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The Effect of Addition of Basil Leaves Extract on the Quality of Nile Tilapia Filet at Low Temperature Storage (5 – 10^{o}C)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This research aims to determine the most effective concentration level of basil leaves extract in preserving nile tilapia filet at low temperatures (5 – 10^{o}C) to obtain optimal shelf life. This research was conducted at the Central Laboratory and Fishery Products Processing Laboratory, Faculty of Fisheries and Marine Sciences, Padjadjaran University started from June until October 2022. The research method used was an experimental method with four treatments by duplo. Fish filets were treated by soaking basil leaves extract with concentrations of 0%, 20%, 25%, and 30% for 30 minutes and stored at low temperature (5 – 10^{o}C). Observations were made on days 1st, 3rd, 5th, 7th, 8th, and 9th for nile tilapia filets with a concentration of 0% while treatment of basil leaves extract with concentrations of 20%, 25%, and 30% was carried out on days 1st, 3rd, 5th, 7th, 8th, 9th, 10th, 11th, and 12th. The parameters observed in this research included the calculation of the total number of

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bacteria (TPC) and degree of acidity (pH). The results showed that the use of basil leaves extract with a concentration of 25% was the most effective concentration because it was able to extend the shelf life until the 11th day with the number of bacteria 4.4 x 10^5 cfu/g and pH value of 6.65.

Keywords: Basil leaves; low temperature; nile tilapia filet; shelf life.

1. INTRODUCTION

Tilapia (*Oreochromis niloticus*) is a commodity that is in great demand by consumers both in the local and export markets [1]. Tilapia in Indonesia is marketed in the form of whole fresh fish and filets. Whole fresh tilapia can be found in both traditional and modern markets, while tilapia filets are generally sold in modern markets. Tilapia in filet form is in great demand because it has many advantages, including being more practical, free of spines and bones, streamlining the production process and improving the quality of processed products [2]. Fish filets also have the disadvantage of not being able to maintain their freshness for a long time because their natural defenses have been damaged in the process of making filets [3]. Changes in freshness quality can take place enzymatically, chemically, and bacteriologically which are influenced by temperature conditions, where the higher the temperature, the faster the freshness quality decreases [4].

One of the efforts to prevent a decrease in the quality of fish filets is to store them at low temperatures and use preservatives. Storage at low temperatures is one of the simplest ways to extend the shelf life of tilapia filets [5]. In addition to storage at low temperatures, giving preservatives is another practical option. Preservation using chemicals should be avoided because it can be bad for health. A food additive is needed as a natural preservative that can extend the shelf life of fish filets by using basil leaf extract. Basil leaves have many benefits, including as medicine, vegetable pesticides, essential oil producers, and vegetables [6]. In addition to these benefits, basil leaves are also easy to obtain and the price is relatively cheap. According to Deviyanti et al. [7] Flavonoid compounds, saponins and tannins in basil leaves have antimicrobial activity that can inhibit bacterial growth.

The concentration of basil leaf extract for fish to be preserved must be considered. Excessive use of basil leaf extract can change the smell, texture and taste of fish. If the level of use of basil leaf extract is less than what it should be, it will have an ineffective impact on maintaining fish quality. Therefore, it is necessary to conduct research on the effect of adding basil leaf extract (*Ocimum sanctum* L.) on the quality of tilapia (*Oreochromis niloticus*) filets at low temperature storage (5 – 10^{o}C).

The purpose of this research was to determine the most effective concentration level of basil leaf extract in preserving tilapia filet at low temperatures (5 – 10^{o}C) to obtain optimal shelf life.

2. MATERIALS AND METHODS

2.1 Time and Place

This research was carried out in July-October 2022 at Central Laboratory and Fishery Product Processing Laboratory, Faculty of Fisheries and Marine Sciences, Padjadjaran University, Jatinangor, West Java, Indonesia.

2.2 Research Materials

The research materials used were live tilapia weighing ± 250-300 g per head of 18 individuals, basil leaves, 96% ethanol, distilled water, physiological 0.9% NaCl, nutrient agar (NA), and buffer solution.

2.3 Research Tools

The tools used in this research were gloves, scissors, blender, measuring cup, beaker glass, coolbox, styrofoam box, cutting board, filet knife, scales, perforated plastic container, plastic container, styrofoam plate, cling wrap, Erlenmeyer tube, tissue towel, perforated plastic, tweezers, petri dish, mortar, pestle, pipette, test tube, test tube rack, rotary evaporator, bunsen, autoclave, incubator, vortex, spatula, tally counter, ph meter, stationery.

2.4 Research Methods and Procedures

The method used in this research is an experimental method with four duplicate treatments. The treatment given was soaking the

nile tilapia filet in a basil leaf extract solution at a concentration of 0% (without soaking the basil leaf extract), 20%, 25%, and 30%. The parameter in this study are the number of microbes measured by the total plate count (TPC) method and degree of acidity (pH).

2.4.1 Basil leaves extraction procedures

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 at a temperature of 52^{o}C and a speed of 45 rpm 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.

After obtaining the initial solution of basil leaves (*Ocimum sanctum* L.), then it was diluted with distilled water to obtain basil leaf extract with concentrations of 0%, 20%, 25% and 30% using the following formula:

$$V_1.M_1 = V_2.M_2$$

Information:

V_1 : Initial extract volume taken
V_2 : Extract volume to be made
M_1 : Initial extract concentration
M_2 : Concentration of the extract to be made

2.4.2 Fileting

Fresh fish were bought from traditional market 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. Nile Tilapia Filet

2.4.3 Applications of basil leaves extracts to filet

Fish filets were soaked in basil leaves extract for 30 minutes according to the concentration of the tested treatment. Soaking was done to determine the effectiveness of adding basil leaves extract to the shelf life of fish filets. 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.

2.5 Parameters Observation

The parameters observed in this research include the calculation of the total number of bacteria (TPC) and the degree of acidity (pH). The total number of bacteria was calculated using the TPC (Total Plate Count) test and the degree of acidity or pH was measured using a pH meter. Observation of test parameters on filets with immersion treatment of basil leaf extract was carried out on storage days 1st, 3rd, 5th, 7th, 8th, 9th, 10th, 11th, and 12th. Filets without immersion in basil leaf extract were observed on day 1st, 3rd, 5th, 7th, 8th, and 9th.

2.5.1 Total Plate Count (TPC)

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 5 x 10^5 cfu/g [8]. 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 [9]:

$$Colonies\ per\ ml = number\ of\ colonies\ per\ cup\ x\ \frac{1}{\text{dilution factor}}$$

2.5.2 Degree of acidity (pH) test

The degree of acidity is one of the chemical factors to determine changes in fish quality. Measurement of the degree of acidity (pH) was carried out to determine changes in pH in tilapia filets due to the activity of enzymes and bacteria during storage. The measurement of the pH value of the filet was determined based on the homogenate in duplicate using a pH meter. The homogenate to be tested was taken from 1 g of tilapia filet which was crushed until smooth, then put into a test tube containing 9 ml of distilled water, shaken until homogeneous. Homogenate was measured with a pH meter that had previously been calibrated with standard buffers of pH 4 and pH 7 [10].

2.6 Data Analysis

The results of observations of the number of bacteria and degree of acidity (pH) were analyzed descriptively and presented in tables and curves based on the number of bacteria counted and degree of acidity during low temperature storage. 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 10^5 cfu/g) [8]. pH measurements were analyzed based on the average pH of fish to see the decrease and increase in fish pH during low temperature storage and related to the results of calculating the total microbial colonies. 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 The Number of Bacteria

The number of bacteria in food is one of the indicators that determine the safety level of food to be consumed [11]. The number of bacteria contained in a food ingredient as much as 5 x 10^5 cfu/g is still considered safe for food material to be consumed [8]. The number 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 research results, the total number of bacteria from tilapia filets on day 1 in each treatment ranged from 2 x 10^3 - 2.8 x 10^3 cfu/g. The highest initial number of bacteria was in the 0% treatment (not given the immersion treatment of basil leaf extract) with an initial number of bacteria 2.8 x 10^3 cfu/g. The higher concentration of basil leaf extract used did not result in a smaller initial number of bacteria. This was proven at a concentration of 25% which had a smaller initial number of bacteria, namely 2 x 10^3 cfu/g compared to a concentration of 30%, namely 2.7 x 10^3 cfu/g. This is in accordance with Shofiani's research [12], which stated that the initial number of bacteria with a concentration of 1.5% basil leaf extract in mullet filet was smaller, namely 1.09 x 10^3 cfu/g compared to concentrations of 3% and 4.5% which had the initial number of bacteria is 2.75 x 10^3 cfu/g and 8.3 x 10^3 cfu/g. The initial large number of bacteria at the highest concentration of basil leaf extract occurred because the bacteria were still in the adaptation phase after being exposed to basil leaf extract containing antibacterial substances. The adaptation or response of bacteria to this pressure is the ability of bacterial cells to fight situations when the bacterial population is briefly exposed to the physical and chemical environment at suboptimal growth rates [13].

Based on Table 1, it was found that the number of bacteria contained in tilapia filets increased with the length of storage time. The increase in the number of bacteria during the shelf life occurs due to the process of autolysis that takes place by enzymes present in the fish's body [4]. The increase in the number of bacteria is also due to environmental conditions that can affect their growth [14]. Autolysis cannot be stopped even at low temperatures [15]. Autolytic enzymes (proteinases) hydrolyze fish proteins to produce peptides and amino acids [13]. The autolysis process will always be followed by an increase in the number of bacteria because all the results of decomposition by enzymes during the autolysis process are suitable media for the growth of bacteria and other microorganisms [4].

Table 1. Total Amount of Tilapia Filet Bacteria by Soaking Basil Leaf Extract During Low Temperature Storage (5-10^{o}C)

Day of Storage	Number of Tilapia Filet Bacteria with Addition of Basil Leaf Extract (cfu/g)			
	0%	**20%**	**25%**	**30%**
1	2.8×10^3	2.4×10^3	2×10^3	2.7×10^3
3	2.4×10^4	2.2×10^4	7.8×10^3	9.3×10^3
5	6.5×10^4	5.7×10^4	4.4×10^4	4.3×10^4
7	**2.4×10^5**	2.2×10^5	7×10^4	7.2×10^4
8	2.9×10^6	3.4×10^5	2.3×10^5	2.3×10^5
9	3×10^7	**5×10^5**	2.9×10^5	3×10^5
10	-	1.5×10^6	3.4×10^5	3.4×10^5
11	-	1.1×10^7	**4.4×10^5**	**4.8×10^5**
12	-	4.5×10^7	2.7×10^6	4×10^6

Note: (-) = No bacterial colony counts were performed

The shelf life of tilapia filets with immersion treatment of basil leaf extract (20%, 25%, 30%) was longer than that of filets without treatment (0%). The number of bacteria in tilapia filets without treatment tended to increase faster than in treated filets. The use of basil leaf extract is effective in inhibiting the growth of bacteria in tilapia filets. This is because filets with basil leaf extract soaking treatment contain antimicrobial substances (flavonoids, tannins, essential oils, saponins, triterpenoids, and alkaloids) which are bacteriostatic (inhibits bacterial growth) and bacteriocidal (kills bacteria) [6].

The mechanism of action of flavonoids is by damaging the bacterial cell membrane in the phospholipid section thereby reducing permeability which results in bacterial damage [16]. The mechanism of action of tannins as an antibacterial is to form complex compounds with proteins through hydrogen bonds, if hydrogen bonds are formed between tannins and proteins, the proteins will be denatured so that bacterial metabolism becomes disrupted [17]. Essential oils have the main content of linalool which has the potential as an antibacterial and belongs to a class of derivatives of phenolic compounds that work to damage cell membranes [18]. Large molecular phenolic compounds are able to inactivate essential enzymes in bacterial cells even at low concentrations. Saponins are antimicrobial compounds because they have the ability to cause leakage of certain proteins and enzymes from cells [19].

The results showed that tilapia filet without treatment only had a shelf life of 7 days, while tilapia filet in the immersion treatment with basil leaf extract had a longer shelf life (9-11 days). Based on administration of several concentrations of basil leaf extract, a concentration of 20% was able to maintain tilapia filet until the 9th day with the number of bacteria 5×10^5 cfu/g. Concentrations of 25% and 30% were able to maintain acceptance limits until the 11th day with the number of bacteria 4.4×10^5 cfu/g and 4.8×10^5 cfu/g. The difference in the number of days at the acceptance limit of the 20% concentration could be due to too little antimicrobial compound making it less effective in inhibiting bacterial growth. Concentrations of 25% and 30% reach the limit of acceptance on the same day. The most effective concentration is 25% because it has more optimal efficiency and function in inhibiting microbial growth. The concentration of 30% is not effective because an increase in the concentration of the extract does not always have a stronger inhibiting effect on bacterial growth. According to Ganiswarna [20], an increase in the concentration of a substance 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.

3.2 Degree of Acidity (pH) Test

Observation of the value of the degree of acidity (pH) is carried out to determine the level of acidity in a product. The value of the degree of acidity (pH) can be used as an indicator in determining the level of freshness of fish because it affects the length of storage. The results of observing the pH value of tilapia filets during low temperature storage are presented in Table 2.

Table 2. Average pH Value of Tilapia Filet Based on Soaking Treatment of Basil Leaf Extract during Low Temperature Storage (5-10^{o}C)

Day of Storage	Average pH Value of Tilapia Filet with Addition of Basil Leaf Extract			
	0%	20%	25%	30%
1	6.55	6.4	6.5	6.55
3	6.35	6.25	6.2	6
5	6.6	6.4	6.25	6.45
7	6.7	6.6	6.35	6.5
8	6.9	6.65	6.4	6.6
9	7.05	6.7	6.5	6.65
10	-	6.8	6.55	6.7
11	-	6.85	6.65	6.75
12	-	7.2	6.8	7.15

Note: (-) = No calculations done

Based on research results, the pH value of tilapia filets tends to decrease at the beginning of storage and then increases fluctuatingly as the shelf life increases. The decrease in the pH value of tilapia filet is because after the fish dies, the blood circulation will stop and the oxygen supply will decrease [21]. The pH value of tilapia filet in each treatment was relatively different, ranging from 6-7.2. The pH value is in the optimum pH range for the growth of spoilage bacteria, namely 6.5-7.5 [12]. The maximum pH value for fresh fish filets is 6.8 [12].

Based on Table 2. it was found that the average pH value of tilapia filet on the 3rd day decreased. According to Afrianto et al. [22], chemical changes in fish meat begin with a decrease in pH that occurs due to the activity of the glucokinase enzyme in the fish's body. This enzyme breaks down glycogen into lactic acid which plays a role in reducing the pH of fish meat. This is in line with the research of Saputra and Tati [23], which stated that the decrease in pH in tilapia filets occurs as a result of the glycolysis process which converts glycogen in the fish's body into lactic acid.

The change from glycogen to lactic acid will affect the pH of the fish so that it directly affects the length of storage. Over time the pH will rise again. This is due to protein and its derivatives being properly decomposed by microbes and enzymatic reactions into alkaline derivatives resulting in an increase in pH [21]. Protein decomposition will produce basic compounds such as ammonia, histamine, tyramine and others [24]. An increase in the pH value of fish meat indicates the activity of proteolytic enzymes that produce ammonia resulting in a change in the degree of acidity (pH) in tilapia filets during storage for 12 days of observation [25].

Based on research results, the pH value of tilapia filets experienced an increase in fluctuations from the 1st day to the last day of observation. This is because the microbes and enzymes contained in the fish meat reform the proteins and fats to produce alkaline compounds [24]. Compounds that are alkaline such as ammonia, trimethylamine, and other volatile compounds can be converted by bacteria into products that can be used as indicators of decay [26].

The pH value of tilapia filet without treatment increased faster than tilapia filet with the addition of basil leaf extract. This is because the basil leaf extract solution contains antimicrobial compounds that are able to inhibit bacterial growth in tilapia filets so that the addition of ammonia can take place more slowly. Tannin is one of the compounds contained in basil leaves that can affect the pH value of tilapia filet. According to Krisanti [27], tannins are phenolic compounds that react with proteins to form insoluble compounds, causing the protein in fish meat to be difficult to break down so that the results of the usually alkaline decomposition will take longer to produce. The mechanism of action of tannins is by making hydrophobic complexes with proteins, inactivating adhesins, enzymes, and cell wall transport proteins thereby disrupting the growth of microorganisms [28].

The acceptance limit for tilapia filet by soaking basil leaf extract at different concentrations based on the pH value, namely at a concentration of 0% has a shelf life of up to the 7th day with a pH value of (6.7). Concentration of 20%, has a shelf life of up to 9th day with a pH value (6.7). Concentrations of 25% and 30% have a shelf life of up to 11 days with pH values (6.65) and (6.75). Based on observations, a

concentration of 25% is the best concentration because it has a stable pH increase and decrease (fluctuation) value and at the storage limit has a pH value that is not too alkaline, namely (6.65).

Adding the concentration of basil leaf extract solution as an antibacterial up to one point will inhibit bacterial growth, adding further concentrations will have the opposite effect because the organic matter contained in the tilapia filet and the basil leaf extract solution are utilized by bacteria as a growing medium resulting in alkaline decomposition results more. This is in accordance with Widiani's research [10], which stated that adding an extract concentration as an antibacterial up to one point would inhibit bacterial growth, further increasing the concentration would be the opposite. Based on the pH value at a concentration of 30% which is equal to (6.75), the high pH value is caused by the excess organic matter content of basil leaves (vegetable protein) so that bacteria are used as a growth medium. The number of bacteria increases resulting in more and more decomposed protein which then causes the pH value to become more alkaline and gives rise to a foul odor. Based on the results of observations, the effectiveness of basil leaf extract will decrease with the addition of concentrations above 25%.

4. CONCLUSION

Based on the research results, it can be concluded that tilapia filet soaking in a solution of basil leaf extract at a concentration of 25% is the most effective and best concentration during low temperature storage (5-10^{o}C). Tilapia filet with a concentration of 25% was able to extend the shelf life up to the 11th day with a bacterial count of 4.4 x 10^{5} cfu/g and a pH value of 6.65.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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