Study of changes in freshness quality of mackerel (*Scomberomorus commerson*) with extract concentration and variation of mangrove leaves

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Abstract. Mackerel is one of the economic and important commodities, but fresh mackerel is a perishable food product that can rot easily. Deterioration in fish quality is inevitable and occurs very quickly in wet products that have high water and protein content. The main cause of fish deterioration process is by the microbial activity found in its body which quickly occurs after death. The research method used was an experimental design with a completely randomized design (CRD) with 2 factors of factorial pattern (*Rhizophora* sp. Leaf Extract & *Avicennia* sp Leaf Extract) and 3 test levels (2%, 3%, and 4%). The aim of its study is to determine the potential of mangrove leaves and the types of phytochemical compounds contained in them and their effectiveness in inhibiting the rate of fish decay. The results that mangrove leaf extract could increase the organoleptic value and inhibit the rate of fish spoilage compared to samples without the addition of mangrove leaf extract. The best treatment was obtained by samples with *Rhizophora* sp mangrove leaf extract by 4% because it could maintain freshness and safe limits for consumption for 8 hours of storage in room temperature compared to control which was only 4 hours.

Keywords: extract concentration; mackerel (*Scomberomorus commerson*); variation of mangrove leaves

1. Introduction
Mackerel is one of the economic and important fish that is consumed by many people, either consumed fresh or processed into derivative products. One of the leading commodities from pelagic fish catches is Mackerel (*Scomberomorus commerson*). Based on data 2006 to 2011 [1] the average catch of mackerel is 3,637 tons/year (44% of the total catch of large pelagics). Meanwhile, fish is a perishable food. The chemical composition of fresh mackerel is water 74.63±0.15%, fat content 0.17±0.04%, protein 20.79±0.19%, and ash about 2.23±0.04%[2]. Deterioration in fish quality is inevitable and quickly occurs in wet products that have high water and protein content, such as fish, milk, and meat. Products with perishable characteristics require particular handling. The main cause of fish quality deterioration process is the microbial activity in its body which quickly occurs after it dies, these changes include autolysis, enzymatic, biochemical, chemical, and microbiological alteration. Before deterioration occurs, fish must be handled or processed properly so it needs efforts so that the fish can be accepted by consumers and proper to consume. One of many efforts that has been done by previous researchers is to add natural antioxidant compounds derived from plants because the use of chemicals such as formalin is strictly prohibited. These studies include the use of mangrove fruits as a natural preservative for fresh fish [3], the use of *Avicennia marina* mangrove
fruits as a preservative for fresh tilapia [4], the use of mangrove leaves (*Sonneratia caseolaris*) as natural preservative for tuna during shelf life [5], Basil leaf extract in maintaining the freshness of scad fish (*Decapterus sp.*) [6] Several studies that have been conducted refer to leaves and fruits utilities as sources of antioxidants and antibacterial which is useful to maintain fish quality during handling and storage.

In this study, natural ingredients as antibacterial used were *Avicennia marina* and *Rhizophora* sp. where there have been many studies that tested the high level of antibacterial bioactive compounds in mangrove leaf compounds. *Avicennia marina* leaf extract contains bioactive compounds that can inhibit the growth of *Staphylococcus aureus* and *Vibrio alginolyticus* bacteria [7]. Antibacterial bioactivity of *Avicennia marina* leaf extract can against *Staphylococcus aureus* and *E. coli* bacteria and control pathogenic bacteria [8]. The mangrove leaves of *Rhizophora apiculata* have bioactivity of active compounds that can inhibit the growth of *Staphylococcus aureus* and *E. coli* bacteria at a concentration of 250 ppm [9]. Based on the above background, the researchers wanted to know the effect of variations in mangrove leaf extract on changes in the quality of fresh Mackerel. The aim of its study is to determine the potential of mangrove leaves and the types of phytochemical compounds contained in them and their effectiveness in inhibiting the rate of fish decay.

2. Material and methods

2.1. Sample preparation

Mackerel with size of 200-250 grams is obtained directly from fishing port and then brought to the laboratory in cold transportation. In this study, no weeding was carried out on mackerel. Samples of leaves of *R. mucronata* and *A. marina* were taken and then washed with water, then wiped by tissue and dried naturally for 3 days (until dry weight was constantly) and smashed into powder. The powder sample was weighed as much as 200 g and extracted sequentially using methanol, ethanol, ethyl acetate, and n-hexane as a solvent for 3 x 24 hours. The filtrate was evaporated using a vacuum rotary evaporator at 40°C to obtain a crude extract [10].

2.2. Test analysis

2.2.1. Phytochemical test. Phytochemical test treatment first weighed as much as 5 leaves powder, then dissolved it with three different solvents (methanol/ethyl acetate/n-hexane) as much as 5 ml in a glass beaker.

a. Alkaloids

The sample solution was added as much as 1 ml of Dragendorf's reagent, observing the changes. If an orange to red-brown color is formed, it indicates the presence of alkaloid compounds.

b. Flavonoids

The prepared sample solution was added as much as 1 ml of concentrated HCl, then 0.20 grams of Mg powder was added. If a yellow, orange or dark red (magenta) color is formed, it indicates the presence of flavonoid compounds. If a positive result is obtained, it is continued with a quantitative test.

c. Saponins

A total of 2.0 mL of the sample solution was put into a test tube and then shaken for a few minutes, if it reacts positively, it will form a stable foam for 15 minutes.

d. Tannins

A total of 1.0 mL of the sample was put into a test tube and then added with a few drops of 5% ferric chloride (FeCl3) reagent if reacted positively, it would produce a brown precipitate.
2.2.2. Organoleptic test [9]. Mangrove leaf extract preservatives will be tested for organoleptic using the scoring method. Organoleptic test using 15 semi-trained panelists. organoleptic test to analyze the appearance of the eyes, gills, meat, texture, and smell of fish.

2.2.3. Moisture content [11]. The sample was crushed and weighed as much as 5 grams in aluminum foil whose weight was known. Then dried in the oven at a temperature of 105°C for 3 hours. Then cooled in a desiccator for 15 minutes and weighed. Then it was reheated for 30 minutes, cooled again in a desiccator and weighed. This treatment was repeated until a constant weight was reached. Weight reduction is the amount of water that is evaporated from the material, with the calculation:

\[
\% \text{ moisture content} = \frac{\text{Dry weight} - \text{Wet weight}}{\text{Dry weight}} \times 100\% \tag{1}
\]

2.2.4. Total plate count [12]. Total microbial analysis used the pour plate method. The sample was weighed as much as 20 grams and then dissolved in 250 ml of distilled water until homogeneous. Then, 1 ml of the sample was put into one of the test tubes containing sterile distilled water to obtain a solution with a dilution of 10-1, then homogenized. 0.1 ml of the sample was pipetted aseptically from tube 1 into tube 2 which contained 9.9 ml of sterile distilled water to obtain a solution with a dilution of 10-3, then homogenized. Repeat until it reaches a dilution of 10-6. After that, 1 ml of each dilution of 10-5 and 10-6 was pipetted into a petri dish and then poured sterile PCA (plate count agar) media which was cooled to ± 50°C for 15-20 ml and shaken to spread the sample. The samples were then incubated for 48 hours. Microorganisms that grow are observed and counted using the formula:

\[
\text{colony per ml} = \frac{\text{Colony Total} \times 1}{\text{Dilution}} \tag{2}
\]

2.2.5. pH test [13]. Before measurement, the pH meter was calibrated using a buffer solution of 7.0. Furthermore, the measurement of the sample solution is carried out by dipping the electrode on the pH meter into the sample solution and leaving it for a while until a stable reading is obtained.

2.3. Research method
The studies were conducted with an experimental design. The data obtained from this study were presented in a quantitative descriptive manner using a 2x3 factorial randomized block design (RAK) with 3 factors (Avicennia sp, Rhizophora sp and control) and 3 test levels (2%, 3%, 4%) with 3 times test (Tajuddin, 2018 modified). Observational data were analyzed using variance test (ANOVA). If the treatment has a significant effect, it is continued by using Duncan's further test to find out the significantly different data. The data was processed using SPSS Version 22 software using ANOVA (Analysis of Diversity Prints). significantly different data (P<0.05) was further tested using Duncan's test.
3. Result and discussion
Samples of natural preservatives used in this study were samples of young *Rhizopora* sp and young *Avicennia* sp mangrove leaves. Figures 1a and 1b are samples of fresh leaves and after undergoing stages after being mashed.

3.1. Main research
The main research consisted of making extracts of young mangrove leaves with 3 formulations namely 2%, 3%, and 4% in 300 ml of water. The three formulations were used and soaked in mackerel for 15 minutes. Each treatment consisted of 2 mackerel fish weighing ± 250 gr. The soaked mackerel is removed from the marinade to be transferred to a clean container and stored at room temperature for 4-16 hours. The test was carried out after the fish was stored for a quarter of an hour for up to 16 hours which included testing the total number of microbes, pH of fish meat, water content and organoleptic appearance (eyes, gills, mucus), texture and aroma. Each treatment consisted of 3 mackerel fish. Soaking using mangrove leaf extract was carried out at room temperature for 15 minutes. The soaked fish were transferred to a clean Styrofoam container with a length of 15.5 cm and a width of 9 cm which had been labeled for storage at room temperature. Observations were made every 4 hours until the fish rotted. Mangrove leaf extract formulations were 2%, 3%, and 4%. Types of mangrove leaves (*Avicennia* sp and *Rhizophora* sp), and storage at 0.4, and 8 hours.

Based on table 1, the results of phytochemical testing of *Avicennia* sp and *Rhizophora* sp mangrove leaf extracts with several different types of solvents, this aims to determine what types of antioxidant or antibacterial compounds are contained in *Avicennia* sp and *Rhizophora* sp mangrove leaf extracts that can be extracted with using different types of solvents, this is because the nature and polarity of each phytochemical compound are different. Table 1 shows the phytochemical compounds found in *Avicennia* sp mangrove leaf species are tannins, steroids, alkaloids, flavonoids, and triterpenoids. While the phytochemical compounds found in *Rhizophora* sp species are tannins, steroids, alkaloids, and flavonoids. According to [14], previous research suggests that *Avicennia* sp. found in Indonesia have secondary metabolite compounds of alkaloids,
phenolics, flavonoids, saponins, tannins, glycosides and triterpenoids which act as virus prevention, inflammation prevention, antioxidant and antibacterial.

Table 1. Phytochemical results from mangrove leaves extract *Avicennia* sp and *Rhizopora* sp.

| Fractions   | Saponins | Tannins | Steroid | Alkaloids | Flavonoids | Triterpenoids |
|-------------|----------|---------|---------|-----------|------------|--------------|
| Methanol    | Negative | Positive | Positive | Positive | Positive | Negative |
| Ethyl Acetat| Negative | Positive | Negative | Positive | Negative | Negative |
| N-Heksan    | Negative | Positive | Negative | Positive | Negative | Negative |

*Avicennia* sp

| Ethanol   | Negative | Positive | Positive | Negative | Positive | Negative |
| Ethyl Acetat | Negative | Positive | Negative | Positive | Positive | Negative |
| N-Heksan | Negative | Negative | Negative | Negative | Positive | Positive |

**Figure 3.** The eye’s score of organoleptic tests.

**Figure 4.** The gill’s score of organoleptic tests.
The triterpenoid phytochemical compounds found in *Avicennia* sp were not found in *Rhizophora* sp. Terpenoids are secondary metabolites with a working mechanism as an antibacterial that acts with porins (transmembrane proteins) on the outer membrane of the bacterial cell wall, forming strong polymer bonds, resulting in the destruction of the porin, the bacterial cell will lack nutrients, resulting in a lack of nutrients, stunted growth or death[15].

3.2. Organoleptic test

The organoleptic test that done to this study were based on on eye, gills, mucus, odor, and texture. The result of this test can be shown from the table below. Based on statistical tests showed that the difference in the concentration of mangrove extract had a significant effect (P <0.05) on the eye appearance value of fresh mackerel when observed for 16 hours, figure 3 shows that the administration of mangrove leaf extract was able to increase the organoleptic value of the eye when compared to the control. The treatment of different types of mangroves extracts also showed the same results, but the highest organoleptic value was obtained in the eye appearance of the *Rhizophora* sp. The longer the observation time, the organoleptic value of the eye also decreases and shows signs of deterioration in quality. One of the characteristics of the decline in

**Figure 5.** The mucus’s score of organoleptic tests.

**Figure 6.** The odor’s score of organoleptic tests.
fish quality is the change in the condition of the eyes of the fish to be set and faded due to the activity of spoilage bacteria [16]. But the addition of mangrove fruit can act as an antioxidant so that the oxidation process that cause quality deterioration can be inhibited [17].

Gills are one of fish freshness parameter because gills are a place that bacteria live and they can cause a damage to fish meat [18]. Based on statistical tests showed that the difference in concentration of mangrove extract had a significant effect ($P <0.05$) on the value of the gill appearance of fresh mackerel when observed for 16 hours, figure 4 shows that the administration of mangrove leaf extract was able to increase the organoleptic value of the gills when compared to the control. The treatment of different types of mangroves extracts also showed the same results, but the highest organoleptic value was obtained from the appearance of the gills from the immersion of Rhizophora sp. The longer the observation time, the organoleptic value of the gills also decreases and shows signs of quality deterioration. One of the causes of the high value of panelists’ acceptance of the gills is the tannin content. Tannins can inhibit bacterial growth. Tannins have the property of being able to shrink the cell membrane so that it interferes with the cell's permeability [19]. It is the same result that shown from it informed that the changes of organoleptic values in fish soaked in mangrove leaf extracts with fish with soaking at 18 hours storage on average were drastically different [18].

Based on Figure 5 shows that the organoleptic value of the appearance of mackerel fish mucus with the administration of mangrove leaf extract has a higher value than the control which is higher. Based on the diagram in figure 5, the control treatment shows that the appearance of mucus does not meet the SNI requirements after 4 hours. Meanwhile, the administration of mangrove leaf extract was able to increase the organoleptic value of mucus compared to the control treatment. While at the 8th hour, both control and treatment of mangrove leaf extract had shown a deterioration in quality with signs of the mucus layer starting to turn cloudy, the white color was a bit dull, less transparent. The characteristics of fresh fish slime are clear, transparent, bright shiny mucus layer [20].

Based on figure 6, the aroma parameter shows that the administration of mangrove leaf extract can improve the aroma of fresh fish, especially when it has undergone a stage of decay. Antimicrobial substances from mangrove leaf extract have chemical compounds that can bind to volatile base components resulting from the metabolism of fresh fish so that it will reduce the fishy and rotten aroma produced by fish as a result of bacterial metabolism. The aroma organoleptic score showed that the level of panelist acceptance of mackerel with the addition of Rhizophora sp extract was higher than Avicennia sp, it was possible that the triterpenoid content in Rhizophora sp was not found in Avicennia sp. Terpenes are a group of abundant hydrocarbon organic compounds produced by various types of plants. Terpenoids are also produced by insects [21]. These compounds generally give a strong odor. In addition, both treatments with the addition of mangrove leaf extract contain phenols so that they are able to maintain the aroma of fresh mackerel by inhibiting the metabolism of bacteria that produce volatile compounds that cause bad odors. The antibacterial activity of phenolic compounds in inhibiting bacteria is by denaturing cell proteins [22]. The hydrogen bonds formed between phenol and protein cause the protein structure to be damaged.

Based on diagram figure 7, the panelists’ acceptance of the texture produced by mackerel with the addition of mangrove leaf extract was higher than the control. Fish meat texture is closely related to muscle and meat fibers, which are mostly made up of protein and muscle cells. Fresh fish has a compact and chewy meat texture. Meanwhile, fish that have experienced quality deterioration and decay have a mushy meat texture accompanied by an increase in the water content of fresh mackerel and the appearance of an unpleasant aroma. In addition to dealing with inversely proportional water content, that the higher the water content of the fish, the panelists’ acceptance of the texture will be lower. In addition to water content, the value of texture organoleptic acceptance is also influenced by the pH of fresh mackerel, that when fresh mackerel loses its flesh cohesiveness, it is a sign of a decline in fish quality. The deterioration of the quality of fish is also influenced by the pH value that the higher the pH value, the higher the rate of decay because pH is an indicator of fish spoilage. The high and low pH of fish depends on the buffering power of the fish
flesh [23]. After the rigor mortis phase begins to end, the fish's pH will rise slowly until it becomes alkaline. This is because of the decomposition of compounds in the fish body due to the decrease in the strength of the buffer. In addition, the flexibility of the fish texture is due to the disconnection of the meat binding tissue and many damaged cell walls. The change in color of the gills to brown is caused by the cessation of blood circulation and oxygen supply from the gills, resulting in a reduction-oxidation reaction.

3.3. Moisture content

The result of moisture content from this study can be shown in the figure 8. Based on figure 8 shows that different concentrations of mangrove extracts showed significant results (P <0.05) on the value of water content. Figure 8 shows that the administration of mangrove leaf extract resulted in a lower value when compared to the control. The higher the water content value in mackerel, the faster the rate of deterioration and spoilage. In this case, indirectly the administration of mangrove leaf extract can slow down the rate of quality deterioration and fish spoilage due to the reduced amount of water used by bacteria to carry out metabolism. Based on the results of the water content test, it was shown that the administration of Avicennia sp. and Rhizopora sp. extracts could prolong the rate of decay in fresh mackerel. The results of the moisture content...
content showed that the rate of decay could be inhibited until the 8th hour and then the trend of water content tended to increase. Mackerel with leaf extract treatment of *Rhizopora sp.* and *Avicennia sp.* had a higher ability to inhibit decay than control. The benefits of *Avicennia sp.* leaf extract as antibacterial and natural preservative of fresh tuna (*Euthynus affinis*) [24]. The results showed that giving 20% extract of *Avicennia marina* leaves could extend the shelf life of fish for 12 hours at room temperature.

### 3.4. pH value

pH value is one of the indicators that used to determine the fish freshness level. The changes of the pH of meat take a big impact in fish spoilage because it affects the autolysis process and bacterial attack [25]. This pH Values of of fresh mackerel with different concentrations of mangrove leaf extract treatment can be shown from the figure 9.

Based on Figure 9 shows that the effect of the concentration of mangrove leaf extract has a significant effect (P <0.05) on the pH value of fresh mackerel. The higher the concentration of mangrove leaf extract applied to fresh fish, it can suppress the rate of deterioration and greater fish spoilage. One indicator of the
freshness of fish is the pH value. The results of bacterial metabolism are nitrogen volatile gases and bases, the bases resulting from bacterial metabolism can result in a higher pH value so that the higher the pH value, the higher the rate of fish spoilage. According to the bar chart, it shows that the administration of mangrove leaf extract can lower the pH value compared to the control. pH is an important and effective indicator to see the quality of meat. The pH value of mackerel fillets will generally increase during storage [26]. This increase is related to the production of alkaline compounds which are alkaline.

3.5. Total plate count (TPC)
The Total Plate Count value of fresh mackerel with different concentrations of mangrove leaf extract treatment can be shown from the figure 10. Based on figure 10, the concentration of mangrove leaf extract has a significant effect on the TPC value of fresh mackerel. The higher the concentration of mangrove leaf extract, the greater its ability to suppress the rate of bacterial decay. The standard TPC of fresh fish is 5.0 x 10^5 colonies/g. The administration of mangrove leaf extract can suppress the rate of bacterial decay so that fresh mackerel can be consumed up to the 8th hour, while without the provision of mangrove leaf extract it can only be consumed until the 4th hour, judging from the TPC value according to SNI standards, which is still below the threshold of 5.0x10^5 colonies/g[12]. The low TPC value in fresh mackerel with mangrove leaf extract was caused by the presence of phytochemical compounds such as phenols, flavonoids, tannins, alkaloids, and triterpenoids. Where these compounds can kill or inactivate microorganisms in several ways. The mechanism of alkaloid compounds, namely alkaloid components, is known as a DNA intercalator and inhibits the topoisomerase enzyme in bacterial cells[27]. The mechanism of steroids as antibacterial causes leakage of liposomes because they interact with cell membrane phospholipids which causes decreased membrane integrity and changes in cell membrane morphology causing cell fragility and lysis. Meanwhile, the mangrove leaf extract of Rhizopora sp species can suppress the growth rate of bacteria more than Avicennia sp. This is possible because in this study the triterpenoid compound was owned by Rhizopora sp but not by Avicennia sp so that the ability of Rhizopora sp was higher to suppress the growth rate of bacteria.

4. Conclusion
Based on the results of the study, it was shown that compared to the control, the addition of mangrove leaf extract was able to improve organoleptic scores and inhibit the rate of deterioration and fish spoilage as indicated by lower TPC, water content, and pH values compared to the control. Mangrove leaf extract of Rhizopora sp species proved to have better performance when compared to Avicennia sp. The best treatment obtained from the research was the use of natural preservatives from Rhizopora species with a concentration of 4%.

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