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Research Article

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Deacetylation degree of chitosan by various bases and its metal adsorption ability related on antioxidant activity

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ABSTRACT

Chitosan is the result of deacetylation process of chitin compounds commonly found in the outer skin of the animal groups crustaceans such as shrimp and crab. The purpose of this research is to obtain scientific data about the comparative degree of deacetylation of chitosan tiger shrimp shells using several strong bases and the ability of chitosan to adsorb ferrous metals as one method of antioxidants testing. The first stage is the produce of chitin from shrimp shells by demineralization, deproteination and depigmentation, the second phase is produce of chitosan with deacetylation used several base at a concentration of 50% (w/v). Chitosan produced from this process is analyzed degree of deacetylation with FT-IR spectrophotometer through analysis functional groups of amine and hydroxyl. Strong base that produces the best degree of deacetylation of chitosan further optimized. Chitosan obtained are then tested antioxidant activity through the ferrous metal adsorption. The amount of metal that can be adsorbed by chitosan was observed using a UV-VIS spectrophotometer. The results showed the degree of deacetylation of chitosan by using NaOH, KOH and $Ca(OH)_2$ respectively by 52.34%, 37.89% and 42.17%. Results of the optimization degree of deacetylation by the use of NaOH 60% (w/v) of 81.94%. Metal ion adsorption test results on chitosan weight of 50 mg, 250 mg and 500 mg respectively 47.65%, 80.07% and 87.00%. It showed that chitosan is able to act as an antioxidant by adsorption of the metal ions.

Keywords: Chitin, chitosan, FT-IR spectrophotometer, UV-VIS spectrophotometer,antioxidants

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INTRODUCTION

Free radicals are unstable molecules that can damage the body's normal system due to its ability to bind electrons, the nature of its reactive free radical is then very quick to react with the protein. Natural compounds are most often found as an antioxidant is a class of polyphenols, such as flavonoids, some are in the form of glycosides unisex aglycone, forms the aglycone can be obtained through a process of fermentation and hydrolysis with a strong acid, such as isoflavone aglycone obtained through fermentation by *Lactobacillus acidophilus* [1] and *Lactobacillus bulgaricus* [2]. However not all sources of antioxidants derived from plants, because there are several compounds that have the characteristics of a free-radical scavengers

Chitin compound has several functional groups, one of which is acetamine ($NHCOCH_3$), so chitin also called polymer acetylglucosamine [3]. This biopolymer is not soluble in water, it is polycationic and its use is limited. This weakness can still be improved by modifying the structure. Chitin structural modifications performed with a strong base sodium hydroxide (deacetylation process) to produce derivatives namely biopolymers β (1,4) -2-amino-2-deoxy-D-glucose-called chitosan. In the process of deacetylation of acetyl group substitution-$NCOCH_3$ with hydrogen to the amine group (NH_2). The amount of substitution that occurs is called deacetylation level or degree of

deacetylation (DD). The success of the process of deacetylation is influenced by the strong base concentration, temperature and duration of the process of deacetylation done [4].

Chitosan is a biological product that is cationic, non-toxic biodegradable and biocompatible. Chitosan has amino (NH_2) relatively large compared to chitin that is more nucleophilic and alkaline. Chitosan crystallinity caused by intermolecular and intramolecular hydrogen bonds lower than chitin, making them easier applied in some reagents. Chitosan is insoluble in water and some organic solvents just as dimethylsulfoxide (DMSO), dimethylformamide (DMF), an organic alcohol solvent and pyridine. Chitosan is soluble in the organic acid or dilute mineral through protonation of free amino groups (NH_2, NH_3^+)at pH less than 6.5. Good solvent to chitosan is formic acid, acetic acid and glutamate acid. Chitosan solubility decreases with increasing molecular weight of chitosan. Parameters that influence the properties of chitosan is the molecular weight and degree of deacetylation. Deacetylation shows reduced degrees of chitin acetyl group into the amino group of chitosan. Measurements degrees deacetylation can use titrimetri HBr, IR spectroscopy [5].

Have conducted further research on hypercholesterolemia effects in vivo in experimental animals rabbits and prove that the chitin from shrimp shells capable of lowering cholesterol of rabbit [6]. Previous research showed that deacetylation degree of chitosan mud crab shell (*Scylla serrata* Forskal) influence cholesterol adsorption as in vitro [7]. Chitosan also been reported to function as chelating for heavy metals from solution, as well as an ion exchanger [8, 9, 10]. Besides, the chitin and chitosan and its derivatives have properties as emulsifiers and thickeners emulsion coagulation [11]. Based research on the ability to lower cholesterol levels it is necessary to be tested as an antioxidant activity with metal adsorption method.

EXPERIMENTAL SECTION

2.1 Chemical Materials

Acetone (p.a, *Merck*), concentrated hydrochloric acid (HCl) (p.a, *Merck*), distilled water, tiger shrimp shells (*Panaeus monodon*), iron (II) sulphate ($FeSO_4$), Potassium thiocyanate (KSCN) (p.a, *Merck*), filter paper (*Whatman)*, standard chitosan (*Sigma Aldrich*), sodium hydroxide (NaOH) (p.a, *Merck*), sodium hypochlorite (NaOCl) (p.a, *Merck*), potasium hydroxide (KOH) (p.a, *Merck*), and calcium dihidroxide ($Ca(OH)_2$) (p.a, *Merck*),.

2.2 Chitosan Preparation

Tiger shrimp shells (*Panaeus monodon*) washed and dried then pulverized and sieved then performed demineralization stage, deproteination, depigmentation and deacetylation into chitosan [12].

2.3 Demineralization Process

Mineral disappearances carried out at a temperature of 25 – 30^oC using 1 M HCl solution with a comparison sample with a solution of HCl = 1:10 (g/ml) with stirring for 120 minutes. Then filtered to take sediment. The precipitate was washed with distilled water until neutral pH, then filtered and dried sediment [12].

2.4 Deproteination Process

This process is carried out at a temperature of 60 - 70°C using 1 M NaOH solution with shrimp powder comparisons with NaOH was 1:10 (g/ml), stirring for 60 minutes. Then the mixture is separated and filtered to take sediment. The precipitate was washed with distilled water until neutral pH, then filtered and dried sediment [12].

2.5 Depigmentation Process

The precipitate results deproteination added acetone then bleaching with NaOCl 0.315% (w/v) for 5 minutes at room temperature. Comparison of solid and solvent 1:10 (w/v). then washed with distilled water until neutral pH then filtered and dried sediment. Then formed chitin [12].

2.6 Deacetylation Process

Chitin that has been resulted in the above process included in NaOH, KOH, and Ca(OH)2 solution with a concentration of 50% at a temperature of 90 - 100°C while stirring at a constant speed for 60 minutes. The result is a slurry was then filtered, washed with distilled waterthen added HCl 1 M to make the pH neutralthenwashed again with distilled water, filtered and dried [12]. Chitosan is obtained weighed and characterized using FT-IR spectrophotometer [13]. Strong base generating the best degree of deacetylation then optimized to obtain better chitosan. To determine the degree of deacetylation (DD) is used Domzy and Robbert line method [12] :

$$DD = 100 - \left[\frac{A1}{A2} x\ 115\right]$$

A = log (Po/P) = absorbance
A1 = Absorbance at wave number 1500-1650cm^{-1} for absorption of amide groups (CH_3COONH^-)
A2 = Absorbance at wave number 3000 - 3500cm^{-1} for absorption of hydroxyl (OH^-, NH_2)

The method used to determine the absorption in the infrared spectra is the method of baseline. With this method, the transmittance at the desired wave number is determined by comparing the distance between the base tape and ribbon peak at the desired wave number, which is mathematically given by equation absorbance which is the negative logarithm of the transmittance, the absorbance can be expressed as follows: [5].

$$A = - \log \frac{P}{Po} = \log \frac{Po}{P}$$

A=Absorbance/uptake
P=The intensity ofthe rest
Po=Initial intensity

2.7 Metal Adsorption Testing

Prepare a solution of iron (II) sulphate ($FeSO_4$) 25 ml, then added the chitosan as much as 50 mg, 250 mg and 500 mg into each solution and stirred for 40 minutes then allowed to stand for 24 hours and the solution is filtered, the filtrate results added 1 drop KSCN, and analyzed by UV-Vis spectrophotometer to determine metal content with the formula [13, 14] :

$$\text{Eff} = \frac{(Co - C1)}{Co} \text{ x } 100\%$$

Eff = efficiency of absorption
Co = concentration of first metal without chitosan added
C1 = concentration of the metal after absorption

RESULTS AND DISCUSSION

Results chitosan preparation specified degree deacetylation using FT-IR spectrophotometer. The resulting spectrum shows similarities with standard chitosan which is also measured on the same instrument.

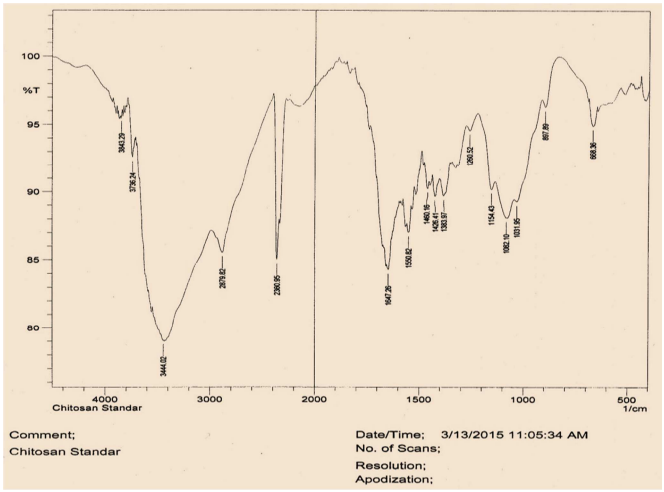

Figure 1. Spectrum of Standards Chitosan by FT-IR Spectrophotometer

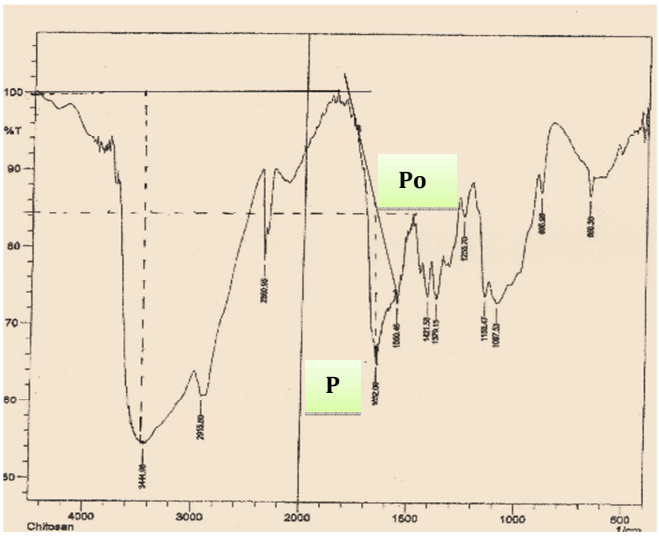

Figure 2.Spectrum and Baseline Method of Deacetylation Degree of Shrimp Shell Chitosan Measurement by FT-IR spectrophotometer

Table1.The degree ofdeacetylation ofthe chitosanthrough thedeacetylationprocessterraced

Concentration 50%	Deacetylation Degree	Concentration 60%	Deacetylation Degree
NaOH	52.34 %		
KOH	37.89 %	NaOH	81.94 %
Ca(OH)2	42.17 %		

Table 2.Results oftestingantioxidantsthrough$FeSO_4$ metal adsorption by the chitosan

Sample (mg)	Absorbance (562nm)	Metal Adsorption (%)	Average (%)
50	0.29	45.99	
50	0.236	56.02	47.65
50	0.317	40.96	
250	0.105	80.44	
250	0.091	83.05	80.07
250	0.125	76.72	
500	0.056	89.57	
500	0.071	86.17	87.00
500	0.079	85.28	

CONCLUSION

Chitosan of tiger shrimp shells have antioxidant activity through metal adsorption method, where the magnitude of the activity is directly proportional to the weight of chitosan.

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