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Original paper

EFFECT OF CHITIN AND CHITOSAN DERIVED FROM CRAB SHELL AND SHRIMP HEAD ON THE UNFROZEN WATER AND DENATURATION OF LIZARD FISH MYOFIBRILS DURING FROZEN STORAGE

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ABSTRACT

The shrimp head and crab shell are rich in chitin and chitosan that can be used as the raw material in various industries. Chitin is composed of 2-acetamido-2-deoxy-D-glucose, whereas chitosan is composed primarily of glucosamine, 2-amino-2-deoxy-D-glucose. Chitin and chitosan can commercially be manufactured in the forms of powder, flake, chitinazed, nitrate chitin and 77-red chitin. Chitin and chitosan is of benefit to neutralize toxicity of polluted water, a pivotal role for strengthening the emulsion system, binding water and fat, advancing the loaf volume of bread, and for binding food drying, purification of apple, beer, wine extracts, etc.

To find out the effect of chitin and chitosan of shrimp head and crab shell on the unfrozen water and denaturation of lizard fish myofibrils during frozen storage, chitin and chitosan were added at certain ratios 0; 2.5 – 7.5 g / 100 g, with non chitin and chitosan treatments as control. Changes of unfrozen water in myofibrils during frozen storage were studied based on the relationship between water content and transition heat, which was determined by Differential Scanning Calorimeter (DSC), whereas Ca-ATPase activity was analyzed using formula introduced by Katoh et. al., (1977).

During frozen storage, chitin and chitosan treatments influenced the amount of unfrozen water and Ca-ATPase activity. Without chitin and chitosan the amount of unfrozen water in myofibrils decreased rapidly, whereas the decrease was moderate when myofibrils received chitin and chitosan. The change in Ca-ATPase activity exhibited a similar tendency to those of the unfrozen water indicating a close correlation between Ca-ATPase activity and amount of unfrozen water.

Key words: chitin, chitosan, myofibrils, unfrozen water, Ca-ATPase

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INTRODUCTION

The shrimp head and crab shell are rich in chitin and chitosan that can be used as raw material in various industries. Darmanto (2002) reported that chitin and chitosan were yielded from crab shell 25% and 65%, and from shrimp head 23,3% and 23% respectively. According to Austin (1988) 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 polymer of 2-Amino-deoxi-D-glucose that contains

acetyl cluster on its monosaccharide. Beside crab shell, other sources of chitin and chitosan are krill, oyster, squid, insect, and fungi.

Based on some researches, chitin and chitosan can be chemically and enzymatically produced. Chitin is produced by demineralization using HCl reagent, followed by deproteinization by using NaOH and heating (Knorr, 1984). Whereas chitosan can be produced by deacetylization of chitin by using NaOH at high temperature.

Chitin can commercially be manufactured in the forms of powder, flake, chitinazed, nitrate chitin, 77-red chitin (Muzzarelli,1987). Biochemistry, pharmacology, food industry, nutrient, enzymology, farming, paper and textile industry, film, and the like intensely demand chitin to run their production (Hirano,1988). Since its capability for dislocating metals, chitosan is beneficial to neutralize toxicity of polluted water. In food industry, chitin plays a pivotal role for strengthening the emulsion system, binding water and fat, advancing the loaf volume of bread, and for binding food dying (Knorr, 1984). Furthermore, chitosan is of benefit for the purification of apple, beer, and wine extracts. Chitin is also capable to binding oxygen that contained in polluted water so thereby assists almost death sunk organisms to keep alive.

Water is a very important component in the chitin and chitosan. It does not only affect taste, texture and flavor, but also determines acceptability, freshness, and durability of the chitin and chitosan. Nozaki et. al., (1991) reported that the state of water plays an important role in the dehydration and frozen storage of myofibril protein. Adjustment and control of water content have now become important measures of quality assurance in the chitin and chitosan processing industry. However, only in recent decades it has been recognized that the chemical, physical, and biological properties and hence, quality and stability of product are related directly to relative humidity of water.

Frozen storage is an excellent method for preventing putrefaction and autolysis of fish meat, but deterioration cannot be avoided under prolonged storage and during thawing, with the meat showing softened texture and losing flavor (Suzuki, 1981). Matsumoto (1979) reported the lowered functional properties such as capacities in emulsifying, lipid binding water holding and gel forming of the long stored fish meat. Preservation of food by freezing not only beneficial toward microbiological aspect but also retards the chemical and biochemical deterioration changes (Powrie, 1973).

Myofibril protein is the protein that forms myofibrils, contains myosin, actin, and regulates protein as tropomyosin, troponin and actinin and so on. Myofibril protein accounts for 66 – 77% of total protein in fish meat, and plays an important role in coagulation and gel formation when fish meat is processed (Suzuki, 1981). Matsumoto *et. al*., (1992) reported that the denaturation of myofibril protein during frozen storage was a result of coagulation, caused by the progressive increase of intermolecullar cross-linkage due to the formation of hydrogen, ionic hydrophobic and/or disulfhydryl bonds. There are many methods of measuring fish protein denaturation during freezing and storage, such as solubility of myofibrillar protein, viscosity, ATPase activity and electron microscopy (Suzuki, 1981). Nozaki *et. al*., (1993) used the Ca-ATPase activity as indicator for quality of myofibrillar protein during frozen storage.

MATERIALS AND METHODS

Sampling protocol

In this analysis Chitin and chitosan were obtained from the crab shell and shrimp head that had been collected from restaurants in Semarang city.

Preparation of chitin

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

Preparation of chitosan

The 20 volumes of 60 % sodium hydroxide were added to the chitin powder while being heated at 130°C for 3 hours. The materials were washed with distilled water until they became neutral. The acetic acid solution (15%) was added and the materials were stirred for 12 hours, centrifuged at 3,800 x g for 30 minutes and the precipitate was washed with distilled water until pH 7.0. Finally the precipitate was lyophilized by using 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 skins 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 for three times. Then, fish meat was pressed out by using hydraulic press until moisture content became 80%. Fish meat was diluted again in 0.1 M KCl reagent and the pH was adjusted to 7.0 with 20 mM trismaleate buffer, then homogenized by waring blender and filtered through a nylon net (#16). Solution of fish meat was added with 1% triton X-100, and kept standing for 30 minutes at 5°C. It was then centrifuged at 3,000 rpm for 10 minutes. Resulted residue was diluted in 0.1 M KCl reagent and the pH was 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 became 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 DSC sample

For the preparation of the DSC sample, 100 g of myofibrils was mixed with 2.5 – 7.5 g chitin and chitosan of crab shell and shrimp head. These myofibrils were then adjusted to pH 7.0 either with 0.01 N HCl or 0.01 N NaOH. Then, 1 g of the sample was placed in a microtube and frozen at -25°C at hourly intervals, samples were taken out for analysis of the unfrozen water and Ca-ATPase activity.

Analysis of Ca-ATPase activity

1 gram of myofibrils protein was dissolved in 19 ml 0.1 M KCl reagent and the pH was adjusted to 7,0 with 20 mM trismaleate buffer. After several times the mixture was homogenized with waring blender. The homogenate was analyzed the for protein, and the other was analyzed for Ca-ATPase activity.

5 ml reagent of mixture consisted 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, 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 was examined by spectrophotometer at 640 nm. Amount of Ca-ATPase activity was determined in micro moles per minute inorganic phosphate (Pi μ mol/minute/mg) in the presence of 1 mM ATP, 100 mM KCl and 5 mM $CaCl_2$ at pH 7.0 with 25 mM trismaleate buffer. The concentration of protein in myofibrils was determined by the burette method with bovine serum albumine as a standard. Myofibrils, one of the main muscle components has an enzymatic activity to split ATP. The Ca-ATPase activity was analyzed by method of Katoh *et. al*. (1977) by using the following formula:

$$L_n \{(1\text{-pi}) \times (6/5) \times (1/31)\} \times (1/5) \times 1 \times (5/A)$$

Pi : Acquired from the regression equation of phosphorus standard solution

A : Acquired from the regression equation of bovine serum albumin standard.

Analysis of unfrozen water

A Differential Scanning Calorimeter (DSC) model SSC-5200 analysis system with plotted type SP-520 (Seiko Electronic Industry, Co.,) was employed to analyze the unfrozen water. A sample of about 20 mg was placed in an aluminum cell, while in another reference cell, 20 mg Al_2O_3 was used to balance the heat capacity of the sample cell. At the first step, sample was frozen at -40°C using liquid nitrogen gas. The sample was then heated to 25°C at the rate of 10°C per minute. The DSC curve for 20 mg of water in the thermogram was obtained by heating, to show endothermic peak which indicated amount of free water in the sample. The amount of total water in the sample of myofibrils was measured by drying at 105°C. Meanwhile the amount of unfrozen water in the sample was calculated by subtracting the amount of free water from the amount of total water. The unfrozen water in the sample was expressed as H_20 g/g dried matter.

RESULTS AND DISCUSSION

There is a relationship between bound water and unfrozen water in the fish myofibrils with the addition of chitin and chitosan shrimp head and crab shell. The relationship can be explained by analysing the state of water (bound water and unfrozen water) using Differential Scanning Calorimeter (DSC) method. The DSC method is based on the Phone (024) 8311525 / (024) 8310965 Fax (024) 8311525 measurement of endothermic or exothermic heat to and from the sample as the temperature is raised continuously (Akahane *et. al*., 1980). The endothermic heat or exothermic one reflects the chemical or physico-chemical changes taking place in the sample. The effect of various concentrations of chitin and chitosan shrimp head and crab shell on the amount of unfrozen water is shown in Fig. 1.

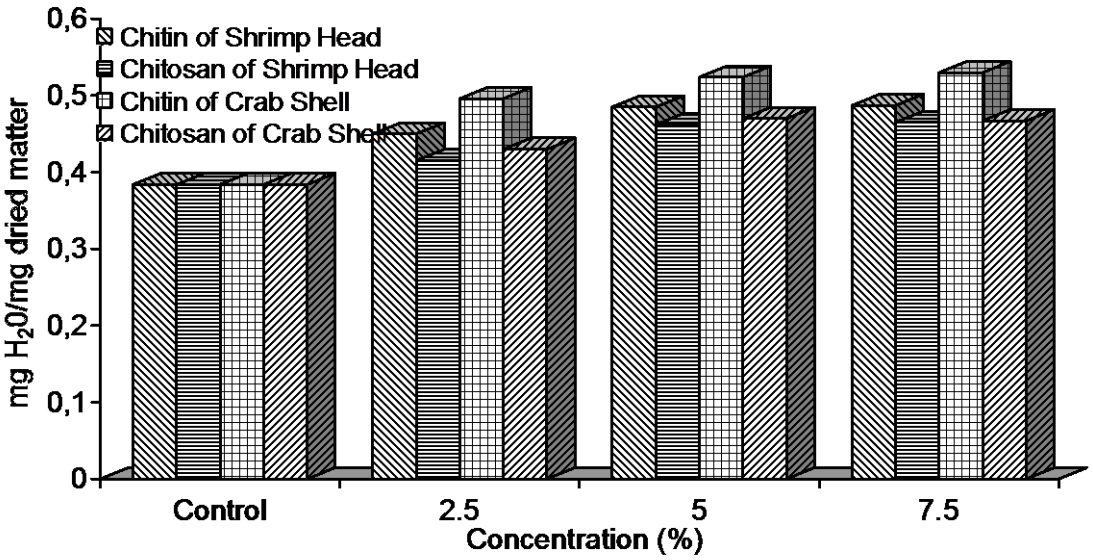

Fig. 1. The Effect of Various Concentration of Chitin and Chitosan Shrimp Head and Crab Shell Added to Myofibrils during Frozen Storage (mg H_20/mg dried matter) on the amount of unfrozen water

Results showed that each chitin and chitosan concentrations had different amounts of unfrozen water. The amount of unfrozen water was the largest in myofibrils with chitin and chitosan crab shell and shrimp head. Likewise, the quantity of unfrozen water in various concentrations of chitin and chitosan shrimp head and crab shell showed that the higher the concentration, the more the quantity of unfrozen water. But at a concentration of 7,5% both with chitin and chitosan shrimp head and crab shell, the unfrozen water tended to decrease. The quantity of unfrozen water in various concentrations and at various chitin and chitosan caused a different state of water in myofibrillar protein during the process of dehydration.

Frozen storage is an excellent method for preventing putrefaction and autolysis of fish muscle, however deterioration cannot be avoided when the storage period is lengthened. This is because when fish has been frozen for a long period, the functional properties such as emulsifying, lipid binding, water holding and gel forming capacities are decreased (Suzuki, 1981).

The effect of chitin and chitosan of crab shell and shrimp head against denaturation of myofibrils during frozen storage was studied through the change of Ca-ATPase activity. Therefore, measuring this activity during frozen storage was hoped to provide a measure of denaturation (Kamal *et al.*, 1989). The Ca-ATPase activity measured as an indicator of the denaturation of myofibrils in the presence of various concentration of chitin and chitosan of crab shell and shrimp head during frozen storage as shown in Fig. 2 – 5. The result showed that during frozen storage Ca-ATPase activity of the control decreased rapidly until the15th day, and then reduced slowly until day 120.

However with the presence of chitin and chitosan of crab shell and shrimp head (2.5 – 7.5 g), Ca-ATPase activity in the myofibril proteins decreased slowly during the subsequent investigation. Generally, the effect of various concentrations of chitin and chitosan of crab shell and shrimp head indicates that the addition of chitin and chitosan of crab shell and shrimp head can suppress the inactivation of Ca-ATPase activity of myofibrils. The results show that the changing Ca-ATPase activity is an indication of the changing in the structure and state of water in myofibrils during frozen storage. The changes in Ca-ATPase activity exhibited a similar tendency to those of unfrozen water, indicating that there is a close correlation between Ca-ATPase activity and the amount of unfrozen water.

Migita, *et. al*. (1960) and Sugiyama *et. al*. (1991), reported that protein denaturation of fish meat caused by drying occurred in the order of falling solubility of myofibrillar protein, a disappearance of concentration of muscle fiber by ATP, and a lowering of myosin ATPase activity. Inada *et. al*. (1992), reported that a change in sedimentation patterns and viscosity indicates that Ca-ATPase activity of myofibrillar protein of storage fish meat occurs faster than the decrease of the solubility of myofibrillar protein. During frozen storage, the viscosity of myofibillar protein decrease parallel with the decreasing solubility and Ca-ATPase activity. Yet the Ca-ATPase activity, when compared with solubility and viscosity, was more sensitive and fell during frozen storage (Noguchi and Matsumoto, 1971).

CONCLUSIONS

The effects of chitin and chitosan derived from crab shell and shrimp head on the unfrozen water and denaturation of myofibrils during frozen storage can be concluded as follows:

1. Addition of chitin and chitosan crab shell and shrimp head suppresses the decrease of Ca-ATPase activity. The chitin showed a better ability than that of chitosan.
2. An increase in concentration of chitin and chitosan crab shell and shrimp head increases the amount of Ca-ATPase activity.
3. During frozen storage chitin and chitosan supressed the falling amount of unfrozen water. In general, in chitin and chitosan shrimp head and crab shell, the unfrozen water amount drastically fell until the 20 days, with a slower decrease until the 120 days.
4. During frozen storage, chitin and chitosan supressed the falling amount of Ca-ATPase activity, but the amount of Ca-ATPase activity still slowly decreases. However, there was a drastic fall without chitin and chitosan.
5. The amount of unfrozen water increases by the adding chitin and chitosan. When chitin and chitosan were not added to myofibrils, the amount of unfrozen water rapidly decreases during frozen storage, whereas the decrease tends to be moderate in myofibrills added with chitin and chitosan.
6. The changes in Ca-ATPase activity exhibit a similar tendency to those of unfrozen water, indicating that there is a close correlation between Ca-ATPase activity and the amount of unfrozen water.

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