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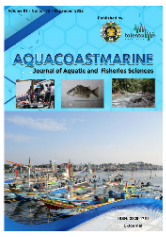

Utilization Study of Halimeda macroloba Extract as Natural Preservative Materials in Skipjack Tuna Meat (Katsuwonus pelamis)

Kajian Pemanfaatan Ekstrak *Halimeda macroloba* sebagai Bahan Pengawet Alami pada Daging Ikan Cakalang (*Katsuwonus pelamis*)

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ABSTRACT

The purpose of this study was to characterize the secondary metabolites in Halimeda macroloba by means of phytochemical screening tests, and to determine the use of the best concentration of Halimeda macroloba extract to maintain the freshness quality of skipjack tuna physically, chemically, and microbiologically. The results of the phytochemical screening test showed that the green macroalgae Halimeda macroloba contains alkaloids, steroids and triterpenoids, saponins, flavanoids, tannins and glucosides. The organoleptic physical test results for the best parameters of meat appearance, aroma and texture were in the A5 concentration treatment (600 ppm) with a value of 8.43, then the chemical test results found that the best protein content and TVB values were found in treatment A5 (600 ppm) with a value of 22.9% and in the A4 treatment (400 ppm) with a value of 9.24 mgN/100g. Meanwhile, the microbiological test results showed that the best TPC value was found in treatment A5 (600 ppm) with a value of 0.5×10^5 col/gram.
Keyword:, *Halimeda macroloba, Skipjack Tuna Meat, Natural Preservative*

ABSTRAK

Tujuan penelitian ini adalah untuk mengkarakterisasi senyawa metabolit sekunder pada *Halimeda macroloba* dengan pengujian secara skrining fitokimia, dan menentukan penggunaan konsentrasi terbaik ekstrak *Halimeda macroloba* untuk mempertahakan mutu kesegaran ikan cakalang secara fisik, kimia, dan mikrobiologi. Hasil pengujian skrining fitokimia diketahui bahwa makroalga hijau *Halimeda macroloba* mengandung senyawa alkaloid, streroida dan triterpenoid, saponin, flavanoida, tanin, dan glukosida. Adapun hasil pengujian fisik secara organoleptik terhadap parameter kenampakan daging, aroma, dan tekstur terbaik pada perlakuan konsentrasi A5 (600 ppm) dengan nilai 8,43, kemudian hasil pengujian kimiawi diketahui bahwa nilai kadar protein dan TVB terbaik terdapat pada perlakuan A5 (600 ppm) dengan nilai 22,9% dan pada perlakuan A4 (400 ppm) dengan nilai 9,24 mgN/100g. Sedangkan hasil pengujian mikrobiologi diketahui bahwa nilai TPC terbaik terdapat pada perlakuan A5 (600 ppm) dengan nilai 0,5 × 10^5 kol/gram.
Kata kunci: Daging Cakalang, Pengawet Alami, *Halimeda macroloba*

1. Introduction

Skipjack tuna is a type of fish from the scombroidae family which is widely used as a local consumption or export commodity. Handling of fresh fish is an important part of the fishing industry supply chain. Once caught, fish are often left at room temperature for a long time which results in decreased quality and post-harvest fish spoilage (Litaay et al., 2020). Some fish producers often add formalin as a preservative with the aim that it has a longer shelf life and does not spoil the fish quickly. People also use ice to slow down spoilage in fish. Given the difficulty of obtaining chemicals that are not harmful, it is better to use natural chemicals derived from plants (Yulisa et al., 2014).

The use of natural ingredients as fish preservatives is very effective in avoiding adverse effects on the health of the body. The content of secondary metabolites from seaweed has the potential to produce various bioactive metabolites with very broad activities as antibacterial, antiviral, and antifungal (Kaimudin et al., 2020). Macroalgae also have a role as a food source for several herbivorous organisms so that they have an important ecological role (Melville, 2005). In addition to its ecological role, macroalgae also have a high enough potential to be utilized. In general, macroalgae are rich in vitamins A, E, C, and niacin. Currently, human consumption of green algae is around 5%, brown algae is 66.5%, and red algae is 33% (Ira, 2018). *Halimeda macroloba* is a macroalgae that has antifungal, antimicrobial and antioxidant properties. *H. macroloba* extract contains biologically active secondary metabolites which include alkaloids, flavonoids, terpenoids, tannins and saponins so that it can be used as a natural preservative. The components in seaweed can prevent the presence of pathogenic bacteria in food which are known as food spoilage (Tjahyaningsih et al., 2013).

2. Methods

2.1. Location and time

This study was conducted in August 2021-January 2022. Sampling is done at different places. Taking skipjack tuna at the Belawan Fish Auction, while taking *Halimeda macroloba* in western waters precisely on pane Island, Central Tapanuli regency, North Sumatra Province.

2.2. Data collection

The research method used is an experimental experiment using complete randomized design with different concentrations of *Halimeda macroloba* extraction solution. The experiment was carried out with three repetitions. The concentration of skipjack tuna soaking with *H. macroloba* filtrate refers to the procedure that Tjahyaningsih et al. (2020) modified by researchers, with *H. macroloba* extract of 200 ppm, 400 ppm, 600 ppm, control (+) without immersion in *H. macroloba* extract and control (-) formalin 1% for 6 hours so that the best immersion results are obtained from giving *H. macroloba* extraction concentrations.

2.3. Secondary metabolites

The phytochemical screening method is carried out by looking at the color test reaction using a color reagent (Kristianti et al., 2008). This test is carried out to determine the content of compounds in plants such as the chemical content of alkaloids, steroids/triterpenoids, saponins, flavonoids, polyphenols, and tannins.

2.4. Organoleptic test

Observation of the physical properties of the samples was carried out on skipjack tuna soaked with *Halimeda macroloba* extract. Observation of the physical properties of skipjack tuna consisted of: appearance, aroma and texture. The observation procedure uses a score sheet that has been prepared beforehand. Organoleptic testing was carried out after immersion according to the SNI 2729: 2013 method using an organoleptic score sheet of fresh skipjack tuna with 30 non-standard panelists.

2.5. Protein

Test for protein levels using the Kjeldal method (AOAC, 2005). Determination of protein content was carried out using the Kjeldahl method, consisting of 3 stages, namely: the destruction stage, the distillation stage and the titration stage.

2.6. Total volatile base (TVB)

Testing the total volatile base (TVB) value was carried out on skipjack tuna fillets. This analysis aims to determine the amount of volatile base compounds formed due to degradation. The work procedure for analyzing TVB levels is divided into the extraction stage, the distillation stage and the titration stage.

2.7. Total plate count (TPC)

Microbiological tests carried out in this study used the TPC method. TPC testing steps according to the method of determining ALT SNI 01-2332.3-2006. Microbial planting uses the pour plate method, the method of planting the test sample into a petri dish and then adding agar media as a place for the sample to live. The principle of the total plate count (TPC) method is to observe the growth of both aerobic and anaerobic microorganisms. Samples were incubated for 24 hours at 35oC. Incubation is a process by which microorganisms will grow and develop at a suitable temperature and time.

2.8. Data analysis

The research data were analyzed descriptively quantitatively by using one way anova where previously the normality and homogeneity of the data were tested using SPSS.

3. Result and Discussion

3.1. Secondary metabolites of Halimeda macroloba

Based on the results obtained that the content of metabolites in green macroalgae *Halimeda macroloba* consists of alkaloids, streoids or triterpenoids, saponins, flavanoids, tannins, and glycosides where the content of these compounds is tested to inhibit bacterial growth (antibacterial). In accordance with Dewinta et al. (2021) which states that *Halimeda macroloba* shows positive results on the content of secondary metabolite compounds based on phytochemical tests adjusted for the adhesion used, *Halimeda macroloba* contains alkaloid and flavonoid compounds.

Tabel 1. Secondary metabolite compound of *H. macroloba.*

Secondary Metabolites	Reactor	Results
Alkaloids	*Bouchardart*	+
	Maeyer	–
	Dragendroff	+
	Wagner	+
Steroids and Triterpenoids	*Salkowsky*	-
	Liebenman – Burchad	+
Saponins	*Aquadest* + Alkohol (96%)	+
Flavanoids	$Fecl_3$ 5 %	–
	Mg (s) + HCl (p)	–
	NaOH 10 %	–
	H_2SO_4 (p)	+
Tannins	$FeCl_3$ 1 %	+
Glucosides	*Mollish*	+

Description: (+) Contains secondary metabolite compounds

(-) Does not contain secondary metabolite compounds

3.2. Characteristic of raw material

Skipjack tuna is a fish with a torpedo-like body shape with characteristics that distinguish it from fish from the family scrombroidae in the form of 5-6 black lines on the body. Skipjack tuna belongs to the family scombroidae, its cigar-like body has two flippers, the front fins are usually short and separate from the rear fins. Skipjack tuna has a mix of gray and also blue body color. The body is not scaly except on the body barbels (corselets). Yield is the most important parameter to determine the economic value and effectiveness of a product or ingredient (Munandar et al., 2016). The yield is something that is important to calculate to find out how much influence the treatment and processing have on the final product. The yield calculation results obtained will show the part of the fish that has high economic value if the percentage value of the yield is higher.

In the yield of skipjack tuna (Figure 2), the yield of skipjack tuna meat is 53%, bones 13%, head 29%, and stomach contents 5%. Meat has the highest yield value among other skipjack fish body parts. This is in accordance with Yusida et al (2020) which states that skipjack tuna has a proportion the yield of meat is greater in comparison bones and innards. Yield of skipjack tuna consists of the following: meat 58%, bone and head 25%, and offal 17%. Skipjack tuna has proportioned the yield of meat is greater in comparison bones and innards.

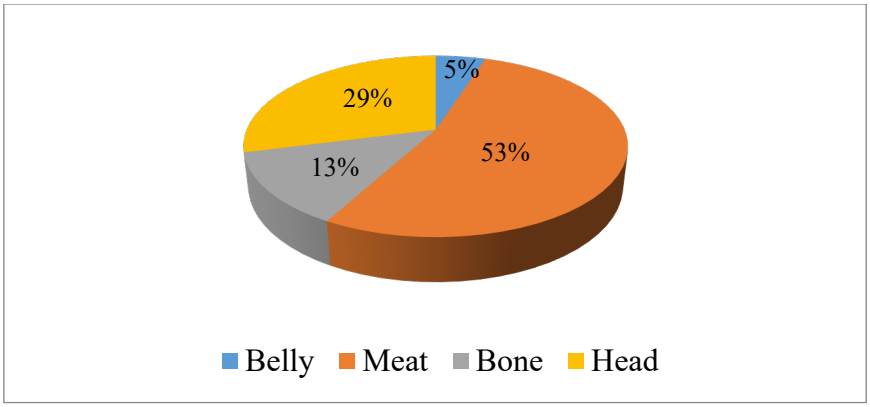

Figure 2. Average yield of skipjack tuna (*Katsuwonus pelamis)*

3.3. Organoleptic value

The organoleptic value was obtained based on the quality level of skipjack tuna (*Katsuwonus pelamis*). The level of acceptance of the sensory quality of skipjack tuna meat was measured based on the preference level of untrained panelists. The minimum number of panelists for non-standard panelists is 30 people. Each panelist filled out the available quality score organoleptic score sheets to provide an assessment of the appearance, smell and texture of skipjack tuna meat with 2 treatment repetitions, A1 (namely control (+) without immersion), A2 (control (-) 1% formalin), A3 (concentration 200 ppm), A4 (concentration 400 ppm), and A5 (a concentration of 600 ppm). The number of panelists in measuring the sensory quality of skipjack tuna meat was 30 panelists.

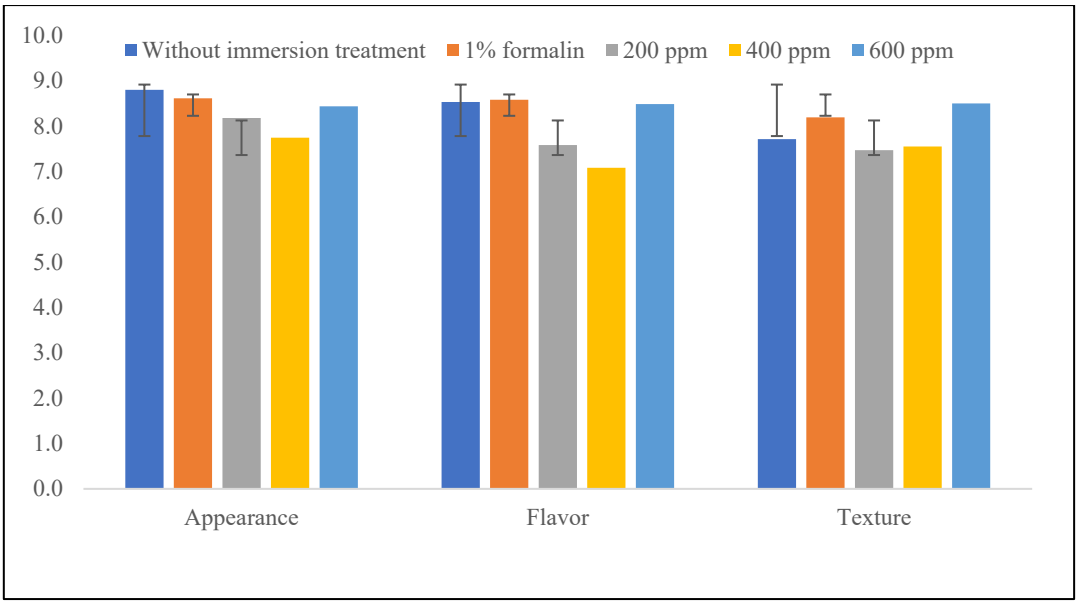

Figure 3. Organoleptic value of skipjack tuna (*Katsuwonus pelamis*)

The average value of organoleptic meat appearance, flavor, and texture of skipjack tuna in the treatment of A1 (without soaking *Halimeda macroloba* extract (+)), A2 (formalin 1% (-)), A3 (200 ppm), A4 (400 ppm), and A5 (600 ppm) is still above the value of 7 so it is still classified/fresh fish category. In accordance with SNI (2006) which states that fish that have an average value of organoleptic above seven is still relatively fresh.

Soaking treatment with different concentrations showed a significant influence on the aroma, texture, and appearance of skipjack tuna meat. Soaking treatment using *Halimeda macroloba* extract in this study also significantly affected ($p<0.05$) the organoleptic value of skipjack tuna. This shows that skipjack tuna soaked with *H. macroloba* extract solution at concentrations of A3 (200 ppm), A4 (400 ppm) and A5 (600 ppm) can maintain the quality of skipjack tuna based on organoleptic tests.

On concentration the highest immersion, namely A5 (600 ppm), obtained the best average organoleptic value the result is close to 9, for the appearance of meat the value is 8.43 illustrates that the appearance of skipjack tuna meat has incisions the flesh is brilliant, and the flesh tissue is very strong, while for the aroma a value of 8.48 was obtained which indicated that the aroma was very fresh, the species specifications were strong, and the texture value was 8.50 which means that the texture of the meat on skipjack tuna dense, compact, and very elastic. The average organoleptic value at skipjack tuna showed that the highest concentration was A5 (600 ppm) maintain the freshness of the meat.

In the treatment of A4 concentration (immersion extract 400 ppm) showed the average value of the appearance of skipjack tuna meat is 7.74 which when rounded will be a value of 8 in the organoleptic table with the characteristics of skipjack tuna meat has the appearance of a brilliant incision specific species and strong meat tissue. It is suspected that skipjack tuna at the time of handling during and after purchase at TPI Belawan is not good, such as too little ice conditions and contamination with outside air, sunlight, and water. In accordance with Naiu et al. (2018) which states that post-harvest fish handling is an important factor in keeping the quality of skipjack tuna fresh, handling such as giving ice with a ratio of 1:1 while during fish handling and weeding, ice is needed with a ratio of ice and fish 1:2.

3.4. Protein

Based on research with concentration treatment provides differences in protein levels in skipjack tuna meat. The average percentage of protein content in skipjack tuna samples was not much different at each soaking concentration. In anova obtained p value > 0.05 which indicates that the administration of the extract concentration does not significantly affect the protein content of skipjack tuna.

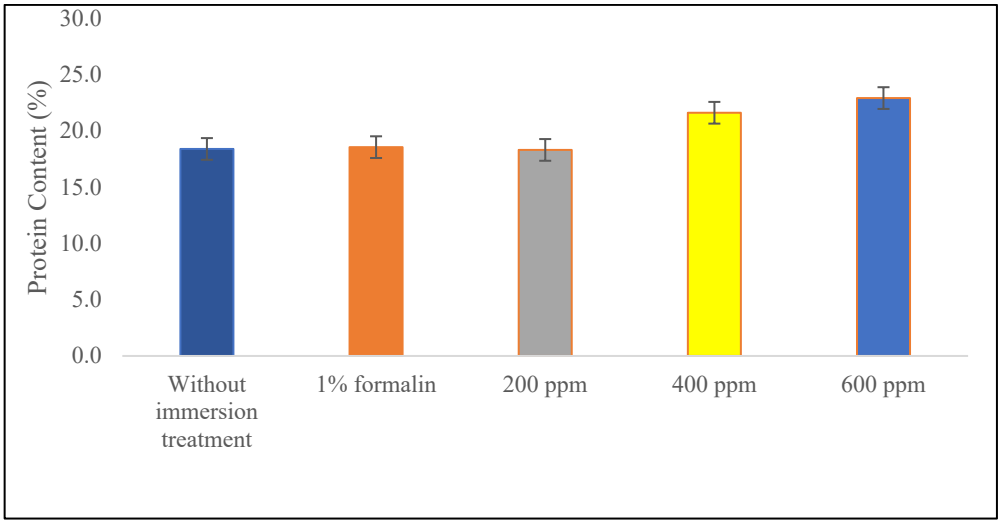

Figure 4. Protein content (%) in skipjack tuna meat (*Katsuwonus pelamis)*

On concentration the highest soaking, namely A5 (600 ppm), obtained the best average organoleptic value, indicating that the highest average protein content was found in the concentration of A5 (600 ppm). Skipjack tuna protein levels after soaking with seaweed extract with a concentration of 600 ppm showed an average amount of the highest protein levels of 22.91%. This is due to the more seaweed added protein levels become increasingly, because seaweed also has protein levels. According to Hartati (2011) the protein content of the material mixed with each treatment showed an increase in protein levels that differ from one treatment to another according to the material used due to the influence of protein content in seaweed is about 20-25%.

3.5. Total Volatile Base (TVB) value

Determination of the total volatile base (TVB) aims to determine the amount of volatile base compounds formed due to protein degradation (AOAC, 1995). TVB (total volatile base) is the result of protein decomposition by bacterial and enzyme activities. The TVB test was carried out after 6 hours of immersion using green macroalgae extraction with different treatments namely A1 (namely control (+) without immersion), A2 (control (-) 1% formalin), A3 (concentration 200 ppm), A4 (concentration 400 ppm), and A5 (a concentration of 600 ppm).

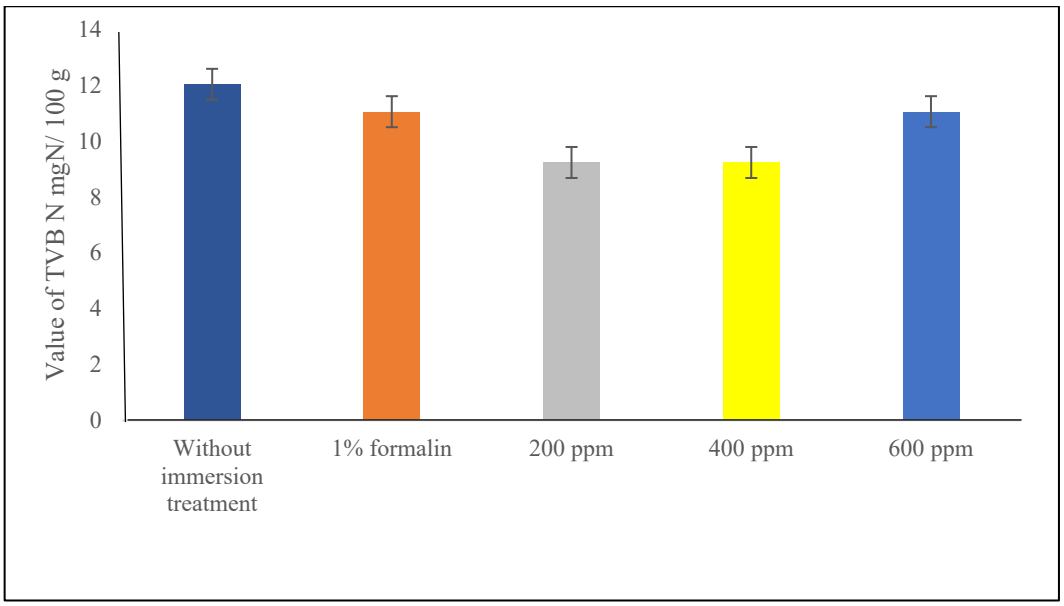

Figure 5. TVB N levels in skipjack tuna meat *(Katsuwonus pelamis)*

The highest average value of TVB levels was found in the control without immersion (+) of 12.24 ± 2.80 mgN/100 g, and the levels of TVB in the immersion with a concentration of 200 ppm 9.24 ± 3.56 mgN/100 g and a concentration 400 ppm was 9.24 ± 3.08 mgN/100 g, while the TVB control (–) value of 1% formalin and 600 ppm concentration obtained in static skipjack tuna was 11.06 ± 1.26 mgN/100 g. The TVB N value in skipjack tuna did not differ much, the TVB N value in treatment A1, which was 12.24 mgN/100 g, was the highest. Meanwhile, the value of TVB N in treatments A2 and A5 has a static value, which is 11.06 mgN/100 g. Treatments A3 and A4 also had a static value, which was 9.40 mgN/100 g and was the smallest TVB N content of all the treatments.TVB N test results soaked using extract solution showed that the more concentration does not significantly affect the value of TVB skipjack fish meat is shown in the calculation of spss with a value of P = 0.80 (P>0.05). According to Fahrul and Metusalach (2014), TVB can only be used as an indicator of feasibility for consumption and not as an index of fish freshness. TVB is a good indicator for decay. The value of TVB in each treatment is still below 20mgN/100g, which means it is still suitable for consumption (fresh), this is in accordance with SNI 4110:2020 which states that the maximum limit of TVB value in fish is a maximum value of 20 mg N/100g.

Skipjack tuna has an average content of TVB reached 12.24 mgN/100g highest in the treatment without soaking, this can be caused by long samples in contact with outside air so that the volatile nitrogen content will increase which results in increased levels of TVB (Susanto et al., 2011). Bacteria in fish play a major role in the increase in TVB after the death of fish. Spoilage bacteria in fish utilize volatile alkaline compounds to respire and multiply (Lestari et al., 2020). Ibrahim (2008) revealed that the increase in TVB N levels due to the increasing number of bacteria in connection with the continuing process of quality deterioration by microorganisms that produce volatile bases such as ammonia.

3.6. Total Plate Count (TPC)

This test was carried out to determine the total bacterial colonies found in skipjack tuna by giving different concentrations and controls. Based on the results obtained, it can be seen that the TPC value in skipjack tuna meat was obtained that the average number of bacterial colonies in treatment A1 (namely control (+) without immersion) was 2×10^5 col/gram, in treatment A2 (control (-) 1% formalin) the number of colonies found was 2.5×10^5 col/ gram, for treatment A3(concentration 200 ppm) the number of colonies was 0.5×10^5 col/gram, the number of colonies in treatment A4(concentration 400 ppm) was 1×10^5 col/gram, there were 0.5×10^5 col/gram in treatment A5 (a concentration of 600 ppm).

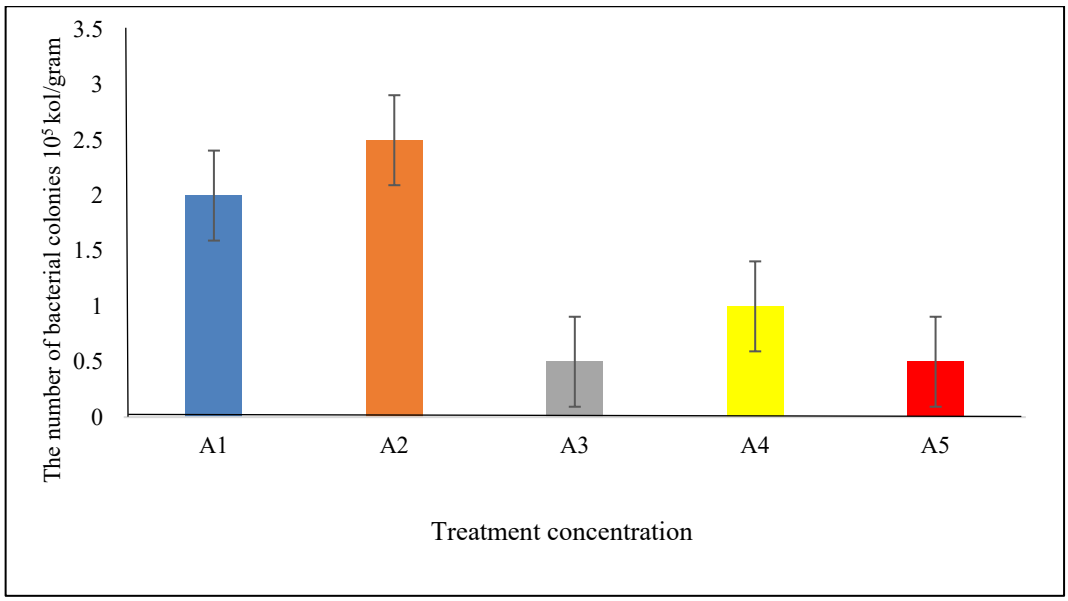

Figure 6. The number of bacterial colonies in skipjack fish meat *(Katsuwonus pelamis)*

It can be seen from the average total number of bacteria counted in the petri dish from the treatment with *Halimeda macroloba* extract concentration, that the petri dish with the higher *H. macroloba* extract concentration had fewer bacterial colonies than the petri dish with control (-) 1% formalin. This shows that there is a tendency that the higher the concentration of *H. macroloba* extract, the fewer the number of bacterial colonies formed.

In the soaking treatment with 1% formalin, the number of bacteria showed an increase in bacterial colonies and was the treatment with the highest average total number of bacterial colonies with the number of colonies found reaching 2.5×10^5 kol/ gram. Where the average number of colonies of these bacteria when viewed from BNJ shows that the treatment of A1 (without immersion extract) is not significantly different from the treatment of A2 (immersion formalin 1%). The growth of bacterial colonies in skipjack tuna meat in A2 soaking treatment increased allegedly due to the amount of formalin concentration of 1% has a low level which resulted in formalin does not work optimally to inhibit the growth of bacteria in skipjack fish meat. This is in accordance with Oessoe and Imelda (2019) which states that the lower the formaldehyde level, the higher the value of bacterial colonies is obtained and vice versa if the value of high formaldehyde levels, the lower the colony value is obtained.

In the soaking treatment with a concentration of 400 ppm obtained the average number of bacterial colonies as much as 1×10^5 kol/ gram is 0.5 higher than the number of colonies at a concentration of 200 ppm. This can be caused by the process of handling skipjack tuna when brought from TPI belawan or post-harvest fish handling. This is in accordance with Nurjanah *et al*. (2011) the most influential factor on the deterioration of fish quality is the post-harvest handling. The increased number of bacteria can be caused by the amount of ice used too little, fish wet with contaminated water from the environment, fish exposed to sun exposure and contamination of the environment or the hands of consumers.

4. Conclusion

The results of research on the study of the potential utilization of *Halimeda maroloba* extract as a natural preservative to replace formalin in skipjack tuna (Katsuwonus pelamis) meat can be drawn several conclusions, namely, soaking skipjack tuna at concentrations of 200 ppm, 400 ppm, 600 ppm is proven to be used as a natural preservative. *H. macroloba* extract influences the total number of skipjack tuna bacteria. Based on the ANOVA test, $p < 0.05$ was also obtained which proved that the administration of *H. macroloba* extract to skipjack tuna had a significant effect. The ability of *H. macroloba* extract was better than formalin as an antibacterial agent in skipjack tuna based on the total plate count (TPC) test. The extract of the green macroalgae *H. macroloba* had a significant effect ($p<0.05$) on the organoleptic value of skipjack tuna. Whereas the chemical parameters which included chemical tests and TVB N tests did not have a significant effect on skipjack meat ($p> 0.05$).

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