APPLICATION OF ALGINATE FROM *Sargassum* sp. AS AN ANTIOXIDANT AGAINST THE FAT OXIDATION PROCESS IN MILKFISH FILLETS DURING COLD TEMPERATURE STORAGE

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ABSTRACT

This study aims to determine the best concentration of alginate from brown seaweed (Sargassum sp.) as an antioxidant against the fat oxidation process in milkfish fillets during cold temperature storage. This research was conducted in July - August 2022 at the Fishery Product Processing Laboratory, Faculty of Fisheries and Marine Sciences, Padjadjaran University. The method used in this study was an experimental method using a Completely Randomized Design (CRD) with four treatments and three replications with a storage time of 6 days. Observations were made on days 1, 2, 4 and 6. The treatments consisted of four treatments namely control treatment A (0%), treatment B (1%), treatment C (1.5%), and treatment D (2%). Parameters observed in this study included thiobarbituric acid (TBA), peroxide value (PV), and pH. The results showed that the TBA values for all treatments ranged from 0.11 to 1.02 mg Mal/kg. PV values ranged from 0.00-28.97 mg eq/kg. Milkfish fillets soaked in alginate concentrations C (1.5%) and D (2%) were the best in inhibiting fat oxidation in milkfish filets as indicated by the lower PV value compared to milkfish filets soaked in alginate concentrations A (0%) and B. (1%). The results of milkfish filet pH measurements ranged from 5.4-6.1. The best treatment was treatment C (1.5%) because it was considered more efficient in the use of materials compared to treatment D (2%).

Keyword : *alginate, antioxidant, fillet, milkfish, oxidation.*

1. INTRODUCTION

Fish is one of the food ingredients that have high nutritional value but has perishable food if it does not go through a good processing process. Fish are easy to experience biochemical, microbiological, and physical changes because in addition to high water content also have fairly high protein, essential amino acids, and unsaturated fatty acids which can reduce the quality of fish during storage (Rosari et al. 2014). Fat oxidation is one of the main causes of fish quality degradation (Azhar and Nisa 2006). The oxidation process occurs in double bonds and results in the formation of short-chain fatty acids, aldehyde compounds, or ketones, thus causing rancidity which can cause a decrease in quality in fish (Yuanita 2006). One of the preventions against rancidity caused by fat oxidation is the use of antioxidants (Rahimabadi and Divband 2012).

According to Dousip et al. (2014), alginate is a product of *Sargassum* sp seaweed. showed lower levels of *Malondialdehyde* (MDA) than negative and positive controls. Alginates have higher antioxidant activity of *Superoxide Dismutase* (SOD) and lower catalase than negative controls. Therefore, alginates have potential antioxidant (in-vivo) activity. Antioxidants found in *Sargassum* sp. not only can be developed in the food sector such as alginate, animal feed, and fertilizer, but it is also able to inhibit the damage caused by free radicals in

products such as fish fillets and fish oil (Prabowo et al. 2013). Fish fillets have perishable properties. The deterioration process must be immediately inhibited so that most fishery products, especially filet, can be maximally utilized, one of which is by developing several ways of preservation (Noviantari et al. 2012). One type of fish that is often processed into filet products is milkfish. According to Junianto (2003), milkfish contains 20.53% protein and 6.73% fat so milkfish is classified as a fish with high protein and moderate fat. Fat in milkfish is a source of unsaturated fatty acids. The content of unsaturated fatty acids is quite high in milkfish and it is very susceptible to oxidation which can cause a decrease in quality. Based on these matters, this study was designed to determine the best concentration of alginate from brown seaweed (*Sargassum* sp.) as an antioxidant against fat oxidation in milkfish fillets.

2. MATERIALS AND METHOD

2.1 Experimental materials

The research was conducted from July to August 2022 at the processing fishery products laboratory of the Faculty of Fisheries and Marine Sciences, Padjadjaran University, and the Food Technology Laboratory of the Faculty of Engineering, Pasundan University. The materials used in this study were: milkfish, alginate flour (Na-alginate) 100 gr (KIMICA EX JAPAN), chloroform ($CHCl_3$), saturated Potassium Iodide (KI) solution, sodium thiosulfate ($Na_2S_20_3$), Amylum indicator, TBA Reagent, 4N hydrochloric acid (4N HCL), and Aquades.

2.2 Experimental method

The research method used was an experimental method using a completely randomized design (CRD) with four treatments and three replications with a storage period of 6 days, observations were made on Days 1, 2, 4, and 6. The treatments carried out in this study were: Treatment A with 0% alginate concentration (control), treatment B with 1% alginate concentration, treatment C with 1.5% alginate concentration, and treatment D with 2% alginate concentration.

In this research, milkfish fillets were soaked in an alginate solution according to each treatment for 1 hour. Making alginate solution is done by mixing the ingredients of aquades 1000 ml and 0 grams of alginate for treatment A, 10 grams for treatment B, 15 grams for treatment C, and 20 grams for treatment D . Mixing the ingredients is carried out until the solution becomes homogeneous using a magnetic stirrer. Then the marinated Fillets are drained and placed in a styrofoam container and then stored in the refrigerator. The main parameters observed were *Thiobarbituric Acid* (TBA), *Peroxide Value* (PV), and pH.

2.3 Data Analysis

The test result data will be analyzed statistically parametric using F test or ANOVA test with a 95% confidence level. If the treatment has a significant effect ($F_{count} > F_{table}$), then proceed with Duncan's multiple range test with a 95% confidence level to find out and determine which treatment has a significantly different effect (Sastrosupadi 2000).

3. RESULTS AND DISCUSSION

3.1 *Thiobarbituric Acid* (TBA)

Based on the results of observing the value of *Thiobarbituric Acid* (TBA) or a secondary product from the fat oxidation process. As shown in Chart -1, all treatments (A, B, C, and D) experienced an increase in the value of malonaldehyde on days 1, 2, 4, and 6. The increase in TBA value occurs due to the decomposition of unsaturated fatty acids found in milkfish fillets. According to Putri et al. (2014), the increase in the amount of decomposition of lipid oxidation products along with the length of storage time can be caused by peroxide as a result of primary oxidation decomposing further into aldehydes, ketones, and alcohols.

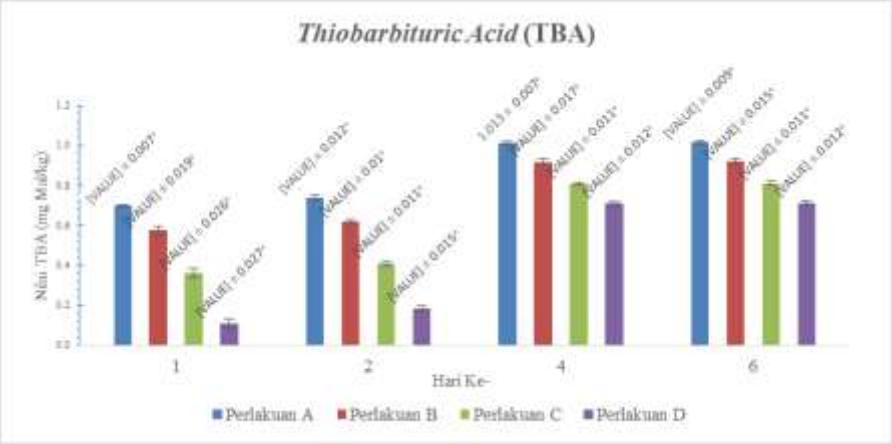

Chart -1: *Thiobarbituric Acid* (TBA) value graph of milkfish fillet samples

Milkfish fillets with 0% alginate concentration (treatment A) had the highest *malonaldehyde* values in each observation ranging from 0.70 to 1.02 mg Mal/kg. The TBA value in all treatments was still below the tolerance limit with an average TBA value in all treatments ranging from 0.11 to 1.02 mg Mal/kg. According to Sallam (2007), the tolerance limit for the highest TBA value that is still acceptable in fish meat is 5 mg Mal/kg.

Table -1 : Average *Thiobarbituric Acid* (TBA) Measurement Results

Treatment	Day-			
	1	2	4	6
A (0%)	0,70 ± 0,007[a]	0,74 ± 0,012[a]	1,01 ± 0,007[a]	1,02 ± 0,009[a]
B (1%)	0,57 ± 0,019[a]	0,62 ± 0,010[a]	0,92 ± 0,017[a]	0,92 ± 0,015[a]
C (1,5%)	0,36 ± 0,026[a]	0,41 ± 0,011[a]	0,81 ± 0,011[a]	0,81 ± 0,011[a]
D (2%)	0,11 ± 0,027[a]	0,19 ± 0,015[a]	0,71 ± 0,012[a]	0,71 ± 0,012[a]

*Note: Different notations show a significant effect at the 95% confidence level (Duncan's multiple range test).

The results of the Analysis of Variance test showed that the differences in alginate concentration in milkfish fillets did not have a significant effect on the TBA value ($F_{count}<F_{table}$ at 95% confidence level), but the lowest malonaldehyde value was obtained in the treatment D (2%), which ranges from 0.11-0.71 mg Mal/kg. This is due to the presence of antioxidant activity in alginates. According to Prawira et al. (2015), the content of flavonoids and phenolic compounds which are antioxidant compounds can reduce the rate of formation of malonaldehyde compounds. Phenolic or polyphenolic compounds can be in the form of flavonoids, cyanic acid derivatives, coumarins, tocopherols, and polyfunctional acids as antioxidant compounds (Rafsanjani et al. 2015). The content of antioxidant compounds from the extract of *Sargassum* sp. can be used to inhibit fat oxidation in fish fillets (Hidayati et al. 2017). Phenolic components can inhibit lipid oxidation by donating hydrogen atoms to free radicals (Septiana and Ari 2012).

The increase in the TBA value is closely related to the increase in the PV value, where the PV value increases followed by an increase in the TBA value. According to Fauzi et al. (2016), the increase in TBA values was caused by a series of oxidation reactions in which the peroxide compounds formed would be degraded along with the long storage time and produce further compounds in the form of hydroperoxides that are unstable and easily broken to produce aldehyde and ketone compounds that can cause rancid odor. The higher the TBA value, the rancid the milkfish filet.

3.2 *Peroxide Value* (PV)

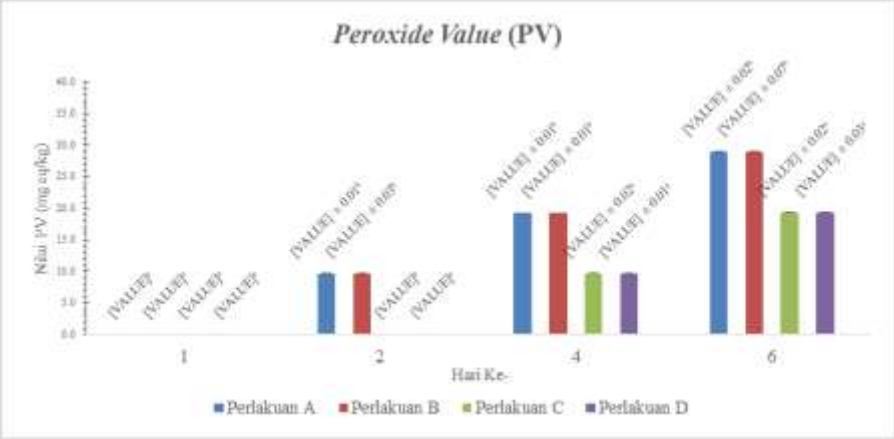

Chart -2: Peroxide Value (PV) value graph of milkfish filet samples

The peroxide value (PV) is the primary product of the fat oxidation process (Husain 2017). According to Khotimah et al. (2013), the content of peroxide compounds is one sign that there is an overhaul or damage to the fat due to the oxidation process (contact with air) which causes rancidity. Based on the average value of Peroxide Value (PV) (Table-2), the average value of PV in all treatments ranged from 0.00 to 28.97 mg eq/kg. PV value of milkfish filet on treatments A (0%) and B (1%) exceeded the tolerance limit on the 4th day, while on treatment C (1.5%) and D (2%) exceeded the tolerance limit on the 6th day. The tolerance limit of the highest peroxide value in fish meat is 10-20 mg eq/kg (Putri et al. 2014).

Table -2 : Average Peroxide Value (PV) Measurement Results

Treatment	Day-			
	1	2	4	6
A (0%)	$0,00^{a}$	$9,65 \pm 0,01^{b}$	$19,25 \pm 0,01^{b}$	$28,97 \pm 0,02^{b}$
B (1%)	$0,00^{a}$	$9,65 \pm 0,03^{b}$	$19,22 \pm 0,01^{b}$	$28,95 \pm 0,07^{b}$
C (1,5%)	$0,00^{a}$	$0,00^{a}$	$9,69 \pm 0,02^{a}$	$19,31 \pm 0,02^{a}$
D (2%)	$0,00^{a}$	$0,00^{a}$	$9,63 \pm 0,01^{a}$	$19,36 \pm 0,03^{a}$

*Note: Different notations show a significant effect at the 95% confidence level (Duncan's multiple range test).

Based on the results of the F ANOVA (Analysis of Variance) test, showed that the differences in alginate concentrations in milkfish fillet immersion had a significantly different effect on PV values on days 2, 4, and 6 ($F_{count}>F_{table}$ at 95% confidence level). Milkfish fillets with alginate concentrations of 0% and 1% showed higher PV values of 9.65 ± 0.01 mg eq/kg and 9.65 ± 0.03 mg eq/kg on day 2, 19.25 ± 0.01 mg eq/kg and 19.22 ± 0.01 mg eq/kg on the 4th day, and 28.97 ± 0.02 mg eq/kg and 28.95 ± 0.07 mg eq/kg on the 6th day compared to milkfish fillets with alginate concentrations of 1.5% and 2%. The decrease in the average PV value occurred in each treatment with the addition of alginate concentration.

According to Ketaren (1986), the increase in peroxide rate is caused by the contact between oxygen and fat (oxidation), where oxidation begins with the formation of peroxide and hydroperoxide, and the peroxide content in fat will increase over time. The lower PV values in treatments C (1.5%) and D (2%) compared to treatments A (0%) and B (1%) are due to the role of the active flavonoid compound from alginate which can inhibit the oxidation process and act as an antioxidant. In accordance with Redha's statement (2016) that flavonoids are one of the groups of phenol compounds that have antioxidative properties and can prevent damage to cells and their cellular components by reactive free radicals. Harikedua (2012) states that antioxidants can inhibit the lipid oxidation process and suppress the increase in peroxide rate. The higher the concentration of antioxidants given the decreased peroxide formed.

Low-temperature storage is also able to inhibit the deterioration of the quality of milkfish filets caused by the fat oxidation process. In accordance with the statement of Santoso et al. (2017) that storage at low temperatures can slow down metabolic activity and inhibit microbial growth, while also preventing chemical reactions and and loss of water content from food ingredients The use of low temperatures in preservation can inhibit changes in original properties in fish such as texture, taste and also smell (Adawyah 2007). Peroxide levels that are high or exceed the standard can cause the destruction of several kinds of vitamins in fatty foods such as vitamins (A, C, D, E, and K and small amounts of B vitamins) (Ketaren 2012). Damage to oil or fat caused by oxidation will result in poisoning in the body and various diseases such as diarrhea, deposition of fat in the blood (artery sclerosis), cancer, and reduced fat digestibility (Namaskara et al. 2017). Fat oxidation in fish not only causes rancidity but also causes a decrease in nutritional value due to reactions between the compounds produced and amino acids. Compounds produced from the fat oxidation process can even cause cancer (Muhammad et al. 2019).

3.3 pH

The results of measuring the pH value of all samples of milkfish filet in all treatments (A, B, C, and D) showed that the average pH value tended to be acidic or less than 7. The average pH value of milkfish filet with treatment A (0%) and B (1%) ranged from 5.5-6.1, then milkfish filet with treatment C (1.5%) ranged from 5.4-6, and milkfish filet with treatment D (2%) ranged from 5. 5-6.

Table -3 : Average pH value Measurement Results

Treatment	Day-			
	1	2	4	6
A (0%)	$5,5 \pm 0,058^{a}$	$5,2 \pm 0,058^{a}$	$6 \pm 0,153^{a}$	$6,1 \pm 0,115^{a}$
B (1%)	$5,5 \pm 0,058^{a}$	$5,2 \pm 0,058^{a}$	$6 \pm 0,058^{a}$	$6,1 \pm 0,1^{a}$
C (1.5%)	$5,4 \pm 0,058^{a}$	$5,1 \pm 0,1^{a}$	$5,8 \pm 0,058^{a}$	$6 \pm 0,058^{a}$
D (2%)	$5,5 \pm 0,1^{a}$	$5,1 \pm 0,058^{a}$	$5,9 \pm 0,115^{a}$	$6 \pm 0,058^{a}$

*Note: Different notations show a significant effect at the 95% confidence level (Duncan's multiple range test).

Based on the F ANOVA (Analysis of Variance) test, the differences in alginate concentrations of 0%, 1%, 1.5%, and 2% in milkfish filet immersion did not have a significant effect on the pH value on days 1, 2, 4, and 6 (Fcount<Ftable at 95% confidence level). The pH value of milkfish fillets in all treatments tended to decrease ranging from 5.1-5.2 on the 2nd day, then tended to increase ranging from 5.8-6 on the 4th day, and tended to increase ranging from 6-6.1 on the second day. 6th day. This is because the glycolysis process is still ongoing after the death of the fish. After all, the enzymes in the fish meat are still active.

The oxygen supply no longer exists when the fish is dead so the formation of glycogen does not occur but there is an overhaul of glycogen into lactic acid, which causes the pH of fish meat to be acidic. According to Afrianto and Liviawaty (2010), pH of fresh fish tends to be neutral and will decrease in the early stages of death caused by the formation of lactic acid which is the result of an overhaul of glycogen. The increase in the pH value was due to the reduced levels of glycogen and ATP so the lactic acid formed from the ATP hydrolysis process decreased and the pH began to rise during storage (Rosari et al. 2014).

Milkfish fillets in all treatments A (0%), B (1%), C (1.5%), and D (2%) until the 6th day showed low pH values or acids ranging from 5.1-6.1. The low pH value will affect the auto-oxidation process in milkfish fillets. The auto-oxidation process occurs at a low or acidic pH range and is highly reactive and unstable. This is in accordance with the statement of Fauzi et al. (2016) that a low pH can increase the rate of auto-oxidation of fish meat thereby affecting the increase in the value of fat oxidation. The addition of alginate which contains phenolic compounds (flavonoids) that function as antibacterial also affects the pH value of milkfish fillets. Suptijah et al. (2008) stated that the use of antibacterial compounds can inhibit bacterial activity so that the decomposition of proteins by bacteria is inhibited and an increase in non-protein nitrogen content which can cause base accumulation is also inhibited.

Storage at cold temperatures (5-10ºC) can also affect the pH value of milkfish fillets. According to Rosari et al. (2014), the low temperature affects changing the pH value of fish meat. The lower the storage temperature, the fish meat will experience a slow change in pH value. The pH content of fish meat is usually between 6.4–6.6 or close to a neutral pH value. If the pH is more than 7 or above the neutral pH value, the fish will be easily damaged, due to low glycogen reserves in fish meat (Anggraini 2018).

4. CONCLUSIONS

Based on the results of the study, it can be concluded that the application of alginate as an antioxidant against the fat oxidation process in milkfish filets at cold temperature storage for 6 days (observations were made on days 1, 2, 4, and 6) with immersion treatment of alginate concentrations A (0%), B (1%), C (1.5%), and D (2%) had no significant effect on TBA and pH values, but milkfish fillets with the immersion of alginate concentrations C (1.5%) and D (2%) gave a significant effect on the PV value compared to the concentrations of alginate A (0%) and B (1%). milkfish filets with the alginate concentrations C (1.5%) and D (2%) were the best concentrations in inhibiting the fat oxidation process in milkfish fillets shown by lower PV values compared to milkfish filets with alginate concentrations A (0%) and B (1%). The results of measuring the TBA value of milkfish fillets from all treatments were still below the tolerance threshold for acceptance of fish meat, which ranged from 0.11-1.02 mg Mal/Kg.

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