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# Effect of Modified Chitosan on the Fat Oxidation of Frozen Filleted Tuna (*Thunnus* sp.)

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**Abstract:- This research aims to obtain the best method for the chitosan modification process by paying attention to fat content, TBA, and color (L, a*, b*). Two different chitosan production protocols, namely: DCMPA (deproteinization - decolorization - demineralization - deacetylation) as a control process and DPMCA (deproteinization - demineralization - decolorization - deacetylation)) as a modification process were used to obtain quality archived frozen tuna during storage. 30 days. The fat and color levels (a* and b*) were lowest in DCMPA using 5% acetic acid solvent. TBA content, color (L) in chitosan using 10% acetic acid solvent. Meanwhile, on the other hand, the fat content, color (a* and b*) of DPMCA chitosan used 10% acetic acid solvent, the TBA content and L value of chitosan were modified using 5% acetic acid solvent. In general, modified chitosan is able to protect tuna fillets from fat oxidation compared to standard.**

***Keywords:-*** *Chitosan, Fileted Tuna, Frozen, Acetic Acid, and Fat Oxidation.*

## I. INTRODUCTION

Omega-3 fatty acids play an important role in human health. Tuna fish contains high levels of omega-3 fatty acids, but is very easily oxidized, especially if the tuna is cut to make value-added food products (Hammond, 2001). A variety of chemical preservatives are used to maintain the durability of these value-added products. Alternative natural preservatives are sought to increase consumer acceptance of the product. However, this treatment also produces shrimp shell waste and increases the use of chitosan.

The use of chitosan as an antibacterial has been widely reported by many researchers. Chitosan has the ability to inhibit the growth of pathogenic bacteria, fungi, yeasts, and viruses. The use of chitosan as a wrapper (film) can also be interpreted preservatives are safe for consumption. The advantage of chitosan is able to form a structure that is more resistant matrix so as to prevent reaction deteorasi food products by inhibiting reactive gases, especially oxygen and carbon dioxide, and can suppress the growth of bacteria and molds as well as control the movement of dissolved solids to maintain the natural color pigments and nutritional products (Krochta, 1992). No. et al., (2002), reported that chitosan with different organic acid solvents have different power resistor. In general, acetic acid, lactic acid, and formic acid is more effective than ascorbic acid and propionate acid. Chitosan showed higher antimicrobial activity for gram-positive bacteria than gram-negative (Jeon et al., 2001)

Based on the description above, it shows that chitosan, which is known as an antibacterial and antioxidant, has been widely applied to beef products, but not to fish. Considering that tuna is an export commodity that is high in histidine and omega-3 content, this fish is easily decarboxylases or oxidized, resulting in a decrease in quality. Therefore, in this research, testing was carried out on tuna fish products, because there were no research reports on this fish.

The aim of this research is to evaluate the effect of several variations of modified chitosan in acetic acid solution on fat oxidation in tuna fillets during 2 months of frozen storage. The fillets will be evaluated periodically every month for parameters of fat content, thiobarbituric acid (TBA), and color (L, a*, b*). According to Barrd et al. (1988), 10 to 20% of damaged tuna landings are caused by bacterial decarboxylase activity or a decrease in omega-3 levels, especially fish preserved using ice during storage.

## II. METHOD

### *A. Chitosan Protocol Process (No et al., 1989)*

- *DP (Deproteinization)*

The process of deproteinization was used by NaOH solution 3.5% (w/w) for 2 hours at a temperature of 65 ° C above stirrer shell to solid with ratio carapac:solution of 1:10 (w / v). Furthermore, the sample was filtered using vacuum suction, and the filtrate was washed with running water for 30 minutes, and dried.

- *DM (Demineralization)*

Demineralization using 1N HCl for 30 minutes at ambient temperature, the ratio of solid:solution of 1:15 (w / v), then the sample is filtered using vacuum suction, and the filtrate was washed with running water for 30 minutes, and dried.

- *DC (Decolorization)*

Decolorization using acetone for 10 minutes and dried for 2 hours at ambient temperature, followed by bleaching using a solution of 0.315% (v/v) sodium hypochloride (NaOCl) (containing 5.25% chlorine) for 5 min at ambient temperature with the ratio of solid: solution 1:10 (w/v), based on a dry basis. Samples were then washed with running water for 30 minutes and dried with a vacuum dryer for 2-3 hours until the powder becomes soft.

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➢ *DA (Deacetylation)*

Deacetylation or remove acetyl groups from chitin performed using an autoclave at a pressure of 15 psi for 30 minutes at 121 ºC using 50% NaOH solution with a ratio of 1:10 (w/v). Chitosan produced neutralized using running water, washed again using distilled water, then filtered and dried at 60 ° C for 24 hours in the oven.

Encoded of Protocol above was DCMPA. In this study there were 4 treatments, where the two protocols and two modified treatment with the different concentrations of acetic acid, so be as follows: DCMPA1, DCMPA2, DPMCA1, and DPMCA2.

- DCMPA = decolorization - demineralization - deproteinization - deacetylation.
- DPMCA = deproteinization - demineralization - decolorization - deacetylation.

*B. Preparation of Filleted Tuna*

Tuna (Thunnus sp) was catches from "Sendang Biru", Malang, Indonesia. Tuna catches (on board) as soon as possible put on the styrofoam with ice trash. Arriving in place of the study, processed to a filet, a total of 108 samples, each weighing approximately 250 g / filet. Furthermore, each fish filet marinated tuna into a solution of chitosan in acetic acid 0.5% and 1% (1:1, w / v) for 3 minutes, and then frozen in a refrigerator at a temperature of - 5 ° C, for 60 days and observed every 30 days.

*C. Experiment Design*

The study evaluated against two concentrations of acetic acid (0.5% and 1%, w / v) and 4 types of modified chitosan. A total of eight treatments was tested on tuna filet. A total of 3 replicates of Tuna fish fillets stored at freezing temperature of -5 ° C for 60 days. Analysis was performed on days 1, 30, and 60.

*D. Implementation*

Make a solution of 0.5% and 1.0% acetic acid, each at 25 L and 250 mL was poured into a plastic boxes for a total of 18 pieces of 0.5% acetic acid, and 18 pieces for 1.0% acetic acid. Furthermore, as many as 18 pieces of tuna filet marinated into a solution of 0.5% acetic acid, and 18 filet into acetic acid 1.0%, respectively for 3 minutes (Hammond, 2001). A total of 36 filet that had been treated, an then inserted into plastic bags and then stored in the refrigerator freezing temperatures -5 ° C for 60 days. Analysis was performed on days 1, 30, and 60.

*E. Chemical Analysis*

➢ *Fat Content*

Fat analysis using soxlet with Hexan as the solvent. 5 grams of fileted tuna wrapped the dried filter paper and weighed, then put 50 ml the filled hexan then heated by water bath (85 to 90 °C) for 4-5 hours. After heating is complete, the sample in filter paper dried in an oven temperature of 85 to 90 °C for 1 hour, then cooled in desicator. Difference in initial weight and final sample weight multiplied by 100% is the fat content of products.

➢ *Color*

The color of fileted tuna was measured using a MINOLTA CR200b spectrocolorimeter (Minolta, Co.., Ltd.., Osaka 541, Japan), which is expressed as L * (lightness), a * (Redness) and b * (yellowness).

➢ *TBA (2-thiobarbituric acid)*

Thiobarbituric acid reaction substance (TBARS) were measured to access oxidized fats, using the method of Buege and Aust (1978). 4 g sample was analyzed in duplicate by entering into a 50 ml test tube Falcon. 16 mL cold buffer (50 mM PO4, 0.1% EDTA, 0.1% propyl gallate) was added to the sample and homogenized for 30 seconds using a Polytron (Kinematica CH-6010 Kriens-Lu; Switzerland) on a set of 5. Polytron washed with running water and distilled water on each sample. 4 mL of 30%. TCA was added and the test tube vortexed for 15 seconds. The mixture was filtered using a paper (Quantitative P8-Fluted, Fisherbrand; Pittsburgh, PA) and 4 mL filtrate pipetted and put into a test tube. 4 mL of 20nm TBA added to each tube, and then shaken in a vortex. At that moment a standard curve prepared TEP, as many as 0, 0.5, 1. O, 1.5, 2.0, and 2.5 mL of standard solution of TEP pipetted into a test tube. Each reaction tube was increased to 4 mL using buffer solution: the TCA (8:2). Furthermore, 4 mL TBA solution was added to each test tube, shaken, and vortexed. Each test tube is used as a determinant of the standard curve, placed into boiling water for 20 minutes, then removed and cooled immediately. Absorbance is read at 530nm using a Beckman DV-64 spectrophotometer (Bekman Coulter, Inc..; Fullerton, CA).

*F. Data Analysis*

Data were analyzed using Microsoft Excel as the mean of independent experiments with standard deviation (SD).

## III. RESULT AND DISCUSSION

*A. Results*

The results of laboratory analysis on fat content, TBA, and color values can be seen in Table 1 to 5.

Table 1 Observations on Fat Content (%) Modified Chitosan Treatment Process

| TREATMENT | OBSERVATION (days) | | |
|---|---|---|---|
| | 1 | 30 | 60 |
| DCMPA_5% | 1,23 ± 0,07 | 2,23 ± 0,08 | 2,65 ± 0,15 |
| DCMPA+10% | 1,90 ± 0,08 | 2,79 ± 0,22 | 3,28 ± 0,09 |
| DPMCA_5% | 1,33 ± 0,13 | 2,34 ± 0,22 | 2,97 ± 0,19 |

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| | | | |
|---|---|---|---|
| DPMCA_10% | 2,25 ± 0,17 | 3,07 ± 0,22 | 3,70 ± 0,17 |

The data in Table 1, showing that the value of the lowest fat content on the observation 1, 30 and 60 days is the control solvent acetic acid 5% (DCMCA, 5%), ie 1.23 ± 0.07, 2.23 ± 0.08, and 2.65 ± 0.15 respectively. The highest fat content at modification products using 10% acetic acid solvent (DPMPA, 10%), ie 2.25 ± 0.17; 3.07 ± 0.22, and 3.70 ± 0.17 respectively. In general, all treatments, both control and modified products at the level of 5% and 10% solvent had fat content tends to increase during storage.

Table 2 Observations on Levels of TBA (%) Modified Chitosan Treatment Process

| **TREATMENT** | **OBSERVATION (days)** | | |
|---|---|---|---|
| | **1** | **30** | **60** |
| DCMPA_5% | 1,55 ± 0,02 | 1,54 ± 0,07 | 0,31 ± 0,02 |
| DCMPA+10% | 1,35 ± 0,05 | 0,67 ± 0,04 | 0,28 ± 0,02 |
| DPMCA_5% | 1,80 ± 0,11 | 0,75 ± 0,06 | 0,45 ± 0,03 |
| DPMCA_10% | 1,17 ± 0,11 | 0,40 ± 0,02 | 0,16 ± 0,01 |

The data in Table 2, showed that the value of the lowest levels of TBA on the observation 1, and 30 and 60 days is the treatment protocol modification using 10 % of acetic acid solvent (DPMCA, 10%), ie 1.17 ± 0.11 ; 0.40 ± 0.02, and 0.16 ± 0.01 respectively. The value of the highest levels of TBA at control products using 5% acetic acid (DCMPA, 5%), ie 1.55 ± 0.02; 1.54 ± 0.07, and 0.31 ± 0.02 respectively. In general, all treatments, both control and modified at the level of 5% and 10% solvents tended to decrease during the storage process.

Table 3 Observations on the Value of L Chitosan Modified Treatment Process

| **TREATMENT** | **OBSERVATION (days)** | | |
|---|---|---|---|
| | **1** | **30** | **60** |
| DCMPA_5% | 55,29 ± 4,13 | 44,86 ± 0,83 | 43,49 ± 2,12 |
| DCMPA+10% | 53,45 ± 4,96 | 42,49 ± 1,44 | 41,91 ± 3,68 |
| DPMCA_5% | 53,60 ± 4,70 | 43,58 ± 1,50 | 43,01 ± 1,14 |
| DPMCA_10% | 52,18 ± 3,42 | 42,04 ± 2,31 | 40,40 ± 1,79 |

The data in Table 3., Showed that the lowest value of the color (L) on the observation 1, and 30 and 60 days is the treatment protocol modification using acetic acid 10% of solvent (DPMCA, 10%), ie 52.18 ± 3.42; 42.04 ± 2.31, and 40.40 ± 1.79 respectively. The highest values in the control using the acetic acid 5% of solvent (DCMPA, 5%), which respectively 55.29 ± 4.13; 44.86 ± 0.83, and 43.49 ± 2.12. In general, all treatments, both control and modified at the level of 5% and 10% solvents tended to decrease during the storage process.

Table 4 Observations on the Value of a * Modified Chitosan Treatment Process

| **TREATMENT** | **OBSERVATION (days)** | | |
|---|---|---|---|
| | **1** | **30** | **60** |
| DCMPA_5% | 5,36 ± 0,24 | 6,22 ± 0,30 | 7,02 ± 0,38 |
| DCMPA+10% | 5,66 ± 0,23 | 6,87 ± 0,53 | 7,45 ± 0,54 |
| DPMCA_5% | 5,45 ± 0,40 | 6,35 ± 0,36 | 7,37 ± 0,43 |
| DPMCA_10% | 5,82 ± 0,09 | 6,93 ± 0,16 | 7,88 ± 0,65 |

The data in Table 4, shows that the lowest value of the color (a *) on the observation 1, and 30 and 60 days is the control treatment using 5% acetic acid solvent (DCMCA, 5%), ie 5.36 ± 0 , 24; 6.22 ± 0.30, and 7.02 ± 0.38 respectively. The highest values in the modification of treatment using 10% acetic acid solvent (DPMPA, 10%), ie 5.82 ± 0.09; 6.93 ± 0.16, and 7.88 ± 0.65 respectively. In general, all treatments, both control and modified at the level of 5% and 10% solvents tended to increase during the storage process.

Table 5 Observations on the Value of b * Modified Chitosan Treatment Process

| **TREATMENT** | **OBSERVATION (days)** | | |
|---|---|---|---|
| | **1** | **30** | **60** |
| DCMPA_5% | 11,17 ± 0,71 | 11,00 ± 0,53 | 12,20 ± 0,53 |
| DCMPA+10% | 11,33 ± 0,93 | 12,27 ± 0,23 | 13,50 ± 1,21 |
| DPMCA_5% | 11,17 ± 0,61 | 11,40 ± 0,17 | 12,53 ± 0,60 |
| DPMCA_10% | 11,90 ± 0,70 | 12,87 ± 0,45 | 13,60 ± 0,95 |

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The data in Table 5, shows that the lowest value of the color (b *) on the observation 1, and 30 and 60 days is the control treatment using 5% acetic acid solvent (DCMCA, 5%), which respectively 11.17 ± 0 , 71; 11.00 ± 0.53, and 12.20 ± 0.53. The highest values in the modification of treatment using 10% acetic acid solvent (DPMPA, 10%), ie 11.90 ± 0.70; 12.87 ± 0.45, and 13.60 ± 0.95 respectively. In general, all treatments, both control and modified at the level of 5% and 10% solvents tended to increase during the storage process.

*B. Discussion*

From the table above, the parameters of the fat and TBA value is an important indicator of fat oxidation. Achieved the highest fat content DCMPA2, as well as the level of the lowest TBA. This suggests that the levels of fat filet of tuna tend to constant product coated with chitosan modified at a concentration of 1%. The product also looks more stable quality changes during storage.

The average fat content of the fish fillet Tuna were 2.65 ± 0.15%, 3.28 ± 0.09%, 2.97 ± 0.19%, and 3.70 ± 0.17% by the end of the storage was higher than salmon (Suvanich et al., 1998). The fat content is lower than 1.6%, as well as the water content (74.3%), and 70% (Nicholas, 2003), but still lower when compared with the results of the study Sigholt, et al. (1997), ie 19%. The differences in both lipid and ash contents can be caused by a species of fish and fishing season.

There was no difference in the value of L *, a * and b * (based STD) among all treatments until end of storage, presumably due to the acetic acid is used at a concentration of 0.5% and 1% is not enough to degrade hemoglobin in meat during storage. Marshall (1998) reported that a direct color after bleaching catfish fillets dipped in 2% acetic acid. Mitsuda et al. (1980) also found that the dip of 1% acetic acid causes early bleaching fillet fish for protein denaturation caused by low pH acidic. Jo et al. (2001) reported that the value of L* higher in pork sausage dipped in chitosan than in controls. However, L* values increased for all treatments during storage. Suvanich et al. (2000) found that the value of L* in the digestive tract of catfish mince unchanged for three months at freezing temperatures (-20 °C). The difference in results may be due to the fact that the digestive tract has a very white meat, while fish Tuna has a characteristic red color.

## REFERECES


[1]. Barrd, P. D., Bennett, R., and Hamilton, M. 1 988. The consumer acceptability of some underutilized fish species, in Food Acceptability, Thompson, D.M.H. (ed), Elsevier, New York, pp. 431-442.

[2]. Chai, T., Chen, C., Rosen, A., and Levin, R.E. 1968. Detection and incidence of specific species of spoilage bacteria on fish. Applied Microbiology 16(11): 1738-1741.

[3]. Chen, C., Liau, W., and Tsai, G. 1998. Antibacterial effects of N-sulfonated and Nsulfobenzoyl chitosan and application to oyster preservation. Journal of Food Protection 61(9): 1 124-1 128.

[4]. Christen, G .L., and Smith, J.S. *Food Chemistry: Principles and Applications.* Science Technology System, California, 2000.

[5]. Cuero, R.G., Duffus, E., Osuji, G., and Pettit, R. 1991. Aflatoxin control in preharvest maize: effects of chitosan and two microbial agents. Journal of Agricultural Science 1 17: 165- 169.

[6]. Darmadji, P., and Izumimoto, M. 1994. Effect of chitosan in meat preservation. Meat Science 38: 243-254.

[7]. Einen, O., and Thomassen, M.S. 1998. Starvation prior to slaughter in Atlantic salmon *(Salmo salar)* 11. White muscle composition and evaluation of freshness, texture and colour characteristics in raw and cooked fillets. Aquaculture 169: 37-53.

[8]. Erickson, M.C. 1993. Compositional parameters and their relationship to oxidative stability of channel catfish. Journal of Agricultural and Food Chemistry 41: 1213-1218.

[9]. Fatima, R., Farooqui, B., and Quadri, R.B. 198 1. Inosine monophosphate and hypoxanthine as indices of shrimp quality. Journal of Food Science 46: 1 125-1129.

[10]. Gonzalez-Rodriguez, M., Sanz, J.J., Santos, J.A., Otero, A., and Garcia-Lopez, M. 2001. Bacteriological quality of aquacultured freshwater fish portions in prepackaged trays stored at 3OC. Journal of Food Protection 64(9): 1399- 1404.

[11]. Hammond, M.D., 2004. The use of chitosan to preserve and extend atlantic salmon quality. The Graduate School The University of Maine.

[12]. Hashimoto, M. 1987. Applications of chitosan as an agent for the polluted wastewater treatment and the removal of nucleic acids and endotoxins.

[13]. Jo, C., Lee, J.W., Lee, K.H., and Byun, M.W. 200 1. Quality properties of pork sausage prepared with water-soluble chitosan oligomer. Meat Science 59: 369-375.

[14]. Knorr, D. 1984. Use of chitinous polymers in food - A challenge for food research and development. Food Technology, 3 8: 85-97.

[15]. Mitsumoto, M., Cassens, R.G., Schaefer, D.M., Arnold, R.N., and Scheller, K.K. 1991. Improvement of color and lipid stability in beef longissimus with dietary vitamin E and vitamin C dip treatment. Journal of Food Science 56(6): 1489-1492.

[16]. Nicholas, T. 2003. Antimicrobial use of native and enzymatically degraded chitosans for seafood applications. MS. thesis, University of Maine, Orono, ME.

[17]. No, H.K., Lee, K.S., Kim, I.D., Park, M.J., Kim, S.D., and Meyers, S.P. 2003. Chitosan treatment affects yield, ascorbic acid content, and hardness of soybean sprouts. Journal of Food Science 68(2): 680-685.

[18]. No, H.K., Lee, K.S., and Meyers, S.P. 2000. Correlation between physicochernical characteristics and binding capacities of chitosan products. Journal of Food Science 65(7): 1 134-1 137.

[19]. Okayama, T., Imai, T., and Yamanoue, M. 1987. Effect of ascorbic acid and alphatocopherol on storage stability of beef steaks. Meat Science 21 : 267-273.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24JUN418

[20]. Ouattara, B., Simard, R.E., Peitte, G., Begin, A., and Holley, R.A. 2000a. Diffusion of acetic and propionic acids from chitosan-based antimicrobial packaging films. Journal of Food Science 65(5): 768-773.
[21]. Ouattara, B., Simard, R.E., Peitte, G., Begin, A., and Holley, R.A. 2000b. Inhibition of surface spoilage bacteria in processed meats by application of antimicrobial films prepared with chitosan. International Journal of Food Microbiology 62: 139-148.
[22]. Reddy, N.R., Solomon, H.M., Yep, H., Roman, M.G., and Rhodehamel, E.J. 1997. Shelf life and toxin development by *Clostridium botulinum* during storage of modified-atmosphere- packaged fresh aquacultured salmon fillets. Journal of Food Protection 60(9): 1055-1063.
[23]. Richards, M.P., Kelleher, S.D., and Hultin, H.O. 1998. Effect of washing with or without antioxidants on quality-retention of mackerel fillets during refrigerated and frozen storage. Journal of Agricultural and Food Chemistry, 46(10): 4363-4371.
[24]. Roller, S., Sagoo, S., Board, R., O'Mahoney, T., Caplice, E., Fitzgerald, G., Fogden, M., Owen, M., and Fletcher, H. 2002. Novel combinations of chitosan, carnocin and sulphite for the preservation of chilled pork sausages. Meat Science 62: 165-177.
[25]. Sanchez-Escalante, A., Torrescano, G., Djenane, D., Beltran, J.A., and Roncales, P. 2003. Combined effect of modified atmosphere packaging and addition of lycopene rich tomato pulp, oregano and ascorbic acid and their mixtures on the stability of beef patties. Food Science and Technology International 9(2): 77-84.