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Production and characterization of Chitosan from shrimp shells waste

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Abstract. This research aims to study the production of chitosan from shrimp shell waste origin and characterize the chitosan quality of shrimp shell waste that includes parameters yield, solubility, intrinsic viscosity, molecular weight and deacetylation degree. Results showed that the treatment of heating temperature and heating time in the process of deacetylation caused significant ($P \leq 0.01$) influence on yield, solubility, intrinsic viscosity, molecular weight and deacetylation degree of produced chitosan. Whereas, the interaction between heating temperature and heating time on the process of deacetylation gave no significant effect on yield, solubility, intrinsic viscosity, molecular weight and deacetylation degree of chitosan produced. The best results in this study were obtained from heating temperature of 100°C and 80 min heating time.
Key Words: Chitosan, heating temperature, heating time, deacetylation degree.

Introduction. Shrimp is one of important fisheries product world wide including Indonesian, this product is mostly exported in frozen condition that has undergone a process of separation of the head and skin (carapace). This separation process will lead to adverse effects of solid waste, it would result in environmental pollution in the form of bad odor and aesthetic damage the environment. However, this fishery by product has an economical value for the chitin and chitosan industry (Budiyanto 1993).

Chitin is biopolymers composed of units of D-glucosamine N-asetil with β (1-4) bound are most often found in nature after cellulose (Patil et al 2000). This biopolymer is a compound result of deacetylation of chitin, composed of units of N-acetyl glucosamine and N glucosamine. The presence of reactive amino groups at C-2 atom and the hydroxyl group at atom C-3 and C-6 on chitosan are useful in a wide application in various industries such as pharmaceuticals, biochemistry, biotechnology, cosmetic, biomedical, paper industry, food and textile industries, and others. Beside that chitosan can be used as an emulsifier, coagulation, chelating agent, and thickener emulsion. In addition, chitosan can also be used as a substitute for formalin to preserve food that serves a relatively safe for consumption (Muzzarelli 1985).

Application of chitin and chitosan in various fields is determined by the characterization of quality includes the deacetylation degree, solubility, viscosity, and molecular weight. Quality of chitosan is mainly determined by the degree of deacetylation, where the deacetylation degree is depending on materials and conditions process such as concentration of alkali solution, temperature, and time (Suhardi 1993).

The objective of the present study was to obtain the optimum conditions of temperature and time of the deacetylation of chitin into chitosan from shrimp shell waste. This study is very important especially for utilization of shrimp waste into economically valuable products.

Material and Method

Experimental design. The randomized design group (RDG) of 3 x 4 with two factors was utilized in this study. The first factor was heating temperature of deacetylation with four levels, namely: 60, 70, 80, and 90°C. The second factor was the heating time of the

deacetylation with three levels *i.e*. 40, 60, 80 (minutes). Combination treatment was 3 x 4 = 12 unit with 2 replications, resulted in 24 experimental units.

Sample preparation. Shrimp shells were collected from the market of Banda Aceh city, Indonesia. The collected shrimp shells were washed with distilled water, and then the samples were sun light dried for 24 h and further dried on the furnace at a temperature of 80°C for 24 h. The dried samples were blended and sieved to 80 meshes. Approximately 20 g of shell powders were used for further analysis.

Phase deproteination. A total of 20 g samples of shrimp shell powders were added to 3.5 % sodium hydroxide as much as 1:5 (w/v). Excerpts stirred over heat and left for 1 hour at 90°C, then the solution was filtered and the residue was washed with tap water until neutral pH, then after the residue was re-dried in a furnace at a temperature of 60°C for 4 hours and resulted in chitin powder.

Stage of demineralization. Chitin powder result of deproteination is then added with 2-N-hydrochloric acid in the ratio 1:5 (w/v), allowed to stand for 1 hour at 90°C to separate the residue from the filtrate, and then the residue was washed with distilled water until neutral pH, then dried in a furnace at 60°C for 4 hours.

Stage of depigmentation. The demineralization of chitin extracted with acetone 1:5 (w/v) for 4 hours in soxhlet, then the residue is bleached with 0.32 % sodium hypochlorite for 5 min at room temperature, then the residue was washed with distilled water until neutral pH and dried in an oven at 60°C for 4 h.

Deacetylation of chitin into chitosan. A total of 5 g of chitin were reacted with 50 ml of 50 % sodium hydroxide, then heated using a hot plate with various temperature (70, 80, 90 and 100°C for 40, 60, and 80 min, respectively), then filtered and the residue was washed until neutral pH and then dried in a furnace with a temperature of 60°C for 4 h.

Statistical analysis. All data were subjected to two-ways analysis of variance (Two-ways Anova), followed by comparison of means using Duncan's multiple range test (Zar 1984). All statistical analyses were performed using SPSS.

Results and Discussion

Yield and solubility. Chitosan yield were ranged from 50.39 to 88.25 % with average of 67.42 %, while the chitin yield was 40 %. The statistical analysis showed that the effect of the heating temperature and heating time of the deacetylation on the chitosan yield were highly significant ($P<0.01$), however the interaction effect between the two factors were not significant ($P>0.05$). Effect of heating temperature of deacetylation on chitosan yield can be seen in figure 1, and the heating time effect of deacetylation on chitosan yield can in figure 2. The Duncan's multiple range tests showed that the highest yield was obtained at heating temperature of 70°C (83.73 %), it was significantly different with 80°C, 90°C and 100°C, while the lower value was found at 100°C (54.65 %). In general, yield values of chitosan were decreased with increasing of heating temperature, probably due to the high temperature will cause the molecular chains of chitosan depolymerization process that eventually then cause decreases in molecular weight of chitosan.

The solubility of chitosan is one of important parameter for quality of chitosan, where higher solubility will produce a better chitosan (Winarti et al 2008). The solubility of chitosan obtained in this study was ranged from 17.43 % to 95.29 % with average of 57.52 %, while the average solubility of chitin was 20 %. The statistical analysis showed that the heating temperature and heating time of deacetylation were significantly affect on production of chitosan ($P<0.01$), however interaction between these factors were not affect significantly ($P>0.05$). Effect of heating temperature of deacetylation on chitosan solubility can be seen in figure 3, and the heating time effect of deacetylation on chitosan solubility in figure 4.

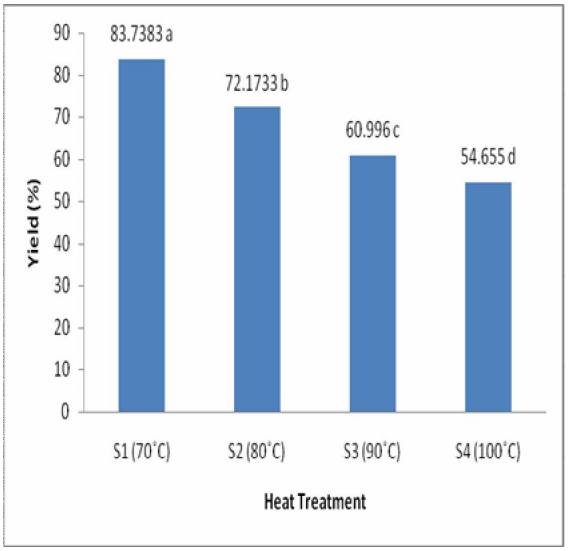

Figure 1. Effect of heating temperature of deacetylation on chitosan yield.

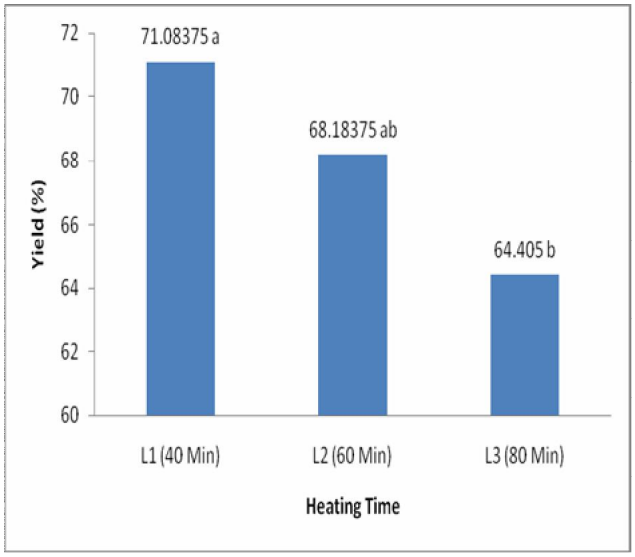

Figure 2. Effect of heating time of deacetylation on chitosan yield.

The solubility of chitosan in acetic acid solution is influenced by temperature and duration of immersion in NaOH solution. Acetic acid is classified as a weak acid class of carboxylic acid containing carboxyl group (-COOH). Carboxyl group contain carbonyl and a hydroxyl group. The boiling point is reached at 118°C and the smell is very sharp (Fessenden & Fessenden 1986). Proportionally increase in solubility is observed with increasing deacetylation degree. This is due to the acetyl group in chitin deacetylation process which will be cut by leaving amine group. H ions on the amine group makes chitosan to easily interact with water through hydrogen bonding. Properties of chitosan is only soluble in dilute acid, such as acetic acid, formic acid, citric acid has been substituted except that chitosan water soluble. Presence of carboxyl group in acetic acid would facilitate the dissolution of chitosan due to the hydrogen interaction between the carboxyl and the amine group of chitosan (Li et al 1997).

In acid solution, free amine group is suitable as polikationic for chelating metal or dispersion. Due to the acidic chitosan polymer with a structure will be straight so it is very useful for flocculation, forming a film or immobilize enzyme (Winarti et al 2008). This is supported by Sanford (1989) in an acid, free amine group of chitosan will be protonated to form cationic amino group (NH_3^+). Cations in the chitosan when reacted with anionic polymers will form a complex electrolyte.

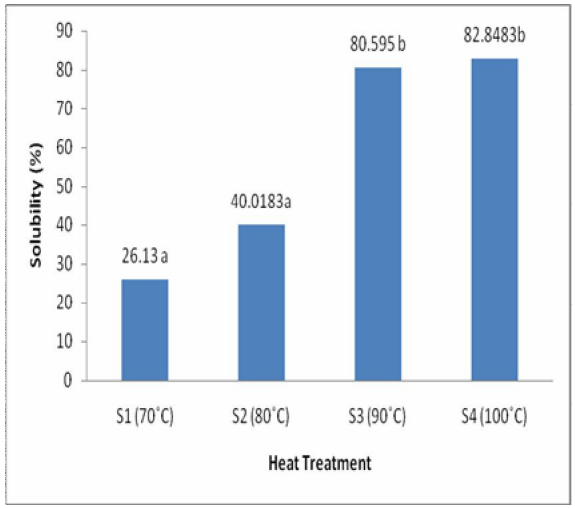

Figure 3. Effect of heating temperature of deacetylation on the solubility of chitosan.

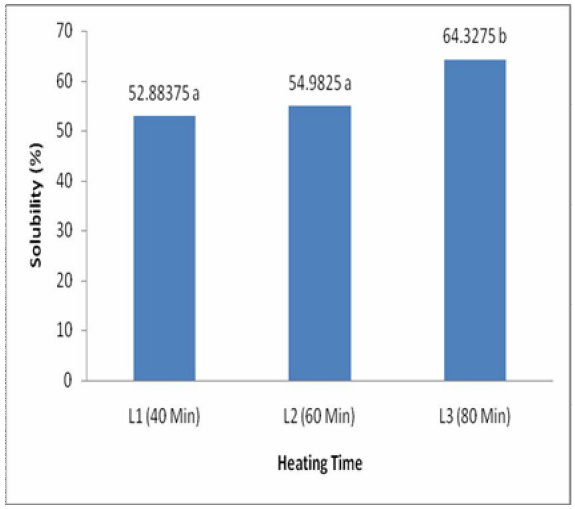

Figure 4. Effect of heating time of deacetylation on chitosan solubility.

Intrinsic viscosity. The quality of chitosan is depending on the intrinsic viscosity, as to find the molecular weight of chitosan intrinsic viscosity value is required, while the intrinsic viscosity is closely related to molecular weight and deacetylation degree. Moreover the study showed that the increase of viscosity is mostly influenced by the rise temperature rather than an extension of time. The increase in viscosity is due to the high acetyl content in chitosan, so the higher the temperature rises, the more soluble is the acetyl, thus increasing the deacetylation degree and viscosity increases (becomes thick like a gel) with increasing temperature. The longer of chitosan deacetylation then higher depolymerization that decreases the viscosity and molecular weight (Bastaman 1989).

Intrinsic viscosity of chitosan obtained in this study ranged from 172.3 to 196.8 ml/g with average of 184.57 ml/g, while the intrinsic viscosity of chitin was 150.9 ml/g. The analysis of variance showed that the heating temperature and heating time of the deacetylation process has affect significantly the value of intrinsic viscosity ($P<0.01$), however, the interaction effect between the two factors was not affected significantly ($P> 0.01$). Effect of heating temperature of deacetylation on the intrinsic viscosity can be seen in figure 5 and the heating time effect of deacetylation on the intrinsic viscosity in figure 6.

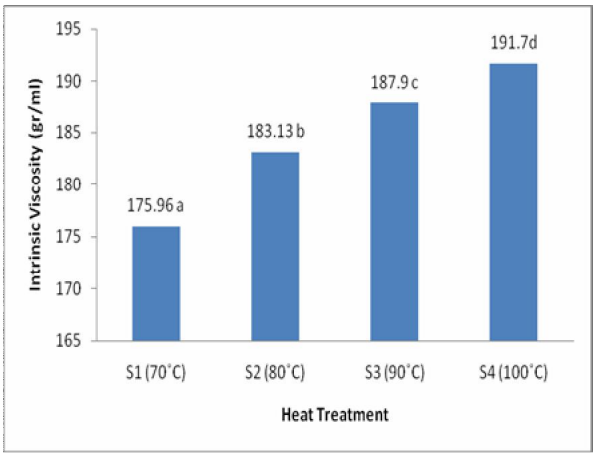

Figure 5. Effect of heating temperature of deacetylation on the intrinsic viscosity.

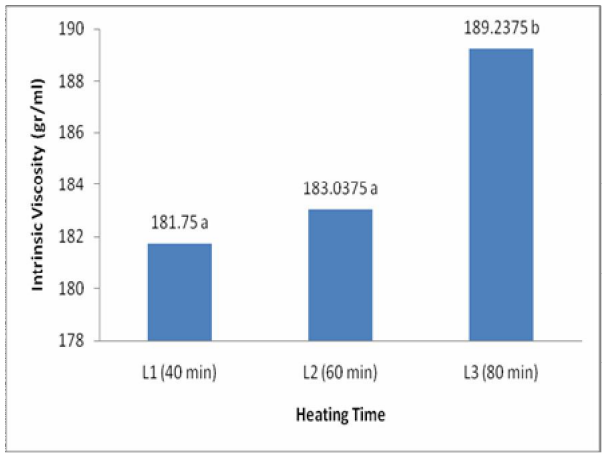

Figure 6. Effect of heating time of deacetylation on the intrinsic viscosity.

Molecular weight. The molecular weight of chitosan is one parameter that can be used as a standard of quality. Due to the lower molecular weight chitosan is appropriately applied as an anti-bacterial, antioxidant and anti-tumor substance. Chitosan having a medium molecular weight is turned out to have anti-cholesterol activity which is higher than the high molecular weight chitosan (Brzezinski et al 2004).

Molecular weight of chitosan obtained in this study ranged from 81948.39 to 110,967.40 g/mol, average molecular weight of chitosan is 92036.83 g/mol, while the molecular weight of chitin is 150.898 g/mol. The results of variance showed that the heating temperature, heating time on the process of deacetylation and interaction between them gives significant effect ($P\leq0.05$) on the resulting molecular weight of chitosan. Effect of heating temperature of deacetylation on the chitosan molecular weight can be seen in figure 7, and effect of heating time of deacetylation on the chitosan molecular weight in figure 8.

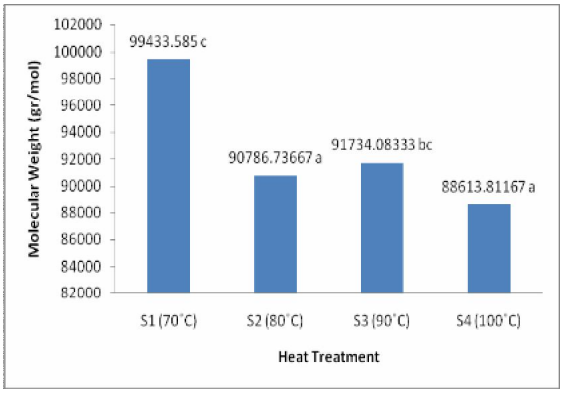

Figure 7. Effect of heating temperature of deacetylation on the chitosan molecular weight.

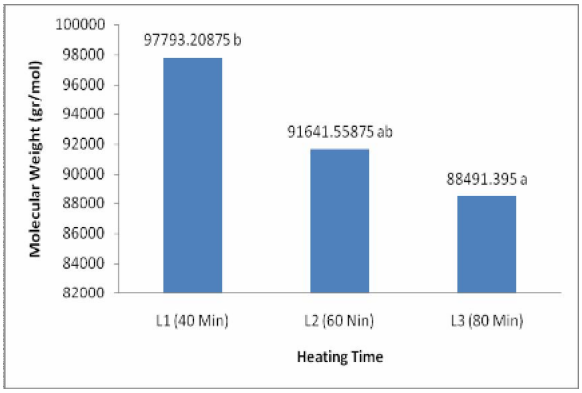

Figure 8. Effect of heating time of deacetylation on the chitosan molecular weight.

Deacetylation degree. Deacetylation degree of chitosan is the most important quality parameter in this study. The deacetylation degree showed that the percentage of acetyl groups can be removed from the chitin to produce chitosan. High deacetylation degree showed that the acetyl groups contained in the chitosan is low. The less chitosan acetyl groups on the interaction between the ions and hydrogen bonds of chitosan will be stronger (Winarti et al 2008).

Deacetylation degree of chitosan obtained in this study ranged from 76.26 to 91.60 % with average overall deacetylation degree was 82.86 %. The results of variance showed that the heating temperature and heating time on the process of deacetylation of chitosan affects highly significant ($P \leq 0.01$) to the deacetylation degree of chitosan produced, while the interaction between the two does not affect significantly ($P > 0.05$). Effect of heating temperature of deacetylation on deacetylation degree of chitosan can be seen in figure 9 and the heating time effect of deacetylation on deacetylation degree of chitosan can be seen in figure 10.

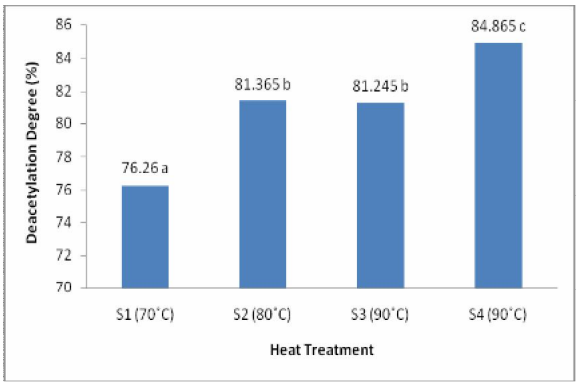

Figure 9. Effect of heating temperature of deacetylation on deacetylation degree of chitosan.

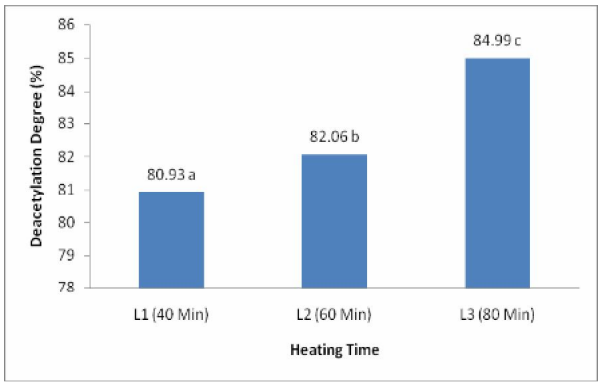

Figure 10. Effect of heating time of deacetylation on chitosan deacetylation degree of chitosan.

Conclusions. Based on the result obtained from research that has been done, it can be concluded that: (i) chitosan yield was 67.42 %, while the chitin yield was 40 %, (ii) the solubility of chitosan was 57.52%, (iii) intrinsic viscosity was 184.57 ml/g, while the intrinsic viscosity of chitin was 150.9 ml/g, (iv) molecular weight of chitosan was 92036.83 g/mol, (v) deacetylation degree of chitosan was 82.86 %.

The best chitosan in this study based on solubility, intrinsic viscosity, molecular weight and deacetylation degree is obtained at a heating temperature of 100°C and 80 min heating time at deacetylation process, while the highest yield is obtained in the

treatment of 70°C heating temperature, and 40 min heating time during the process of deacetylation.

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