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MAKING CHITOSAN EDIBLE COATING FROM MARINE INVERTEBRATES WASTE AND ITS APPLICATION AS NATURAL PRESERVATIVE IN SALTED FISH PROCESSING

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ABSTRACT:

Chitosan is a cationic polysaccharide which can be used as food preservative and applied as edible coating. This research aimed to determine chitosan level for salted fish preservation, to determine change of dried fish included microbiological analysis (TPC), chemicals (Aw), TBA, proximate and organoleptic test. for several weeks. The results of this study are 1.5% chitosan treatment appearance better than the usual treatment of formalin and salting, salted fish products with 1.5% chitosan treatment did not different with salinity and formalin treatment, fungi was not seen after 6 weeks of storage of 1.5% chitosan treatment, whereas in normal salting the 4th week already visible, durable power of salted fish in the treatment of chitosan may be preserved up to 3 months whereas durable power of normal salted fish was 2 months and formalin treatment 3 months 2 weeks.

Keywords: Edible coating, invertebrates, preservative, salted fish

INTRODUCTION

Industrial salted fish processing in some areas in Indonesia using formaldehyde as an ingredient in the manufacture of salted fish. The use of formaldehyde by the processor is intended as a preservative and additive yield of dried fish produced. The government could not take action against the processors that use formalaldehyde, because it has no proper evidence to preserve anchovies except formalin. The processor request sought for a solution to resolve the issue.

Targeted results that obtained are detailed information about the utilization of the waste marine invertebrate, the shrimp shell as an edible coating for natural preservation in processing salted fish replace synthetic materials formalin, such information includes how to make and manufacturing products of edible coating from shrimp shell waste and data quality salted fish that use edible coating.

In Indonesia, fish meat can be used to some products, such as beef jerky, meatballs, shredded, sausages, flour, silage, surimi, boiled, and salted fish. Their skin is tanned and made shoes, purses, belts and bags; fins for soup ingredients; teeth are used for wall decoration and heart for fish oil (Fisheries Research Institute, 1992).

According to Ronsivalli *et al.* (1978), in America, fish meat has been canned, smoked and made "sausage". Meat can also be made of fish fillet or "steak" as fresh or frozen. Bones can be made adhesive, whereas intestine material was extracted from intestine. In Japan, salted fish, dried fish and smoked fish are the a traditional foods. Famous fish in Japan is "hoshizame" (*Mustelus manzo*) that is eaten raw with vinegar and beans.

MATERIALS AND METHODS

Materials that used in this study were divided into two groups, each material for the production of edible coatings (chitosan) and materials for making salted fish. The tools used in this study were divided into two groups, each tools for making salted fish and chitosan, and tools for the analysis of the quality of the product.

Making Edible Coating: The process of making edible coating (chitosan) generally consisted of three stages, they were demineralization, deproteinization, and deacetylation. First of all shrimp waste washed and dried, and then blended. The next step was the process of demineralization with 1N HCl in the ratio 1:7 at a temperature of 90°C for 1 hour. Then, separation step with distilled water until neutral pH. The process carried out by the addition of proteinase 3.5N NaOH in the ratio 1:10 at the temperature of 90°C for 1 hour, then filtered to obtained chitin. After chitin was formed, the next step was adding NaOH deacetylation 50% with a ratio of 1:20 at 140°C for 2 hours.

Further separation and washing with distilled water until neutral pH and dried to get chitosan in powder form.

RESULTS AND DISCUSSION

Preparation of chitosan and its characteristics: The results of the characterization of chitosan were 7.54% water content and 0.75% ash content, meet the quality standards set by the PROTAN chitosan Laboratories, that is ≤ 10% water content and <2% for ash content respectively. The degree of deacetylation of chitosan was 75.42%, meet the quality standards set by the chitosan PROTAN Laboratories, i.e ≥ 70%.

Making Coating Formulation: Determination of appropriate edible coating formulation as coating materials in the manufacture of salted shark fish based on the hedonic scale organoleptic included appearance, color, texture, aroma and taste of the chitosan concentration of 0, 0.5, 1, 1.5, 2, 2, 5 and 3%. Kruskal-Wallis analysis showed that the appearance of the seven treatments (coating chitosan concentrations of 0, 0.5, 1, 1.5, 2, 2.5 and 3%), did not exert a significantly different level of preference panelists about sightings of salted shark fish.

Advanced Multiple Comparison Test, showed that the seven treatments (coating chitosan concentrations of 0, 0.5, 1, 1.5, 2, 2.5 and 3%), did not exert a significantly different level of preference panelists shall taste the salted fish shark. Treatment of chitosan coating 1.5 and 2% concentrations gave a significantly different affect on the level of fondness panelists about salted shark fish color than the concentration of chitosan coating 0, 0.5, 1, 2.5 and 3% treatments. The treatment of chitosan coating 2% concentration gave a significantly different affect on the level of joy in texture and flavor panelists salted fish than the concentration of chitosan 0, 0.5, 1, 1.5, 2.5 and 3% coating treatments.

From the analysis of the range, there was a real difference of treatment affecting of chitosan concentration and storage time. If followed by Least Significant Difference test, the result that 1.0%, 1.5% and 2.0% concentration of chitosan significantly different from both the control (normal salting and acetic acid treatment). From the organoleptic and microbiological tests concluded that the best formulation for the manufacture of edible chitosan coating was 1.5% concentration. It is mean to make 100 ml of edible coating required need: 1.5 g chitosan, 1 ml acetic acid and 99 ml of water. 1.5 grams chitosan could be used to coating 1 kg of dried fish.

Analysis and Utilization of Edible Coating in Salted Fish Processing: The analysis of dried fish included microbiological analysis, chemical and organoleptic test, microbiological analysis (Total Plate Count bacteria), chemicals (value of water activity [Aw], Thio Barbituric Acid [TBA]), proximate and organoleptic test (appearance, taste, smell, and consistence) done at 0, 2, 6 and 8 weeks. The analysis of TVB, total fungi and TBA was carried out at 0, 3, 6, 9 and 12 weeks.

The total value of the mold: Histogram of the average value of the logarithm of total salted shark fish mold was presented in Figure 1.

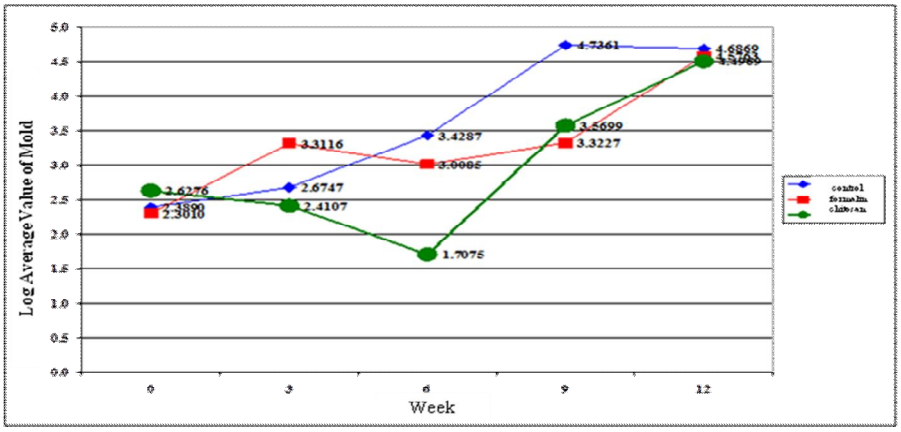

Figure 1: Histogram of the average value of the logarithm of total salted shark fish mold

The average value of the logarithm of total salted shark fish molds with chitosan coating treatments lower than formalin immersion treatment and control. It can be concluded that chitosan treatment was better to suppress the growth of fungi compare to other treatments. The low growth of fungus on fish salted shark fish with chitosan coating treatments through the inhibition of mold growth was caused by the activity of natural polikation chitosan (El Ghaouth *et al.*, 1991).

Based on visual observations during 12 weeks of mold growth in salted shark fish, showed that the controls on the storage-6 weeks, starting to look the growth of mold. For the treatment of chitosan coating and soaking formalin, at weeks 0^{th}, the 3^{rd} and the 6^{th}, had not seen the growth of mold and mold growth began to look at 9 weeks of storage.

According to SNI-salted fish molds required should be negative. Based on observations, mold activity that caused salt fish visually damage is *Dun*, that is the process of decay is characterized by gray spots, usually on the side of salted fish meat and brown to dark brown, it was found that the shelf life of fish salted shark control, chitosan coating and immersion consecutive formalin for 2 months, 3 months and 3 months 2 weeks.

Value Total Volatile Bases (TVB): Based on statistical tests concluded that treatment with chitosan coating can reduce the value of TVB when compare with controls but higher than the formalin treatment. Interaction between formalin immersion treatment at week 0, week 3, week 6, week 9 and week 12 gave significantly different values compared to the control at week 9. Interaction between chitosan coating treatment at week 3, week 9 and week 12 gave significantly different values compared with controls at week 9. The interaction between the controls at week 0 gives values significantly different compared with controls at week 9. Treatment of chitosan coating affect the value of TVB salted fish shark. Chitosan has effectiveness in inhibiting mold that produces proteolytic enzymes that play a role in the degradation of proteins that then influence the decay of meat and production of volatile nitrogen (Darmadji and Izumimoto, 1994). Histogram of the average value of Total Volatile Bases (TVB) salted shark fish is presented in Figure 2.

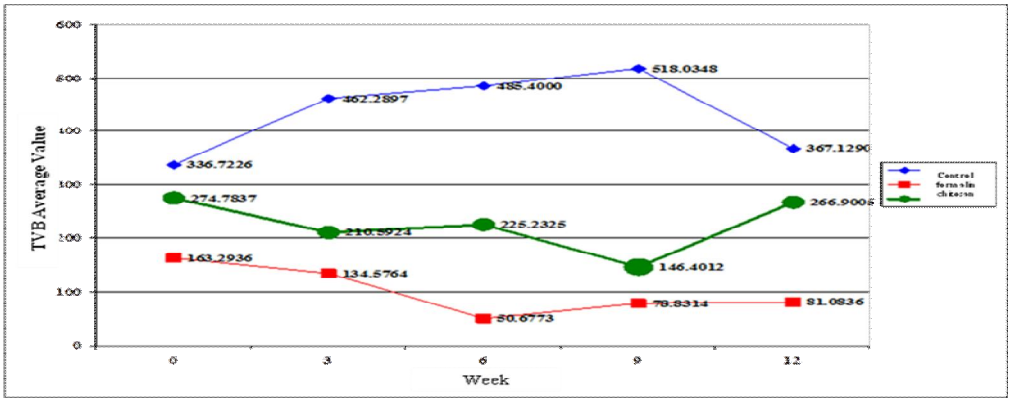

Figure 2: Histogram of the average value of Total Volatile Bases (TVB) salted shark fish

TVB range of fish that had undergone salting and drying is still suitable for consumption ranges from 100-200 mg% N/100 g (Connell, 1990). Increasing the value of TVB during storage thought to be caused by the formation of ammonia and increased levels of trimethyl amine (TMA) (Tazwir *et al.*, 1998). High levels of urea in fish shark, that is by 2.0 - 2.5% of total meat, should be overhauled by the activity of mold bases primarily evaporated ammonia affecting normality content of TVB (Stansby *et al.*, 1968). The increase in TVB during storage is also suspected to be caused by the decomposition of proteins by a mold that grows on the product being volatile bases nitrogen (Zaitsev *et al.*, 1969).

Value Barbituric Thio Acid (TBA): The increase in TBA values during storage was still relatively low and were in a standard TBA values for categories of food products that are still good quality. The product had a good quality was still TBA values fewer than 3 mg malonaldehyde/kg sample (Anonymous, 1991). Based on the analysis of variance showed that

the coating factor, storage time and interaction between the coating and storage time has significantly different influence on the value of TBA salted shark fish. According to HSD test, chitosan coating treatments, soaking formalin and controls gave significantly different values. Treatment of chitosan coating in suppressing influence on the value of TBA salted shark fish compared to controls. The process of fat oxidation caused by the contact between O_2 and the amount of fatty acid was inhibited by the low malonaldehyde content of chitosan and resulting low TBA values. In addition, chitosan can inhibit the activity of mold during storage leading to the hydrolytic rancidity. Histogram average Barbituric Thio Acid (TBA) shark salted fish was presented in Figure 3.

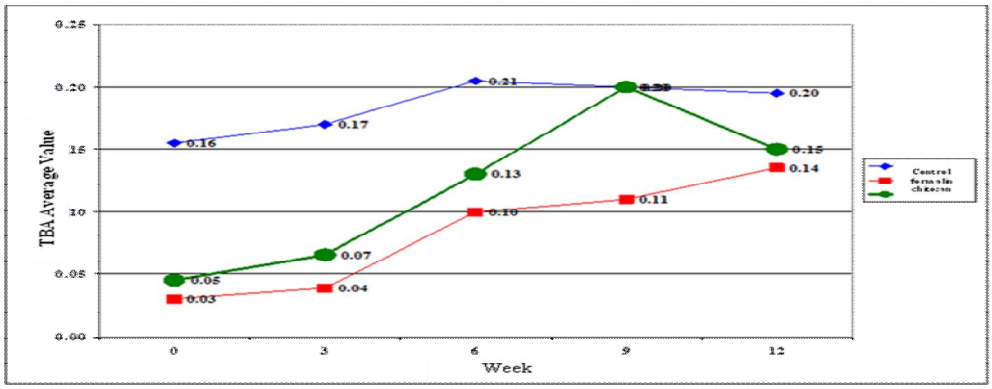

Figure 3: Histogram average Barbituric Thio Acid (TBA) salted shark fish

Value of water activity (Aw): The average value of water activity (Aw) salted shark fish storage at the beginning of the end of the 8th week of storage increased. The average value of the water activity at the beginning of storage (week 0) for every salted shark fish treatment controls, formalin, and chitosan coating respectively at 0.75, 0.732, and 0.728. While at the end of the 8th week of storage the average value of water activity (Aw) for salted fish shark control of 0.76, salted shark fish with formalin immersion at 0.7525 and shark fish salted with chitosan coating of 0.744.

Based on the analysis of variance showed that the factor coating and storage time significantly different influence on the value of aw shark salted fish, while the interaction between the factor coating and storage time did not give a significantly different effect. further test HSD, it was found that the water content of 1.5% chitosan coating treatment significantly different from control moisture. The 1.5% chitosan treatment was able to contribute to lowering the value of aw during storage. Improvement and fluctuations of water activity (Aw) allegedly caused by environmental humidity fluctuations during storage. Graph of the average value of water activity (Aw) shark salted fish was presented in Figure 4.

Total bacterial: The results of the statistical analysis Total Value Plate bacteria showed that the chitosan coating treatments were better than formalin treatment and control in suppressing the growth of bacteria until at week 8. Value (TPC bacteria) until the 8th week of chitosan coating treatments and formalin treatment was in accordance with ISO Standard-salted fish that was less than 1 x 10^5.

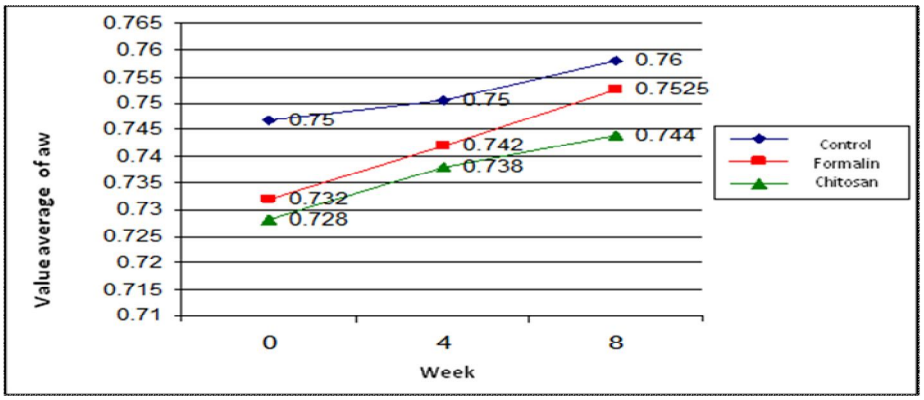

Figure 4 : Graph of the average value of water activity (Aw) shark salted fish

The ability to suppress bacterial growth due to the positively charged chitosan has polikation inhibiting the growth of bacteria and fungi (Allan and Hadwiger, 1979; El Ghaouth *et al.*, 1991). This causes the shelf life of salted fish given chitosan treatment can last up to 3 months compared with the salting anchovies which only last up to 2 months. Logaritmic total value of cucut salted fish bacteria during storage was presented in Figure 5.

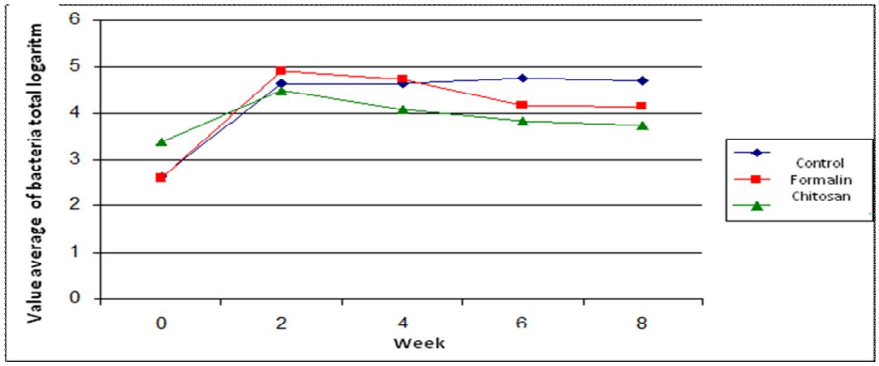

Figure 5 : Logarithm total value of salted shark fish bacteria during storage

Organoleptic hedonic quality test

Sightings: Kruskal-Wallis analysis of the results of the hedonic quality shark sightings salted fish on the 4th week of storage showed that the shark fish salted with chitosan coating significantly different and better than the hedonic quality shark sightings salted fish with 2% formalin coating and salted fish shark control. The storage of the 8th week showed that the appearance of shark fish salted with 1.5% chitosan coating better and significantly different sightings salted shark with formalin and control. Sightings of salted fish in 3 treatments can be seen in Figure 11. Sightings chitosan treatment better than other treatments due to chitosan coating forms protective barrier on the surface (Jiang and Li, 2001). The average value of hedonic quality test against sightings salted shark fish by coating chitosan ranged between 7-8. Descriptively, this value lies in the category intact, clean, a bit dull to intact, clean, less tidy, glowing by type. Salted shark fish sightings control at week-0 the average value 7.2 to the storage of the 2nd week of hedonic quality score shark sightings salted fish control to 6.8. Salted Shark fish with formalin immersion has an average value of hedonic quality sightings at week-0 storage of 7.3, the 2nd week of 7.0, and the 4th week average hedonic quality sightings dropped to 6, 7. Based on the statistical analysis of Kruskal-Wallis for hedonic quality sightings salted shark during storage obtained significantly different results between treatments coating with storage time ($P < 0.05$), meaning that both chitosan coating treatments, formalin, control or influence the quality of shark sightings salted fish during storage. Sightings of cucut salted fish during storage in 8th weeks with three treatments was presented in Figure 6.

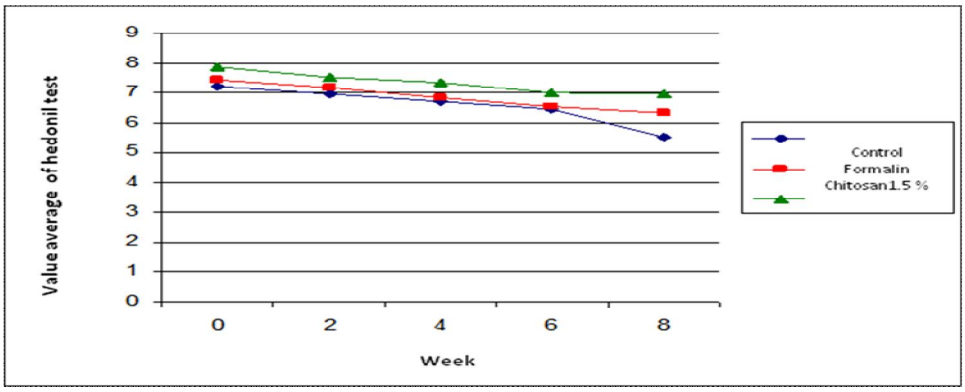

Figure 6 : Sightings of salted shark fish during storage in 8th weeks with three treatments

Odor: The average value of hedonic quality test shark salted fish odor control at the beginning of storage (week 0) of 7.4 and the 2nd week of the average value of 6.7. In the salted shark fish with 2% formalin immersion hedonic odor quality at the beginning of storage (week 0) of 7.2, the 2nd week of 7.0 and 4th week of storage the average value of 6.7. For salted shark fish with chitosan coating had odor hedonic quality score of 7.3 (week 0), 7.1 (week 2), and 6.4 (week 4). Kruskal-Wallis analysis resulted odor hedonic quality on the 4th week of storage, fish salted shark with chitosan coating significantly different from controls salted fish shark.

Treatment with formaldehyde during storage got organoleptic value lower than the chitosan coating treatment and odor control for the influence of formaldehyde that was still felt. While chitosan treatment gave better results than other treatments at week 8 because of the nature of the chitosan were odorless and can suppress the growth of odor-causing bacteria. The average value of hedonic quality tests on fish shark smells salty with each chitosan coating, formalin and control at the beginning of storage (week 0) ranges between 7.2-7.5. Kruskal-Wallis analysis resulted odor hedonic quality during storage against salted shark fish showed significantly different results in each treatment ($P < 0.05$), meaning that the control treatment, formalin, and chitosan coating on making salted fish shark exert a significant different to the smell of fish salted shark during storage**.** Average value of hedonic odor in cucut salted fish during storage was presented in Figure 7.

Sense: The average value of hedonic quality test taste the salty shark fish ranged from 6.8 to 7.2 (week 0). Descriptively, this value lies in the bad category, specific type, a little extra flavor to the category of very tasty, specific type, without any extra flavor. During storage the average shark salted fish hedonic quality tends to decline. Salted shark fish control has an average rating of 7.2 flavor hedonic quality (week 0), 6.8 (week 2), and 6.4 (week 4). Salted shark fish with formalin immersion had an average rating of 7.0 flavor hedonic quality (week 0), 6.7 (week 2), and 6.1 (week 4). Salted shark fish with chitosan coating had an average rating of 7.1 flavor hedonic quality (week 0), 6.7 (week 2), and 6.5 (week 4). Kruskal-Wallis analysis showed the quality of hedonic taste in salted shark fish and any influence of different treatments during storage. From the multiple comparison tests further treatment of chitosan coating on anchovies gives a better taste compared to the control and treatment of formalin in the 8th week of storage. Chitosan treatment did not cause a strange taste and was not significantly different from the other treatments at week 2, 4 and 6. This is because the fresh chitosan (Jeon *et al.*, 2002).

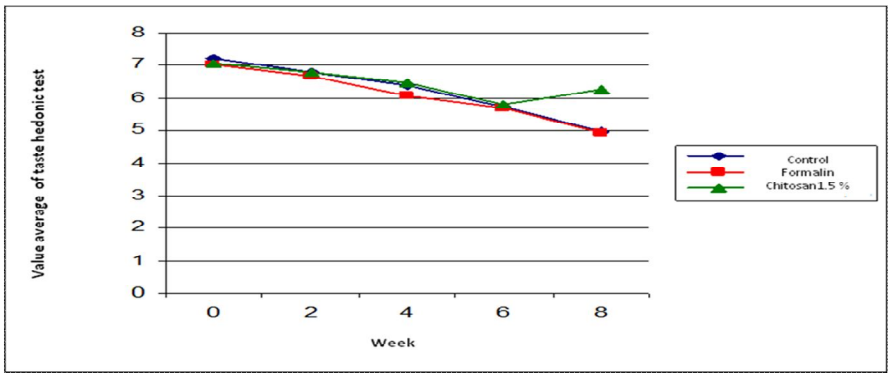

Figure 7 : Average value of hedonic odor in cucut salted fish during

Consistency: The average value of hedonic quality test consistency on shark salted fish at the beginning of storage ranged from 7.4 to 7.9 (Figure 4). Descriptively, the value lies in the category of too hard, not brittle until solid category, compact, less flexible dry. During storage until the 8th week, the average value of hedonic quality test consistency salted shark fish was declining.

For salted shark fish control has an average value of hedonic quality consistency of 7.4 (week 0) and 6.9 (week 2). In the salted shark fish with 2% formalin immersion has an average value of hedonic quality test consistency of 7.6 (week 0), 7.3 (week 2), 7.1 (week 4), 6, 9 (week 6), 6.7 (week 8). Kruskal-Wallis analysis of the results of testing the consistency of hedonic quality salted shark fish during storage, for each treatment showed a significantly different effect ($P < 0.05$). Average value of consistency in salted shark fish during storage is presented in Figure 8.

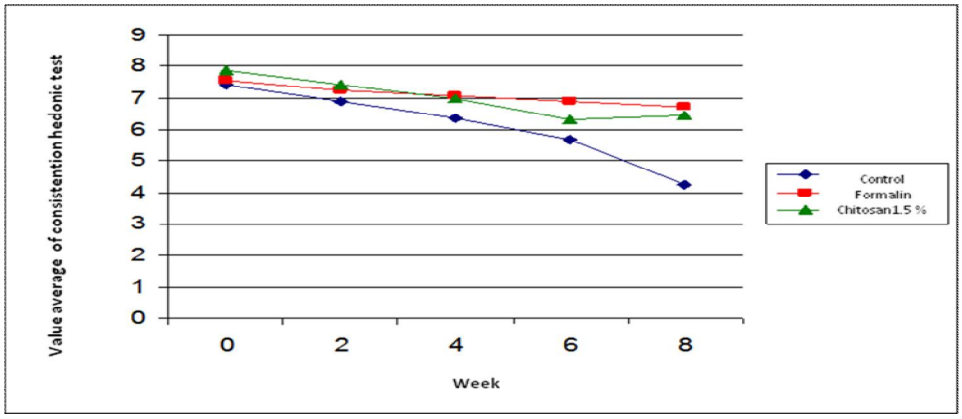

Figure 8: Average value of consistency in cucut salted fish during storage

Test of Proximate

Water content: The average value of fish salted shark water levels at the beginning of the storage until the end of the 8th week of storage has decreased. The water content of salted shark fish with chitosan coating on the storage at the beginning of 44.197%, while at the end of the 8th week of storage water level reached 49.83%. When compared with SNI maximum moisture content of 40%, meaning salted fish products with chitosan treatment still does not meet the standards set.

Based on the analysis of variance showed that the coating and storage time factors affected fish shark salted water content, while the interaction of the coating with storage time factor was not significantly different influence on levels water of salt shark fish. Of further test HSD, it was found that the storage of weeks 4, 6 and 8 levels of salt water fish shark with 2% formalin immersion significantly different from control water content, whereas at week 4, 6, and 8 levels of salt water fish shark 2% formalin did not differ significantly with water

content of 1.5% chitosan. Average value of water content of salted shark fish during storage is presented in Figure 9.

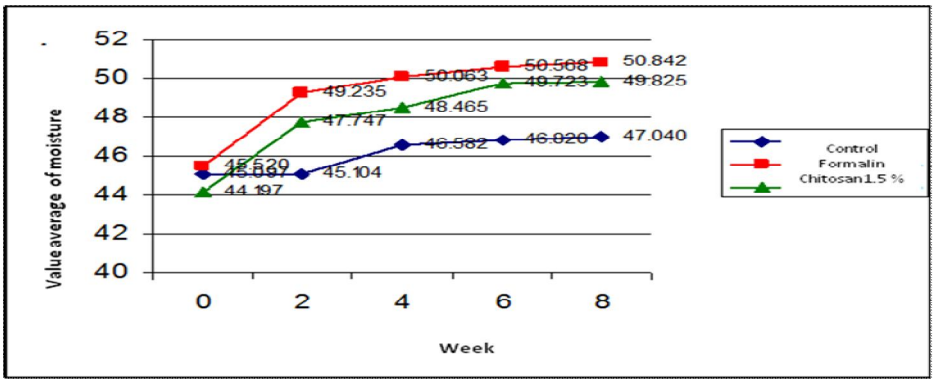

Figure 9 : Average value of water content of salted shark fish during storage

Levels of gray: In this study, measurement of ash content salted fish shark performed a total of 2 times, at the beginning and end of the storage-12 storage weeks. Graph the average ash content shark salted fish is presented Figure 10.

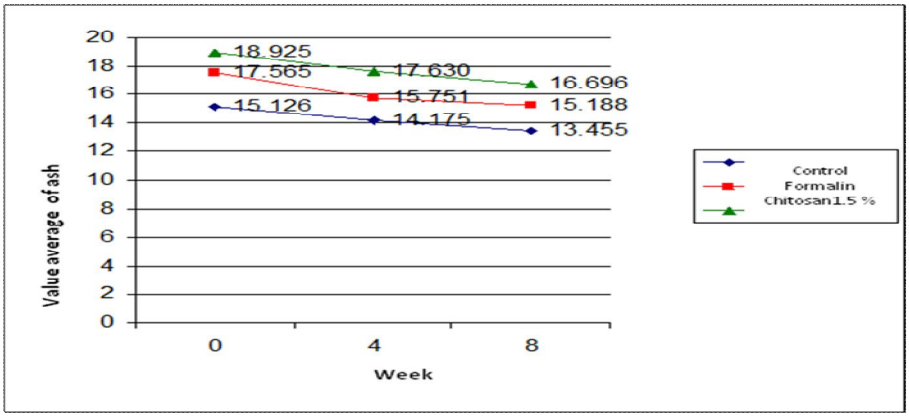

Figure 10 : Graph the average ash content shark salted fish

At the beginning of storage (week 0) average ash content for salted shark fishing controls, formalin, and chitosan coating amounted to 15.126%, 17.565% and 18.925%, 15.188%, while at the end, and chitosan coating for 16.696%. The average value of salted shark ash storage at the beginning to the end of storage has decreased.

Levels of protein : In this study, measurement of protein content salted shark fish performed 3 times, at the beginning of storage (week 0), week 4, and the end of the 8th week of storage. The average value of salted shark fish protein levels early in storage until the end of the 8th week of storage has decreased. The analysis of variance showed that the factor coating and storage time and interaction between the coating and storage time significantly different influence on levels of protein salted fish shark. Of further test HSD, it was found that treatment of formalin immersion significantly different chitosan coating treatments and control, as well as significantly different chitosan coating with and control. Protein content at higher chitosan coating treatment compared with controls.

Levels of fat: In this study, measurement of fat content of salted shark fish performed a total of 3 times, at the beginning of storage (week 0), week 4, and the end of the 8th week of storage. Graph the average fat content of salted shark fish is presented in Figure 11.

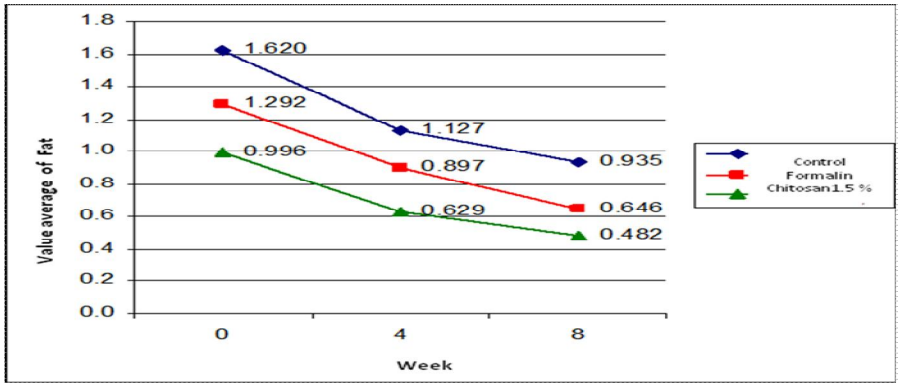

Figure 11 : Graph the average fat content of salted shark fish

The average value of shark salted fish fat levels at the beginning of the storage until the 8th week of storage has decreased. Based on the analysis of variance showed that the coating factor significantly different influence on fat content salted shark fish, while the storage time and the interaction between these factors and the old coating. Of further test BNJ, found in fat salted shark fish with chitosan coating significantly different fat content of salted shark fish control, while the fat content in salted shark fish with formaldehyde immersion was not significantly different to the fat content of salted shark fish control and chitosan.

CONCLUSION

Edible coating formulations manufacture as a natural preservative need 1.5% chitosan. Products with natural preservatives producing salted fish conformed to ISO-dried salted fish (SNI-2721-1992). In hedonic organoleptic quality test until the 8th week of storage showed that 1.5% chitosan treatment appearance better than the usual treatment of formalin and salting. In taste tests, salted fish products with 1.5% chitosan treatment did not differ by treatment with salinity and formalin. In fungi test, 1.5% chitosan treatment until 6 weeks of storage fungi have not seen, whereas in normal salting the 4th week already visible. In TPC (Total Plate Count) test bacteria is still below the standard anchovies 1 x 10^5. Durable power of salted fish in the treatment of chitosan can be preserved up to 3 months and 2 months salting and formalin treatment 3 months 2 weeks.

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