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Microstructures Evaluation of Fish Fillets Tuna (*hunnus albacares*) Coated with Chitosan from Waste Shell Vannamei Shrimp (*Litopenaeus vannamei*)

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Abstract: Fish is a food that is easily damaged; do to either the influence of environmental factors or the biochemical reactions that occur in food, especially tuna fish fillet. Tuna fish is one of the important species that have high economic value which is influential in the international market trade. Generally, Tuna fish is consumed in the fresh form, canned or frozen. Some research has shown that the use of chitosan as a coating can maintain the physical, chemical and microbiological, but changes in the microstructure of the characteristics of the filet can only be seen under the microscope. The aim of the study was to determine the microstructure of Tuna fish fillets coated with chitosan from vannamei shrimp shell waste at the various treatment of solvent, stored at room temperature and low temperature (-10^{0}C). The results showed that the microstructure of Tuna fish filet coated by chitosan extracted with different stages stored at room and low temperature showed different microstructures.
Keywords: Chitosan, tuna fillet, microstructure, room temperature, low temperature.

Introduction and Experimental

Shells of shrimp produced from the canning industry cannot be utilized fully, so alternative methods are needed to increase the economic value of the shells of shrimp, among which are chitin or chitosan product[1,2]. Chitosan is made from shrimp shells and can be used in the chemical industry as drugs and supplements, fat thickener, and the metal absorber material for the manufacture of cosmetics[3,4].

Tuna is one of the important fish species that have high economic value which is influential in the international market trade. Generally, tuna is consumed in the fresh form, canned and frozen. Fat in tuna is rich and it is known as unsaturated fatty acid components or poly unsaturated fatty acid (PUFA) highly susceptible to fat oxidation of hydrogen peroxide. Oxidation of fats occurs in materials during storage and with heat and the final product during the storage period specified. Oxidative damage affecting the organoleptic characteristics including flavor and aroma makes the product not good for consumption[5,6]. Fresh fish can be extended by adding antibacterial compounds and antibiotics[7]. The antibacterial compounds can diffuse into the surrounding environment and inhibit or stop the growth of bacteria. Materials such as tetracycline antibiotics have been banned for health reasons, therefore it is no longer an effective antibiotic substance used in the handling of fish catches. The use of natural materials can be used as a solution that is not harmful to health[8].

Chitosan is a polymer of glucosamine that has many benefits and applications. One of the uses and applications of chitosan is as antibacterial agents. Antibacterial ability of chitosan because of the NH_3 groups of glucosamine can interact with the surface of bacterial cells that are negatively charged[9]. Characterization of chitosan according to Suptijah[10], is a cationic polymer which has a monomer amount of about 2000-3000 monomer, not toxic to the value of LD_{50} = 60 g / kg body weight. Chitosan has a molecular weight of about 800 kDa. Chitosan can interact with materials that have a load such as proteins, anionic polysaccharides, fatty acids,

bile acids and phospholipids. Chitosan has the characteristics which is including the physical, biological and chemical that can be degraded and updated and have the nature of non-toxic, making it safe for use[11]. Chitosan has the ability to form a gel that also acts as a reactive component, chelating, binders, absorber, stabilizers, film-forming, purification, flocculants and coagulants[12].

Limited information is available in the literature on the effect of Tuna fish fillet on the microstructure quality, especially on texture and microstructure of muscle. The object of this research was to describe the microstructure of Tuna fish fillets coated with chitosan from vannamei shrimp shell waste at the various treatment of solvent, stored at room temperature and low temperature (-10^0C) by scanning electron microscope.

Material

The raw materials used in this study are the shell of vannamei shrimp (*Litopenaeus vannamei*) which were obtained from Sidoarjo city, Indonesia. Yellow fin tuna was obtained from the Port of "Sendang Biru", Malang, Indonesia. The method used in this research is descriptive method. In this study, chitosan was used as a coating on the surface of the of tuna fish fillets at room temperature and low temperature storage.

Chitosan preparation.

The processing of chitosan is done to get a natural preservative to be used in preliminary testing. The processing of chitosan refers No and Meyer[13] and Suptijah[8].

Microstructures test of Tuna fish fillets coated by chitosan with different solvent

a. Manufacture of chitosan to form a film using an organic acid solvent, namely acetic acid 1 % and formic acid 1 %. There are two types of chitosan processing; namely DMPA (decolorization-demineralization-deproteinasi-deacetylation) and DPMA (decolorization-deproteinasi-demineralization-deacetylation).
b. The concentration of chitosan used in this study was 1.5%. A total of 1.5 grams of chitosan DPMA and DMPA dissolved into 100 ml of acetic acid 1 % of concentration.
c. The fillets was then inserted into polypropylene plastic and stored at room temperature and at low temperature (- 10^OC). Evaluation was done at 1 and 7 days for room temperature storage, but 1 and 60 days for low temperature storage.
d. Muscle cell was evaluated using SEM (Scanning Electron Microscope).

Result

Microstructure test of Tuna fish fillets coated by chitosan at the acetic acid solvent.

The results of the microstructure of Tuna fish fillets coated by chitosan at the 1 % of asetic acid solvent can be shown on figure 1, 2, 3 and 4 as below.

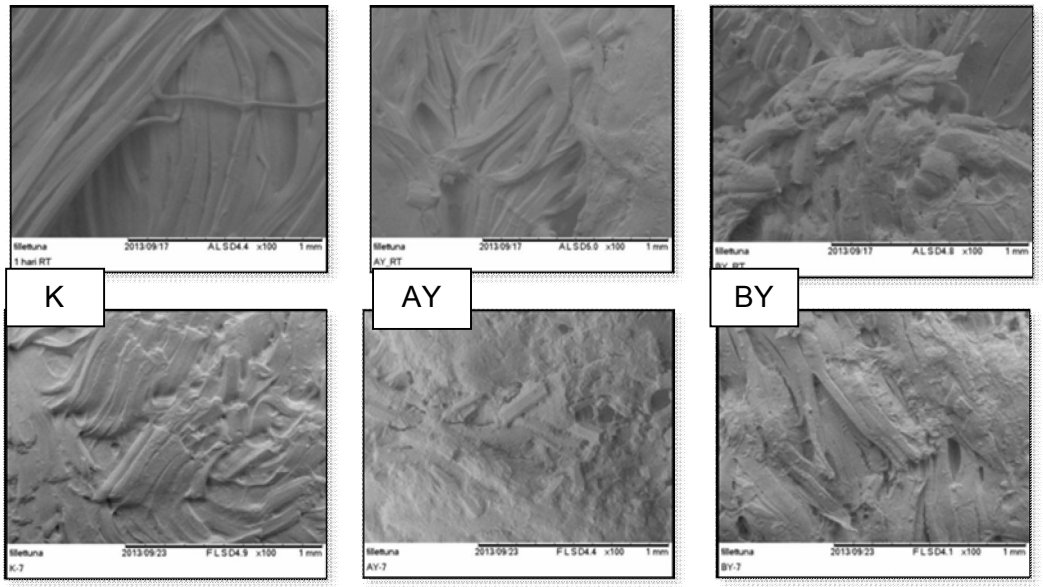

Figure 1. SEM of tuna fish fillet treatments at 1 (up) and 7 days (below) of storage room temperature. K = without chitosan; AY = Chitosan by DPMA processed with 1% of acetic acid solvent; and BY = Chitosan by DMPA processed with 1 % of acetic acid solvent. (100 x magnification).

Microstructures analysis performed using SEM (Scanning electron microscopy) with treatment samples at 1th day and 7th days storage at room temperature (figure 1 and 2) has shown the difference for K, AY and BY treatments. In the control treatment 1th day storage, tuna fish fillet fibers separate and there is no fiber or fiber layer that coats the surface of the meat, while the 7th days treatment showed weakening of the strength of the fibers of the meat which is marked by the release of fibers due to the decay process. In contrast to the treatment of AY and BY coated with chitosan concentration of 1.5%, it is indicated that a thin layer that surrounds the fillet and the meat fibers clump together. At AY treatment using chitosan A (DPMA) and the solvent acetic acid concentration of 1% shows that the structure of the fillet surface is more densed and looks more compact than the control treatment. In the treatment using chitosan BY (DMPA), fillet surface structure shows fibers separate meetings and fewer when compared to the control treatment.

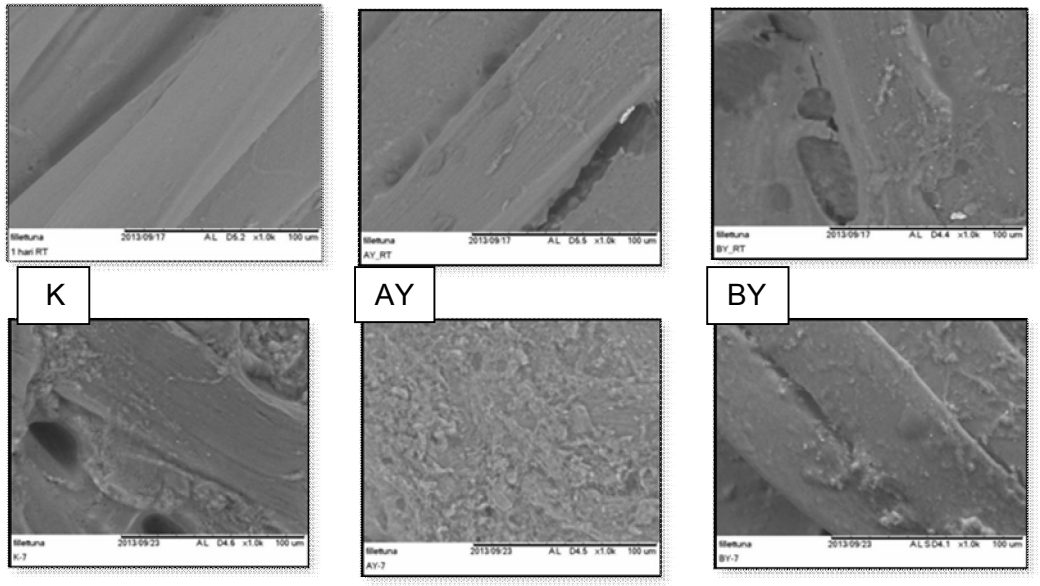

Figure 2. SEM of tuna fish fillet treatments at 1 (up) and 7 days (below) of storage room temperature. K = without chitosan; AY = Chitosan by DPMA processed with 1% of acetic acid solvent; and BY = Chitosan by DMPA processed with 1 % of acetic acid solvent. (<u>1000 x magnification</u>).

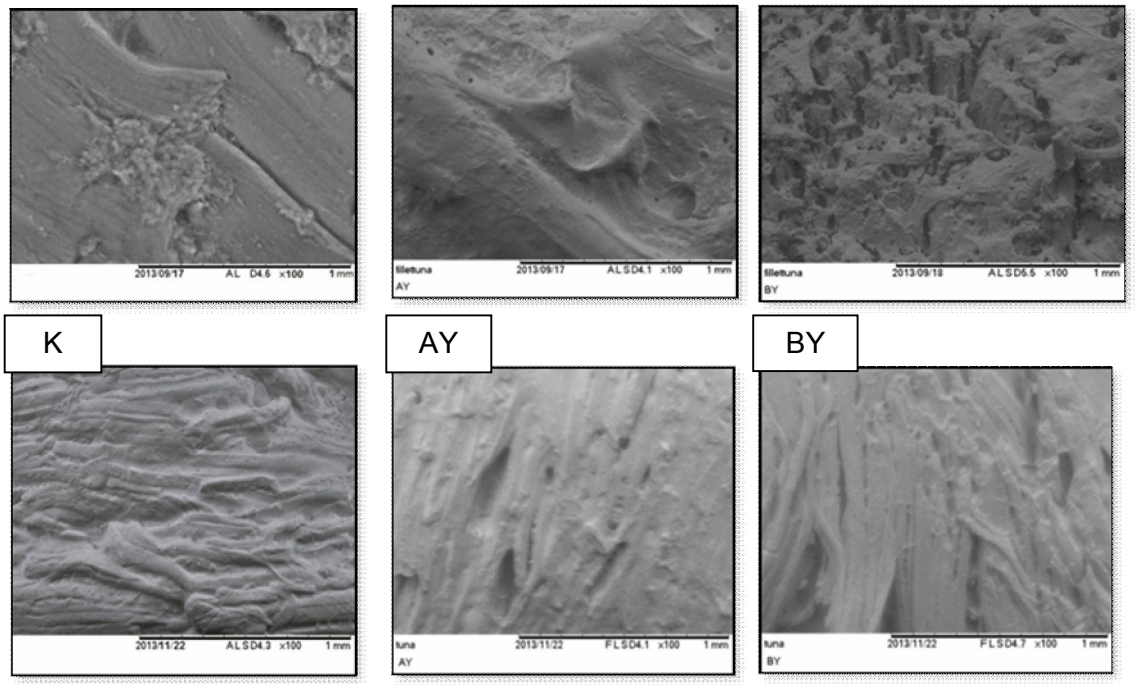

Figure 3. SEM photos of tuna fish fillet treatments 1 (up) and 60 days (below) of storage at low temperature (-10 ^{0}C). K = without chitosan; AY = Chitosan by DPMA processed with 1% of acetic acid solvent; and BY = Chitosan by DMPA processed with 1 % of acetic acid solvent. (<u>100 x magnification</u>).

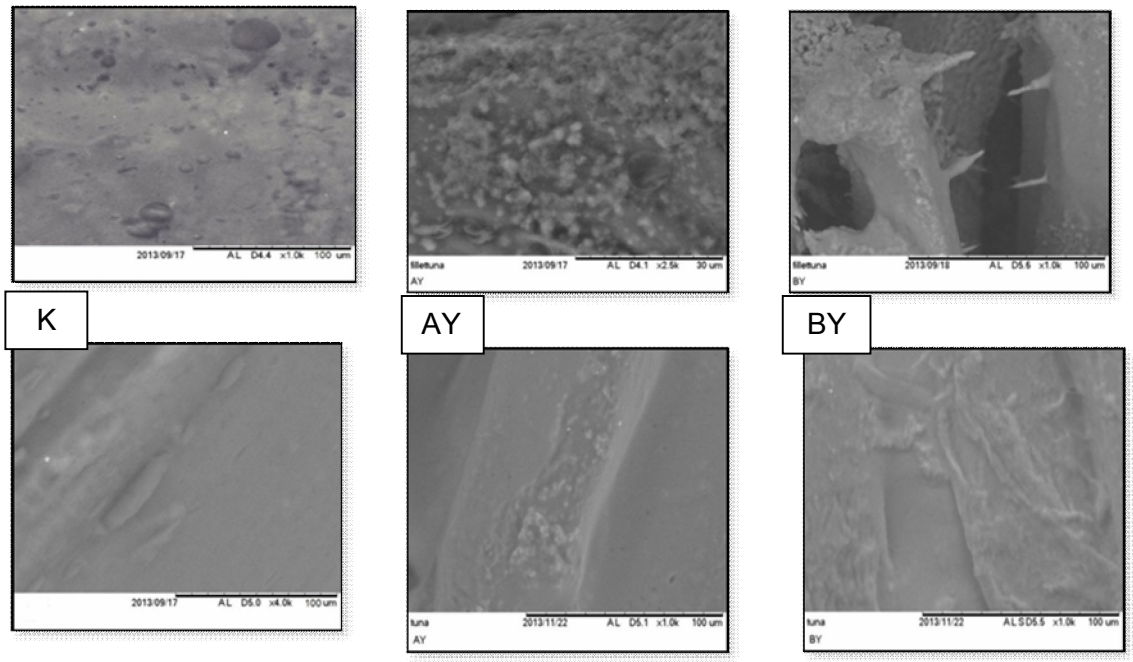

Figure 4. SEM of tuna fish fillet treatments at 1 (up) and 60 days (below) of storage low temperature (-10 ^{0}C).. K = without chitosan; AY = Chitosan by DPMA processed with 1% of acetic acid solvent; and BY = Chitosan by DMPA processed with 1 % of acetic acid solvent. (1000 x magnification).

Microstructure test of Tuna fish fillets coated by chitosan at the formic acid solvent.

Analysis of the microstructure of Tuna fish fillets coated with chitosan dissolved with 1% formic acid, was only evaluated at the low temperature storage. The products stored at room temperature cannot be evaluated because of damage. In detail can be seen in figure 5 and 6 as below.

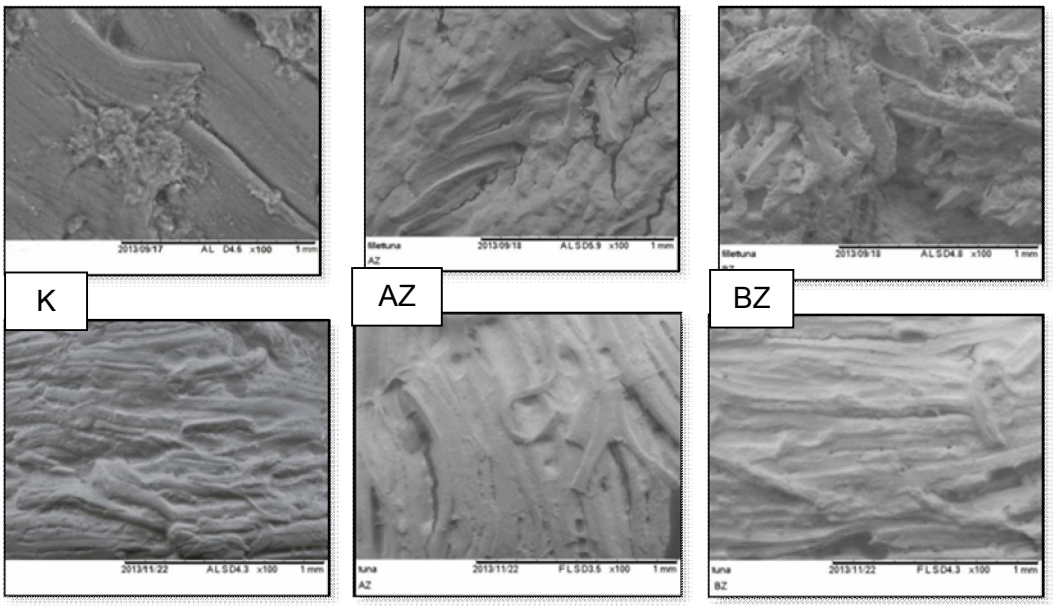

Figure 5. SEM of tuna fish fillet treatments at 1 (up) and 60 days (below) of storage low temperature (-10 ^{0}C). K = without chitosan; AZ = Chitosan by DPMA processed with 1% of formic acid solvent; and BY = Chitosan by DMPA processed with 1 % of formic acid solvent. (100 x magnification).

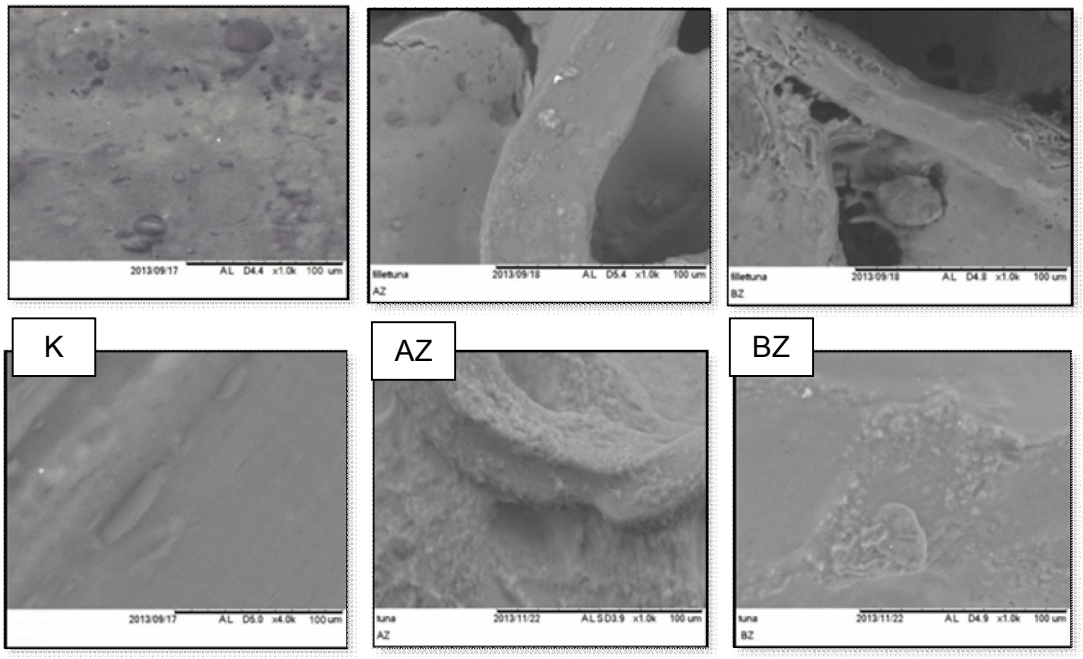

Figure 6. SEM of tuna fish fillet treatments at 1 (up) and 60 days (below) of storage low temperature (-10 ^{0}C).. K = without chitosan; AY = Chitosan by DPMA processed with 1% of formic acid solvent; and BY = Chitosan by DMPA processed with 1 % of formic acid solvent. (1000 x magnification).

Figures 5 and 6 above show that the microstructure of a Tuna fish fillet of one and 60 days of storage at low temperatures showed the difference of muscle. In the control treatment (without using chitosan) with a storage time of one day it is shown that a fillet surface is smooth, meat fibers are not independent of each other and the absence of a transparent layer that covers the surface of the fillet; while in 60 days storage the layers fillet is evenly, fiber-statements meat fused to one another.

Discussion

Microstructure test of Tuna fish fillets coated by chitosan at the acetic acid solvent.

Based from figure 1 and 2 it is shown that the layer on the surface of the fillet. Chitosan coating on the shrimp are coated white colored chitosan covering the surface of the shrimp. Treatment AY 1 day storage Showed fillet surfaces were coated with chitosan and sticking to each other, whereas the 7-day storage, tuna fish fillet surface was coated by the gel with densities shown by Fig 6. BY treatment on 1 day of storage showed uneven structure of the meat and meat fibers roomates coagulates while the 7-day storage, the meat fibers was coated by chitosan agglomerate. The structural change in the muscle of Tuna fish fillets was coated by chitosan at room temperature storage produced by protein degradation [14].

The results showed that only the surface of the fillet is visible, while myofibril protein constituent of fish meat is not visible. Based on research (Chantarasataporn[15] and Chen[16] the results of the microstructure of the processed shrimp coated with chitosan showed the protein structure myofirbril formed Z stripe pattern while the results showed chitosan coating the surface of the fillet of tuna, protein structure myofibril on tuna fish fillet is not evident. The results showed that chitosan is able to coat the fillet of tuna until 7 days storage at room temperature and extend the durable power of tuna fish fillets.

Results of analysis using SEM with 100x magnification (figure 3), shows that there are differences in each treatment which were suspected of chitosan able to coat the surface of the fillet of tuna to extend the shelf life, and is able to inhibit the decline in the quality of tuna fish fillets. The first day of the storage control treatment showed that the structure is not compact and the fibers of the meat apart, while the 60-day treatment, the surface uneven fillet, and the cooling temperature (-10^{0}C) shows the surface of the meat that is bumpy and belong together. In the treatment AY 1 day storage, Tuna fish fillet was covered by a layer of chitosan so that the meat fibers are not visible at the time of observation, while the 60th day storage of meat fiber agglomerate

which is covered by a layer of chitosan. Ayala [17] and Souza [18] stated that muscle samples the formation of ice crystals during the freezing process produced abundant clear spaces occupied by liquids at the interstitial spaces and inside the muscle fibres.

BY storage at one day treatment, meat fibers are numerous and there are clots that covered most of the surface of the fillet; while the 6 days of storage, tuna meat clot fiber and each fiber fused with each other. Allegedly the treatment AY and BY, chitosan covered the entire surface of the fillet of tuna and with low Temperatures causing agglomerate chitosan coating that surrounds the tuna fillet.

Chitosan apart also functions as antibacterial barriers which inhibits the activity of bacteria in a split macromolecular complex and use it for the metabolic process that will eventually lead to decay products. Chitosan has antibacterial mechanism in the bacterial cell wall where the OH groups of chitosan binds to the bacterial cell wall polysaccharides in the positive charge.

The observation using SEM 1000x magnification showed that the coating is able to coat the surface of the fillet of tuna. The first day of the storage control treatment showed that the structure is not compact and there are holes on the surface of the fillet, while the 60-day treatment, the surface of the fillet show that there is damage due to weakening of the muscles of meat that allegedly caused the cooling temperature (-10^0C) that causes ice crystals entry of tuna fish into fillets. Treatment AY, storage of 1 day showed fiber layer fillet of tuna coated chitosan looks like flakes of water a gel along the surface of the fillet, while in storage 60 days, the surface of the meat fibers showed a coating that sticks to the outside of the fiber, allegedly chitosan was coated and adsorbed until the inside of the fillet. In the treatment of BY, storage of 1 day showed chitosan lining up gets the fillet of tuna, meat fibers appear thick layer that lines like blobs of gel, while in storage 60 days, meat fibers are not visible, where all surfaces fillet was covered with chitosan were also affected by annealing temperature so that the fibers of meat covered with chitosan as a barrier.

Microstructure test of Tuna fish fillets coated by chitosan at the formic acid solvent.

The observation of Tuna fish filet on the formic acid coated by SEM with a magnification of 100x shows that the first day of the storage control treatment has structure that is not compact and the fibers of the meat is apart, while the 60-day treatment, the surface uneven fillet, and the cooling temperature (-10^0C) shows the surface of the meat that is wavy. AZ and BZ on 1 day storage, has a surface that is uneven and there are holes on the surface of the fillet, but in both treatment some meat fibers coagulates by a layer, which allegedly led to the accumulation of meat fibers are chitosan. While the 60-day storage, treatment AZ clots meat fibers and was coated by chitosan. As for the BZ treatment, surface treatment fillet clumped together like AZ.

Control treatment showed that the structure is not compact and there are holes on the surface of the fillet, while the 60-day treatment, the surface of the fillet show that there is damage due to weakening of the muscles of meat allegedly due to cooling temperatures (-10^0C) which causes the ice crystals into fillets of tuna. In the treatment of AZ with a magnification of 1000x, one fiber wrapped meat perfectly by chitosan. As for treatment BZ using 1000x, layer that surrounds the fillet looks clear and transparent where the gel is coated by chitosan that surrounds the tuna fillet. Both treatments shown no difference compared to using 100x magnification, fiber meat looks uneven and the treatment of AZ showed the clear gel spots. This situation allegedly occurred because of formic acid as a solvent is not perfect in the gel forming, so the fat fraction in filet partially out of the surface of the product. Sigurgisladottir[19] suggest that the lipids were presumably released from fat cells, and droplets were freely coating between the muscle fibers. Kim[20] Stated that Chitosan showed different film properties when different solvents and degrees of deacetylation were used to prepare the film-forming solutions. The structure or size of acids, as counter ions, may have influenced the intramolecular and intermolecular interactions[21].

Conclusion

Chitosan is able to protect the decline in the structure quality of tuna fillets until 7 days at room temperature storage and 60 days at low temperature storage. The use of 1% acetic acid solvent in the order DMPA and DMPA process has shown that Tuna fish fillet structure is more compact compared with 1% formic acid solvent. The use of formic acid 1% with DMPA process sequence is unable to coat filet perfectly.

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