Potential various mangrove fruit extract as a bacterial growth resistor in *Euthynnus affinis*

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Abstract. This research study effect of various mangrove fruit submersion as a bacterial growth resistor is in *Euthynnus affinis* in the process of preserving the fish. The designed used in this research was with the experimental design using 4 treatments (*Avicennia* sp., *Sonneratia* sp., and *Bruguierra* sp.) mangrove extract with submersion fruit: water (3:1). The parameters being observed included the organoleptic test, total plate count number test, pH test, moisture content and protein content. The result of the study showed that the lowest total bacterial colonies were found in the treatment of *Avicennia* sp. mangrove's fruit extract, which was 7.78x10⁵ col/gr. The use of mangrove fruit extract in this study was able to extend the shelf life for fresh fish for one week with refrigerated temperature.

Keywords: fish; mangrove fruit; shelf life; TPC

1. Introduction

Fresh tuna is one of the important commodities in Indonesia and the fish that is most often consumed by the public, both consumed in fresh and processed form. Fresh tuna is a fish that belongs to the family Scombridae. Biogenic amines are found in many fishery products, including Scombridae fish, also known as scombrotoxin. The formation of biogenic amines occurs due to the decarboxylation of amino acids by bacteria. Bacteria capable of decarboxylation of amino acids include Enterobacter, Clostridium, Hafnia, Klebsiella, Lactobacillus, Photobacterium, Proteus, Pseudomonas and *Vibrio* spp. [1]. *Citrobacter* spp., *Serratia* spp., and *Morganella morganii* [2]. One of the factors for the formation of the amino acid histidine into histamine is storage conditions, storage temperature and bacterial contamination. Therefore, there is a need for special handling of fresh fish, especially the Scombridae family so that when handling histamine toxins are not formed. Things that need to be considered are temperature control and suppression of the rate of microbial metabolism.

Mangrove fruit is a source of antioxidants that have been widely used for various fields of health, food, and beauty. Several studies that utilize the components and bioactive compounds found in mangrove fruit include examining the bioactive content of mangrove fruit (*Sonneratia alba*) in the form of alkaloids, flavonoids, phenolics, tannins, saponins, steroids and terpenoids [3], fruit extracts. *Rhizophora* sp. as an antibacterial against pathogenic bacteria of freshwater fish [4], the antioxidant potential of *Bruguierra gymnorrhiza* mangrove fruit [5], the antibacterial activity of extract of api-api mangrove fruit (*A. marina*) [6], the antibacterial role of mangrove fruit *Rhizophora stylosa* and *A. marina* on larval vibriosis of mangrove crab (*Scylla serrata* Forskal 1775) [7]. After that, conducted a study on mangrove fruit regarding the effect of bioactive compounds of *Avicennia marina* mangrove fruit on the oxidation level of red tilapia (*Oreochromis niloticus*) fillet during cold storage [8]. Based on some of the studies above, it shows that mangrove fruit from several species has the potential as antibacterial and antioxidant which can be applied to the preservation of fresh fish to improve quality.
and maintain nutrients during the shelf life. Therefore, to determine the extent of the antibacterial effectiveness of each mangrove fruit, this study applied three different types of mangrove fruit, namely *Avicennia* sp., *Bruguiera* sp. After that, a study on mangrove fruit was conducted on the effect of bioactive compounds of *Avicennia marina* mangrove fruit's oxidation level of red tilapia (*Oreochromis niloticus*) fillet during cold storage *Bruguiera* and *Sonneratia* sp. used to maintain the quality and preserve fresh tuna (*Euthynnus affinis*) for one week.

2. The research methods

2.1. The making of extract mangrove fruit solution

Procedure to making of extract mangrove fruit solution:

1. The mangrove fruit were washed clean, and weighted (2,500 gr for two treatments), then mixed with sterile aquadest with the different various of mangrove fruit (*Sonneratia* sp., *Bruguiera* sp., and *Avicennia* sp.) with 3 (mangrove fruit): 1 (water) concentration of solution, and

2. The extract mangrove fruit solution that has been filtered then being used to soak the fresh *Euthynnus affinis*. Every total weight of the fish of every treatment was 1,500 gr each. Then from the submersion the cold chain system was applied, the soaking was carried out for 12 hours before being storage in the cooler at a temperature of less than 5°C, in a large container so that all fish surfaces get a same treatment and all of the fish bodies were submerged in the extract solution.

2.2. Procedure of storing the *Euthynnus affinis* fish in cool box

1. The *Euthynnus affinis* that has been submerged then stored for a week in a cool box, with the addition of ice and the fish were 3 (mangrove fruit): 1 (water). To keep the temperature stable so it would not be more than 5°C, stored the fish in one week in a cool box made of Styrofoam,

2. During the storage, ice had to change every 8 hours so that the temperature stays less than 5°C, and

3. Then the quality of fish was being measured everyday which consisted of degree of acidity (pH), moisture content, protein content, organoleptic and Total Plate Count (TPC).

2.3. Testing

2.3.1. Moisture content test moisture. Content can be determined by the heating method is to weigh a sample of 2 grams that has been dried by inserting it into the oven at a temperature of 105°C for ± 4 hours, after that it is inserted into a desiccator for 30 minutes with 3 repetitions, weighed as weight. The calculated based on the following equation [9]:

$$W(\%) = \frac{(FW - IW)}{(FW)} \times 100\%$$

$W$ : Weight (%) Moisture Content

$FW$ : Final weight

$IW$ : Initial weight

2.3.2. Protein concentration test. Method for measuring protein content was carried out using the Kjeldahl method. The principle of analysis of this method includes destruction, distillation, and titration. The principle of protein content analysis using the Kjeldahl method is to determine protein from carbon-containing materials and convert nitrogen into ammonia. Ammonia reacts with acid to form ammonium sulphate, then ammonia is absorbed in boric acid solution (Merck). The HCl titration step can determine the amount of nitrogen contained in the sample [10].
2.3.3. Organoleptic test. Mangrove leaf extract preservatives will be tested using the scoring method. Organoleptic test using 15 semi-trained panelists. Organoleptic testing in this study, in the form of testing in terms of the appearance of the eyes, gills, meat, texture, and smell of fish [11].

2.3.4. pH test. Before taking measurements, the pH meter was calibrated first using a buffer solution (buffer) 7.0. Next, 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 [12].

2.3.5. TPC test. Total microbial analysis uses the pour plate method. The sample was weighed as much as 20 gr and then dissolved in 250 ml of distilled water until homogeneous. After that, put 1 ml of the sample 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 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 grew were observed and counted using the formula [13].

\[ C/ml = N \times \left( \frac{1}{d} \right) \]  

\[ C/ml \] : Colonies per ml  
\[ N \] : Number of colonies on plates  
\[ d \] : Dilution

The data obtained from this study were presented in a 3x6 factorial completely randomized design (CRD) with 4 factors (Avicennia sp. (A), Bruguiera sp. (B), Sonneratia sp. (S) and control) and 7 levels. Test (days 0,1,2,3,4,5, and 6) with 3 replications. Observational data were analyzed by means of variance test (ANOVA). If the treatment has a significant effect, it is continued by using Duncan's post hoc test to find out the data that is significantly different. The data was processed using software SPSS Version 16 using ANOVA. (P<0.05) was further tested using Duncan's test.

3. Results and discussion
3.1. Organoleptic test
Based on the results of the graph in figure 1 shows that the organoleptic value with treatment of mangrove fruit extract showed a higher organoleptic value than the control without the addition of mangrove fruit. The organoleptic value during 6 days of storage decreased. The decrease in organoleptic value occurs because of the process of fish decay rate which causes decreased sensory quality. This is also supported by an increase in the TPC value because the rate of bacterial damage is characterized by a high amount of TPC, an increase in pH, and water content. The increase in water content causes the texture and appearance to be damaged, incomplete, mushy, water holding capacity decreases, and gel ability is lost. In line with this, the organoleptic value is also declining, but it is within the prescribed period, a decline in the value of aroma due to the onset of the stench caused by the rate of spoilage bacteria that produce alkaline volatile, ammonia and H_{2}S causes the stench of fish, other than the colour of the ball the eyes become redder and gradually turn pale. With the addition of mangrove fruit treatment, it can improve the organoleptic quality of fresh tuna by inhibiting the growth of bacteria that cause fish damage. The organoleptic value of fish meat during the study decreased, both soaked in fruit macerate and mangrove fruit powder [14]. This indicates a decrease in fish freshness during cold storage [15]. Changes in the texture of fish meat become soft and tender due to the autolysis process which causes changes in the meat, such as the meat becomes soft and easily separated from the bone. Although mangrove fruit contains phytochemical compounds that can act as antibacterial but along with the length
of storage, the number of spoilage bacteria in fish is increasing and their activity is increasing so that at
the end of storage, fish meat changes in texture.

3.2. Moisture content
Based on figure 2 above, it shows that the water content of the fish has a significant effect (P<0.05) on
the different treatment of mangrove fruit compared to the control. The graph of water content in fresh
tuna for 6 days shows that there is an increase in the water content value, while compared to the control
water content, it shows a higher value than the water content value in the treatment of mangrove fruit.

Figure 1. The organoleptic value of fresh tuna (Euthynnus affinis) with treatment of mangrove fruit
during storage. A is fish with Avicennia sp. fruit extract treatment, B is fish with Bruguierra sp. fruit extract
treatment, S is fish with Sonneratia sp. fruit extract treatment, and K is control (without treatment).

Figure 2. The moisture content of fresh tuna (Euthynnus affinis) with treatment of mangrove fruit
during storage. A is fish with Avicennia sp. fruit extract treatment, B is fish with Bruguierra sp. fruit extract
treatment, S is fish with Sonneratia sp. fruit extract treatment, and K is control (without treatment).
extract. This means that fresh fish given mangrove fruit extract was able to reduce the value of water content compared to control (without the addition of mangrove leaf extract). It is possible that the water content binds to antibacterial compounds such as phenols, tannins and saponins, thereby reducing the value of the water content. Water content is closely related to the quality of a food ingredient, the higher the water content, the quality of the fish will rapidly decline, this is because water is a suitable medium for bacterial growth, but along with the high use of extracts. basil as a source of antioxidants, the water content decreases [16].

3.3. Protein content

Based on figure 3 above, it shows that the value of protein content showed a significant difference (P<0.05) to the treatment of mangrove fruit during storage for 6 days. The longer the shelf life, the lower the protein content. The treatment of mangrove fruit was Avicennia sp. spable to maintain protein value during the 6-day shelf life compared to the control and treatment of Sonneratia sp. and Bruguiera sp. The decrease in fish protein levels during storage is closely related to the work of protein-degrading enzymes and spoilage bacteria that break down proteins into smaller molecules[14]. The reshuffle will
be followed by an increase in the number of spoilage bacteria in the fish and the appearance of an off odor.

The role of mangrove fruit extract on the protein content of fresh fish is as an antioxidant, which can prevent fat damage, protein denaturation and free radical scavengers. Antioxidants can protect protein components that are easily damaged so that they can be maintained throughout the shelf life. Components that can maintain and protect the damage to protein content are tannins, phenols, and flavonoids that are able to ward off free radicals. In general the components identified in the three mangroves are tannins, saponins, and steroids [17]. This shows that water can be used to extract the phytochemical components of mangroves, namely tannins, saponins, and steroids.

3.4. pH value
Based on the graph, figure 4 shows that the pH value of fresh fish during storage has increased. The highest pH value was indicated by the control treatment without the addition of mangrove fruit. While the lowest pH value was indicated by the addition of Avicennia sp. The optimum pH for bacterial growth is 7-7.5. Fresh fish has a pH of around 6.8 [18]. The three species of mangrove fruit showed that they could maintain a low pH compared to the control where pH was an indicator of fish freshness. The high pH of fresh fish during storage indicates that bacterial metabolism has occurred and the rate of fish spoilage has occurred, where the products of bacterial metabolism are in the form of alkaline volatile compounds that accumulate so as to increase the pH of tuna. Most bacteria live at neutral to slightly alkaline pH [19]. In acidic conditions, bacterial growth can be inhibited, but some bacteria can live even in acidic conditions. The high and low pH is influenced by the active substance of curry leaves, namely flavonoids which function as antibacterial and antioxidant [20]. The factor that most influences changes in the pH of fresh fish is the length of storage. During storage there is a tendency to increase the pH along with the rate of fish spoilage. The increase in pH may also be due to the development of

Figure 5. The total plate count of fresh tuna (Euthynnus affinis) with treatment of mangrove fruit during storage. (a) value TPC control and give treatment mangrove fruit, and (b) value TPC of give treatment mangrove fruit with different variations. A is fish with Avicennia sp. fruit extract treatment, B is fish with Bruguierra sp. fruit extract treatment, S is fish with Sonneratia sp. fruit extract treatment, and K is control (without treatment).
psychrophilic bacteria which can cause the formation of volatile bases. The pH value of tilapia meat at room temperature storage for 12 hours is still acceptable [21]. This means that the pH value at 12 hours of storage still includes the pH of fresh fish where the fish can still be consumed. Storage for 18 and 24 hours there was an increase in the pH value that had passed the pH limit of fresh fish where the fish did not meet the criteria for consumption anymore. Increasing the pH value of fish meat is closely related to glycogen reserves in the fish body and how to kill fish.

3.5. Total plate count value
Based on figure 5 shows that the TPC value for 6 days of storage has increased. In the treatment of giving mangrove fruit is able to suppress the rate of bacterial decay and can still be consumed until the 6th day. While the control treatment was not able to inhibit the rate of bacterial decay only until the 4th day. According to SNI (2013), the standard value of total bacteria for fresh fish is 5.0 x 10⁵ CFU/g. On the 4th day the total bacterial value in fresh tuna has exceeded 5.0x10⁵ CFU/g. All treatments with mangrove fruit extract showed their potential as antibacterial because they were able to inhibit the rate of decay by suppressing the total number of bacteria that remained below the standard of total bacteria in Fresh Fish. The use of mangrove fruit mase rate on tilapia is thought to be able to extend shelf life because mangrove fruit mase rate contains phytochemical compounds that can act as antibacterial or anti-microbial [22].

The ability of mangrove fruit to inhibit bacterial growth is due to the presence of antibacterial and antioxidant compounds such as flavonoids, tannins, alkaloids, phenols, triterpenoids and saponins. While the most effective administration of mangrove fruit extract was Avicennia sp. because it was able to suppress the highest bacterial growth rate, with the lowest total bacterial value on day 6 of 7.78x10³ CFU/g. Some literature states that phytochemical compounds have antibacterial properties that can act as growth inhibitors and kill bacteria by various mechanisms. The mechanism of action of alkaloids as antibacterial is thought to be through inhibition of cell wall synthesis which will cause cell lysis so that the cell will die. Damaged cell walls also cause secondary metabolites to enter deeper and damage bacterial membranes [23]. The mechanism of terpenoids as antibacterial is to react with porin (transmembrane protein) on the outer membrane of the bacterial cell wall, forming a strong polymeric bond, resulting in the destruction of the porin. Damage to the porin which is the entrance and exit of the compound will reduce the permeability of the bacterial cell wall which will result in the bacterial cell being deprived of nutrients, so that bacterial growth is inhibited or dead [24]. The mechanism of action of flavonoids as antibacterial is to form complex compounds with extracellular and dissolved proteins so that they can damage bacterial cell membranes and are followed by the release of intracellular compounds [25]. In addition, phenol compounds (flavonoids) and their derivatives easily form protein complexes through hydrogen bonds. The H⁺ ion from the complex can damage the phosphate group of the bacterial membrane so that the phospholipid molecules break down and cause the membrane shape cannot be maintained [26]. The mechanism of action of saponins as antibacterial is to reduce surface tension, resulting in increased permeability of cell leakage and resulting in the release of intracellular compounds. If it continues, then this can cause bacterial cells to lyse and die. While the mechanism of action of tannins as antibacterial is to inhibit the reverse transcriptase enzyme and DNA topoisomerase so that bacterial cells cannot be formed [25]. The antibacterial activity of tannins is related to its ability to inactivate enzymes and interfere with protein transport in the inner layer of cells [24].

4. Conclusions and suggestions
Based on the results of the study showed that the application of mangrove fruit types was active in inhibiting bacterial growth compared to controls. The best treatment in inhibiting bacterial growth and quality degradation was tuna with the addition of mangrove fruit Avicennia sp. which could inhibit bacterial growth until the 6th day with a total bacterial value of 7.78x10³ CFU/g while at the same time the total value of bacteria without mangrove additions amounted to 7.11 x 10⁵ CFU / g. In addition, mangrove fruit was Avicennia sp. also able to maintain protein damage from denaturation and oxidation with the highest value of protein content on day 6 was 16.33% while the treatment without the addition
of mangrove fruit was 13.46%. Based on the results of the study, it is known that *Avicennia* sp. has a higher effectiveness in inhibiting bacterial growth compared to *Bruguiera* sp. and *Sonneratia* sp.

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