to approach or meet standards. Data in this research were analyzed descriptively and presented in tables and figures.

3. RESULTS AND DISCUSSION

Chitosan product results in this study has a different yield. The results yield chitosan are shown in Table 1.

| No. | Treatment | Yield Percentage |
|-----|-----------|------------------|
| 1.  | D3        | 40.35            |
| 2.  | D4        | 37.27            |
| 3.  | D5        | 33.15            |
Table 1, shows that it has gone through four stages of the process for the manufacture of chitosan are deproteinization, demineralization, bleaching and deacetylation. At this stage of deproteinization which aims to break the bond between the protein and chitin by adding sodium hydroxide. Through deproteinization phase, proteins are extracted in the form of Na-proteinat, where the Na\(^+\) ions bind to the end of the negatively charged protein chain so as to precipitate. Chitosan is obtained by deacetylation of the chitin process. Deacetylation is the process of converting the acetyl group (-NHCOCH\(_3\)) on chitin into an amine group (-NH\(_2\)) on the addition of high concentrations of NaOH. Chitin deacetylation reaction is essentially an amide hydrolysis reaction of \(\alpha\) (1-4) -2-acetamide-2-deoxy-D-glucose. Mahatmanti (2001) suggested that the hydrolysis reaction of chitin like Figure 3. Reflux in this process produce a gel. By the fourth process resulted in a severe reduction of waste shrimp were due to the removal of minerals and proteins contained in the shrimp waste. Chitosan product produced in this research can be seen in Figure 4.

Figure 4. Chitosan product of this research.

Characterization of chitosan and shrimp shells include: moisture, ash, fat and protein compared with commercial chitosan and standard laboratory Protan Inc. Chitosan structure test results of studies done by FTIR.
Based on Figure 5, the acquisition of chitosan lower water content shrimp shells. This is due to the drying process of shrimp shell waste before made of chitosan, while shells not done drying. With the reaction of chitosan with sodium hydroxide that are hygroscopic lead compound chitin in shrimp shell waste transformed into chitosan. Therefore the process of drying and reaction with sodium hydroxide, then the water content contained in the shells to be low.

Chitin have the binding force of the water molecules is stronger than chitosan. More the number of water molecules bound to the shrimp shell chitin is used as a compound, as compared to the chitosan may be caused by the persistence of the protein that surrounds the chain chitin because the cluster peptide potential protein chains bind water molecules. After going through the deacetylation with strong base solution concentration and high temperature, then the proteins that still surrounds chitin chains apart and cause the water holding capacity of the chitosan molecules decreases. As said Lesbani et al., (2011) that chitosan water content is smaller than shrimp shell due to chitin transformation process into chitosan using sodium hydroxide which is hygroscopic compound so chitosan water content is smaller than shrimp shell.

The water content of chitosan research 15.26% (D3), 10.15% (D4) and 9.66% (D5) is greater than the commercial chitosan at 9.45% and 9.28% Protan laboratory standards. It is possible during the drying process of chitosan, the temperature and time of drying used in this study have not been optimized so the high moisture content in the product of chitosan.
The results of ash content in Figure 6 shows that the ash content of 5.02% shrimp shells, chitosan amounted to 3.18% (D3), 1.14% (D4) and 0.10% (D5). Ash is an indication of component inorganic compounds contained in the raw material chitin and chitosan. The ash content is a measure of the success of the demineralization process of the raw material chitin isolation (Dewi and Fawzya 2006 in Dompeipen dkk, 2015). The ash content can be used as a quality parameter of chitosan. The lower the ash content, the higher the degree of chitosan purity, and vice versa. The results showed that the ash content in the presence of demineralization waste shrimp shells into chitosan, the minerals contained in shrimp shells decreases. Mineral loss in shrimp shell waste is indicated by ash from 5.02% to 3.18%, 1.14% and 0.10%. In accordance with the opinion of Lekka et al., (2001) which stated that inorganic minerals can be removed by treatment of acids and bases.

Lesbani et al., (2011) the stated that the ash content indicates oxides of metals and minerals contained in a material. The high ash content of a material to identify the high content of oxides of metals and minerals contained in these materials. The ash formed an oxides metal or metal fire. Ash content 3.18% chitosan research (D3), 1.14% (D4) and 0.10% (D5) is greater than the commercial chitosan at 0.48% and Protan standard of <0.5%. It is possible that the minerals contained in chitosan still a lot on the treatment D3 and D4. For the treatment of D5 has lost mineral accordingly, so that the resulting ash content is smaller than the standard commercial and Protan chitosan.
The results of the fat content in Figure 7 shows that the presence of demineralization and deacetylation shrimp waste into chitosan, the minerals contained in shrimp shells decreases. Mineral loss in shrimp shell waste is indicated by the fat content of 2.47% to 2.02% (D3), 1.71% (D4) and 0.29% (D5). As said by Aldes et al., (2011) that the presence of demineralization caused the removal of mineral and oxides in material. With the loss of minerals and oxides in the material causes decreased fat content in the material.

Chitosan fat content of research results for 2.02% (D3), 1.71% (D4) and 0.29% (D5) is smaller than the commercial chitosan at 2.31%. These results compared to standard laboratory Protan were greater for D3 and D4 treatment, while identical result for the D5 treatment.

Figure 7. The fat content derived from chitosan product.

Figure 8. The protein content derived from chitosan product.
The results in Figure 8, protein content of 14.85% shrimp shells. The protein content of chitosan by 3.34% (D3), 1.93% (D4) and 0.69% (D5). The value of protein obtained show that the presence of deproteinization process waste shrimp shells into chitosan, the protein in shrimp shells decreases. Decreased protein in shrimp shell waste into chitosan indicated by the protein content of 14.85% to 3.34% (D3), 1.93% (D4) and 0.69% (D5).

As said by Sukardjo and Mawarni, (2011) that the higher the concentration of NaOH and temperature, protein separation processes more effective. The concentration of NaOH used in this research was 3.5% NaOH. The protein content of the research results 3.34% (D3), 1.93% (D4) and 0.69% (D5 larger than the commercial chitosan at 0.48% and Protan standard <0.5%.

From the results obtained for the water content, ash, fat and protein look very significant difference for deacetylation process 3 hours, 4 hours and 5 hours. In the deacetylation process within 5 hours, the result meets the laboratory protan standard compared to the deacetylation process at 3 hours and 4 hours. This is due to the deacetylation process of 5 hours of acetyl group in chitin completely decomposed into chitosan.

Chemical test results chitosan by using FTIR in order to identify the structure of chitosan production, in particular the functional groups of organic compounds. Chitosan was tested by FTIR is chitosan in the treatment of deacetylation in 5 hours. IR spectrophotometry provide maximum peaks as clear as good as the minimum peak, as shown in Figure 9.

Based on the FTIR spectra, it can be estimated that there has been a change of chitin into chitosan. Chitosan obtained spectra are shown in Figure 9, inform their absorption bands at wave number 3,591.46 cm\(^{-1}\) as a result of vibration range of the group -OH. Absorption width and shift wave number-OH group is due to the overlap with the NH group of amina. Uptake at wave number 2831.50 cm\(^{-1}\) indicates a group of alkane C-H stretching vibration of demonstrating -CH\(_2\) group. The loss of a methyl group (-CH\(_3\)) are tied to the amide (-NHCOCH\(_3\)) can be determined from the loss of absorption at the wave number 2,962.66 cm\(^{-1}\) and the loss of group C=O amide (-NHCO-) is known from the loss absorption band contained in wavenumber 1,581.63 cm\(^{-1}\) and 1,508.33 cm\(^{-1}\). Typical uptake of chitosan look at wave number 1666.50 cm\(^{-1}\) shows the N-H bending vibration
of the amine (NH$_2$) (Silverstein et al., 1981). Absorption band at number 1045.42 cm$^{-1}$ shows the stretching vibration -C=O- group. Sharp uptake at wave number 894.97 cm$^{-1}$ showed that mineralsilika still present on chitosan despite a lower intensity.

Degree of deacetylation (DD) is the percentage of acetyl groups were successfully removed during the process deproteination chitin, where chitin by treatment by adding NaOH 60% are caused hydrolyzed acetyl group of acetamide group in chitin. The degree of deacetylation can be determined from the spectrum of IR absorption spectroscopy with the base-line method. The highest peak is recorded and measured from the base line is selected. Comparison of wave numbers between the absorption band of amide (1,655 cm$^{-1}$) with hydroxy absorption band (3,450 cm$^{-1}$). Results DD of chitosan with a deacetylation treatment for 5 hours (D5) of 83.25%.

Figure 9. Chitosan FTIR results.
4. CONCLUSION

Based on the results of this study concluded that the shrimp waste containing chitosan amounted to 40.35% in the deacetylation process for 3 hours, 37.27% in deacetylation for 4 hours and 33.15% in deacetylation for 5 hours. Typical IR absorption spectra of chitosan look at wave number 1,666.50 cm⁻¹, showed the presence of NH₂ uptake which proves the process of deacetylation. Chitosan is best contained in the deacetylation process time for 5 hours, with water content 9.66%, 0.10% ash, 0.29% fat, 0.69% protein and 83.25% degrees deacetylation. The characterization results are compared to a standard chitosan Protan Laboratory Inc., which meet is the ash, fat, protein and degree of deacetylation, while the water content does not meet the standards. The effect of time of deacetylation of the chitosan characterization results are very significant corresponding test results Analysis of Variance.

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