EFFECT OF DIFFERENT CONCENTRATIONS OF ALGINATE FROM SEAWEED *Sargassum* sp. ON THE COMPOSITION OF PEROXIDE VALUE (PV), THIOBARBITURIC ACID (TBA) VALUE, AND pH IN MILKFISH FILLETS DURING COLD STORAGE

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ABSTRACT

This study aims to determine the effect of different alginate concentrations from Sargassum sp. as an antioxidant on the composition of Peroxide Values (PV), Thiobarbituric Acid (TBA) values, and pH in milkfish fillets during cold storage. This research was conducted from July to August 2022. The research was carried out at the Fishery Product Processing Laboratory, Faculty of Fisheries and Marine Sciences, Universitas Padjadjaran. Thiobarbituric Acid (TBA), Peroxide Value (PV) and pH analysis was carried out at the Food Technology Laboratory, Faculty of Engineering, Pasundan University. The method used in this research 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 treatment namely control treatment A (0%), treatment B (1%), treatment C (1.5%), and treatment D (2%). 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, milkfish, oxidation..*

1. INTRODUCTION

Milkfish is one of the fish that has high economic value and is an important cultivation commodity because it tastes delicious and the price is affordable by all levels of society. Milkfish is one of the most widely cultivated types of consumption marine fish in Indonesia. The development of milkfish cultivation technology in the community is inseparable from its comparative and strategic advantages because it can be cultivated in brackish water, sea, fresh water, is tolerant to changes in environmental quality, has mastered the enlargement and hatchery technology in the community, and is resistant to disease (Handayani et al. 2019). Milkfish is usually processed into processed products such as milkfish presto, milkfish with thorns, milkfish brains, meatballs, nuggets, shredded or filet (Rachmawati et al. 2016). According to Junianto (2003), milkfish contains 20.53% protein and 6.73% fat so that 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 which is quite high in milkfish is very easy to undergo oxidation which can cause a decrease in quality.

Fat oxidation is one of the main causes of the decline in fish quality (Azhar and Nisa 2006). The oxidation process occurs at the double bond and results in the formation of short chain fatty acids, aldehydes or ketones, causing rancidity which can lead to a decrease in fish quality (Yuanita 2006). One way to prevent rancidity caused by fat oxidation is by using antioxidants (Rahimabadi and Divband 2012).

Antioxidants are compounds that can prevent oxidation reactions by donating electrons to free radicals so that they can overcome excess free radicals (Tiwari et al. 2013). Antioxidants are divided into two, namely natural and synthetic antioxidants. Natural antioxidants are mostly isolated from natural sources such as plants. Natural antioxidants are found in plant parts such as wood, bark, roots, leaves, fruit, flowers, seeds and pollen. Synthetic antioxidants are made and synthesized by humans for commercial purposes, there are five types of antioxidants for food, namely Butyl Hydroxy Aniso (BHA), Butyl Hydroxyl Toulen (BHT), propyl gallate, Tertiary Butyl Hydroxy Qunion (TBHQ) and tocopherols. Synthetic antioxidants turn out to be both toxic and carcinogenic, so the food and drug industry is now turning to developing natural antioxidants (Palupi and Martosupono 2009).

Antioxidants can inhibit the process of fat oxidation. The use of antioxidants in food by soaking, spraying and combining them with packaging materials (Afrianto and Liviawaty 2010). Antioxidants obtained from natural products are plant secondary metabolites such as alkaloids, phenolics and flavonoids. Flavonoids that have antioxidant activity include flavones, flavonols, isoflavones, catechins, and chalcones (Harikedua 2012). According to Koivikko (2008) on the brown algae *Sargassum* sp. florotanin was found, which is a phenolic compound that acts as a source of antioxidants. *Sargassum* sp. is a type of brown seaweed that has not been used optimally. Antioxidants found in brown algae Sargassum sp. not only can it be developed in the food sector such as alginate, fodder and fertilizer, but it is also able to inhibit damage caused by free radicals in products such as fish fillets and fish oil (Prabowo et al. 2013). Based on this, this research was conducted to determine the best concentration of alginate from *Sargassum* sp. as an antioxidant based on Peroxide Value (PV), Thiobarbituric Acid (TBA), and pH of milkfish filet.

2. MATERIALS AND METHOD

2.1 Experimental materials

This research was conducted from July to August 2022. The research was carried out at the Fishery Product Processing Laboratory, Faculty of Fisheries and Marine Sciences, Universitas Padjadjaran. Thiobarbituric Acid (TBA), Peroxide Value (PV) and pH analysis was carried out at the Food Technology Laboratory, Faculty of Engineering, Pasundan University. The materials used in this study were: milkfish fillets, alginate (KIMICA EX JAPAN), aquades, chloroform ($CHCl_3$), saturated Potassium Iodide (KI) solution, sodium thiosulfate ($Na_2S_20_3$), Amylum indicator, TBA Reagent, and 4N hydrochloric acid (4N HCL).

2.2 Experimental method

The method used in this research 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 which referred to Hidayati et al.'s research. al. (2017). The percentage of alginate addition refers to the research by Hidayati et al. (2017) and Husni et al. (2014) are as follows: treatment A: 0% Alginate concentration (control), B treatment: 1% Alginate concentration, C treatment: 1.5% Alginate concentration, and D treatment: 2% Alginate concentration.

The procedure in this study was the preparation of alginate solution which was carried out by weighing the alginate according to the treatment, then the process of mixing the ingredients using a magnetic stirrer was carried out until the solution became homogeneous which was then placed in a container. Milkfish filets were soaked in a solution according to each treatment for 1 hour. Milkfish filet that has been soaked, drained and placed in a styrofoam container then stored in the refrigerator.

2.3 Data Analysis

The data from the test results will be analyzed statistically parametrically using the F test or ANOVA test with a 95% confidence level. The treatment had a significant effect if ($F_{count} > F_{table}$) then continued Duncan's multiple range test with a 95% confidence level to find out and determine which treatment had a significantly different effect (Sastrosupadi 2000).

3. RESULTS AND DISCUSSION

3.1 Thiobarbituric Acid (TBA)

Thiobarbituric Acid (TBA) is a secondary product of the fat oxidation process. Measurement of the TBA number is used to measure secondary products from lipid oxidation and shows the level of rancidity (Husain 2017). Measurement of the content of malonaldehyde compounds in a food ingredient can be used as a benchmark to determine antioxidant activity (Prawira et al. 2015).

Based on the results of observations of the Thiobarbituric Acid (TBA) (Chart -1), all treatments (A, B, C and D) experienced an increase in malonaldehyde values on days 1, 2, 4, and 6. The increase in TBA values occurred due to 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 decomposes further into aldehydes, ketones, and alcohols.

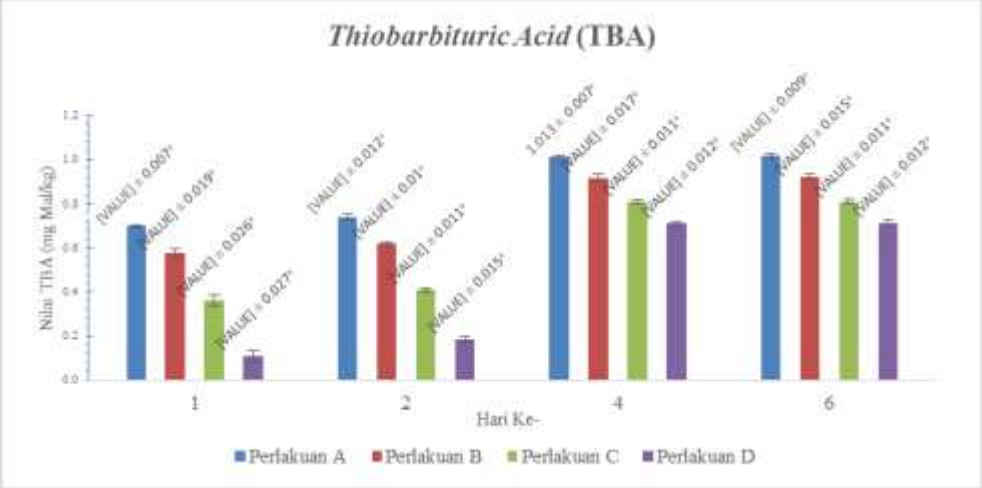

Chart -1: Thiobarbituric Acid (TBA) value graph of milkfish fillet samples

Milkfish filet with 0% alginate concentration (treatment A) had the highest malonaldehyde value in each observation, ranging from 0.70 to 1.02 mg Mal/kg compared to 1% alginate (B) immersion treatment ranging from 0 .57-0.92 mg Mal/kg, (C) 1.5% ranging from 0.36-0.81 mg Mal/kg, and (D) 2% ranging from 0.11-0.71 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 et al. (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 F ANOVA (Analysis of Variance) test, showed that differences in alginate concentration in milkfish fillet immersion 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 treatment D (2%), which ranges from 0.11-0.71 mg Mal/kg. This is due to the antioxidant activity of alginate.

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 which can be in the form of flavonoids, cyanic acid derivatives, coumarins, tocopherols and polyfunctional acids can function 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 degrade over time of storage and produce further compounds in the form of hydroperoxides which are unstable and break easily to produce aldehydes and ketones which can cause a rancid odor. The higher the TBA value, the rancid the milkfish filet will be.

3.2 *Peroxide Value* (PV)

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 a sign that there is an overhaul or damage to fat due to an oxidation process (contact with air) which causes rancidity. Based on the results of measuring the average Peroxide Value (PV) (Chart -2), treatments A (0%) and B (1%) had an average PV value of 0 mg eq/kg on 1st day and began to increase to 9.65 ± 0.01 mg eq/kg and 9.65 ± 0.03 mg eq/kg on the 2nd day, 19.25 ± 0.01 mg eq/kg and 19.22 ± 0.01 mg eq/kg on 4th day, and 28.97 ± 0.02 mg eq/kg and 28.95 ± 0.07 mg eq/kg on 6th day. While in treatment C (1.5%) and D (2%) the average PV value was 0 mg eq/kg on 1st day and 2nd day and began to increase to 9.69 ± 0.02 mg eq/ kg and 9.63 ± 0.01 mg eq/kg on the 4th day, and 19.31 ± 0.02 mg eq/kg and 19.36 ± 0.03 mg eq/kg on the 6th day.

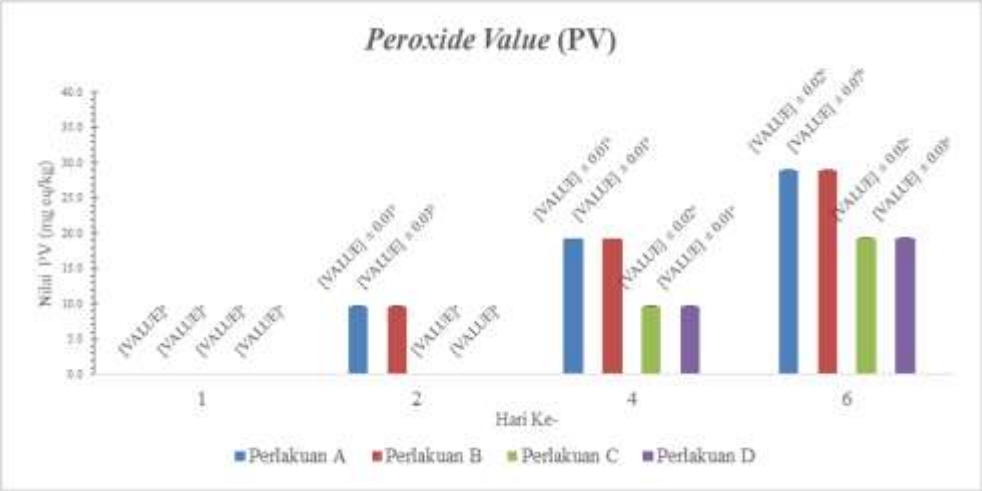

Chart -2: Peroxide Value (PV) value graph of milkfish filet samples

The average value of PV in all treatments ranged from 0.00-28.97 mg eq/kg. The PV value of milkfish fillets in treatments A (0%) and B (1%) had exceeded the tolerance limit on 4th day, whereas in treatments C (1.5%) and D (2%) it had exceeded the tolerance limit on 6th day. The tolerance limit for the highest peroxide value in fish meat is 10-20 mg eq/kg (Putri et al. 2014).

Based on the results of the analysis of variance F ANOVA (Analysis of Variance) test, showed that 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). Soaking 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 2nd day, 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.

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

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

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

According to Ketaren (1986) the increase in peroxide numbers is due to contact between oxygen and fat (oxidation), where oxidation begins with the formation of peroxides and hydroperoxides, peroxide levels in fats will increase with 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 is able to inhibit the oxidation process and act as an antioxidant. In accordance with Redha's statement (2016) that flavonoids are a group of phenolic compounds that have antioxidative properties and can play a role in preventing damage to cells and their cellular components by reactive free radicals. Harikedua (2012) stated that antioxidants can inhibit the process of lipid oxidation and can suppress the increase in peroxide numbers. The higher the concentration of antioxidants given the decreased peroxide formed.

The inhibition of the fat oxidation process in milkfish fillets is due to the presence of flavonoid active compounds in alginate which play a role in inhibiting the formation of free radicals at the initiation stage. The initiation stage begins with free radicals which react with unsaturated fatty acids to produce lipid free radicals which are highly reactive and unstable because they have one or more unpaired atoms. The active compounds of flavonoids will immediately donate their electrons to free radicals, thereby changing the free radicals into a more stable and unreactive form because the free radicals have found their atomic partners. Peroxide compounds formed from the reaction of lipid free radicals with oxygen in the propagation stage can be reduced and will eventually inhibit a series of oxidation processes (Fauzi et al. 2016).

Low temperature storage is also able to inhibit the decline in the quality of milkfish fillets 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 loss of water content from food ingredients. The use of low temperatures in preservation can inhibit changes in the original properties of fish such as texture, taste and smell (Adawyah 2007).

Peroxide levels that are high or exceed standards can cause the destruction of several kinds of vitamins in fatty foods such as vitamins (A, C, D, E, K and small amounts of B vitamins) (Ketaren 2012). Oil or fat damage caused by oxidation will result in poisoning in the body and various diseases such as diarrhea, deposition of fat in the blood (artero sclerosis), cancer, and reduced fat digestibility (Namaskara et al. 2017). Oxidation of fat 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.

Based on the analysis of variance F ANOVA (Analysis of Variance) test, differences in alginate concentrations of 0%, 1%, 1.5% and 2% in the immersion of milkfish fillets did not have a significant effect on the pH value on days 1, 2, 4 , and 6 ($F_{count}<F_{table}$ 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 because the enzymes in the fish meat are still active.

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).

The oxygen supply is no longer there when the fish is dead so that the formation of glycogen does not occur but there is an overhaul of glycogen into lactic acid, this is what causes the pH of fish meat to be acidic. According to Afrianto and Liviawaty (2010) fresh fish has a pH that 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 the breakdown of glycogen. The increase in pH value was due to the reduced levels of glycogen and ATP so that 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 a low or acidic pH value 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) which function as antibacterial also has an effect on 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), low temperature has an effect on changing the pH value of fish meat. The lower the storage temperature, the fish meat will change the pH value slowly. In fish meat, the pH content is usually between 6.4–6.6 or close to a neutral pH value. If the pH is > 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 this study it can be concluded that the effect of differences in alginate concentrations from *Sargassum* sp. as an antioxidant on the composition of peroxide value, TBA value, and pH in milkfish fillets in cold storage for 6 days (observations were made on days 1, 2, 4, and 6) by immersion treatment with alginate concentrations A (0%), B (1 %), C (1.5%), and D (2%) had no significant effect on TBA and pH values, but milkfish filet soaked with alginate concentrations C (1.5%) and D (2%) had a significant effect. significantly to the PV value compared to the concentrations of alginate A (0%) and B (1%). Milkfish fillets soaked in alginate concentrations C (1.5%) and D (2%) were the best concentrations in inhibiting the fat oxidation process in milkfish fillets as indicated by the lower PV value compared to milkfish filets soaked in alginate 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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