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The Effect of Garlic Extract Addition on Tilapia Skin Gelatin Based Edible Coating Towards Antimicrobial Properties and Fish Meatball's Shelf Life

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ABSTRACT

The purpose of this research is to determine the best concentration and effect of garlic extract incorporated on tilapia skin gelatin-based edible coating to preserve and extend the shelf life of fish meatball at cold storage. This research was conducted from May 2018 until October 2018 on Fisheries Product Processing Laboratory of The Faculty of Fisheries and Marine Sciences, Universitas Padjadjaran. The garlic extract conducted with maceration method with ethanol 96% was performed at Organic Chemical Laboratory FMIPA Universitas Padjadjaran, and for TPC test, inhibition test of edible coating Tilapia's fish-skin with garlic extract was conducted in Microbiology and Molecular Biotechnology Laboratory of Faculty of Fisheries and Marine Sciences Universitas Padjadjaran. The method used is experimental method with 5 treatments which is meatballs without edible coating. The addition of garlic extract was done with various concentration at 0%, 1%, 1.5%, 2%. The observations were Total Plate Count (TPC) test, inhibition zone test against *Pseudomonas aeruginosa*, and pH value. The results showed that the best treatment is 2% garlic extract with TPC value 1.1×10^5 cfu/g during 14 days of storage and concluded that it could extend the shelf life of meatball until 14 days, pH value was 7.03 and inhibition zone against *P. aeruginosa* was 7.01 mm. The addition of garlic extract on tilapia skin gelatin-based edible coating could extend the shelf life of fish meatballs until 14 days on cold storage.

Keywords: edible coating, extract, garlic, pH values, shelf life, tilapia gelatin, TPC, *Pseudomonas aeruginosa*, *Staphyllococcus aureus*, *Salmonella typhimurium*, *E. coli*

1. INTRODUCTION

The fishery product processing industries are growing from every year. Data export of fisheries products in 2015 compiled by the Ministry of Maritime Affairs and Fisheries (KKP) showed the number 974,55 thousand tons. This situation caused many processing industries in the fisheries sector. One of the processing industries engaged in fisheries is the tilapia filet industry. In 2010, tilapia production amounted to 464,191 tons, increasing in 2013 to 1,110,810 or almost tripling. In average the production of tilapia has increased significantly with the average rise 34.85% (KKP 2014). The increase in production is due to the increasing demand of tilapia in the domestic and export markets. The tilapia filet industry produces waste such as bone, head, skin, scales and leftover washing water. The waste is rarely utilized and wasted. The use of waste is just for making crackers from tilapia skin and feed ingredients.

Tilapia fish skin and bones can be utilized as gelatin. This potential can replace gelatin from cow or pork skin, where pig gelatin is banned by the majority of Muslims in Indonesia. Research on extraction of gelatin from fish skins and bones has been carried out as much as in salmon, sharks and gelatin from catfish skin (*Pangasius sutchi*) (Mahmoodani *et al.* 2014).

Gelatin is a derivative product from animal bones and skin's collagen hydrolisis. Gelatin was already used on every dairy products and non-dairy products. Gelatin on dairy products had been used as stabilizer, binder, thickener, emulsifier, gelling agents, and edible food packaging material (edible coating).

Edible coating is an edible thin layer, formed with dipping, spraying technique on food surface which could protect the food and increase the additive value. The other purpose of edible coating is to regulate humidity, gases and fats transfer from product, to be additive substance and nutrient (Campos *et al.* 2011). Edible coating which is added with antimicrobial can protect from microbiological damage and extend product shelf life.

The ability of edible coating to protect food can be applied on meatball products, because meatballs only last short time at room temperature. Metballs sold in traditional markets tend to be placed at room temperature with a less hygienic environment. This causes the meatballs fall into short shelf life due to microbiological damage.

Edible coatings added with natural antimicrobial compounds also had function as the product preservatives. The preservative commonly added to meatballs is a chemical such as benzoate. Other preservatives, such as borax and formaldehyde, are harmful. There must be a natural preservative that is safe for health as a substitute for artificial preservatives in fish meatballs. One of the natural preservative that is often used is garlic extract. Garlic (*Allium sativum* L.) is a natural antimicrobial material that has been widely applied in various food products. Garlic contains alicin compounds that are bactericidal and can inhibit the growth of bacteria such as *E. coli* and *Staphyllococcus aureus*. The combination of garlic oil and chitosan on edible films can improve the antimicrobial ability of chitosan films.

Edible coatings/films that are applied to food products are usually added natural bioactive ingredients to extend shelf life and inhibit microbial activity of product. The natural ingredients are as follows: garlic extract/oil, peppermint oil, citronella (Yanwong and Threepopnatkul 2015), oregano oil, lavender (Martucci *et al.* 2015). Garlic extract can inhibit bacterial growth in fish meatballs such as *E. coli, S. aureus* and *Salmonella typhimurium*.

Based on this explanation, it is necessary to study the effect of addition garlic extract on tilapia edible coating gelatin on antimicrobial properties and shelf life of fish meatballs.

2. MATERIALS AND METHODS

The research consists of several steps which are: making tilapia skin gelatin, making garlic extract, making fish meatballs, making edible coatings, applying edible coating on fish meatballs, and observe the meatballs. Tilapia skin gelatin was characterized by determined viscosity and pH value. Edible coating was analyzed concerning the inhibition zone against *Pseudomonas aeruginosa*. The meatballs on storage were observed by Total Plate Count Analysis and pH value.

2. 1. Making of Tilapia Fish Skin Gelatin

Tilapia skin gelatin was made by using an acid method based on Trilaksani *et al.* (2012) with 3% of acetic acid. The stages of making gelatin include degreasing, washing and boiling, cutting the skin, demineralizing, neutralizing pH, extracting, filtering, drying the filtrate, and grinding the gelatin.

2. 2. Making of Garlic Extract

Garlic extract was made by maceration method based on Karina (2013). The steps are peeling and washing of the garlic, drying, soaking in 96% ethanol, filtering and evaporating the filtrate to become garlic extract.

2. 3. Making Edible Coating with Garlic Extract

Edible coating was made from tilapia skin gelatin with the addition of garlic extract. Edible coating was made from tilapia skin gelatin with the addition of garlic extract. The manufacturing process includes dissolving plasticizers, adding gelatin, adding garlic extract, and adding distilled water to an edible coating solution. Gelatin edible coating formulations with garlic extract are presented in Table 1. Preparation of edible coating of fish gelatin was based on Junianto *et al.* (2012) with modification.

Table 1. Edible Coating Formulation.

Materials	Concentration
Aquadest	600 ml
Sorbitol	5 % (b/v)
Gelatin	10 % (b/v)
Garlic Extract	1%; 1.5%; 2% (v/v)

2. 4. Making of Fish Meatballs

The meatballs was made from tilapia's meat. Procedures for making fish meatballs was based on SNI (2014). The formulation of ingredients for making tilapia meatballs is based as follows.

Table 2. Fish Meatballs Formulation.

Materials	Percentation
Tilapia Meat	A kg
Tapioca	10% A
Garlic	3% A
Pepper	0.03% A
Onion	2.5 % A
Salt	3% A
Sugar	0.02% A
Ice cubes	20% A

2. 5. Edible Coating Application on Products

The mixture of edible coating gelatin for tilapia and garlic extract was applied to fish meatballs based on the Hadi method (2008). Meatballs were dipped in edible gelatin solution with garlic extract for 60 seconds, then they were placed in styrofoam and wrapped in plastic wrap to cover the entire surface. Next they were placed at a low temperature (5-10 ºC), then 15 days physical observation and microbiology observation were conducted on day 1, 4, 7, 8, 9, 10, 11, 12, 13, 14, 15 and 16. The method used in this research is an experimental with 4 treatments with garlic extract concentration used as follows:

1) Treatment A: 0% without the addition of garlic extract
2) Treatment B: 1% concentration of garlic extract from solution
3) Treatment C: 1.5% concentration of garlic extract from solution
4) Treatment D: 2% concentration of garlic extract from solution.

2. 6. Characterization of Tilapia Fish Skin Gelatin

2. 6. 1. Yield of Gelatin

The gelatin that has been obtained is then calculated for the yield. Calculation of the yield of gelatin was done using the AOAC method (1995). Dry gelatin weight compared to the weight of fresh skin (skin that has been cleaned of leftover meat and fat layer):

$$\text{Yield} = \frac{dry\ gelatin\ weight}{fresh\ skin\ weight} \times 100\ \%$$

2. 6. 2. Viscosity of Gelatin

The viscosity test based on Ward & Courts (1977) was carried out as follows. Samples were prepared with concentration of 6.67% and diluted with distilled water, viscosity then

measured with a Brookfield Viscometer at a temperature of 60 ºC at 60 rpm using spindle number. The results were multiplied by conversion factors; for spindle 1 the conversion factor is 1. The value of viscosity is presented in units of centipoises (cP).

2. 7. Edible Coating's Antibacterial Test (Disc-diffusion Method)

The testing of the antibacterial activity of edible coating was carried out by the disc-diffusion method. The bacteria tested was *Pseudomonas aeruginosa* which is one of the fish spoilage bacteria and pathogens in humans. The inhibiton zone is calculated by the following formula.

Inhibition zone = diameter of clear zone − diameter of paper disc

2. 8. pH Measurement

pH testing on the product aims to determine the acidity of the meatballs produced. The pH value is measured using a pH meter based on Suzuki (1981).

2. 9. Total Plate Count Analysis

Analysis of Total Plate Count (TPC) was carried out to determine the number of microbes in fish meatballs coated with tilapia edible coating gelatin with garlic extract during low temperature storage. Total plate count test based on observation of microbial counts was carried out on the 1st, 4th, 7th, 8th, 9th, 10th, 11th, 12th, 13th, 14th, 15th and 16th storage days. The formula for calculating microbial populations is as follows:

$$\text{Colonies per gram} = \text{number of colonies per plate} \times \frac{1}{\text{dillution factor}}$$

3. RESULTS

3. 1. Yield of Tilapia Skin Gelatin

The yield of gelatin produced was 10.67%. The weight of the fish skin that processed is 225 grams and the resulting gelatin weight is 24 grams. These results are the research where the red snapper skin soaking in 3% acetic acid concentration resulted in yield between 10-16% depending on the length of soaking the skin. Using the skin of catfish with soaking acetic acid of pH 3 for 12 hours resulted in an yield of 9.63%.

There are several factors that influence the yield of fish skin gelatin during processing which are the percentage of acid used, skin immersion time, and skin extraction temperature. The longer the immersion time and the concentration of the acid, the lower yield produced; this is due to the occurrence of continued hydrolysis and some of gelatin was also degraded and decreased the yield.

The extraction temperature affected the production of gelatin, the temperature of 80 ºC was optimal temperature for gelatin extraction. If the temperature is too high, the protein in gelatin will be denatured.

3. 2. Viscosity of Tilapia Skin Gelatin

The viscosity value of the tilapia skin gelatin is 13.50 cP at 60 rpm. This value was not in accordance with the standard A-type gelatin according to GMIA (2012). The viscosity of gelatin is influenced by the concentration, temperature, pH, and acid used. The lower the temperature of the gelatin solution, the higher the concentration of gelatin and also higher the viscosity. High viscosity is thought to be due to the temperature which continues to decrease during measurement which affects the viscosity value. One may state that the fish gelatine can be distinguished from bovine and porcine gelatin based on its physical properties, which is high viscosity solution.

3. 3. pH Value of Tilapia Skin Gelatin

The pH value of tilapia skin gelatin produced was 5.2. This value is in accordance with GMIA standard (2012) for the category of A-type gelatin which ranges from 3.8 to 6.0. The pH value of gelatin from the skin of red snapper with immersion of 3% acetic acid for 18 hours is 5.45. The lower gelatin pH value can be caused by repeated washing with water after the immersion process has not been able to remove all the acids in the collagen tissue of fish skin so that there is residual acetic acid carried away during extraction.

3. 4. Amount of Microbes and Shelf Life

The amount of microbes in fish meatballs during the 16 day storage period is presented in Figure 1.
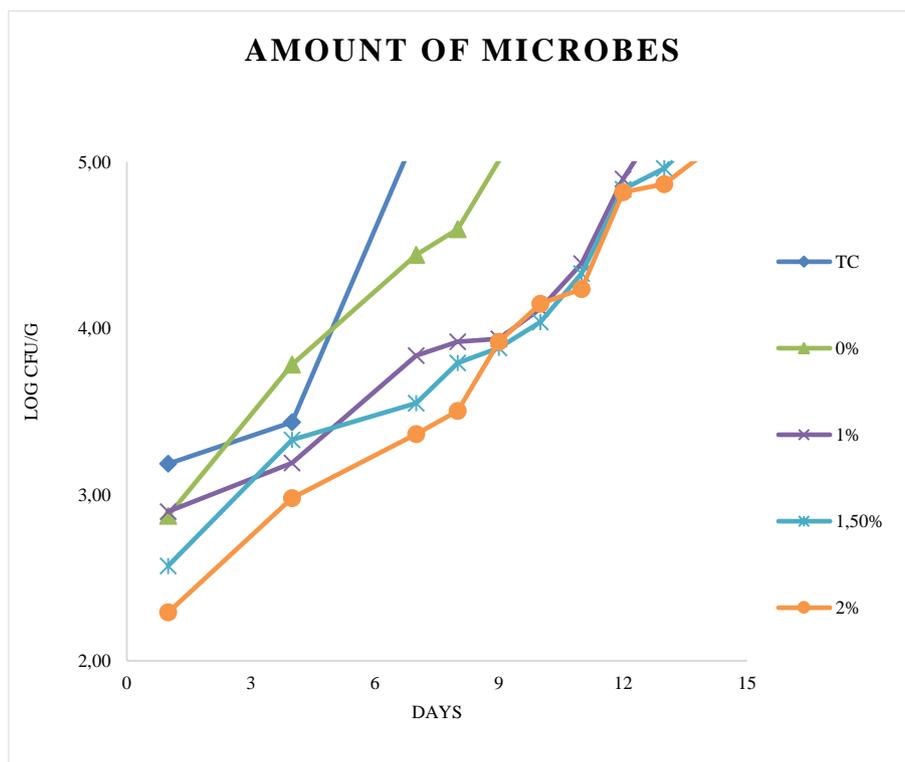

Figure 1. Graph of Amount of Microbes on Fish Meatballs during Storage

Based on Figure 1, the number of microbes in each treatment increased during storage period. On the first day of observation, the number of bacteria in each treatment ranged from 2.0×10^2 - 1.5×10^3 cfu/g. The treatment without edible coatings (TC) had the highest amount with 1.5×10^3 cfu/g. This shows that edible coating can protect products and inhibit microbial growth. Edible coating functions as a barrier to control water transfer, oxygen uptake, and lipid transfer. The number of bacteria in all treatments on the first day is still within safe limits SNI (2014) which is 1×10^5 cfu/g.

The number of bacteria on the 7th day tends to increase high. That increase occurred in the treatment without edible coating (TC) with 1.5×10^5 cfu/g. The number of bacteria has exceeded the SNI safe limit for the total plate count (TPC) of fish meatballs, which is 1×10^5 cfu/g. The number of bacteria in the treatment 0%, 1%, 1.5%, and 2% concentration of garlic extract is still below the SNI safe standard. Fish meatballs that are not coated with edible coating can only last for 7 days. The treatment using edible coating without extract (0%) shows a value of 4.0×10^4 cfu/g and still within the SNI safe limit. This value is higher than the treatment of edible coating garlic extract at 1%, 1.5%, and 2% concentration. This is because antimicrobial substances in garlic extract can inhibit microbial growth. Edible coating of tilapia fish can extend the shelf life of fish meatballs at low temperatures.

The increasing number of bacteria is caused by the transfer of water vapor from the environment which increases microbial activity. The intrinsic factor that causes the growth of microorganisms is the presence of barrier or inhibiting substances. The barrier that plays role here is the presence of edible coating which is able to inhibit bacterial growth in fish meatballs. Edible protein-based films such as gelatin have high permeability to water vapor. Edible films derived from proteins and polysaccharides are the best material in blocking the entry of oxygen. This is because both of these materials have large hydroxyl groups. This hydroxyl group makes strong polymeric bond interactions and limits the rate of oxygen transmission.

The number of bacteria on day 8, 9, 10, 11, until 15th day continues to increase. Edible coating treatment with 0% garlic extract on the 9th day had reached the safe limit of microbial contamination in meatballs with 1.5×10^5 cfu/g. Using edible carrageenan coating in meatball products allows to maintain a shelf life of up to 12 days at 5 ºC. Different result of the number of bacteria can be caused by the conditions of fish meat or poor sample preparation prior to storage such as bacterial contamination when meatballs will be packed.

The treatment of edible coating with 1% garlic extract can maintain the shelf life of meatballs until 13th day with a microbial amount of 1.9×10^5 cfu/g. The treatment of 1.5% and 2% garlic extract maintains the shelf life of meatballs until 14th day with the number of microbes each 1.6×10^5 cfu/g and 1.1×10^5 cfu/g. However, the treatment of 2% garlic extract produces fewer microbes. 1% garlic extract on carrageenan edible coating can extend the shelf life of fish meatballs at 5 ºC for 15 days with the number of microbes $1,2 \times 10^6$ cfu/g, according to SNI 1996. The addition of garlic extract to edible coating can extend the shelf life of fish meatballs at low temperatures because they are antimicrobial. Garlic extract contains many bioactive substances such as *allin, allicin*, disulfide, trisulfide, flavonoid and essential oils (Kemper 2000). Allicin can penetrate bacterial cells and destroy sulfurhidril groups in bacteria so the cell wall of bacteria undergoes lysis and its metabolism is inhibited. Generally, edible coating of gelatin with garlic extract can extend the shelf life of fish meatballs microbiologically. The higher the garlic extract used (2%), the longer the shelf life of fish meatballs. The effectiveness of antimicrobial work relates exponentially where the higher the antimicrobial concentration will provide good effectiveness.

3. 5. pH Values

Observation of pH values was carried out for 14 days with a pH meter. The pH value of fish meatballs during the observation is presented in Figure 2.
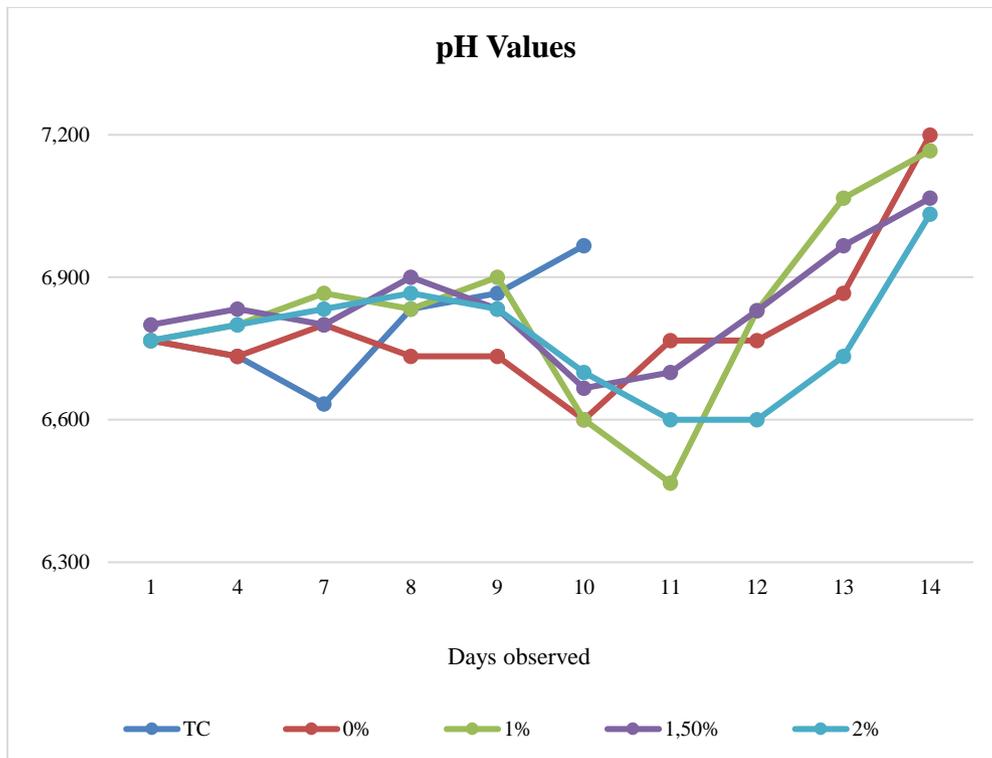

Figure 2. pH Values of Fish Meatballs during Storage

Based on the Figure 2, the pH value of fish meatballs decreases and rises according to the length of storage. The pH value at the beginning of storage ranges starts from 6.8 to 6.7 and decreases to 6.6 - 6.4. Decreasing pH values indicates anaerobic and aerobic microbial activity in fish meatballs. The decreased pH value in the treatment without edible coating (TC) was 6.63 and faster occurred on the 4th day compared to other treatments. The pH value in treatment 0% and 1.5% of garlic extract resulted in decrease, respectively 6.6 and 6.67 on the 10th day. Decreased pH value at treatment 1% and 2% are the lowest on day 11 with the value of 6.40, and 6.60, respectively. Meatball coated with edible coating can inhibit the decrease of pH in fish meatballs. Using carrageenan edible coating and garlic extract produced a pH value during the storage period at 5-15 ºC between 6.0 - 7.0.

The decrease in pH value is affected by an increase of acid in the product. Enzymes from microbes that metabolize compounds in fishery products will overhaul carbohydrates into lactic acid which decreases pH. Microbes on fish meatballs are coming from contamination with the external environment such as air, processing environment, and meatball storage area shortly before being coated with edible coating. Bacteria in fish meatballs break down carbohydrates into lactic acid and acetic acid. Bacterial genera such as *Bacillus* sp. and *Pseudomonas* sp. metabolize carbohydrates into lactic acid, acetate, formic acid, 2,3-butandiol, CO_2, and H_2.

Treatment without edible coating (TC) increased pH on the 9th day with value of 6.87 and continued to increase at the following day. Treatment of 0%, 1%, 1.5%, and 2% experienced a rise in pH each on the 11th, 12th, 11th, and 13th days with a value of 6.77; 6.83; 6.83; 6.73, respectively. The 2% garlic extract concentration is the best treatment that could inhibit the change of pH value. The final pH value is 7.03 on the 14th day. The increase in pH value in each treatment was caused by metabolism of proteolytic bacteria originating from outside / contamination with fish meatball products and metabolize single amino acids to produce keto acids, fatty acids, H_2, CO_2, NH_3, H_2S, amine, indole, putrescine, cadaverine, and histamine. This metabolic product is related to damage or decrease in food quality such as bad odor and cause health problems. The resulting ammonia is alkaline and increases the pH value and number of bacteria, especially decomposing bacteria because a high pH value is a suitable medium for them. Increased pH value is an indicator of spoilage in fish meatballs.

Edible coatings in this case could inhibit the changes of pH value. The pH value that changed earlier indicates higher microbial activity and spoiled faster, while edible coating tilapia skin gelatin with garlic extract could inhibit the change of pH value and meatballs spoilage until the 14th day. Edible coating can inhibit the transfer of water vapor and oxygen to products, which can inhibit microbial activity and metabolism. Edible coatings from gelatin can reduce the migration of oxygen, moisture, fat, or carry bioactive materials. The addition of garlic extract to edible coating can inhibit microbial activity and lower oxygen migration can reduce the pH value changes in fish meatballs.

3. 6. Antimicrobial Activity of Edible Coating

The bacteria tested in the inhibition zone in this research are *Pseudomonas aeruginosa*, a spoilage bacteria on fish and fish meatballs. The values of the diameter of the inhibitory zone are shown in Table 3.

Table 2. Inhibition Zone of *edible coating* with garlic extract.

Bacteria	Inhibition Zone (mm)			
	0% (control)	1%	1.50%	2%
P. aeruginosa	0.11	4.24	5.80	6.89
	0.15	4.32	4.56	7.12
Mean	0.13	4.28	5.18	7.01

The observation of inhibition zones for each treatment is documented in Figure 3. The difference in the concentration of garlic extract affects the inhibition zone. The higher garlic extract is added, the greater inhibition zone produced. The highest inhibition zone is 2% concentration of garlic extract with a diameter of 7.01 mm, while the lowest diameter of the inhibition zone is at concentration of 0% (control) with 0.13 mm. This shows that the addition

of garlic extract to the edible coating of fish gelatin can inhibit the growth of spoilage microbes such as *Pseudomonas aeruginosa*. Garlic extract contains allicin which can inhibit bacterial activity.
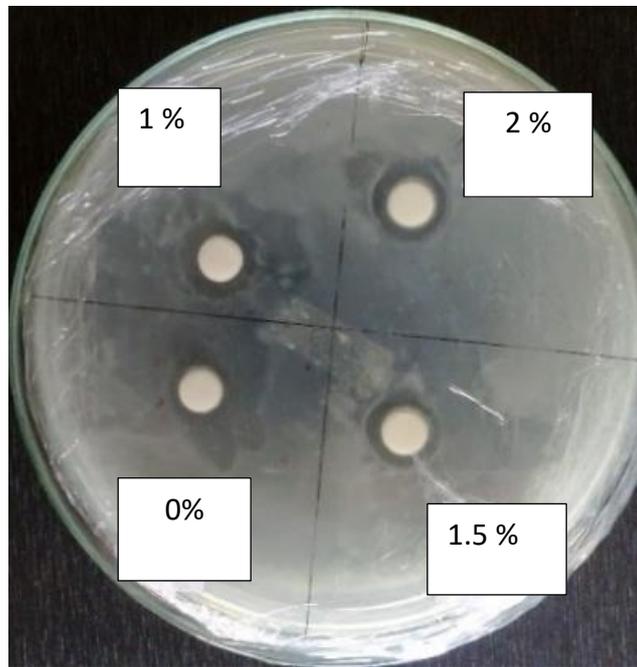

Figure 3. Inhibition Zone of *Edible Coating* with Garlic Extract.

Garlic extract with ethanol as solvents could get chemical compounds such as alkaloids, tannins, phenolics, flavonoids, and triterpenoids. Other contents of garlic extract are antibacterial substances such as allicin and essential oils. The mechanism of allicin in inhibiting bacterial activity such as *P. aeruginosa* relies on penetrating the bacterial cell wall because of its high permeability. The thiol group in allicin reacts with enzymes containing sulfurhidril which composing the cell membrane, then destroys the sulfhidril group on bacterial constituents so that the bacterial cell wall experiencing lysis and metabolism are inhibited. Essential oils work by inhibiting the formation of bacterial cell membranes. Flavonoids work by denaturing bacterial proteins, inhibiting bacterial enzyme activity and disrupting bacterial metabolism.

The addition of 2% garlic extract in 1% chitosan edible coating solution can increase the inhibition zone of *P. aeruginosa* bacteria with a diameter of 8.93 mm inhibition zone. Gull *et al.* (2012), state that garlic ethanol extract 0.5 mg/mL can inhibit *P. aeruginosa* bacteria with diameter of 13.3 mm inhibition zone. The study showed that garlic extract was able to inhibit *P. aeruginosa* bacteria with inhibition zone diameter of 19.45 mm at concentration of 200 µg/mL. The inhibition zone produced in this research ranges from 4-7 mm. The concentration of the test solution used is different and affects the diameter of inhibition zone. The higher concentration of extract, the greater inhibition zone produced. The sensitivity of *P. aeruginosa* to edible coating gelatin garlic extract was classified as moderate. The strength of inhibition zone diameter on antibacterial antibiotics was determined if inhibition zones >20 mm were grouped into bacteria that were very sensitive to antimicrobial assays, inhibitory zones ranging

from 10-20 mm classified as sensitive, if inhibitions zones from 5-10 mm are moderate and inhibition zone <5 mm is classified as weak. However, garlic extract added to edible coating of tilapia gelatin has proven the antibacterial activity against the bacteria *P. aeruginosa.*

4. CONCLUSIONS

The addition of garlic extract can extend the shelf life of fish meatballs in cold/low temperatures (5 ºC). The addition of 2% concentration of garlic extract resulted in the best value of TPC (Total Plate Count), pH value and diameter of inhibition zone. The TPC value of the 2% garlic extract treatment was 1.1×10^5 cfu/g for a shelf life of 14 days and extended the shelf life of the meatballs to 14 days. The treatment is the best for inhibiting the change of pH value during storage with the final pH value of 7.03 on the 14th day, and the inhibition zone diameter against *Pseudomonas aeruginosa* was 7.01 mm.

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