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The Effect of Concentrations of Basil Leaves Extract as Natural Preservatives in Mullet Fillet on Bacterial Growth in Low-Temperature Storage

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Authors' contributions

This work was carried out in collaboration among all authors. Author SS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors Junianto and IR managed the analyses of the study. Author EA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

This research aimed to know the shelf life of mullet fillet with basil leaves extract treatment in different concentration based on the amount of bacteria contained on mullet fillet during low temperature storage. The research was conducted at The Central Laboratory and The Laboratory of Fishery Product Processing, Padjadjaran University, Jatinangor. The method used in this research was experimental with four treatments by duplo. Basil leaves extract treatment concentrations were given in 0%, 1.5%, 3% and 4.5% concentrations, soaked for 30 minutes and stored at low temperature (5-10℃). The observations for grey mullet fillet with 0% concentration (without soaking on basil leaf extract treatment) were made on the 1^{st}, 3^{td}, 5^{th}, 6^{th}, 7^{th}, 8^{th} and 9^{th} day of research. The observations for 1.5%, 3% and 4.5 concentrations were made on the 1^{st}, 3^{rd}, 5^{th}, 6^{th}, 7^{th}, 8^{th}, 9^{th}, 10^{th}, 11^{th} and 12^{th} day of storage period. The parameters observed in this research was the amount of bacteria. The result of research showed that the use of basil leaf extract in concentration of 3% on mullet fillet during low temperature storage has the longest shelf life, that was until 11 days with total amount of bacteria about 4.55 x 10^6 cfu/g.

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Keywords: Bacteria; low temperature; basil leaf extract; mullet fillet.

1. INTRODUCTION

The mullet fish (*Mugil cephalus*) is one of the Mugilidae family which is spread in estuaries, ponds, rivers, and coastal waters both in tropical and subtropical regions [1]. Mullet fish has a relatively high selling price compared to other estuary species [2]. Mullet fish is one of the economical fish that has the potential to be developed due to the high market demand. Mullet fish is produced from catches by fishermen or anglers. The price of mullet fish is around Rp.23,000,-30,000/kg with relatively high market demand [3]. Based on observations, the prices of mullets in TPI (local fish market) Taman Jaya, Banten ranged from Rp.12,000-15,000/ kg while in TPI Muara Angke reached to Rp.25,000-Rp 30,000/kg depending on the number of catches [4]. The proximate composition of mullets consists of 73% water, 20% protein, and 2.5% fat [5].

Some mullet fish can be made into filet. Filet-shaped meat is more practical to cook because the meat is free from bones that will not be used in the cooking. However, filet-shaped fish meat no longer has a protective layer so that the growth of microorganisms is easier to occur in a short time. High water content and nearly neutral pH condition in the meat can be easily digested by the enzyme autolysis which causes fish meat to become a good medium for the growth of bacteria. Fish meat has very little binding material (tendons) so it is very easily digested by the autolysis enzyme. The results of autolysis become a suitable medium for the growth of microorganisms [6]. The changes in the quality of freshness can take place enzymatically, chemically and bacteriologically with organoleptic quality decreases followed by temperature conditions, where the higher the temperature, the faster the decline in freshness quality [7].

One way to preserve the quality of filet is by storing filet at low temperatures and use preservatives. Storage with low temperatures is one of the simplest attempts to extend the shelf life of fish. Low-temperature storage can slow down metabolic activity and inhibit microbial growth, while also preventing chemical reactions and loss of water content from food [8]. In addition to low temperatures storage, the provision of preservatives is another practical effort of choice. These days, many food producers use preservatives with affordable prices without considering the impact of its use on consumers' safety. Preservatives contain antimicrobials that help extend the shelf life. Antimicrobials can be obtained from natural ingredients such as fruits or vegetables.

Some of the natural ingredients have antimicrobial substances that can be used as preservatives. One of them is basil leaf. Basil leaves contain active compounds that can play a role in the process of preserving fish meat. The active compounds in the basil leaves are essential oils, phytosterols, alkaloids, phenolic compounds, tannins, lignin, starches, saponins, flavonoids, terpenoids, and anthraquinones so that they can be used as natural preservatives because they can inhibit microbial activity [9].

The use of natural ingredients as preservatives is closely related to the content of antibacterial substances in them. The content of compounds in natural materials is more antibacterial which can precipitate enzymes released by microbes that inhibit microbial activity [5]. One of the natural ingredients that almost has the same active compounds like basil leaf is palliasse leaf which its leaf extract gives an influence on the freshness of mackerel during room temperature storage. The result says that the concentration of 3% gives better results than concentrations of 2% and 4%, which has a shelf life of eight hours long with a total microbial 5×10^5 cfu / g and a pH value of 5.4 [10]. Thus, the potential of active compounds in basil leaf will give some results to the shelf life of mullet filet. The ability of antibacterial substances depends on the amount of concentration given, the higher the concentration the ability of the active compound will be higher.

The purpose of this research is to determine the concentration of basil leaf extract that is most effective in extending the shelf life of mullet filet at low temperatures.

2. MATERIALS AND METHODS

2.1 Research Site

This research was conducted from November 2019 to February 2020 at Central Laboratory and The Laboratory of Fish Product Processing Faculty of Fisheries and Marine Sciences, Padjadjaran University, Jatinangor, West Java, Indonesia.

2.2 Research Matterials

The ingredients used in this study were 23 of mullet fish with weights ranging from 100-125 g, basil leaf extract, physiological NaCl 0.9%, distilled water, nutrient agar, 96% ethanol and hand sanitizer.

2.3 Research Tools

The tools used in this study were gloves, scissors, blenders, 500 ml measuring cups, beaker cups, coolboxes, boxes, cutting boards, filet knives, two kilograms capacity scales, hollow plastic containers, plastic basins, plates, plastic wrap, erlenmeyer tubes, tissue towels, perforated plastic, tweezers, digital scales with a precision of 0.1 g, petri dishes with a diameter of 11.5 cm, mortar and pestle, 10 ml volume pipette, test tubes, test tube racks, bunsen, oven, incubator, spatula, colony counter, tally counter, pH meter, 25 ml and 50 ml measuring cups, and stationery.

2.4 Research Methodology

The method used in this study is an experimental method with four duplicate treatments. The treatment given was soaking the mullet filet in a basil leaf extract solution at a concentration of 0% (without soaking the basil leaf extract), 1.5%, 3%, and 4.5%. The parameter in this study is the number of microbes measured by the total plate count (TPC) method.

2.5 Research Procedures

2.5.1 Basil leaves extraction

Basil leaves were cut from the branches and washed thoroughly before dried for three days. The dried leaves were placed into a container then filled with ethanol 96% to begin the maceration process. The sample was filtered out every 24 hours to get the ethanol extract and evaporated by using the rotary evaporator to separate the leaf extract and ethanol. The extract was collected and placed into a bottle. This step was repeated until the ethanol extract was no longer has a green color, which means all the low molecular weight compounds have been extracted.

2.5.2 Filleting

Fresh fish were bought from local fisherman and stored in the *coolbox* filled with ice to preserve its quality. The fish were brought to the laboratory immediately. Fish were cleaned from scales and washed thoroughly, then sliced across the back of the head and base of the tail, then formed at an angle to the rib cage. The meat was sliced from the head to the base of the tail along the dorsal fin, the incision is only as deep as the spine towards the abdomen. The meat opened and sliced following the shape of the rib cage. Filet-shaped meat was washed thoroughly with cold water at 10°C to remove impurities and blood residue.

Fig. 1. Mullet fillet

2.5.3 Applications of basil leaf extracts to filet

Fish filets were soaked in basil leaf extract for 30 minutes according to the concentration of the tested treatment. Soaking was done to determine the effectiveness of adding basil leaf extract to the shelf life of fish fillets. After the filet is immersed, the filet is drained for 15 minutes and placed on a plate that has covered with tissue paper towels and perforated plastic and then packed using cling wrap. The packaged filet is stored in a refrigerator with a temperature range of 5 - 10°C.

2.6 Observed Parameters

The parameter in this study is the number of microbes measured by the total plate count (TPC) method. One of the causes of spoilage in fishery products is the emergence of spoilage or bacterial microorganisms that change the characteristics of the product so that it can no longer be consumed. The total plate count method is used to determine the total number of aerobic and anaerobic microorganisms in fishery products. Bacterial colonies were grown by the pouring method, incubated at 35 ℃ ± 1 ℃ for 48 ± 2 hours. The calculation is carried out during the observation period until it reaches the limit of acceptance of bacterial colonies in fresh fish, which is 10^6 cfu/g [11]. If the bacterial colony has passed this limit, then the making of bacterial samples is stopped. For the calculation of the number of microbial colonies, the following formula is used [12]:

$$N = T \quad Cc \quad x \frac{1}{d \quad f} \tag{1}$$

Note:

N = Total Colony per ml

2.7 Data Analysis

The results of observations of the number of bacteria were analyzed descriptively and presented in tables and curves based on the number of bacteria counted. Calculations of the number of spoilage microbes using the Total Plate Count (TPC) method are compared with the limit of microbial acceptance of food products that are safe for consumption (5 x 10^6 cfu/g) [13]. A descriptive analysis method is done to compare the similarities and differences of two or more facts of the object under study based on a particular frame of mind by describing, to find the elements, then analyzing and drawing conclusions.

3. RESULTS AND DISCUSSION

3.1 Results

The number of bacteria in food is one of the indicators that determine the safety level of food to be consumed. The number of bacteria contained in a food ingredient as much as 10^6 cfu g is still considered safe for food material to be consumed [11]. The amount of bacteria contained in the mullet filet after being soaked in a basil leaf extract solution for 30 minutes and stored at low temperatures is presented in the following Table 1.

Based on the results of the study, the initial number of bacteria obtained from the mullet filet in each treatment was 3.03 x 10^3 cfu/gr, 1.09 x 10^3 cfu/gr, 2.75 x 10^3 cfu/gr, and 8.3 x 10^3 cfu/gr. The highest initial number of bacteria was at 0% treatment (not soaked in basil leaf extract dilution) with the initial number of bacteria 3.03 x 10^3 cfu/gr. The higher concentration of basil leaf extract given does not result in a smaller initial number of bacteria. This is proved at the concentration of 1.5% the initial amount of bacteria that is 1.09 x 10^3 cfu/g compared to the concentration of 3% and 4.5% which has an initial number of bacteria 2.75 x 10^3 cfu/gr and 8.3 x 10^3 cfu/gr. This is because the bacteria are still in the adaptation phase after the exposure to basil leaf extract which contains antibacterial substances. Bacterial response to pressure is the ability of bacterial cells to fight conditions when bacterial populations are briefly exposed to the physical and chemical environment at suboptimal levels of growth [14].

Fish spoilage is caused by fish meat degradation due to enzyme activity, biochemical changes, and the growth of microorganisms [15]. When a fish dies, the enzymes found in the fish begin to actively degrade fish meat into simpler substances, and microorganisms found in the contents of the stomach, gills, and skin multiply rapidly. Bacteria begins to produce sulfur-containing products that cause unpleasant and toxic odors [16]. Bacteria also changes the appearance and physical properties of some fish components [15].

Tabel 1. The number of bacteria in mullet filet based on treatment during low-temperature storage

Storage days-	Basil Leaves Extract Concentration (%)			
	0	**1.5**	**3**	**4.5**
1	3.03×10^3	1.09×10^3	2.75×10^3	8.3×10^3
3	1.89×10^5	3.08×10^4	2.43×10^4	2.9×10^4
5	9.3×10^5	2.94×10^5	5.3×10^4	7.2×10^4
7	4.1×10^6	2.9×10^6	3.65×10^5	3.1×10^5
8	2.9×10^7	3.85×10^6	5.6×10^5	6.85×10^5
9	1.2×10^8	9.3×10^6	2.75×10^6	4.55×10^6
10	-	1.37×10^7	3.45×10^6	3.2×10^6
11	-	1.28×10^7	4.55×10^6	5.4×10^6
12	-	3.35×10^7	2.8×10^7	1.08×10^7

Note:
(-) = Bacterial colonies measurement was not done

An increase in the number of bacteria during the shelf life occurs due to the process of autolysis that takes place due to the activity of enzymes contained in the body of fish [7]. The process of autolysis cannot be stopped even at low temperatures, this process begins together with a decrease in pH. Autolytic enzymes hydrolyze fish protein to produce peptides and amino acids [14]. These compounds are substrates that provide nutrients for bacterial propagation [17]. Usually, the process of autolysis is always followed by an increase in the number of bacteria, because all the breakdown of enzymes during the autolysis process is a very suitable medium for the growth of bacteria and other microorganisms [7].

Microbes that play a role in the destruction of food are bacteria, which have existed since fish were alive [18]. Bacteria are microorganisms that live normally on the surface of the body and intestines of healthy fish. Bacteria grow on the body of live fish as normal living creatures, but when the fish die and the metabolism stops automatically the bacteria break down the protein contained in the fish's body so that decay occurs. Bacteria utilize proteins found in fish meat and will be broken down into simpler compounds through the autolysis process. Bacteria break macro compounds in fish meat such as protein, carbohydrates, and fats in fish meat through enzymatic processes with the help of oxygen (aerobic) [19].

The final product of an overhaul that can occur in the form of H_2S and other sulfides such as sulfide, mercaptan, and indole are products that show the decaying process. Bacteria will secrete enzymes that breakdown compounds and tissues causing the meat becomes damaged and rots. Bacteria will cause a bitter taste, foul odor, mucus which was originally bright becomes opaque [20].

The growth of bacteria in dead fish increases due to the condition of the fish that allows bacteria to grow and develop due to the results of degrading protein which is a good substrate for bacterial growth [21]. The number of bacteria increases with the duration of storage due to the optimal environment for bacterial growth which causes bacteria to grow optimally [22].

The temperature used to store the mullet filet in this study is low (5-10 ℃). The number of bacteria usually decreases during low temperatures (cold/freezing), but the decrease only occurs in thermophilic and mesophilic bacteria [20]. Besides, at low temperatures and an increase in intracellular solid concentration results in physical and chemical changes in bacterial cells and fungi that cause decay [23]. When the temperature conditions are low, the growth of spoilage bacteria and biochemical processes that take place in the body of the fish leads to slower quality decline [24]. The higher the storage temperature, the faster the metabolism of bacteria, but storing at low temperatures slow down the bacterial metabolism, so the quality can be maintained.

The results of this research show that the number of bacteria during storage of the mullet filet has increased, indicating that bacteria can still live at low temperatures. The type of bacteria that lives and grows is thought to be a psychrophilic bacterium because these bacteria grow optimally at temperatures of 5-10 [25]. Groups of bacteria that can damage food at low temperature are psychophilic and psychrotrophic bacteria groups [19]. Psychrotrophic bacteria can grow at a minimum temperature (-4 - 5℃), optimum (25 - 30℃), and maximum (30-35) [26]. Also, psychrophilic bacteria can grow at temperatures below 5℃, but rapid growth occurs at temperatures of 10 - 25℃ even at higher temperatures [14]. During low-temperature storage the bacteria will grow with a doubling time of about 1 day and will reach an amount of 10^8 - 10^9 cfu/g of meat or skin cm^2 after 2-3 weeks [27].

The damage in fish meat is mainly caused by the activity of bacteria [28]. The main microbes that cause rot in fishery products are *Pseudomonas* sp., *Archrobacter* sp., *Flavobacterium* sp., *Coryneform* sp., and *Micrococcus* sp. which can grow at an optimum temperature of 5-10 (psychophilic bacteria) [20]. The results of observing the number of bacteria found in the mullet filet which was inoculated in a petri dish were found to be uniform, white and in one colony there was a branching and circular shape.

The shelf life of a mullet fillet with treatments is calculated to be longer when compared with a filet without treatment. Besides, the number of bacteria in the mullet without treatment tends to increase faster than the fillet with treatment. This happens because the treated filet contains antimicrobial substances (tannins, flavonoids, saponins, triterpenoids, and alkaloids) which are bacteriostatic (inhibiting bacterial growth) and bactericidal (killing bacteria). The chemical properties of basilostatic and bacteriocidal basil leaves are alcohol, volatile oil, and phenol [29]. The phenol group is known to have an antimicrobial activity which is bacteriocidal but not sporicidal [30]. Large molecular phenol compounds are capable of activating essential enzymes in bacterial cells even in very low concentrations. Basil extract contains quite a lot of phenol compounds including tannins and flavonoids.

Antimicrobial compounds such as flavonoids work through several mechanisms, namely inhibiting the synthesis of nucleic acids, inhibiting the function of bacterial cytoplasmic membranes and inhibiting energy metabolism [31]. This mechanism occurs due to the reaction between lipids and amino acids with alcohol groups in flavonoids so that the cell walls are damaged and cause these compounds to enter the nucleus of bacterial cells. The difference in polarity between lipids and DNA compilers with alcohol groups in flavonoid compounds causes a reaction so that the nucleus of bacterial cells will be damaged and lysis. Flavonoids also cause bacterial cell damage, enzyme inactivation, and cell leakage [32], which are bacteriostatic which work by inhibiting bacterial cell wall synthesis [33].

Tannins have plasmolytic properties that shrink the cell walls or cell membranes that have been lysed due to flavonoid compounds. This causes tannin compounds to easily enter the bacterial cell so that the cell cannot carry out live activities and its growth is stunted or even dead. Tannins also can inhibit bacterial growth by inactivating enzymes. Condensed tannins bind to bacterial cell walls so that the activity of protease enzymes that break down proteins is inhibited [34]. The continuation of bacterial activity depends on the action of the enzyme. If the enzyme work is disrupted, bacteria will need relatively large amounts of energy to carry out their activities, so that the energy used for bacterial growth is reduced. If this lasts a long time then the bacterial activity will be inhibited and lead to lysis.

The results showed that the mullet filet without treatment only had a shelf life of 7 days, while the mullet filet with treatment gave a longer shelf life (9-11 days), but this was not directly proportional to the concentration used. Based on the administration of several concentrations of basil leaf extract, a 1.5% concentration can extend the shelf life of mullet filet for 9 days in low-temperature storage. While concentrations of 3% and 4.5% can extend the shelf life for 11 days.

The difference in the number of days at the receiving limit of 1.5% concentration can be due to antimicrobial compounds that are too small so that they are less effective in inhibiting bacterial growth. While concentrations of 3% and 4.5% do have the same acceptance limit for bacteria, the most effective concentration is a concentration of 3%. This is because of its efficiency and optimal function to inhibit microbial growth. As for the 4.5% concentration, it becomes ineffective because the addition of the extract concentration does not always provide a stronger bacterial growth-inhibiting effect. The increase in the concentration of material will be followed by an increase in the inhibition of bacterial growth, but at the maximum concentration, there will be a decrease in the inhibition of bacterial growth [35]. The addition of concentration does not provide a longer shelf life due to the content of nitrogen and protein compounds contained in extracts at a certain amount utilized by bacteria and spur growth [36]. Bacteria can fix nitrogen and collect it in the form of compounds in their cells [37].

The growth of bacteria continues to occur because, during the storage period, organic material contained in the filet or living media is still available. The bacteria that grow on food are heterotropic, which requires organic substances in their growth [38]. Metabolism of heterotrophic bacteria uses protein, carbohydrates, fats, and other organic components as a source of carbon and energy for growth. The presence of organic material can reduce the effectiveness of antimicrobial substances by activating antimicrobial agents or protecting microbes from the effects of antimicrobial substances [37]. This is why the treatment of mullet filet treatment of 4.5% becomes ineffective.

The mechanism of action of antibacterial agents in inhibiting growth is influenced by several factors including antibacterial concentration, storage time, ambient temperature, environmental pH, and bacterial properties including age, type, and condition of bacteria [12]. Antibacterial inhibits the growth of the number of bacteria with a certain period, but not all bacteria can be inhibited growth. Bacteria that can survive will quickly multiply and the bacteria will become resistant to antibacterial.

Bacteria become resistant because they separate themselves genetically, then bacteria can grow into new bacteria that are resistant because of the process of mutation and gene transfer to other bacteria. The mutation itself is the occurrence of protein modification, namely a decrease in the affinity of bacterial protein bonds with antibacterial. Protein will be resistant to loss of efficiency due to the mutase. Some mutases cause bacteria to produce enough chemicals (enzymes) to inactivate antibacterial [39]. This shows that increasing the concentration of basil leaf extract does not always provide a better bacterial growth inhibitory effect.

Based on the observation of the number of bacteria, it can be seen that the basil leaf extract can inhibit the growth of bacteria because the mullet filet treatment can last longer than the mullet filet without treatment.

3.2 Discussion

Fish spoilage depends on several factors namely the type of fish, the condition of the fish, the level of fatigue, the size of the fish, way of handling fish, and the temperature of storage [40]. Fish quickly damages or decays because there are internal and external factors that influence it happens. Internal factors that trigger rapid decay occur in the fish meat are fish species, the condition of the fish when it dies, and chemical composition in the meat namely high water, protein, and fat content. Each type of fish has a different chemical composition so that the speed reaches the rigor stage is different. Fish with high-fat content will more quickly experience decay [41]. High-fat content in fish triggers fat oxidation which causes rancidity [40]. Fat oxidation forms peroxide and ketone compounds which affect the appearance and odor of fish [42]. The oil and fat content in food affects the rancidity of taste and aroma. External factors that trigger spoilage are the location of handling, season, and method of catching [43]. External factors such as how to catch, facilities, handling processes, and transit time. The rate of deterioration in the quality of injured or bruised fish is higher compared to fish with the intact physical condition [5]. The worse the condition of fish, the faster the decay process occurs. Fish that die due to flounder or jostling decompose faster than fish that die instantly [44]. The damaged surface of the fish body facilitates bacterial penetration [45].

Fish is known as a commodity that has high nutritional value but is easy to rot because it contains high protein content with free amino acids used for the metabolism of microorganisms, ammonia production, amino biogenic, organic acids, ketones, and sulfur components. The high protein content in fish triggers protein denaturation, causing changes in fish [20]. Protein denaturation is the process of breaking hydrogen bonds, bonding salts, and forming folds of molecules so that the structure of proteins is damaged [46]. The process of decay occurs due to the presence of enzymes produced by bacteria and damage nutrients in fish meat [47]. The bacterial activity can cause various biochemical and physical changes that cause overall damage called rotting [48]. The process of change that occurs in the body of the fish is caused by the activity of enzymes, microorganisms, and oxidation in the body of the fish [44].

After a fish die, various processes of physical and chemical changes take place more quickly, all changes eventually lead to decay [7]. Ways to slow down fish spoilage are including cooling and storing it in ice. Besides, efforts are needed to preserve these foods so that they can be consumed in conditions that are still suitable for consumption. Preservation or processing of fishery products aims to inhibit or stop the activity of substances and microorganisms that can cause spoilage (deterioration of quality) and damage [49]. The preservation that is generally used to maintain fish is by cooling, salting, drying, and increasing the temperature of the substance [50]. This research uses a cooling method that is storing the filet at a temperature of 5-10 ℃ and adding a basil extract solution.

Mullet filet stored in low temperatures will continue to suffer a deterioration in quality. One cause of deterioration in quality is bacterial activity. The bacterial activity can be inhibited by storing mullet filets at low temperatures, but low-temperature storage still has a limitation of relatively short shelf life. The main cause of fish damage during low-temperature (cold) storage is the activity and growth of psychotropic bacteria. Besides, bacteria that also live at low temperatures are mesophyll bacteria, which can live at a minimum temperature of 5-10 ℃ [5].

Giving a basil leaf extract solution can inhibit bacterial activity. This is because the basil leaves contain an antibacterial form of essential oils, phytosterols, alkaloids, phenolic compounds, tannins, lignin, starches, saponins, flavonoids, terpenoids, and anthraquinones [9]. Flavonoids, saponins, tannins, and terpenoids in sequence also play a role in inhibiting bacterial growth. This compound will inhibit bacterial growth. Flavonoids, alkaloids, terpenoids, saponins, and tannins are soluble in water solvents [51], so the five compounds have a role in inhibiting bacterial growth in fish filets. Flavonoids and other antibacterial compounds can work together to inhibit the growth of resistant bacteria at once [31]. Giving a basil leaf extract solution will bind to fish meat protein compounds making it more difficult to be overhauled either by enzymes from fish or bacteria. As a result of the disruption of bacterial activity, the mullet filet with treatment has a longer shelf life compared to the control mullet filet.

Antibacterial compounds in basil leaves have different properties in their mechanism of inhibiting bacterial activity. Alkaloids interact with bacterial DNA, which damages DNA by binding nitrogen bases between the composition of amino acids so that bacteria lysis [34]. Flavonoids are antimicrobial compounds that are effective in inhibiting the spread of microorganisms [34]. The ability of flavonoids as an antibacterial is shown by inhibiting nucleic acid synthesis, cytoplasmic membrane function, and energy metabolism [31]. Saponins interact with the lipid layer and lipopolysaccharide on the bacterial outer membrane so that the integrity of the bacterial cell wall is damaged [52]. Tannins form complexes with proteins so that the activity of the protease enzyme is inhibited [31]. Steroids/Triterpenoids can damage bacterial membranes, but these compounds are reported to only be able to inhibit bacteria as much as 30% of total microorganisms.

This study discusses and observes how the condition of the mullet filet treated with the addition of a basil leaf extract solution which has a concentration of 1.5%, 3%, and 4% stored at low temperatures (5-10 ℃) for 12 days viewed from the number of bacteria. The mullet filet with treatment has a longer shelf life of around 7-11 days compared to the mullet filet without treatment which only reaches a shelf life of six days. The mullet filet without treatment has a shelf life of two days faster than the mullet filet with 1.5% treatment or faster than the 3% and 4.5% treatment.

The main parameters in determining the shelf life of the mullet filet can be seen from the results of the test of the number of bacteria. The shelf life of a mullet filet is a grace period or a time interval for the mullet filet that can be stored while still being consumed. The mullet filet in this study has a shelf life which is influenced by internal factors such as enzymes in fish meat and external factors such as temperature. The higher the storage temperature the faster the bacterial metabolism, but if stored at low temperatures the metabolism is inhibited, so freshness can be maintained. This is supported by [42] that low-temperature conditions make the growth of bacteria in the body of the fish can be slowed down so that the freshness of the fish is maintained longer. A good source of nutrition can be obtained if the condition of the fish is fresh.

Mullet filet treated with soaking it in basil leaf extract solution during storage at low temperatures has undergone a process of change. These changes have included changes in physical, chemical, microbiological, and organoleptic characteristics that cause changes in quality during storage. Changes in the quality of the mullet filet cannot be stopped but can be inhibited. Inhibiting changes in the quality of mullet filet in this study utilizing low temperatures and antimicrobial solutions of basil leaf extract.

Antibacterial compounds that synergize the inhibited growth of decomposing bacteria in the mullet filet. Disruption of antibacterial also inhibits the activity of enzymes in bacterial cells through the inhibition of oxidative phosphorylation [53]. This causes the quality of the mullet filet given a basil leaf extract solution will be maintained longer. Flavonoids and other antibacterial compounds can work together to inhibit the growth of resistant bacteria at once [31].

Fish is one source of animal protein that is easily damaged caused by bacteria, yeast, and fungi. The main cause of fish damage is spoilage microorganisms that naturally damage fresh fish after death and fish meat has few tendons, so the process of spoilage in fish meat is faster than other animal or animal products [28]. Protein in fish bodies is very easy to undergo decay and fish are very easy to experience denaturation (damage) of protein that occurs because fish meat has little binding material (tendons). High protein content in fish triggers rapid damage to fish meat which causes a foul odor, color, taste, texture that changes [54]. Changes that arise in the process of deterioration in the quality of fish such as the smell of rotten, and the presence of mucus.

The number of bacteria is an important parameter to determine the freshness level of the mullet filet. Mullet filet without treatment and mullet filet with 1.5%, 3%, and 4.5% treatment showed differences in their acceptable limits. The mullet filet without treatment reached the limit of acceptance of fresh fish specifications on the 6th day with the number of bacteria 3.1 x 10^6 cfu/g. Whereas the mullet fish treatment 1.5%, 3%, and 4.5% reached their respective acceptance limits, namely on the 8th, 12th, and 12th days, with a relatively equal number of bacteria also reaching 10^6 cfu/g. The mullet filet 1.5% treatment has a different acceptance limit which is four days faster than the other two treatments (3% and 4.5%).

The number of bacteria found in fish depends on the environment where the fish are caught [49]. The bacteria commonly found in fish are *Pseudomonas* sp., *Alcaligenes* sp., *Sarcina* sp., *Vibrio* sp., *Flavobacterium* sp., *Serratia* sp., and *Bacillus* sp. Various factors that influence the growth of microorganisms in food, among others, are determined by the physicochemical characteristics of the food (intrinsic factor), and the storage environment conditions (extrinsic factor) [55].

The determination of the best treatment in this study was determined from the treatment with the smallest number of microbes. Based on the results of the study, the best and most effective shelf life of mullet filet is mullet filet treatment of 3%, because it can inhibit the growth of spoilage bacteria with a storage duration of 12 days.

If the antimicrobial agent matches the ideal dose (3%), it will work optimally for preserving the mullet filet, but by reducing or exceeding the ideal dosage, the work of the antimicrobial substance will not be optimal for preserving fish, such as at concentrations of 1.5% and 4.5 %. Besides, the concentrations of 1.5% and 4.5% indicate a shelf life of 7 days and 11 days, although the shelf life of concentrations of 3% and 4.5% are the same, the effectiveness of 3% concentration is much better than the concentration of 4.5%. The treatment concentration of 1.5% and 4.5% becomes ineffective because the 1.5% antimicrobial concentration contained is less than optimal so it is not enough to inhibit bacterial growth. Whereas the 4.5% antimicrobial concentration is not optimal enough because it is too much when compared to the number of bacteria so that it has the potential to make the bacteria resistant to antimicrobials and make the bacteria can continue to grow in the presence of organic material from basil leaves (vegetable protein) or more nutrients, the existence will be a medium or nutrition that can be used by bacteria that can still survive for energy sources and growth during filet storage.

The 3% concentration is the best in inhibiting deterioration in quality and extending the shelf life of the mullet filet. A 3% concentration can extend the shelf life of the mullet filet until the 11th day with the number of bacteria of 4.55 x 10^6 cfu/g.

4. CONCLUSION

Basil leaves extract can used to preserve fillet because it contains antimicrobial compounds such as essential oils, phytosterols, alkaloids, phenolic compounds, tannins, lignin, starches, saponins, flavonoids, terpenoids, and anthraquinones those inhibit microbial activity. Smaller amount of antimicrobial concentration is less than optimal so it is not enough to inhibit bacterial growth, while the higher concentration has the potentials to make the bacteria resistant to antimicrobials. Based on the results of this study it can be concluded that the use of basil leaves extract with a concentration of 3% in mullet filet during low-temperature storage has the longest shelf life of 11 days with a total bacterial value of 4.55 x 10^6 cfu / g.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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