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THE EFFECT OF CHITIN AND CHITOSAN OF CRAB SHELL ON WATER SORPTION OF ISOTHERM AND DENATURATION OF MYOFIBRILS DURING DEHYDRATION PROCESS

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ABSTRACT

Indonesian shrimp production is estimated at approximately 342,000 tons per year, followed by crab production at the level of more than 200,000 tons annually. Apparently, 50 – 60% out of the total production consists of waste in the form of crab shell. Crab shell is rich in chitin, chitosan, and cellulose. The United States, Japan and other developed industrial countries have used chitin, chitosan, and cellulose as raw material for various purposes, such as toxic waste processing, water purification, enzyme immobilization, skin and hair cosmetics, bone connecting, biomedicine, paper and textile industry, pharmacology, film, food industry, feed and others

Chitin (C8H13NO5) is a Poly-β-N-Acetyl-D-GlucoSamine standing for a natural biopolymer, which constructs the shells of crab species. Chitin cannot be examined as a pure essence, since it is melt with rich texture of protein, CaCO3, fat pigmen, and small amount of metals. In order to fabricate Chitosan, one should demolish the Acetyl cluster of Chitin by employing strong alkalis. Chitin makes up the combination between Poly (N-acetyl-2-amino-2-deoxy-β-D-gluco-piranosa) and N-acetyl-2-amino-2-deoxy-D-glucopiranosa.

To find out the effect of chitin and chitosan from crab shell on the water sorption isotherm of myofibrils protein during dehydration process, chitin and chitosan from crab shell were added to myofibrils protein at the ratio of ratio 2,5 – 7,5 g / 100 g, homogenized, and afterwards dried in a dessicator. After some time, moisture content, water activity (Aw), Ca-ATPase activity, and proximate were analyzed. Mono layer water was analyzed according to Brunauer's method (1968), multi-layer water was analyzed according to Bull's method (1944), whereas Ca-ATPase activity was analyzed using the formula introduced by Katoh et. al. (1977).

The result of the analyses shows that a higher concentration of chitin and chitosan on myofibrils resulted in higher amount of mono-layer and multi-layer water. The presence of different amount of mono-layer and multi-layer water indicates that the state of water changing occurs on myofibrils protein which in turn affects its quality. Likewise, the increase of chitin and chitosan concentration suppresses the decrease of acceleration of Ca-ATPase activity.

Key words: Chitin, chitosan, monolayer water, multilayer water, myofibrils protein, and Ca-ATPase

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INTRODUCTION

Annually, the Indonesian fisheries industry undergoes enormous improvements, especially in fishing technique, aquaculture, and fish processing. According to Ilyas (1993), the Indonesian shrimp production is estimated at 342,000 tons per year, followed by crab production at the level of more than 200,000 tons annually. The increase of shrimp and crab production also increases the amount of waste, which, if not handled seriously, will cause environment pollution. Johnson and

Peniston (1982) noted that 55 – 60% out of the total crab production consits of waste/garbage, which are in the forms of crab shell. If disposal of crab shell are neglected, it will cause an increase of Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD), and, as a consequence, causes environmental pollution.

In Indonesia, crab shell is used as one of the most important raw materials to produce cattle feed. On the contrary, the United States and Japan have utilized crab shell as raw material to produce the chitin and chitosan. According to Austin (1990) chitin or Poly-β-N-Acetyl-D-Glucosamine with chemical composition of C8H13NO5, is analytically composed of C = 47.29%; H = 6.45%; N = 6.89%; and O = 39.37%, whereas chitosan is a polymer of 2-Amino-deoxi-D-glucose which contains acetyl cluster in its monosaccharide. Besides crab shell, other sources of chitin and chitosan are krill, oyster, squid, insect and fungi.

Based on some research results, chitin and chitosan can be produced by chemical and enzymatic methods. Chitin is produced by demineralization using HCl reagent, then deproteinization by using NaOH and heating (Knorr, 1984). Whereas chitosan can be produced by deacetylization of chitin using NaOH at high temperature. Chitin and chitosan have specific natural characteristic, namely non-toxic, bioactivity (have active capability in living cells), biodegradability (naturally reducible) also have the capability in absorbing fat. Hirano (1998) stated that chitin and chitosan have attractive characteristics, and consequently the product is useful for the interest of industry, for example in farming, water purification, heavy metal binding agent, food industry, and so on.

MATERIALS AND METHODS

The research materials of chitin and chitosan was obtained from the crab shell that had been collected from restaurants in the city of Semarang . Mean while the myofibril protein (Mf) was isolated from lizard fish.

Methods

Preparation of Chitin

The crab shells were immersed in 20 volumes of 2N hydrochloric acid for 48 hours, changed twice in 24 hours intervals. After 48 hours, the materials were washed in distilled water and adjusted to pH 7.0, and than mixed with 20 volumes of 1 N sodium hydroxide and heated at 98°C for 36 hours. After heating, the materials were neutralized using distilled water. Finally, the materials were dried using a fan and fractured into chitin powder.

Preparation of Chitosan

The 20 volumes of 60 % sodium hydroxide were added to the chitin powder while heating at 130°C for 3 hours. The materials were washed with distilled water until they became neutral. Then, acetic acid solution of 15% was added and the materials was stirred for 12 hours and centrifuged at 3,800 x g for 30 minutes and the precipitate was washed with distilled water until it reached a pH of 7.0. Finally the precipitate was lyophilized using a fan and powdered by mortar to produce chitosan.

Preparation of Fish Myofibrils

The myofibrils were prepared from lizard fish meat. After cutting off the head and removing viscera, the skin and bones were removed, then fish meat was washed in 0,1 M KCl reagent with the pH adjusted to 7.0 with 20 mM trismaleate buffer. This washing process was repeated three times. Then, fish meat was pressed out using

hydraulic press in order to reach a moisture content of 80%. Fish meat is then diluted again in 0.1 M KCl reagent and the pH adjusted to 7.0 with 20 mM trismaleate buffer, than homogenized by a Waring blender and filtered through a nylon net (#16). Solution of fish meat is then mixed with 1% triton X-100, and kept for 30 minutes at 5°C. Then it was centrifuged at 3,000 rpm for 10 minutes. The resulting residue was diluted in 0.1 M KCl reagent and the pH adjusted to 7.0 with 20 mM trismaleate buffer and was centrifuged again at 3,000 rpm for 10 minutes. This process was repeated until supernatant become clear. The residue was diluted in cold distilled water and centrifuged at 5,000 rpm for 20 minutes. The result was myofibrils that was used as research material.

Preparation of Sample

There are four treatments in conducting this research. Chitin and chitosan of crab shell weighing (0; 2.5; 5; 7.5) g each are added to 100 g myofibrils. The pH of the mixture was adjusted to 6,8 by adding HCl or NaOH before sealing it in a cellophane bag, and put in a desiccator. After the mixture reached moisture content of 10%, a vacuum desiccator was used. Myofibrils without chitin and chitosan was used as a control and treated by a similar procedure.

Analysis of Water Content

Analysis of water content was done by measuring the initial weight and final weight of myofibrils protein after being put in the oven at 105°C for 18 hours or when the weight of myofibrils protein became constant.

Analysis of Water Activity (Aw)

Analysis of water activity was done by using oil monometer (Akiba, 1961) at 10°C. Water activity was determined by the following formula:

$$Aw = \frac{P}{Po} \times \frac{RH}{100}$$

where:
Aw : water activity
P : sample vapour pressure
Po : water vapour pressure
RH : relative humidity

Analysis of Ca-ATPase Activity

One gram of myofibrils protein was dissolved in 19 ml 0.1 M KCl reagent and the pH adjusted to 7.0 with 20 mM trismaleate buffer; after several times the mixture was homogenized with waring blender. The homogenate was analyzed for protein, and other was analyzed for Ca-ATPase activity.

Five ml reagent of mixture consisting of 3.5 ml CA-ATPase reagent; 1 ml sample and 0.5 ml 10 M ATP were put in to waterbath at 25°C for 5 minutes. After the reaction took place, the reaction was stopped by adding 1 ml TCA 30%. In the next process, 1 ml reagent was taken and added with 2 ml amonium molidate and 0,5 ml elon reagent, and then kept for 45 minutes. The mixture was filtered and its filtrate examined by spectrophotometer 640 nm.The amount of Ca-ATPase activity was determined in micro moles per minute inorganic phosphate (Pi μ mol/minute/mg).

Analysis of Data

Analysis of Water Sorption Isotherm

Water sorption isotherm in a material is the relationship between moisture content and water activity at certain temperature. According to Rockland and Steward (1981), water sorption isotherm is divided into three sections, namely monolayer water, multilayer water and capillary water. To determine the amount of monolayer water, Brunauer (1968) suggested the following formula:

$$[1 / v] \times [Aw / (1-Aw)] = [1/(Vm.C)] + [(C-1)/(Vm.C] \times Aw$$

$$\downarrow$$

$$M1(\%) = (100.Vm) / (1+Vm)$$

Whereas Bull (1944), determined the amount of multilayer water by the following formula :

$$M2 = (M1 / Aw) \times 100\%$$

where

V : volume of absorbed water
C : constants
Vm : monolayer water (g/g of dried matter)
Aw : water activity
M1 : monolayer water
M2 : multilayer water

Analysis of Ca-ATPase

Myofibrils protein is one of the protein components of fish. This protein can be broken down to ATP by enzyme activity. Therefore, during the dehydration process, it is necessary to analyze Ca-ATPase activity (Kamal, 1989). Nozaki (1993) determined the amount of Ca-ATPase activity during dehydration process by the following equation:

$$\text{Ca-ATPase activity} = \text{Ln}\{(1-pi) \times (6/5) \times (1/31)\} \times (1/5) \times 1 \times (5/A)$$

where

Pi : Acquired by the equation of the regression of phosphorus standard solution
A : Acquired by the equation of the regression of bovine serum albumin as a standard

RESULTS AND DISCUSSION

Table 1. Chemical Composition and Properties of Chitin and Chitosan Crab Shell

Chemical Compositin	Chitin (%)	Chitosan (%)
Moisture content	16.70	7.90
Crude protein	< 0.10	< 0.10
Crude lipid	2.80	2.50
Crude ash	4.90	5.50
Degree of deace-tylation	5.40	0.10
Viscosity	1.04	1.35
Yield	25	65
Particle size	< 1 mm	< 1 mm
Color	Ivory	Ivory

Table 1 shows general chemical composition of chitin and chitosan prepared from crab shell. Moisture content accounted for about 16.70% in chitin and 7.90% in chitosan crab shell. Crude protein accounted for less than 0.10%, crude lipid accounted 2.80% in chitin and 2,50% in chitosan. Other component of degree of deacetylation accounted 5.40% in chitin and 0.10% in chitosan. The moisture content of chitin and chitosan were more than 7.90% This was possibly related with the fan used during lyophillze. Besides, the degree of acetylation of chitin and chitosan was about normal. Suhardi (1993) reported that the degree of deacetylation was 10.5% in crab shell.

A water sorption isotherm of materials represents the relationship between moisture content and water activity of myofibrils at a particular temperature. In accordance with this relation, the water activity of myofibrils without or with 2.5-7.5% chitin and chitosan during dehydration process was plotted in Fig. 1 and 2. Both water sorption isotherm shows sigmoidal curve which have two bindings within the 0.17 - 0.21 and 0.61- 0.71 range of water activity.

Water activity of myo-fibrils treated with various concentration of chitin and chitosan decreased remarkably throughout the dehydration process , which were

lower than that of the control for the same moisture content.

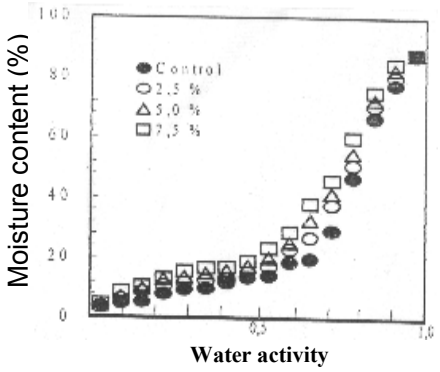

Fig 1. Effect of various concentration of chitin from crab shell on water sorption isotherm of lizard fish myofibrils during dehydration process

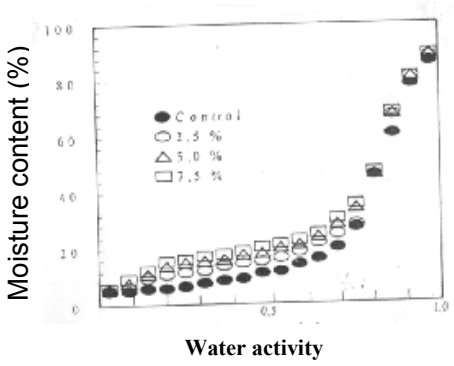

Fig 2. Effect of various concentration of chitosan from crab shell on water sorption isotherm of lizard fish myofibrils during dehydration process

Characteristic of water state in myofibrils based on water sorption isotherm (M1, M2, Aw1, Aw2, Ca-ATPase activity, sorption surface area) of chitin and chitosan is shown in table 2 and 3. The result showed that the various concentrations of chitin and chitosan had influenced the increase in the amount of monolayer and multilayer water and sorption surface area of myofibrils. In this study the water sorption isotherm patterns of the control and the myofibrils containing various concentration of chitin and chitosan were similar to those of carp actomyosin (Nakano *et al*., 1979).

Table 2. Amount of monolayer and multilayer water, sorption surface area of lizard fish myofibrils added to various concentration of crab shell chitin and remaining Ca-ATPase activity of the myofibrils coresponding with monolayer and multilayer water

Chitin	Monolayer water*1		Aw_1*2	Ta_1*3	Multilayer water*4		Aw_2*5	Ta_2*6	M_2/M_1	S*7
	M_1*8	M_d*9			M_2*8	M_d*9				
Control	4.94	0.052	1.711	23.90	22.15	15.29	6.900	52.40	4.48	0.148
2,5%	7.52	0.075	0.281	36.10	29.40	20.09	0.694	56.20	3.90	0.256
5,0%	8.27	0.084	0.289	37.02	50.10	20.70	0.706	59.60	3.63	0.307
7,5%	8.88	0.086	0.328	40.10	30.60	27.23	0.710	59.80	3.44	0.335

*1 Estimated by B.E.T. analysis
*2 Water activity of the sample at the M_1 point
*3 Remaining myofibril relative Ca-ATPase activity (%) of the sample at M_1 point
*4 Estimated by Bull's analysis
*5 Water activity of the sample at the M_2 point
*6 Remaining myofibril relative Ca-ATPase activity (%) of the sample at M_2 point
*7 Sorption surface area (m^2/mg) of the sample
*8 Moisture content (g/100g of sample)
*9 Moisture content (g/g of dried matter)

Table 3. Amount of monolayer and multilayer water, sorption surface area of lizard fish myofibrils added to various concentration crab shell chitosan and remaining Ca-ATPase activity of the myofibrils coresponding with monolayer and multilayer water

Chitosan	Monolayer water*1		Aw_1*2	Ta_1*3	Multilayer water*4		Aw_2*5	Ta_2*6	M_2/M_1	S*7
	M_1*8	M_d*9			M_2*8	M_d*9				
Control	4.94	0.052	1.171	21.10	19.20	15.29	6.90	57.10	3.88	0.180
2,5%	9.80	0.085	2.10	26.40	22.41	20.10	6.98	59.00	2.28	0.290
5,0%	10.50	0.108	2.40	31.60	23.10	21.06	7.04	60.16	2.20	0.380
7,5%	12.40	0.116	2.56	36.10	24.00	21.64	7.16	62.01	1.93	0.410

*1 Estimated by B.E.T. analysis
*2 Water activity of the sample at the M_1 point
*3 Remaining myofibril relative Ca-ATPase activity (%) of the sample at M_1 point
*4 Estimated by Bull's analysis
*5 Water activity of the sample at the M_2 point
*6 Remaining myofibril relative Ca-ATPase activity (%) of the sample at M_2 point
*7 Sorption surface area (m^2/mg) of the sample
*8 Moisture content (g/100g of sample)
*9 Moisture content (g/g of dried matter)

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It has been shown that there is a correlation between the activity of enzymes and the moisture content of the food. A certain minimal amount of water is necessary for enzyme activity that increases with increasing moisture content. The water activity is expressed as a function of sorbed water in foodstuff. The first part of the isotherm, up to the lower inflection point, which corresponds roughly to the completion of monolayer of absorbed water, represents a region of very firm water binding due to the interaction of water molecules directly with surface polar groups of the absorbent.

Based on the theory above, this research is aimed at investigating the effect of various concentration of chitin and chitosan on the Ca-ATPase activity of myofibrils during dehydration process. Results of this research showed that various concentration of chitin and chitosan resulted in different degrees of Ca-ATPase activity. Ca-ATPase activity decreased rapidly at water activity level of 0.5 in chitin and chitosan, and then decreased slowly when water activity reached over 0.5 (Fig. 3 and 4). The hampered effects on dehydration associated with activation of myofibrillar Ca-ATPase differed among the various concentrations of chitin and chitosan.

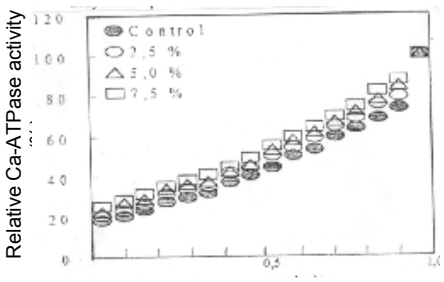

Fig 3. Effect of various concentration of chitin from crab shell on myofibril Ca-ATPase activity of lizard fish myofibrils during dehydration process

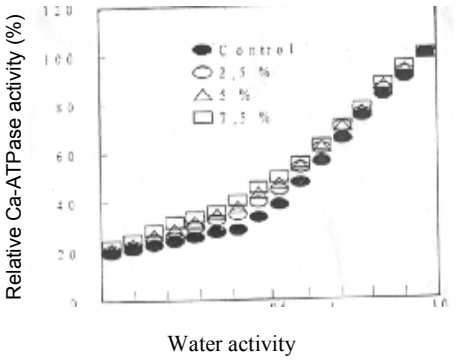

Fig 4. Effect of various concentration of chitosan from crab shell on myofibril Ca-ATPase activity of lizard fish myofibrils during dehydration process

However, the effect of chitin and chitosan on myofibrillar Ca-ATPase activity and those water sorption isotherm curves showed a similar tendency. Chitin or Poly-β-N-Acetyl-D-Glucosamine stands for a natural biopolymer that constructs the shrimps shell and the yeast and fungus partition. Moreover, it is a complex chain of polysaccharide that is prognoses and is constructed from the combination of C-2 and the cluster of amino-acetyl. Likewise, chitosan, which is β-1,4 poly-D-Glucosamine was deacetylated from chitin. A study conducted by Lang and Clausen (1990) concluded that the hampered effects of various concentration of chitin and chitosan on dehydration and water binding capacity were parallel with the higher concentration of chitin and chitosan. On the other hand, myofibrils Ca-ATPase activity was higher in myofibril containing chitin and chitosan that more markedly decreased water activity. In other words, chitin and chitosan that caused more markedly incorporation of water structure in myofibrils had more markedly hampered effects on denaturation of myofibrils.

From the above, it was found that the suppressive effect on denaturation of myofibrils resulting from dehydration was deferred according to the various concentration of chitin and chitosan, and there is a correlation between the suppressive effect and the state of water in myofibrils. The result suggests that the suppressive effects on denaturising are likely to be attributed to the stabilization of the hydrated water surrounding myofibrils by the addition of chitin and chitosan.

CONCLUSIONS

The effects of chitin and chitosan on the water sorptiom isotherm in myofibrillar protein during the dehydration process can be concluded as follows:

1. The addition of chitin and chitosan to myofibrillar protein during the dehydration process results in an increase of water sorption isotherm.
2. During the dehydration process of chitin and chitosan crab shell, different amounts of monolayer water, multilayer water, and sorption surface area were found. An increase in chitin and chitosan concentration is parallel with an increase in monolayer and multilayer water.
3. The addition of chitin and chitosan crab shell suppresses the decrease of Ca-ATPase activity. The chitin shows a better ability than chitosan.
4. An increase in concentration of chitin and chitosan crab shell will increase the amount of Ca-ATPase activity.
5. The addition of chitin and chitosan results in a decrease in water activity, increase in the monolayer water or multilayer water and increase in sorption surface area, indicating the changes in the state of water in myofibrillar protein.

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