

Study of Preservation Using Lactic Acid Bacteria from Fermentation of Cabbage (*Brassica oleracea* var. *Capitata*) to Shelf Life of Fresh Tilapia (*Oreochromis niloticus*)

Nesya Joza Amanda, Lukman Hidayat*, and Wuri Marsigit

Agricultural Technology Department, Agriculture Faculty-Bengkulu University

Jalan W.R Supratman, Kandang Limun, Bengkulu, Indonesia

*Corresponding Author : lukman_hidayat@unib.ac.id

ABSTRACT : This study aims to determine the best concentration of Lactic Acid Bacteria (LAB) solution from fermented cabbage to tilapia fish during storage. This study used a completely randomized design with the percentage of addition of LAB solution as a treatment (0%, 2%, 4%, 6%). The results of this study indicate that the LAB solution concentration has a significant effect on the physical, chemical, and microbiological quality of fresh fish where the higher the concentration, the higher the resulting value. The LAB solution concentration as the best preservative was found in the LAB solution with a concentration of 6%. The results of the physical properties on the observation of the eyes, gills, meat, and texture met the SNI requirements, namely 7 to the 12th observation hour at a concentration of 6%, but on mucus and odor at the 12th observation hour at a concentration of 2%, 4% and 6 % still fulfills the SNI requirements, namely 7. The chemical properties at pH until the 24th hour of storage were 6.33 and protein content 21.88%. The microbiological properties of tilapia by giving LAB 6% solution at the 12th hour observation still met the maximum limit of microorganism growth with a value of the number of colonies / plates 4.2×10^5 cfu / g.

Keywords: Fermented cabbage, fresh fish, LAB solution

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INTRODUCTION

Production of aquaculture, seeding and ornamental fish in Bengkulu over the past two years reached Rp 3,497,595,369,463. This type of fish production is dominated by tilapia production of 13,877,768 tons, followed by carp with production of 873,473 tons, while other commodities in the form of *gracillaria verrucosa*, gurame, catfish, mujair, catfish and *vaname* Shrimp (Bengkulu Today, 2020).

Tilapia also has a high nutritional content such as protein, calcium,

phosphorus and iron. The nutritional content of tilapia can be used to fulfill people's nutrition. The weakness of tilapia is that it is easily damaged compared to poultry and mammal meat. This is due to the high water content of 79,70%, pH (5.8-7), and fish meat nature which is easily digested by autolysis enzymes, thus causing delicate meat to serve as the best medium of growth for decaying bacteria that can cause physical, chemical and microbiological damage (Adawyah, 2007). This makes public interest in consuming

tilapia become reduced, so fresh tilapia need natural preservation, so that the fish remains durable when in transportation (export), until it arrives in the hands of consumers, the fish is still worthy to consumpt.

The use of preservatives aims to improve the appearance, taste, texture and prolong the shelf life, so that the shelf life of fish will be longer. Formalin in Indonesia itself is still used as a preservative in food, especially fresh fish. The use of such substances is a form of abuse of the function of formalin. Formalin is not allowed in food or beverages, because in the long run it can trigger the development of cancer cells. One alternative to overcome the problem of formalin use is the use of natural preservatives (Yusmidiarti *et al*, 2013).

One alternative in preventing the decline in the quality of tilapia is to use the addition of natural ingredients in the form of a solution of Lactic Acid Bacteria or commonly called LAB that can be a natural preservative for fish. LAB is a bacterium that produces bacteriosis that can slow the growth of bacteria (Savadogo, *et al*, 2006).

Cabbage (*Brassica oleracea*) is one of the plants that are widely grown in the highlands, with an abundant amount of production and low prices. Preservation with fermentation of cabbage leaves that produce lactic acid can extend the shelf life of tilapia. The results of previous studies were similar to 300 grams for 460 minutes or 7.40 hours, and there was a difference in the length of the shelf life of tilapia with soaking lactic acid fermented results (Yusmidiarti *et al*, 2013). The results of Edam's research (2018) found that LAB solution from cabbage fermentation is one of the natural preservative alternatives. The best variation of lactic acid fermentation process from cabbage is the length of fermentation of 8 days with the addition of

NaCl 3% and obtaining the highest lactic acid content of 2.6%.

MATERIALS AND METHODS

This research was conducted in July-August 2020 at the Laboratory of Bengkulu University. The tools to be used are jars, petri dishes, ose needles, shakers, digital pH meters, coloni counters. The materials used in this study were fresh tilapia, cabbage, Sodium Agar, Nacl, MRS Agar, MRS Broth, 30% sodium hydroxide, 0.1 N hydrochloric acid, red methyl indicator, 0.1 N sodium hydroxy solution, catalyst (selenium mixture), alcohol, concentrated sulphuric acid, litmus paper, axle, black plastic, Polypropylene plastic (PP), tissue, aluminum foil, cling wrap, cotton wool, and rubber bracelet.

The design used in this study was Completely Randomized Design (CRD) with a single factor. The treatment used is LAB solution-concentration from cabbage fermentation, namely tilapia without soaking LAB solution (N0), LAB solution 2% (N1), LAB solution 4% (N2), LAB solution 6% (N3). This study was conducted by observing the freshness level of tilapia using subjective (organoleptic), objective (pH, protein content, and TPC (Total Plate Count). Organoleptic tests, pH and TPC tests on fish were conducted at the 0th, 6th, 12th, 18th and 24th hour observations after 120 minutes of immersion. Protein levels, however, are only tested at the 0th and 24th hours of each treatment.

The manufacture of cabbage fermentation solution refers to the research of Yusmidiarti *et al*, 2013), weighed cabbage as much as 400 gr / sample, washed thoroughly after it is sliced with a size of ± 2 cm, then sterilized in a jars with alcohol by rubbing using tissue. Then, in a jar, add 1000 ml of clean water to the jar, after which add a saline solution with a

concentration of 3% (30 g of salt) into the jar, then stir well and then the jar is covered with black plastic and tied using a rubber band, last incubated for 8 days at room temperature.

RESEARCH AND DISCUSSION

Number of Lactic Acid Bacterial Cultures from cabbage fermentation

Lactic acid bacteria (LAB) is one type of bacteria capable of producing metabolite compounds as antibacterial. The main role of these bacteria is to preserve foodstuffs by producing lactic acid, acetic acid, ethanol, CO₂, and bacteriocins (Desmazeaud, 1996).

The cabbage fermentation solution on the 8th day used to make LAB culture has a pH of 3, which means it has acidic properties. While on the first day of manufacture the solution of fermentation of cabbage pH shows 6.9 which almost has neutral properties. Rising acid levels are not always accompanied by decreased pH because there are other ingredients that affect the pH, such as ammonia.

Table 1. Number of Lactic Acid Bacteria from cabbage fermentation solution

Dilution	Replication 1	Replication 2
10 ⁻¹	Can't Be Counted	Can't Be Counted
10 ⁻³	76 x 10 ³ cfu/ml	64 x 10 ³ cfu/ml
10 ⁻⁵	1	2

Based on LAB obtained from the fermentation of cabbage obtained LAB culture with 3 kinds of dilution, namely 10⁻¹, 10⁻³, and 10⁻⁵. Dilution of LAB culture is done with 2 repeats of dilution. LAB used in this study is LAB with dilution of 10⁻³, because in this dilution obtained a better LAB than LAB with dilution of 10⁻¹ and 10⁻⁵. LAB 10⁻¹ is a bacteria that can not be calculated because there are many bacteria, while LAB 10⁻⁵ obtained a very little LAB.

The amount of LAB from the fermentation of cabbage can be seen in Table 4 and the LAB image can be seen in Figure 1.



10⁻¹

10⁻³

10⁻⁵

Figure 1. Lactic Acid Bacteria with Some Dilution

Physical Properties

Physical properties are measured using consumer acceptance. Organoleptic properties are one of the parameters used to determine consumer acceptance of food products organoleptic testing carried out by method (ranking system) on 6 panelists from students, conducted at the observation of the 0th hour, 6, 12, 18, and 24 hours of observation after the storage of fish given LAB solution. Panelists are instruments or tools used to assess the

quality and sensory properties, parameters of organoleptic characteristics covering the eyes, gills-color, mucus, meat, smell-odor and texture.

Eye Appearance

The eyes are a key indicator of freshness seen by consumers when buying fresh fish. Fresh fish has the characteristics of a convex eyeball, bright and clear white, not bleeding with black pupils and rotten fish eyeballs in the form of concave and murky (Junianto, 2003).

Table 2. Organoleptic mode value of tilapia eye

Concentration LAB	Observation Hours				
	0	6	12	18	24
0%	9	7	6	3	1
2%	9	7	6	5	1 and 3
4%	9	7 and 8	6 and 7	3, 5 and 6	3
6%	9	8	7	5	3

The value of mode in organoleptic eye on Table 6 is at the observation of the 0th hour at all concentrations of 9. Observation of the 6th hour at concentrations of 0% and 2% is 7, while at concentrations of 4% are 7 and 8, and concentrations of 6% are 8. Then at the observation of the 12th hour at a concentration of 0% and 2% that is 6, while at a concentration of 4% that is 6 and 7, and at a concentration of 6% that is 7. Observation of the 18th hour at a concentration of 0% is 3, concentration 2% is 5, concentration 4% is 3, 5, and 6, concentration is 6% that is 5. After that at the observation of the 24th hour at a concentration of 0% that is 1, concentrations of 2% namely 1 and 3, concentrations of 4% and 6% that is 3.

Fish that rapidly experience a decline in organoleptic quality is fish at a concentration of 0% while slow quality changes occur in fish soaked with a

concentration of LAB solution of 6%. Fresh fish quality standard (SNI 01-2346-2013) shows that the decline of organoleptic value of the eye until the 12th hour at the concentration of LAB solution of 6% can still be tolerated because it is still in organoleptic condition of fresh products where the value is 7. This indicates that the use of LAB solution as a natural preservative of fish is very good to maintain the quality of fish in fresh condition to the hands of consumers. While at concentrations of 0%, 2% and 4% organoleptic values of the eyes have reached the limit of rejection and are not yet qualified ie rather concave eyeballs, cloudy corneas, gray pupils

Gill Appearance

The color of fish gills is one of the indicators to determine the quality of freshness of fish. Fresh fish has a bright red gill color, while fish that are not fresh have a dark brown gill color

Table 3. Organoleptic mode value of tilapia gills

Concentration LAB	Observation Hours				
	0	6	12	18	24
0%	8 and 9	7	5	3	1 and 3
2%	9	7	5	3 and 5	3
4%	8 and 9	7	5	5	3
6%	9	8	7	5	3

The mode values in the gill organoleptic in Table 7 observations at the 0th hour concentration 0% and 4% namely 8 and 9, Concentration 2% and 6% are 9. Observation of the 6th hour at Concentration 0% 2% and 4% is 7, while in Concentration 6% is 8. Then at the 12th hour observation at Concentration 0%, 2%, and 4% which is 5, while in Concentration 6% is 7. Observation of the 18th hour at Concentration 0% is 3, Concentration 2% is 3 and 5, Concentration 4% and 6% is 5. After that on the observation of the 24th hour at Concentration 0% namely 1 and 3, Concentration 2%, 4% and 6% namely 3.

The rate of decrease in the organoleptic value of the gills varies for each Concentration treatment of LAB solution used. This indicates the inhibition of the deterioration of organoleptic quality of fresh tilapia gills by LAB solution (0%) experience quality deterioration faster. According to Sevik (2007), gills are one of the internal organs that can accelerate the decay process. The gills are soft and moist so it becomes the ideal place for bacteria to grow. In the gills bacteria grow rapidly which causes odor changes and discoloration. The color-changing gills and slimy are indications of poor quality of fish. But due to the antibacterial properties of

LAB solution, quality deterioration in LAB solution treatment can be inhibited compared to without LAB solution treatment (0%).

Fresh fish quality standard (SNI 01-2346-2013) shows that the decline of gill organoleptic value until the 12th hour in LAB solution concentration 6% can still be tolerated because it is still in organoleptic condition of fresh products where the value is 7, this indicates that the use of LAB solution as a natural preservative of fish is very good to maintain the quality of fish in fresh condition to the consumer, while at Concentration 0% , 2%, and 4% of the gill organoleptic value has reached the limit of rejection and is not yet eligible i.e. the color of the gills gray or grayish-brown with a lumpy white mucus.

Appearance of Surface Mucus

Mucus in fish serves as an antifungal and anti-bacterial capable of protecting fish from infection, since its slippery properties also make it more difficult for parasites to stick to the skin of fish. Shiny fish scales of its kind, the surface of the body is not slimy or thin slimy with clear and diluted mucus, scales do not easily come off, the stomach is dense and intact

Table 4. Organoleptic mode value of tilapia mucus

Concentration LAB	Observation Hours				
	0	6	12	18	24
0%	8 and 9	7	6 and 7	3	1 and 3
2%	9	7	7	3	3
4%	9	8	7	3, 5, and 6	3
6%	9	8	7	6	3

The mode values in mucus organoleptics in Table 8 observations at the 0th hour at Concentration 0% are 8 and 9, Concentration 2%, 4%, and 6% are 9. Observation of the 6th hour at Concentration 0% and 2% is 7, while in Concentration 4% and 6% is 8. Then at the observation of the 12th hour at Concentration 0% namely 6 and 7, Concentration 2%, 4% and 6% namely 7. Observation of the 18th hour at Concentration 0% and 2% namely 3, Concentration 4% which is 3 5, and 6, Concentration 6% is 6. After that on the observation of the 24th hour at Concentration 0% namely 1 and 3, Concentration 2%, 4% and 6% namely 3.

According to Murniyati and Sunarman (2000), that in the process of decomposition of fish occurs hypermia stage that is fish mucus detached from the

glands in the skin, forming a thick clear layer around the body of the fish and is an excellent substrate for bacterial growth. Fresh fish quality standard (SNI 01-2346-2013) shows that fish mucus in all LAB solution concentration at the 12th hour storage still meets organoleptic value requirement of at least 7.

Meat

Fish that are still fresh have the appearance of bright meat, not dull, but this appearance will be reduced, the more gloomy fish color due to the onset of mucus as a result of further biochemical processes and the development of microbes. According to Adawyah (2007), one of the decaying bacterial activities is seen in fish meat. Changes in the color of fish meat start from around the spine due to high temperatures for a long time.

Table 5. Organoleptic mode value of tilapia meat

Concentration LAB	Observation Hours				
	0	6	12	18	24
0%	8	7	5 and 6	3	3
2%	8 and 9	8	6	5	3
4%	9	7	6	3 and 5	1, 3, and 5
6%	9	7 and 8	7	5	3

The value of mode in organoleptic meat in Table 9 observation of the 0th hour at Concentration 0% is 8, Concentration 2% is 8 and 9, Concentration 4% and 6% is 9. Observation of the 6th hour at

Concentration 0% and 4% is 7, while in Concentration 2% is 8, and Concentration 6% is 7 and 8. Then at the observation of the 12th hour at Concentration 0% namely 5 and 6, Concentration 2% and 4% namely

6, and 6% that is 7. Observation of the 18th hour at Concentration 0% is 3, Concentration 2% and 6% is 5, Concentration 4% is 3 and 5.. After that at the 24th hour observation at Concentration 0%, 2%, and 6% namely 3, Concentration 4% namely 1, 3, and 5.

The influence of LAB solution on tilapia is able to inhibit the deterioration of organoleptic quality of tilapia meat, where the organoleptic value of fish meat at Concentration 0% is easier to experience the decay process quickly. According to Naufalin (2005) antimicrobials are able to work as a good barrier between the environment and meat so as to minimize contamination. The difference is due to the difference in the amount of Concentration substances in the Concentration solution of LAB solution used, fish meat is said to be fresh only reached 12 hours storage at

Concentration 6%. Fresh fish quality standard (SNI 01-2346-2013) shows that 12 hours storage at Concentration 6% still meets organoleptic test requirement of at least 7. While Concentration 0%, 2% and 4% are not yet eligible, namely dull meat incisions, and less strong meat tissue.

Odor

Fishy smell of fish is one of the indicators to determine the quality of freshness of fish. Fishy smell is caused by reduced freshness of fish due to ammonia, trimethylamin, volatile fatty acids and oxidation results of fatty acids. Components of meat quickly rot, since the main components of fish meat come from different types of amino acids. Generally fish meat contains more unsaturated fatty acids so it is easier to be rancid.

Table 6. Organoleptic mode value of tilapia odor

Concentration LAB	Observation Hours				
	0	6	12	18	24
0%	8	8	6	3, 5 and 6	1
2%	8	7	7	6	1
4%	8 and 9	8	7	6	1
6%	9	8	7	6	1

The value of mode in organoleptic odor in Table 10 observation of the 0th hour at Concentration 0% and 2% is 8, Concentration 4% is 8 and 9, Concentration 6% is 9. Observation of the 6th hour at Concentration 0%, 4% and 6% is 8, while in Concentration 2% is 7. Then at the observation of the 12th hour at Concentration 0% namely 6, Concentration 2%, 4% and 6% that is 7. Observation of the 18th hour at Concentration 0% namely 3, 5, and 6, Concentration 2%, 4% and 6%

which is 6. After that at the observation of the 24th hour on all Concentration that is 1.

The rate of decrease in organoleptic odor value varies for each Concentration treatment of LAB solution used. The longer the storage time causes the organoleptic value of fish odor to decrease. The smell of fish treated with lactic acid gives the smell of acid from lactic acid and not the smell because the fish is decayed.

The influence of the use of LAB solution is able to inhibit the onset of odor in fish. Acidic substances contained in LAB

solution can prevent the formation of side compounds resulting from denaturation of proteins that cause fishy odor so that fishy smell can be disguised or can not be detected by the human sense of smell, this condition is seen in Concentration 0% which is more fishy than Concentration 6%. Based on Aprianti research (2011) acid can disguise the smell arising from fresh fish caused by reduced number of microbes.

The difference is caused by the difference in the number of Concentration substances in the Concentration LAB solution used. (explain the effect). Referring to (SNI 01-2346-2013) fresh fish shows that fish that are declared still fresh and still meet the organoleptic value of fish

at the 12th hour storage at Concentration 2%, 4%, 6% i.e. at least 7, while Concentration 0%, 2% and 4% are not yet eligible, namely strong fish acid odor.

Texture

The texture of fish meat is one part of the fish's body that can be used as a parameter of freshness of fish. The texture of fresh fish is still supple or stiff with a chewy, supple meat texture and when pressed quickly recovers. Organoleptic value of fish texture is seen higher in higher concentration of LAB solution, but organoleptic value at all levels concentration of LAB solution decreases along with length of storage.

Table 7. Organoleptic mode value of tilapia texture

Concentration LAB	Observation Hours				
	0	6	12	18	24
0%	8	7	6	3	1
2%	8 and 9	8	5 and 7	5	3
4%	8 and 9	7	5	3, 5, and 6	3
6%	9	8	7	5	1 and 5

The value of mode in organoleptic texture in Table 8 observation of the 0th hour at Concentration 0% is 8, Concentration 2% and 4% is 8 and 9, Concentration 6% is 9. Observation of the 6th hour at Concentration 0% and 4% is 7, while in Concentration 2% and 6% is 8. Then at the 12th hour observation at Concentration 0% which is 6, Concentration 2% is 5 and 7, Concentration 4% is 5 and Concentration 6% is 7. Observation of the 18th hour in Concentration 0% namely 3, Concentration 2% and 6% namely 5, Concentration 4% is 3, 5, and 6 and Concentration 6% is 5. After that at the 24th hour observation at Concentration 0% namely 1, Concentration

2% and 4% namely 3, and Concentration 6% which is 1 and 5.

The decrease in organoleptic value of texture in fish during storage is due to the activity of microorganisms that affect the state of fish texture. There is a change in texture that occurs softening of visible finger marks when pressed, easily tearing the meat from the spine.

Storage at low temperatures is known to slow down the process of quality deterioration and prolong the life of tissues in foodstuffs by inhibiting the activity of enzymes and decay bacteria. However, some decaying bacteria are able to survive at low temperature storage because this process inhibits growth not to kill or stop

microorganisms altogether. Therefore lactic acid bacteria are used as preservatives to improve the texture of fish (Dwihandita, 2009).

The rate of decrease in texture organoleptic value varies for each Concentration treatment of LAB solution used. This indicates the inhibition of organoleptic quality deterioration of fresh tilapia texture by LAB solution, where the addition of

LAB solution is able to maintain the quality of tilapia meat texture compared to treatment without LAB solution. The decay process that occurs in fish causes the texture of the fish is not compact and becomes soft.

This is due to the autolysis process by enzymes that cause changes in fish meat. The use of LAB solution can make the texture of fish become more compact and dense due to acid binding water from the body of the fish, but the texture changes slightly softer along with the

length of storage caused by the acid strength of the LAB solution is decreasing so that the connective power of the water decreases as a result of little by little water entering the fish meat so that the texture of the fish softens. Fresh fish quality standard (SNI 01-2346-2013) shows that the texture of fish observation at the 12th hour at Concentration 6% still meets the requirements of organoleptic test standards of at least 7, while Concentration 0%, 2% and 4% are not yet qualified, namely rather soft and less elastic.

Chemical Properties

Effect of LAB solution on Tilapia pH

pH is the degree of acidity used to express the acidity or wetness of a solution. The more acidic a solution is, the smaller the acidity or pH. Changes in the pH of fish meat is very large role because it affects the process of autolysis and bacterial attack. Tilapia pH test results can be seen in Figure 2.

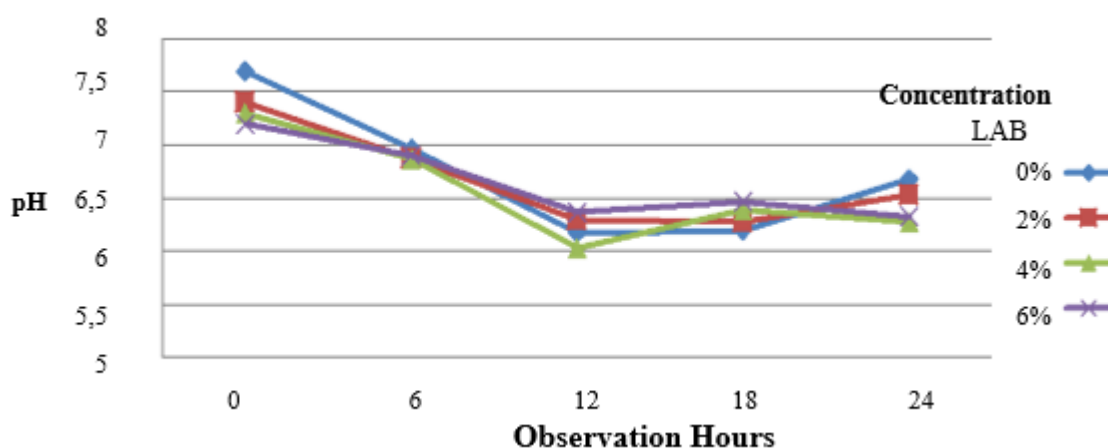


Figure 2. Graph of the effect of LAB solution on tilapia pH

Figure 2 shows the effect of observation time and concentration of solution on pH. During the 24 hours of storage time the pH value of the fish changes. The pH value of fish in the 0th to 12th Observation Hours continues to decline. At the observation clock to 0 pH the highest fish is 7.7 (LAB 0%) and the

lowest pH value is 7.2 (LAB 6%). Storage of the 6th hour pH value of fish then decreased with the lowest pH value found in the Concentration LAB solution of 2% and 4% of 6.87 and the highest value is in the Concentration of LAB solution of 0% which is 6.97. While in the storage of the 12th hour pH of fish with the highest value

is in the Concentration LAB solution of 0% which is 6.37 and the lowest value is in the Concentration of LAB solution of 4% which is 6.03. Storage of the 18th hour pH of fish with the highest pH value of 6.47 in concentration of LAB solution is 0% and the lowest value is in the Concentration of LAB solution of 6% which is 6.2. Storage of the 24th hour from the measurement of the

highest fish pH value of 6.68 with Concentration LAB solution 0% and the lowest pH value is in the Concentration of LAB solution of 4% which is 6.28.

LAB solution in this study can generally lower pH. This can be seen in Figure 2 which shows the pH value of tilapia that are not given LAB solution has a higher pH value over time.

Table 8. Average pH of Tilapia during storage and DMRT Test at 5%

Concentration LAB	Observation Hours				
	0	6	12	18	24
0%	7,7 ^a	6,97 ^a	6,17 ^a	6,2 ^a	6,68 ^c
2%	7,4 ^a	6,87 ^a	6,3 ^a	6,28 ^a	6,53 ^{bc}
4%	7,3 ^a	6,87 ^a	6,03 ^a	6,39 ^a	6,28 ^a
6%	7,2 ^a	6,9 ^a	6,37 ^a	6,47 ^a	6,33 ^{ab}

Note : The numbers followed by the same letters in the same column are not significant according to the 5% DMRT Test.

Based on Table 12, tilapia pH ANOVA test results show that all Concentrations in each Observation Hours have no real effect except in the 24th Observation Hours. In the observation of the 24th hour gave a real influence of LAB concentration with DMRT test results At the 24th hour, namely at Concentration 0% real different to Concentration 2% , 4% and 6% and vice versa, it is suspected in observation hours to 24 pH fish experienced.. Observations made on fish pH parameters showed that the administration of LAB solution in fish can lower the pH. Handling fish before storage can affect the stability of fish pH, with the addition of LAB solution as a natural preservative of fish can be used to maintain the stability of fish pH. The pH value in fish for all concentrates and Observation Hours meets the pH quality requirement, this is supported by Ilyas (1983) explaining the pH value for fresh

fish is about 5.8-7. The pH value that qualifies fresh fish is fish at the 24th hour observation on all concentration LAB solution.

Protein Content

Protein is an essential food substance for the body. This is because protein serves as fuel in the body, building and regulating the body (Winarno, 2002). Protein is one of the macronutrients has an important role in the formation of biomolecular. Proteins are formed by various amino acids, which contain elements of carbon (C), hydrogen (H), oxygen (O), through peptide bonds (Tejasari, 2005). Determination of cell size, the main component of water enzymes that is biolicalisator as a metabolic reaction in the body (Cakrawati and Mustika, 2012). The things that can cause denaturation in proteins are heat. pH, pressure, electrical flow, and the presence of chemicals

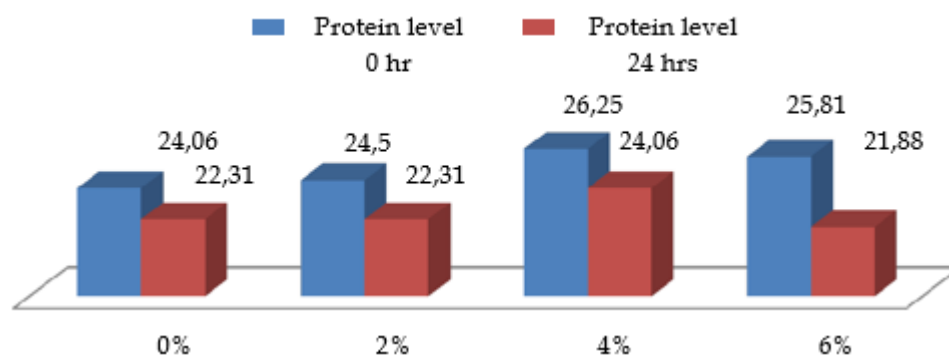


Figure 3. Graph of the effect of LAB solution on tilapia protein content

Figure 3 shows that protein levels in tilapia soaked in each difference concentration solution LAB 0%, 2%, and 4% increased at the 0th hour to the 24th hour, but Concentration 6% decreased from the 0th hour and 24th. This is because the higher concentration, the more acidic it is that causes damage to protein levels. The highest protein content at the 0th hour is at Concentration 4 % with a value of 26.25% and the lowest is at Concentration 0% with a value of 24.06%. Then at the 24th hour observation the highest protein content is Concentration 4% with a value of 24.06% and the lowest at Concentration 0% and 2% with a value of 22.31%. Based on the results of this study, protein levels in tilapia decreased in each Concentration LAB solution and the highest protein content was found in Concentration 4% both in the 0th Observation Hours and the 24th hour.

The occurrence of increased protein levels is caused by cabbage waste containing lactic acid bacteria that can inhibit the growth of other bacteria, by producing such proteins called bacterioine (Alfusia., *et al*, 2012 *in* Edam, 2018). One widely known example of bacterioine is nisin produced by *Lactobacillus lactis* sp. Lactic acid bacteria can also be said to be preservatives anti-microbial substances cause protein damage. The higher concentration of NaCl, the content of lactic acid produced is increasing, so that the protein in fish meat can be maintained

high. The ability of a preservative to inhibit the growth of microbes influenced by several factors, including concentration of preservatives, physical and chemical properties of food including water content, pH, type and number of compounds in it, environmental temperature and storage time (Fardiaz, 1989).

Microbiology Properties

Properties of microbiology measured using Total Plate Count (TPC) method. According to Alfusia, *et al* (2012) *in* Edam (2018), the occurrence of increased protein levels is caused by cabbage waste containing lactic acid bacteria that can inhibit the growth of other bacteria, by producing such proteins called bacterioine. One widely known example of bacterioine is nisin produced by *Lactobacillus lactis* sp. Lactic acid bacteria can also be said to be preservatives anti-microbial substances cause protein damage. The higher concentration of NaCl, the content of lactic acid produced is increasing, so that the protein in fish meat can be maintained high. The ability of a preservative to inhibit the growth of microbes influenced by several factors, including concentration of preservatives, physical and chemical properties of food including water content, pH, type and number of compounds in it, environmental temperature and storage time (Fardiaz, 1989)

Table 9. Total Plate Count (TPC) test results of fish during storage

No	Treatment	0	6	12	18	24
1	0%	8×10^4	$9,8 \times 10^4$	$6,1 \times 10^5$	2×10^6	$2,3 \times 10^6$
2	2%	$5,5 \times 10^4$	$9,9 \times 10^4$	$5,7 \times 10^5$	$1,7 \times 10^6$	$2,1 \times 10^6$
3	4%	$5,2 \times 10^4$	$9,4 \times 10^4$	$5,6 \times 10^5$	$1,5 \times 10^6$	$1,9 \times 10^6$
4	6%	$4,0 \times 10^4$	$8,5 \times 10^4$	$4,2 \times 10^5$	$1,3 \times 10^6$	$1,8 \times 10^6$

Table 9 shows that the highest initial number of bacteria is at Concentration 0% LAB solution which is 8×10^4 cfu/g. The higher concentration of LAB solution causes the number of bacteria to be smaller, it is caused by the difference in the number of Concentration substances in the Concentration LAB solution used. This is evidenced in concentration 6% LAB solution lower bacteria amount of 4.0×10^4 cfu / g compared to Concentration 2% and 4% LAB solution that is 5.5×10^4 cfu / g and 5.2×10^4 cfu / g. Observation of the 6th hour with dilution of 103 to dilution of 104 the highest number of bacteria is found in concentration 2% LAB solution is 9.9×10^4 cfu / g and the lowest number of bacteria is found in concentration 6% which is 8.5×10^4 cfu / g. Observation of the 12th hour with a dilution of 103 to dilution of 104 the highest number of bacteria is found in concentration 0% LAB solution is 6.1×10^5 cfu / g and the lowest number of bacteria is in concentration 6% which is 4.2×10^5 cfu / g. Observation of the 18th hour with a dilution of 103 to dilution of 104 the highest number of bacteria is found in concentration 0% LAB solution is 2×10^6 cfu / g and the lowest number of bacteria is found in concentration 6% which is 1.3×10^6 cfu / g. Observation of the 24th hour with a dilution of 103 to dilution of 104 the highest number of bacteria is found in concentration 0% LAB solution is 2.3×10^6 cfu / g and the lowest number of bacteria is found in concentration 6% that is 1.8×10^6 cfu / g.

Research on the influence of lactic acid concentration of cabbage fermentation results on total plate numbers (TPC) in fresh fish shows that the total number of plate numbers in fresh fish as a control has a high total number of plate numbers because without soaking lactic acid solution and storage at room temperature that can result in the growth of bacteria so fast growing that it results in damage and decay in fish. TPC in fresh fish that has been preserved using lactic acid resulting from fermentation of cabbage shows alt is getting lower. These natural preservatives usually have an acidic pH that ranges from pH 4.5-4.6, as well as lactic acid from the fermentation of cabbage. Organic acids that serve to inhibit the growth of bacteria in food.

Lactic acid produced can lower the pH and increase the acid so that it can serve as an ingredient to inhibit the growth of microorganisms. The addition of lactic acid bacteria can lower the pH of fish meat, can slow the growth of decaying bacteria so that the decomposition of proteins by decaying bacteria is inhibited.

Lactic acid bacteria can serve as food preservatives because it is capable of producing organic acids, lowering the pH of its environment and excreting compounds capable of inhibiting pathogenic microorganisms such as H₂O₂, diasetil, CO₂, acetaldehyde, d-isomers of amino acids and bactericin (SNI 01-2729-2006). Several generations that produce bacteroine are *Lactobacillus*, *Lactococcus*, *Streptococcus*, *Leuconostoc*, *Pediococcus*,

Biidobacterium and *Propionibacterium*. Lactic acid bacteria produce a protein called bacteroïne. One widely known example of bacteroïne is nisin, produced by *Lactobacillus lactis* ssp. Nisin can inhibit the growth of several bacteria, namely *Bacillus*, *Clostridium*, *Staphylococcus*, and *Listeria*. Bacterial compounds produced by lactic acid bacteria can be useful because it inhibits pathogenic bacteria that can damage food or harm human health, so food safety is more guaranteed

The growth of microorganisms in fish is influenced by intrinsic and extrinsic factors. Intrinsic factors include acidity (pH), water activity (aw), biological structure, and nutrient content in ingredients. Extrinsic factors include storage temperature, relative humidity, as well as the type and amount of gas in the environment (Arpah, 2001). During the storage time, fish are placed in a space of temperatures of 20-40°C which is the optimum temperature for the growth of microorganisms and the growth of microorganisms is also affected by the amount of gas in the air. Most kapang is mesophilic i.e. growing well at room temperature.

The values obtained showed a decrease in the value of TPC by increasing the Concentration of LAB solution used so as to suppress the increase in the total number of bacteria up to the 12th hour in the Concentration of LAB solution extract 6%. This indicates the inhibition of the increase in the value of fresh tilapia TPC by concentration of LAB solution, where the higher the Concentration LAB solution used is able to maintain the value of tilapia TPC compared to the treatment without concentration LAB solution (0%). This is because LAB solution has the ability as an antibacterial because in LAB solution there are active clusters that bind to microbes, so as to inhibit the growth of microbes.

Quality standards SNI Total Plate Number (TPC) fish 01-2332.3-2006 establish microbial contamination in fish has a maximum limit of microbial contamination that is ALT 5.0×10^5 colonies / g, *Escherichia Coli* <3 APM/g, *Salmonella* <3 APM/g, *Vibrio Cholera* Negative /25g, *Vibrio Parahaemolyticus* <3 APM/g. In accordance with the standard SNI Total Plate Number (ALT) tilapia with the provision of LAB solution 6% at the observation of the 12th hour still meets the maximum limit of growth of microorganisms with the value of the number of colonies / cup 4.2×10^5 cfu / g. While in Concentration 0%, 2% and 4% have not met the standard limit that has been set because bacteria are already greater than 5.0×10^5 cfu/g.

CONCLUSIONS

The use of LAB solution as a preservative of physical properties (eyes, gills, meat, mucus, smell and texture of tilapia) in the observation of eyes, gills, meat, and texture qualifies SNI which is 7 to 12th Observation Hours at Concentration 6%, but on mucus and odor in the 12th Observation Hours at Concentration 2%, 4% and 6% are still eligible for SNI of 7, and for the chemical properties of tilapia pH shows that, fish observation at the 24th hour is of a real effect, but the protein content in tilapia soaked in each difference Concentration LAB solution 0%, 2%, and 4% increased at the 0th hour to the 24th hour, but concentration 6% decreased from the 0th and 24th hour. This is because the higher concentration, the more acidic it is that causes damage to protein levels. The use of LAB solution as a preservative affects the microbiological properties of tilapia during storage. The values obtained showed a decrease in the value of TPC by increasing the Concentration of LAB solution used so

as to reduce the increase in the total number of bacteria up to the 12th hour in the Concentration of LAB solution by 6%. Concentration of LAB solution as preservative is best found in LAB solution with Concentration 6%. The results of physical properties in the observation of eyes, gills, meat, and texture meet sni requirements, namely 7 to the 12th Observation Hours at Concentration 6%, but in mucus and odor in the 12th Observation Hours at Concentration 2%, 4% and 6% still eligible SNI of 7. Chemical properties at the pH until the 24th hour of storage obtained 6.33 and protein levels of 21.88%. Microbiological properties of tilapia with the provision of LAB solution 6% at the 12th hour observation still meets the maximum limit of growth of microorganisms with the value of the number of colonies / cup 4.2×10^5 cfu / g.

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