

*Application of Belimbing Wuluh Leaves (*Averrhoa Bilimbi*. Linn) to Maintain the Quality of Fish Komu (*Auxis Rochei*) in Terms of Histamine Levels*

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Abstract - Maluku has marine products in the form of fish that are very abundant, so it needs handling in terms of processing of fishery products to be used with good quality. Histamine is one of the most toxic chemicals found in the body. In this study resulted in preservation of komu fish (*auxis rochei*) by using belimbing wuluh leaves (*averrhoa bilimbi*. linn) where the histamine content formed will be tested measure. Two treatments were performed with preservation for 7 hours and no preservatives as a comparison. Initial histamine data was calculated using fresh fish. Histamine levels were tested using uv-vis spectrophotometry. In fish samples without using belimbing wuluh leaves (control) containing histamine levels of 16.23%, and with belimbing wuluh leaves histamine levels ranged between 14.03% - 9.16%. The level of histamine in each treatment is not the same, the more giving of belimbing wuluh leaves to the komu fish used, the smaller the histamine levels obtained.

Keywords - Belimbing Wuluh Leaves (*Averrhoa Bilimbi*. Linn.), Histamine, Komu Fish (*Auxis Rochei*).

I. INTRODUCTION

Fish is a group of foods that are very easily damaged, unlike other meat products. The deterioration of the quality of fish that is rapidly taking place after a dead fish is caused due to several factors including the occurrence of an enzymatic process, or the presence of decomposing microorganisms that develop in the body parts of the fish [1]. Fish products are very interesting because they function as a source of protein, vitamins, minerals and fat. Fish are very easy to damage so that proper processing and packaging can help maintaining the quality of fish. Worldwide, various preservation techniques are followed, ranging from simple storage, cold, pressure modification and electromagnetic field applications [2]. Microbial decay is the main mechanism that affects the quality of fresh fish. Compounds with a distinctive smell like trimethylamine

(TMA), various nitrogen (TVB-N) and sulfuric compounds, aldehydes, ketones, and esters produced by various microorganisms during fish decay [3].

Damage to fish begins with the occurrence of autolysis caused by enzymes originating from the fish itself followed by microbiological, physical, or chemical damage. Autolysis damage to fish begins as soon as the fish dies. The process of deterioration in fish includes physical, chemical, and organoleptic changes with sequences ranging from changes in pre-rigormortis, rigormortis, enzyme activity, microbial activity, and oxidation. All of these changes will lead to decay [1]. Dead fish will quickly enter the pre-rigor stage as indicated by anaerobic glycolysis and decreased adenosine triphosphate (ATP) and creatine phosphate. Pre-rigor is the event of the release of mucus from the glands below the skin surface. This mucus released consists mainly of

glucoprotein and mucin which are ideal media for bacterial growth. The released mucus forms a thick, clear layer around the fish's body. This release of mucus from the mucous glands is a natural reaction of fish that are dying of unpleasant conditions. The amount of mucus released and covering the body can be very large up to 1-2.5% of body weight [1].

Rigormortis is a result of a series of complex chemical changes in the fish muscle after his death. After the fish die, the blood circulation stops, the oxygen supply decreases so that there is a change in glycogen to lactic acid. This change causes the pH of the body of the fish to decrease, followed by a decrease in the amount of adenosine triphosphate (ATP) and the inability of muscle tissue to maintain elasticity. The rigormortis phase is characterized by changes in the pH of the body of the fish to 6.2-6.6 from initially pH 6.9-7.2. The initial pH value of fish is very dependent on the number of available glycogen and buffering power in fish meat. The strength of buffering in fish meat is caused by protein, lactic acid, phosphoric acid, TMAO, and volatile bases. The length of the rigormortis phase in fish lasts for several hours or days, depending on a number of factors such as species, size of fish, physical condition of fish, level of fish fatigue and storage temperature. The process of rigormortis is characterized by muscle spasms in a few hours so that the fish become stiff because changes in nucleotide compounds due to cessation of supply of oxygen and energy after the fish die. Stage rigormortis is an indicator of the quality of fish that needs to be considered for consumption. The complete rigormortis stage is characterized by the occurrence of the process of autolysis and microbial growth and enzyme activity after several days at a temperature of around 5° C [1].

The autolysis stage begins with the breakdown of fish constituents into other compounds with a smaller molecular weight. The breakdown of the constituent fish tissue will result in a decrease in organoleptic properties such as odor, taste, texture, and sometimes color. The process of enzymatic decomposition of tissue (autolysis) runs on its own after the fish die with a complex mechanism. Enzymes that play a role in the autolysis process, including proteolytic enzymes and lipolytic enzymes contained in the body of the fish immediately launch its action, breaking down proteins and fats into simpler compounds such as amino acids and fatty acids. Autolysis in fish is more dominated by proteolytic enzymes because the protein content in fish meat is far more than the fat content. In the stomach of the fish found proteolytic enzymes pepsin and trypsin. Meanwhile, in fish meat found proteolytic enzymes

kathepsin. The optimum temperature for autolysis is 40°C and stops at 65°C. At a temperature of -14°C autolysis is inhibited, Microbiological damage began intensively after the rigor mortis process was complete. The bacteria that were originally only in the gills, stomach contents, and fish skin began to enter the muscles and break down energy source compounds such as proteins, fats, and carbohydrates into decomposing compounds in the form of indole, skatol, mercaptan, ammonia, sulfide acid, and etc. This microbiological damage is the heaviest and is considered the most responsible for decomposing fish, both fresh and processed. Other microbiological damage that needs to be considered is that which causes disease, poisoning, or allergies in consumers even though fish do not experience decay [1].

Efforts to prevent fish decay are often done by cooling but there are also by naughty traders using chemical preservatives or often known as formalin. In the interior of the Moluccas, especially on the island of Seram, preserving corpses is often done using herbs from plants. One of the plants that is often used is the belimbing wuluh plant (*Averrhoa bilimbi*. Linn). The belimbing wuluh plant is one of the plants used as natural medicine. Belimbing wuluh leaves have pharmacological activities which are for pain relief and as anti-inflammatory. The belimbing wuluh plant has chemical constituents, namely: potassium oxalate, flavonoids, pectin, tannin, gallic acid and ferulic acid. The natural chemical content found in the leaves of the belimbing wuluh which is thought to have anti-inflammatory activity are flavonoids and saponin. Based on the description above, the purpose of this study is to look at the application of belimbing wuluh leaves (*Averrhoa bilimbi*. Linn) in maintaining the quality of comu fish (*Auxis Rochei*) in terms of histamine levels.

II. RESEARCH METHODS

A. Materials and Tools

The materials used in this study include; Sulfanilic Acid, Chloride Acid, Sodium Nitrite, Sodium Chloride, Sodium Anhydrous Sulfate, Sodium Phosphate Monohydrate, Sodium Carbonate, n-butanol, Histamine Dihydrochloride, Aquades, Whatman Filter Paper No. 42, Fish samples and Wuluh xxbelimbing leaves. The tools used in this study include; Glassware (Pyrex), Analytical Balance, Blender, Electric Heating (Cimarec 2), Refrigerator (LG), Sentrifuge (200-Heraeus Labofuge), UV-Vis Spectrophotometer (UV-1700 Pharmaspec - Shimadzu).

B. Sample preparation

Commu fish that have been taken from the place of sale are made two treatments, namely in the presence of preservatives and those that are not. The preserved fish is put into a place containing leaves of starfruit with a weight of 100, 200, 300, 400 and 500 grams of starfruit leaves for 7 hours at room temperature. After 7 hours, the fish meat is washed and the dorsal (skinless) part is taken from the body part of the commu fish, then thinly sliced. The same is done also for the meat of commu fish which is still fresh as an introduction.

C. Manufacture of *p*-phenildiazonium sulfonate reagent

A mixture of 1.5 mL sulfanyl acid 0.9% (b / v) in concentrated HCl and 1.5 mL of 5% (b / v) NaNO₂ was cooled by soaking in ice water for 5 minutes. 6 mL of 5% NaNO₂ solution was added and allowed to stand for 5 minutes. Then, the reagent is stored in the ice bath and then left for 12 hours and ready for use.

D. Histamine extraction

Fish samples were thinly sliced and weighed about 5 grams. The sample was homogenized with 20 mL of 0.85% (b / v) NaCl solution for 2 minutes using a blender. Then put into a 75 mL centrifuge tube and centrifuged at 5300 rpm for 1 hour. The supernatant formed was made into 25 mL with 0.85% NaCl solution. Extract results were used for further analysis. In the test tube, 1 mL of extract was diluted to 2 mL with 0.85% NaCl solution and 0.5 gram salt mixture (containing 6.25 g of anhydrous Na₂SO₄ added 1 g of Na₃PO₄·H₂O). The tube is shaken to mix evenly. Then add 2 mL of *n*-butanol and shake it as hard as possible for 1 minute and let stand for 2 minutes and then shake a little to cause damage to the protein gel. The tube is then shaken for a few minutes and centrifuged at 3100 rpm for 10 minutes. Butanol located at the top (about 1 mL) is transferred into a clean and dry tube. Then evaporated to dry completely. The residue was crushed in 1 mL of distilled water and then reacted with reagent *p*-phenildiazonium sulfonate.

E. Spectrophotometric analysis

In a test tube that is clean with 5 mL 1.1% Na₂CO₃ solution is added slowly to 2 mL reagent *p*-phenildiazonium sulfonate and mixed. Then added 1 mL of residual solution obtained from the extraction process into the tube. The absorbance of the resulting color is measured immediately after 5 minutes at a wavelength of 497.8 nm using distilled water as blank. The histamine concentration in the sample was obtained from a standard curve for absorbance measurements at 497.8 nm with regression analysis.

III. RESULTS AND DISCUSSION

The measurement results of standard histamine absorbance can be made standard absorbance vs standard concentration curves which can be seen in Figure 1. Based on the calibration graph made, it can be seen that the graph is a straight line. These results indicate that the greater the concentration the greater the absorbance value. Analysis of histamine content in comu fish (*Auxis rochei*) was carried out using the calibration curve method. The absorbance value of the sample solution for each treatment is plotted onto the calibration curve or in the calibration curve equation obtained.

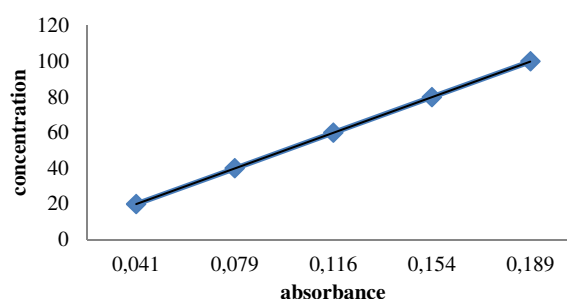


Figure 1. Standard concentration vs. absorbance curve standard

Based on the results of data analysis, histamine levels in commu fish (*Auxis rochei*) were obtained using a combination of belimbing wuluh leaf preservatives stored for 7 hours using room temperature. In the sample of commu (*Auxis rochei*) without using belimbing wuluh leaves (control) containing histamine levels of 16.23%, samples of commu fish using 100 gr belimbing wuluh leaves containing histamine content of 14.03%, commu fish samples using 200 gr belimbing wuluh leaves containing histamine levels of 11.73%, samples of commu fish using 300 gr belimbing wuluh leaves containing histamine levels of 11.01%, samples of commu fish using 400 gr xxx leaves contains histamine 11.98%, commu fish using 500 gr belimbing wuluh leaves containing 9.16% histamine content.

Degradation of the quality of fish muscles is usually associated with changes in physical, chemical, biochemical and microbiological activities of fish. Fish experience quality changes as a result of autolysis and bacterial activity [2]. In biochemical processes, enzymatic reactions are generally related to the initial freshness of fish, while microbial activity is related to the decay process. So that the initial process is related to biochemical or enzymatic freshness and the latter is related to bacterial freshness or decay [4].

In samples of fish containing containing *E. coli* bacteria [5], but different for each type of fish. For freshwater fish fillets (*Sparus aurata*) there are dominant decay microorganisms namely *Brochothrix thermosphacta* and Lactic Acid Bacteria (LAB) [3]. Storage conditions also affect the type of microorganism, for example in conditions of aerobic storage, *Shewanella putrefaciens* as decomposing organisms for cold fish from the northern sea [3]. Pathogenic microorganisms such as *Listeria monocytogenes*, *Salmonella* and *Staphylococcus aureus* are common for seafood and terrestrial muscle food. However, other microorganisms such as *Clostridium botulinum* type E, *Vibrio* and *Aeromonas* species are more common for seafood products [1].

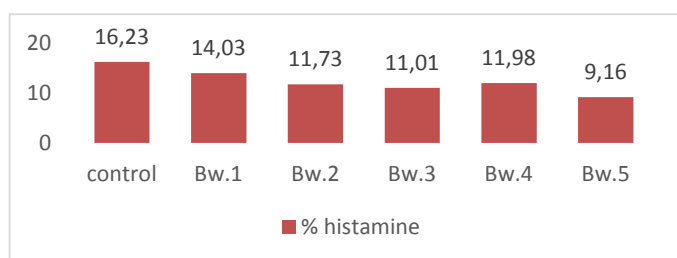


Figure 2. Graph of the effect of treatment on histamine levels.

Figure 2. shows that the treatment of giving belimbing wuluh leaves (*Averrhoa bilimbi*, Linn.) in commu fish (*Auxis rochei*) has histamine levels ranging from 16.23% - 9.16%. The highest level was found in the control sample while the lowest was found in the Bw.5 sample. In the graph it can be seen that, the histamine levels in each treatment are not the same, the more the belimbing wuluh leaves are given to the commu fish used, the lower the histamine levels obtained.

The belimbing wuluh plants have chemical contents, namely: potassium oxalate, flavonoids, pectin, tannin, gallic acid and ferulic acid. The natural chemical content found in the belimbing wuluh leaves which is thought to have anti-inflammatory activity are flavonoids and saponins. Research has been carried out on the chemical content of belimbing wuluh leaves (*Averrhoa bilimbi* L) on extracts and powders. The results of the examination concluded that the extract obtained showed the presence of tannins and phenolic groups (protocatric acid gallic acid, gentisic acid and ferulic acid) and 95% ethanol extract showed the presence of flavonoids. Tanin has antibacterial properties by precipitating bacterial proteins, because tannins are thought to have the same effect as phenolic compounds.

Furthermore, research on the antibacterial activity of the starfruit leaves (*Averrhoa bilimbi* L) against *Staphylococcus aureus* and *Escherichia coli* bacteria and the detection of active compounds by bioautography so that the results of ethanol and water extracts have antibacterial activity against *S.aureus*, whose growth can begin to be inhibited the administration of ethanol extract with a level of 40% and extract of water with a concentration of 60%. The ethanol and water extracts do not show antibacterial activity against *E. coli*.

IV. CONCLUSION

Utilization of belimbing wuluh leaves (*Averrhoa bilimbi*, Linn) as a natural preservative for commu fish (*Auxis rochei*) can reduce histamine levels, the more belimbing wuluh leaves are used, the lower the histamine levels obtained. In fish samples without using belimbing wuluh leaves (control) containing histamine levels of 16.23%, and with belimbing wuluh Leaves histamine levels ranged between 14.03% - 9.16%.

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