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Original paper

UTILIZATION OF CHITOSAN PREPARED FROM SHRIMP SHELL AS FAT DILUENT

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ABSTRACT

Shrimp shell waste from seafood restaurants and canned shrimp industries is potential to be used as chitosan source. This material contains 18.1% of chitin which could be converted into chitosan through demineralization, deproteination and deacetylation process. Chitosan is fine chemical used to adsorb fat from body, heavy metal absorbent, fat diluent and medicine. This research looked into the prospect of chitosan from shrimp shell as fats dilluent. The aims of this research are to study the influence of NaOH concentration on preparing chitosan from shrimp shell, and evaluate the performance of chitosan produced as fat diluent. As indicators, the purity of chitosan and the percentage of fats diluted are measured. This investigation was conducted in two steps involving the preparation of chitosan and the process optimation of fats dillution using chitosan. In this case, the NaOH concentration was varied from 20% to 60% with the step size of 10% to obtain highest quality of chitosan. Whereas, in the second step, the time of fats dilution and chitosan quality were varied to obtain optimum condition of fats dillution. The dillution time was varied from 10 to 30 minutes with incremental of 5 minutes. While, the chitosan quality was varied based the result of preparation step. The results indicated that the highest chitosan quality of 82.45% could be obtained in the percentage of NaOH of 60%. Meanwhile, in the fats dillution process, it showed that the highest percentage of fats dilluted is 96.57% that could be achieved at time dillution of 10 minute and chitosan quality of 82.45%.

Key words: Crab shell, deproteination, demineralization, deacetylation, chitosan

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INTRODUCTION

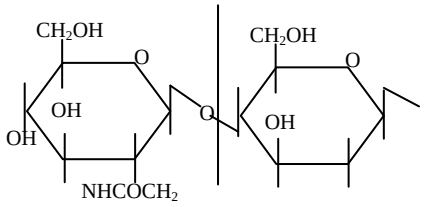
Shrimp shell dispossed from canned shrimp industry has not been maximally exploited. As consideration, one unit canned shrimp industry with product capacity of 10 tons per day could disposse 1-3 tons shrimp shell per days. The shell is part of shrimp that covers 30-40% of total weight of shrimp (Suhardi, 1993). At present, this material is only used as cattle feed which has low economical value. Hence, it need an alternative method to improve the shrimp shell utilization.

One alternative method to utilize shrim shell is as chitin or chitosan. Chitin is a fine chemical in shrimp shell with the percentage of 18.1% (Suhardi, 1993). Chitin could be converted to chitosan through deacetylation process. The primary molecular stucture of chitosan is 2-deoxy-2-(acetylamino) glucose (Suhardi, 1993). This units are combined by 1-4 glycoside linkage, forming a long chain

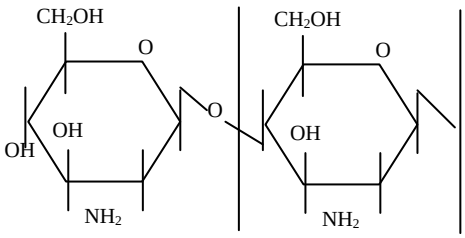
linear polymer. These fine chemical are very useful in chemical industries as a medicine and drugs supplement, fat coagulant, metal adsorption and cosmetic (Hanafi et al, 2000). However, the process from chitin of shrimp shell to chitosan still needs more investigation especially on the process condition to obtain high quality of chitosan product. The aim of this research is to determine optimal condition of chitosan production from shrimp shell and to evaluate the performance of chitosan product to adsorb fat.

Chitin and Chitosan

Chitin is a polymer formed primarily of repeating units of beta(1-4) 2-acetamido-2-deoxy-D-glucose or N-acetyl-glucosamine. Its structure resembles that of cellulose, except that the hydroxyl groups in position 2 have been replaced by acetylamino groups. While the chitosan is a polymer derived from chitin through deacetylation process. The chitosan is formed by repeating units of beta (1-4) 2-amino-2-deoxy-D-glucose or D-glucosamine. Figure 1 shows the chitin and chitosan molecular structure (Suhardi, 1993, and Hanafi et al, 2000).



Chitin



Chitosan

Fig. 1 The Structure of Chitin and Chitosan

Chitin could be isolated from shrimp shell through two steps process namely deproteination and demineralization (Milka et al, 2003). The deproteination is a process to reduce protein content by extraction process using strong alkali solution in the concentration of 3 to 6 N (Hanafi, et al 2000). Occasionally, the alkali used for this process is sodium hydroxide, sodium carbonate, potassium hydroxide or potassium carbonate. However, the use of sodium hydroxide is preferable due to its lower cost (Djaeni, 2003).

After deproteination process, the solid product is washed by aquedest to reduce remaining alkali in solid. Product is then reacted with hydrochloric acid (HCl) 20% to remove $CaCO_3$ content. In this step, the $CaCO_3$ combined with HCl to form $CaCl_2$ which is soluble in water. The mixture was then filtered to obtain solid chitin. This material was then washed using aquadest and dried to obtain solid chitin.

Chitin from the above process could be converted to chitosan compound through deacetylation process. The acetylation is a process to remove acetyl groups (CH_3CHO^-) bounded on amine groups in chitin compound by adding NaOH in the concentration of 20 to 50% (Milka et al, 2002). Here, the acetyl groups is reacted with NaOH producing sodium acetic. The main product, deacetylated chitin popular as chitosan is yielded in solid phase of mixture. The chitosan is then separated form the mixture by filtration process. The cake, wet chitosan is then dried to obtain dry chitosan with the purity of 70-80% (Hanafi et al, 2000). Chitosan is fine chemical that is used for medicine, fat coagulation, and heavy metal absorbent. The price of chitosan with the purity of 70% in world market could achieve US $ 750/kg (Djaeni et.al, 2002).

Source of Chitin

The source of chitin in the nature is very wide as well as cellulose source. Chitin could be found in marine animals such as fish and crustacean shell. It could be also obtained from protozoa cell and insect (Suhardi, 1993). In crustacean shell, the chitin content could achieve 20-80% in dry weight. While, in the insect and protozoa, the chitin content are in the range of 16-75% (Muzzareli, 1997).

Other sources of chitin are fungi and algae. In this organism, the chitin is bounded with poly-saccharide in fungi cell. The content of chitin in fungi could achieve 45%. Recently, one of a large potential as chitin source is *Aspergillus's niger* that contains high sufficient of chitin approximately 45% from organic materials (Suhardi, 1993).

Chitosan from Chitin

Chitosan is modified natural carbohydrate polymer derived from chitin which occurs principally in animals of arthropods. The primary molecular stucture is 2-deoxy-2-(acetylamino) glucose (Suhardi, 1993). This units are combined by 1-4 glycoside linkage, forming a long chain linear polymer, as presented in Figure 1. Removal most of the acetyl groups of chitin by treatment with strong alkaly yielding chitosan (Padmono and Nasution, 1998).

Related with the application and uses, the Chitosan has many special properties such as insoluble in water, organic acid, and high reactivity with amine primer groups. Chitosan is able to react with alkil, acetyl, sulphite, and carboxilyc. It is also stable in radiation effect and it has high ability to adsorb fat and heavy metal. At present, many potential products using chitosan have been developed, including flocculating agents for removal of traces of heavy metals from aqueous solutions, coating to improve dyeing characteristics of glass fibers, wet strength additives for paper, adhesive, photographic, and printing applications (Peniston and Johnson, 1980,; Campbell, 2000).

MATERIALS AND METHODS

The research was conducted in two steps involving the preparation of chitosan from shrimp shell, and the dillution of fat using chitosan product in varied grade. In this case, the shrimp shell was obtained from five seafood restaurants located in Semarang.

Preparation of Chitosan from Shrimp Shell

Figure 2 shows the preparation of chitosan from shrimp shell that involved four main steps, namely, size reduction, deproteinization, demineralization, and deacetylation.

The side reduction step was objected to reduce the size of shrimp shell up to 16 mesh. In this step, shrimp shell was milled using hammer mill and shrimp shell powder is manually screened at 17 and 16 mesh. The product remained on 16 mesh screen is taken 100 grams to be processed in the next step namely deproteination (Djaeni et al, 2002).

The shrimp shell powder was then added by 2 N of NaOH in the ratio of (1:6) to obtain chitin. The process is carried out in stirred tank mixture at 70^{o}C for 1 hour. After that, the mixture is then cooled and filtered. The deproteinated shrimp shell is washed by pure water and dried until 10% water content (Djaeni et al, 2002; Hanafi et al, 2000; and Efrina and Rafiah, 1999). The deproteinated shrimp shell is reacted with hydrochloric acid to remove $CaCO_3$ content. The process is done in stirred-mixer for 2 hours at 70^{o}C. Here, $CaCO_3$ was converted to $CaCl_2$ that is soluble in water. The mixture is then filtered by vacuum filter to separate solid and liquid

phase. The solid phase was washed by pure water and then dried in 90 to 100^{o}C for 2 hours to yield dry chitin. Meanwhile, the filtrate is neutralized and disposed.

The dry chitin is a raw material to produce chitosan through deacetylation process. The chitin powder is mixed with high concentration of NaOH to remove acetyl groups bounded in amine groups of chitin. Here, the acetyl reacted with NaOH to form sodium acetic (Mekawati, et al, 2000). The sodium acetate will disolve in solution, while deacetylated chitin namely chitosan could be obtained as solid product. The mixture was separated by vacuum filter to obtain chitosan as solid phase (cake). The cake was washed by pure water and then dried in electric oven for 2-4 at 105^{o}C. The dried chitosan is weighed using electrical balance. The chitosan product is analyzed for water content and ash content to determine the purity of chitosan.

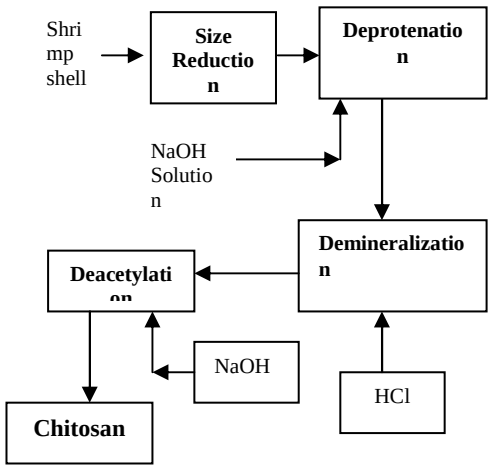


Fig. 2 Schematic Diagram of Chitosan Production

In this investigation, the quality of chitosan was also analyzed using FTIR and deacetylation grade (Milka and Patana, 2002).

Fat Adsorbtion Using Chitosan

The aims of this step are to evalaute performance of chitosan on fat dillution. In this investigation, the chitosan product is dilluted in 2% of acetic acid solution in the concentration of 2 gram per 100 gram solution. This mixture is used to reduce fat on beef fat sample in the ratio sample to chitosan mixture 1:2. The dilluted fat is evaluated based on the difference of fat content in sample fed with sample out of process using following equation.

$$X = ((F_f-F_o)/F_f).100\% \quad (1)$$

Where:
X is the percentage of dilluted fat (%)
F_f is the content of fat in feed (gram)
F_o is the fat content after process (gram)

Determination of Process Variables

This investigation is focused in two objectives. Firstly, it is adressed to study the effect of sodium hydroxide concentration (%) on quality of resulted chitosan. The grade of chitosan is determined based on deacytilation grade (Suhardi, 1993). In this research, the NaOH concentration was varied in the range of 20-60% with the step size of 10%. When the concentration of NaOH is added below 20%, the OH^- concentration on the mixture is too low. Hence, the acetyl groups removed from the bound is hampered. As a results, the quality of chitosan is too low with the long of operation time. Conversly, If the percentage of NaOH added is upper than 60%, the OH^- concentration in the mixture is very high and it may attack the chitin polymer. In addition, higher NaoH concentration will make difficulty on chitosan purification, and more NaOH will be lost in final washing to obtain chitosan product.

While, the second, is to study the influence of time and chitosan grade on fat

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dillution. The time is adjusted on 10, 15, 20, 25, and 30 minutes. Whereas, the grade of chitosan is determined based on the results of chitosan preparation in different NaOH concentration. As a response, the percentage of fat dillution is estimated using Equation 1 (Milka and Patana, 2002).

RESULTS AND DISCUSSION

The results of this investigation are presented in Table 1 and 2. While the discussion related with investigation results is thoroughly described in Section 4.1 for operation time influence and Section 4.2 for NaOH concentration effect.

The Influence of NaOH concentration on Chitosan Yield

Based on the Table 1, It indicated that the deacetylation grade is influenced by NaOH concentration. Acetyl groups bounded in chitin is difficult to be removed. So, it needs high concentration of NaOH and temperature. In this case, the increase of NaOH concentration address to enhance the deacetylation grade where the highest deacetylation grade (82.45%) could be reached at NaOH concentration of 60 %. The percentage of NaOH more than 60% was not observed since the process become inefficient especially in final washing to obtain chitosan product. In this case, more NaOH will be disposed and more purified water has to be required to get chitosan product. However, in the future works, the percentage of NaOH upper than 60%, is needed to be investigated in order to get optimal condition on chitosan preparation.

Table 1. The Effect of NaOH Concentration on Chitosan Grade

NaOH Concentration (%)	Deacetylation Grade (%)
20	67.30
30	75.55
40	77.79
50	80.86
60	**82.45**

The Influence of Time Operation and Chitosan Quality on Fats Dilution

Table 2 presents the influence of chitosan grade on fats dillution. The performance of chitosan on diluting fat improves as well as deacetylation grade and time of dillution enhances. Refer to Table 2, the percentage of fats dilluted becomes greater when dillution time prolonged. However, the influence of time does not affect significantly as indicated in the low incremental fat reduction. Averagely, at the same chitosan grade, the incremental time prolonged in the step of 5 minute will improve 2-2.5% of fat dilluted.

Table 2. The Effect of Chitosan Grade on Fats Dillution

Chitosan Grade (%)	Dillution Time (minute)	Dilluted Fat (%)
67.30	10	73.19
	15	73.61
	20	74.19
	25	74.35
	30	75.09
75.55	10	80.30
	15	80.78
	20	81.12
	25	82.70
	30	83.38
77.79	10	84.06
	15	84.74
	20	85.30
	25	86.34
	30	87.30
80.86	10	91.79
	15	92.40
	20	93.09
	25	93.88
	30	94.14
82.45	**10**	**96.57**
	15	96.78
	20	96.85
	25	97.06
	30	97.42

Meanwhile, the percentage of fats dilluted also equals to deacetylation grade where the increase of deacetylation will dillute higher fats as depicted in Table 2. This is because, in high deacetylation grade, the number of amine groups on chitin is greater that makes the fats dillution being higher. Amine groups will give positive ion that will catch the fats on mixture. The incremental of deacetyltion grade increasing, will dillute the fats in the conversion ratio of 1 to 2. It means that when the deacetylation grade is enhanced in 1%, the fats dilluted will increase 1-2%. In this investigation, it could be also concluded that the maximum of fats dillution is 96.57% that could be reached at time dillution of 10 minutes with chitosan grade of 82.45%.

CONCLUSSION

The results indicated that the highest chitosan grade of 82.45% could be obtained in the NaOH concentration of 60%. Meanwhile, in the fats dilution process, it showed that the highest percentage of fats diluted is 96.57% that could be achieved at time dilution of 10 minute and chitosan grade of 82.45%.

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