The Effect of Concentrations of Basil Leaves Extract as Natural Preservatives in Mullet Fillet

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Authors’ contributions

This work was carried out in collaboration among all authors. Author J designed the study. Author SS performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author 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 concentrations based on the number of bacteria contained on mullet fillet during low-temperature storage. The method used in this research was experimental with four treatments in double measurements. Mullet fillets were soaked for a half-hour in different concentrations of Basil leaves extract (0%, 1.5%, 3%, and 4.5%) and stored at low temperature (5-10°C). The parameters observed in this research were the number of bacteria, pH measurements, and weight loss. 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 fillet during low-temperature storage has the longest shelf life of 11 days with the amount bacteria of 4.55 x cfu/g, pH value of 6.55 and weight loss value of 20.00%.

Keywords: Shelf life; low temperature; pH; mullet fillet.

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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]. It fetches a high price compared to other estuary species [2]. So, it 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 (1.6-2.09$) with relatively high market demand [3]. Based on observations, the prices of mullets in TPI (local fish market) Taman Jaya, Banten ranged from Rp.12,000-15,000/kg (0.83-1.04$) while in TPI Muara Angke reached to Rp.25,000-Rp 30,000/kg (1.74-2.09$) 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 the fillet. Fillet-shaped meat is more practical to cook because the meat is free from bones that mostly will not be used in the cooking. However, fillet-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) which makes meat becomes a more suitable medium for the growth of microorganisms [6].

One way to preserve the quality of fillet is by storing fillet at low temperatures and use preservatives. Storage with low temperatures is one of the simplest attempts to extend the shelf life of fish. Changes in the quality of freshness can take place enzymatically, chemically, and bacteriologically, followed by a decrease in organoleptic which is influenced by temperature conditions, where the higher the temperature, the faster the decline in freshness quality [7]. 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. Most of the preservatives are antimicrobials that assist to extend the shelf life.

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 due to its antibacterial activity. The content of compounds 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 $5 \times 10^5$ cfu / g and a pH value of 5.4 [10]. Thus, the potential of active compounds is responsible for the extension of the shelf life of mullet fillet. 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 fillet at low temperatures.

2. MATERIALS AND METHODS

2.1 Research Materials

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

2.2 Research Methodology

The method used in this study is an experimental method with four duplicate treatments. The treatment given was soaking the mullet fillet in a basil leaf extract solution (solid extract of basil leaves ethanol extract and aquades as the solvent) at a concentration of 0%, 1.5%, 3%, and 4.5%.
2.3 Research Procedures

2.3.1 Basil leaves extraction

Basil leaves were cut from the branches and washed thoroughly and dried in shadow for three days. The weight of leaves used in the extraction was 4.917 kg. 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.

2.3.2 Filleting

Fresh fish were bought from local fishermen and stored in the thermocol box filled with ice and 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. Fillet-shaped meat was washed thoroughly with cold water at 10°C to remove impurities and blood residue.

2.3.3 Applications of basil leaf extracts to fillet

Fish fillets 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 fillet is immersed, the fillet is drained for minutes and placed on a plate that has covered with tissue paper towels and perforated plastic and then packed using cling wrap. The packaged fillet is stored in a refrigerator with a temperature range of 5 - 10°C.

2.4 Observed Parameters

2.4.1 Total bacteria

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 °C ± 1 °C 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]:

$$ N = \text{Total Colonies} \times \frac{1}{\text{dilution factor}} $$

Note:
N = Total Colony per ml

2.4.2 pH measurements

The measurements are carried out to determine the chemical changes during storage. A sample of 1 gram of fish meat is crushed until smooth,
then put into a test tube containing 9 ml of distilled water, shaken until homogeneous. Homogenates were measured by a pH meter that had previously been calibrated with a standard buffer of pH 4 and pH 7 [13].

2.4.3 Weight loss

The weight loss in the mullet fillet is a decrease in the weight of the mullet fillet that occurs while stored after going through the process of immersion in the concentration of basil leaf extract. The decrease in weight in the fillet is generally caused by the loss of water content in the fish fillet meat itself during the storage process. Great weight loss during storage can be caused by the moisture present in the material leaving the surface of the material and heading into the surrounding air through a process of condensation of water vapor [14]. The amount of weight loss in the fillet can be calculated with the following equation [15]:

\[
\text{Weight Loss} = \frac{W_t - W_0}{W_0} \times 100\% \tag{1}
\]

Notes:
Wo = Fillet weight on the first day of storage
Wt = Fillet weight on the measurement day of storage

2.5 Data Analysis

pH measurements were analyzed based on the average pH per gram of fish to see the decrease and increase in pH of fish during low-temperature storage. Weight loss calculation is done on the first day for all fish fillet samples and compared with the calculation on the specified test day.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Total bacteria

The research about the effect of concentrations of basil leaves extract as natural preservatives in mullet fillet showed that it can extend the shelf life of the fillet for 11 days in the concentration of 3% in low-temperature storage according to bacterial limit in product [16]. The amount of bacteria during storage is shown in Table 1.

Basil leaf extract solution can inhibit bacterial activity. This is because the basil leaves contain an antibacterial form of essential oils, phytochemicals (tannins, lignin, starches, saponins, flavonoids, terpenoids, and anthraquinones [9]). 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 fillet with treatment has a longer shelf life compared to the control mullet fillet.

Based on the results of the study, the initial number of bacteria obtained from the mullet fillet in each treatment was $3.03 \times 10^2$ cfu/gr, $1.09 \times 10^2$ cfu/gr, $2.75 \times 10^2$ cfu/gr, and $8.3 \times 10^2$ cfu/gr. The highest initial number of bacteria was at 0% treatment with the initial number of bacteria $3.03 \times 10^2$ 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 $1.09 \times 10^3$ cfu/g compared to the concentration of 3% and 4.5% which has an initial number of bacteria $2.75 \times 10^3$ cfu/gr and $8.3 \times 10^3$ 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 [17].

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 [18]. 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 [19]. Bacteria can fix nitrogen and collect it in the form of compounds in their cells [20].

The result of the research showed that the use of basil leaf extract in a concentration of 3% on mullet fillet during low-temperature storage has the longest shelf life, that was until 11 days with the total amount of bacteria about \(4.55 \times 10^6\) cfu/g [16].

If the antimicrobial agent matches the ideal dose (3%), it will work optimally for preserving the mullet fillet, 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 fillet storage [21].

### 3.1.2 pH measurements

The results of observing the acidity or pH measurements of the mullet fillet during low-temperature storage are presented in Table 2. Based on the table, the pH value of the mullet fillet during storage at low temperatures fluctuates but tends to rise with a pH ranging between 6.5 - 7.3. The pH value is in the optimum pH range for the growth of spoilage bacteria which is in the range of 6.5 - 7.5 [12]. Generally, as soon as the fish dies the pH of the meat of the fish will be near neutral, which is around 6.8 to neutral, causing the breakdown of glycogen which produces lactic acid thereby increasing the acidity of the meat and causing the pH of the meat to decrease [15]. The pH measurement is an important indicator in determining the level of freshness of the fish because it affects the period of storage. After the fish die, the value of the acidity or pH value tends to increase [16]. The results showed the pH value of the mullet fillet tended to decrease at the beginning of storage and then increased with fluctuation with increasing storage period.

Chemical changes in fish meat begin with a decrease in pH that occurs due to the activity of the glucokinase enzymes in the body of the fish [23]. These enzymes remodel glycogen into lactic acid which plays a role in reducing the pH of fish meat [15]. The magnitude of the decrease in pH depends on the amount of initial glycogen found in fish muscle [9]. The more glycogen reserves are converted to lactic acid, the lower the pH value, causing muscle tissue to be unable to maintain its flexibility [7].

The change from glycogen to lactic acid will affect the pH of fish so that it directly affects the storage period. Over time, the pH will increase again, this is because the protein and its derivatives are decomposed both by microbes and enzymatically into alkaline derivatives increasing pH [24]. The decomposition of the protein will produce basic compounds such as ammonia, histamine, thiamine, and others [23]. An increase in pH in fish meat during storage indicates the activity of proteolytic enzymes that produce ammonia, resulting in changes in the degree of acidity in the mullet fillet during storage within 14 days of observation [26].

The pH value affects the growth of bacteria in fish meat because fish meat is a good substrate for bacterial growth. The components contained in fish meat can provide a source of energy for bacterial growth, including the pH of fish flesh that is suitable for its growth [5]. The lower the pH value, the bacteria will actively decrease the pH of the environment towards acidic, while the higher the pH value (towards the alkali) the decomposing bacteria will be more active in remodeling proteins and fats [27]. Bacteria such as proteolytic bacteria, gram-negative rod-shaped bacteria, cannot grow on acidic foodstuffs [28].
Table 1. The number of bacteria in mullet filet based on treatment during low-temperature storage

<table>
<thead>
<tr>
<th>Storage days</th>
<th>Basil Leaves Extract Concentration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>3.03 x 10^3</td>
</tr>
<tr>
<td>3</td>
<td>1.89 x 10^5</td>
</tr>
<tr>
<td>5</td>
<td>9.3 x 10^5</td>
</tr>
<tr>
<td>7</td>
<td>4.1 x 10^6</td>
</tr>
<tr>
<td>8</td>
<td>2.9 x 10^7</td>
</tr>
<tr>
<td>9</td>
<td>1.2 x 10^8</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>-</td>
</tr>
</tbody>
</table>

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

Table 2. The pH values of mullet fillet based on treatments during low-temperature storage

<table>
<thead>
<tr>
<th>Storage days</th>
<th>pH Values on Mullet Fillet in Every Treatment during Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>6.5</td>
</tr>
<tr>
<td>3</td>
<td>6.25</td>
</tr>
<tr>
<td>5</td>
<td>6.45</td>
</tr>
<tr>
<td>7</td>
<td>6.7</td>
</tr>
<tr>
<td>8</td>
<td>6.95</td>
</tr>
<tr>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: (-) = pH value measurement was not done

The pH value of the environment or media where fish live will affect the activity and weakness of macromolecules such as enzymes, thus inhibiting growth and spoilage bacteria [29]. Bacteria grow well in dead fish because the pH of fish flesh is between 6.4 - 6.8 [30]. Bacteria are more susceptible to pH than fungi or yeast, especially pathogenic bacteria [31].

Based on the results of the examination of basil leaf extracts used in this study have a pH value that is classified as slightly acidic. So that value can affect the pH of the fillet. Naturally, the pH in fish will decrease at the beginning of the storage period and then rise until the pH reaches [32].

Changes in the pH of fish meat are caused by the process of autolysis (enzymes) and bacterial attack [22]. The decay process will be followed by an increase in bacterial growth and this condition will also be followed by an increase in pH [33]. Spoilage began to occur due to the increased base content of compounds in meat. In this condition, the pH of the fish rises slowly and the more purine and pyrimidine compounds that are formed will further accelerate the increase in fish pH [24]. An increase in pH due to the formation of alkaline compounds such as ammonia resulting from the process of protein overhaul in fish meat by enzymes and spoilage bacteria [31].

Based on the results of the study it was found that the pH value of the mullet filet increased on average with slight fluctuations starting from day one to the end of observation. It happened because enzymes derived from fish meat and microbes make changes to proteins and fats to produce alkaline compounds [25]. The pH value of fish meat gradually becomes alkaline due to the formation of volatile compounds such as ammonia, trimethylamine, indole, and others [25]. Marine fish and freshwater fish contain trimethylamine oxide which is degraded by some bacteria into trimethylamine [34].

Trimethylamine is formed from the reduction of trimethylamine oxide by enzymes derived from fish meat or microbes. The reshuffle of trimethylamine oxide to trimethylamine is an
important reaction from enzymatic fish damage [31]. Fish that are not fresh have a high meat pH (alkaline) compared to fresh fish. This is due to the emergence of basic compounds such as ammonia, trimethylamine, and other volatile compounds [35]. Compounds such as amino acids, glucose, lipids, trimethylamine oxide, and urea can be converted by bacteria into products that can be used as indicators of decay [36].

Soaking mullet fillets in basil leaf extract and stored in low temperatures can slow the increase in pH value. This is because at low temperatures the growth of bacteria becomes slow, so that protein overhaul lasts a long time, so the pH of fish is difficult to reach its basic nature. 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 [24].

Besides, also because the basil leaf extract solution contains antimicrobial compounds that can inhibit the growth of bacteria in the mullet fillet so that the accumulation of ammonia can take place more slowly. Tannins contained in basil leaves can be one that affects the pH value of the mullet fillet. Tannin is one of the phenol compounds that react with proteins to form insoluble compounds, causing an overhaul of alkaline remodeling to be produced longer [9].

Tannins work by making complex compounds hydrophobic with proteins, inactivating adhesin, enzymes, and cell wall transport proteins that interfere with the growth of microorganisms [30]. If the growth of bacteria or microorganisms is disrupted, the overhaul of protein by bacteria will be less than optimal, so the pH of acidic fish meat can be maintained longer because of the lack of simple metabolites of protein (amino acids).

A neutral to alkaline pH is a good condition for the growth of spoilage bacteria. The degree of acidity (pH) is related to the number of bacteria that grow because as bacterial growth increases, it causes flesh overhaul by bacterial activity which then produces alkaline compounds [5]. The pH value that has the best characteristics is the 3% treatment because it has a stable increase and decrease (fluctuation) pH value and at the storage, the limit has a pH value that is not too alkaline that is equal to 7.3.

The decrease in quality is caused by the activity of microbes and enzymes that overhaul complex compounds found in the mullet fillet (carbohydrates, proteins, fats) into simple compounds that are alkaline and cause a foul odor. One of the simple compounds is ammonia, ammonia compounds produced by the activity of spoilage bacteria will produce alkaline compounds so that the pH of fish meat increases [5]. The increase in pH value continued until the last day of observation, despite fluctuations. The pH value is still in the same main number. This is because enzymes derived from fish meat and microbes make changes to proteins and fats to produce basic compounds [31].

The high protein content of fish meat will trigger damage quickly, causing a foul odor, color, taste, texture that changes. The compounds produced by bacteria will cause a bitter taste, foul odor, mucus that was originally bright to blurry [31]. Bacteria contained in food can simultaneously produce compounds that are acidic and basic. Thus, the pH value is strongly influenced by dominant compounds [37].

Mullet fillet treated with soaking in basil leaf extract solution was still accepted until the 8th and 12th day, while the mullet fillet without treatment was received until the 6th day. The mullet fillet 4.5% treatment has a higher degree of acidity (pH) (6.75) compared to the 3% treatment fillet, only (6.70). This shows that the addition of the concentration of basil leaf extract solution as an antibacterial up to one point will inhibit bacterial growth but further addition will be the opposite because the organic material contained in the mullet fillet and the basil leaf extract solution is used by bacteria to grow resulting in an overhaul result more alkaline.

This is in line with [13] that the addition of extract concentrations as an antibacterial to one point will inhibit bacterial growth but further additions will be the opposite, also by [26] which shows that the immersion treatment of manga leaf extract with a concentration of 30% provides the longest shelf life compared to the concentrations of 10%, 20%, and 40%. Based on the pH value at a concentration of 4.5%, which is (7.45), this high pH value is due to the excess organic matter content of basil leaves (vegetable protein) so that bacteria are used as an energy source for growth, hence the amount of microbes increases as a result of protein which also decomposes more and more, the lottery causes the compounds to be alkaline out or the pH value will be very alkaline and cause a foul odor. So the effectiveness of basil leaf extract solution will
increase with the addition of concentrations above 3%. Based on observations it can be concluded that the pH of the mullet control and the mullet treatment of 1.5%, 3%, and 4.5% continues to increase with a slight fluctuation until the end of storage, but the increase in pH of the mullet fillet with fairly stable treatment with a close-range compared to the untreated fillet mullet which is quite far in the range.

3.1.3 Weight loss

Weight loss is one of the factors that indicate physical changes in fish meat. This physical change is caused by microorganisms that grow in food [5]. The results of observations of the weight loss of the mullet fillet during low-temperature storage are presented in Fig. 2.

The decrease in weight in the fillet is generally caused by the loss of water content in the fish fillet itself during the cooling process. Great weight loss during cooling can be caused by the moisture present in the material leaving the surface of the material and heading into the surrounding air through the process of condensation of water vapor [14]. Besides, weight loss occurs because of the transpiration process, where weight loss is greater at high temperatures.

![Fig. 2. Weight loss during low-temperature storage](image-url)

**Table 3. Weight loss during low-temperature storage**

<table>
<thead>
<tr>
<th>Days</th>
<th>0 (Control)</th>
<th>1.5</th>
<th>3</th>
<th>4.5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fillet Weight</td>
<td>WL (%)</td>
<td>Fillet Weight</td>
<td>WL (%)</td>
</tr>
<tr>
<td></td>
<td>Wo</td>
<td>W'</td>
<td></td>
<td>Wo</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>13</td>
<td>11</td>
<td>18.18</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>13</td>
<td>15.38</td>
<td>9</td>
</tr>
<tr>
<td>7</td>
<td>13</td>
<td>11</td>
<td>18.18</td>
<td>9</td>
</tr>
<tr>
<td>8</td>
<td>15</td>
<td>14</td>
<td>7.14</td>
<td>9</td>
</tr>
<tr>
<td>9</td>
<td>8</td>
<td>7</td>
<td>14.29</td>
<td>10</td>
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<tr>
<td>10</td>
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<td>12</td>
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<tr>
<td>12</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>13</td>
</tr>
</tbody>
</table>

*Notes: Wo = Initial fillet weight; SB (%) = Weight loss; Wt = Fillet weight on measurement day; (-) = Measurement was not done*
Low-temperature storage can reduce the speed of respiration and transpiration [8]. Water that disappears in the transpiration process causes the material to lose weight. Water lost or evaporated from the material depending on the temperature and humidity of the environment [38]. Weight loss during fillet storage at low temperatures occurs due to denaturation and autolysis processes. The denaturation process can occur due to heating or decreasing pH [39]. After undergoing denaturation, the protein which was originally elastic will turn into hard, compact, and less elastic. Thus, the protein in the mullet fillet is no longer able to maintain the liquid it contains so that it drips as a drip.

Based on the figure shows that the weight loss in the mullet fillet during low-temperature storage has increased and decreased (fluctuating). The pattern of increasing and decreasing continued to occur in all treatments, the highest weight loss results on the 7th day was found in the untreated mullet fillet (0%) of 18.88 compared to the fillet with successive treatments of only 11.11, 11.11, and 8.33. This result is due to the number of bacteria found in the mullet fillet with less treatment so that the reshuffle caused by bacteria and the number of drips that come out is also less. The percentage of small weight loss shows that protein still can bind water so that free water in meat does not come out. The lowest weight loss results on the 10th day were found in the fillet of mullet treatment of 3% (10.00), whereas in the treatment of 4.5% the weight loss was (25.00).

The increasing weight loss during storage occurs because of the process of protein damage that causes the release of water bonds in fish. Damage to proteins by enzymes from the fish's body and by bacteria will cause a reduction in the strength of the constituent meat is holding water [9]. The overhaul of protein by enzymes derived from mullet fish into simpler components will cause the protein function as a binding of body fluids to decrease [40] and the fluid will come out of the tissue [5] resulting in weight loss. Then the increase in weight loss will cause an increase in spoilage bacterial populations.

Temperature affects weight loss, with the use of low temperatures, bacterial growth can be inhibited and the activity of protein remodeling by bacteria will take longer so that it results in lower weight loss [9]. When storage at low temperatures, antimicrobial substances contained in the fillet soaked with a basil leaf extract solution can inhibit bacterial growth, causing an overhaul caused by bacteria less than the control mullet fillet, which has the potential for more bacteria to grow. Along with the increasing number of spoilage bacteria, it will cause an increase in weight loss [15]. Bacteria in fish meat will remodel the connective tissue (protein) that functions to hold water in the meat [32].

The use of immersion of mullet fillet using basil leaf extract solution causes weight loss but can keep the fillet from losing a lot of water. That is because the basil leaves contain antimicrobial compounds such as tannins and flavonoids that play a role in inhibiting the growth of microbes by interfering with the formation of membranes or cell walls. Membrane or cell wall that is not formed or not fully formed can inhibit growth and even kill bacteria [41]. Tannin compounds that bind to proteins will produce insoluble compounds and cause protein in the meat to be overhauled by bacteria or enzymes [9] because the difficulty of protein which is overhauled by bacteria causes the strength of water-binding capacity to increase and causes weight loss to be lower.

The percentage of weight loss is in line with the number of bacteria found in the mullet fillet ie the longer the shelf life, the weight loss will continue to increase and the number of bacteria will increase. The weight loss results in this study have increased and decreased by 0.0%-50.00%. The weight loss percentage for all treatments based on the acceptance deadline is in the range between 4.76%-50.00%. A relatively low percentage of weight loss indicates that there is still a lot of protein in fish meat that has not been broken down by enzymes and still can bind water so that free water in the flesh does not come out much.

Based on the results of the weight loss parameter, it is found that the percentage of weight loss has increased and decreased until the acceptance deadline, which is on the 11th day. The weight loss percentage of the 3% treatment fillet mullet at the acceptance deadline is 20.00. Depreciation of weights is in line with the decrease in the ability to bind water that occurs during storage. The amount of drip formed causes weight loss to increase. Changes in fish meat water content can be known based on changes in weight that occurs.
4. CONCLUSION

Basil leaves extract can be 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. A smaller amount of antimicrobial concentration is less than optimal so it is not enough to inhibit bacterial growth and bacterial activities, 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 fillet during low-temperature storage has the longest shelf life of 11 days with the pH value of 6.55 and weight loss value of 20.00%.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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