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## Cell-Free Supernatant Antimicrobial From Pediococcus Pentosaceus BAF715 As Mackerel Fish Ball Bio-Preservative

**Running tittle:** Cell-Free Supernatant Antimicrobial From …

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**Abstract**

Antimicrobials produced by lactic acid bacteria are natural preservatives (bio-preservatives) that can be used for food preservation. This study aimed to determine the effectiveness of the cell-free supernatant antimicrobial of *Pediococcus* *pentosaceus* BAF715 as a bio-preservative of mackerel fish ball (*Scomberomorus commersoni*) at cold storage (5°C).Mackerel fish balls were soaked in cell-free supernatant antimicrobial for 30 minutes, then stored at cold temperatures for 0, 2, 4, 6, and 8 days. The results showed that the cell-free supernatant antimicrobial of *Pediococcus pentosaceus* BAF715 as a bio-preservative could maintain the physical quality of fish balls for up to 6 days of storage, while in quality control of fish balls without soaking with the cell-free supernatant antimicrobial, the physical quality could only be maintained for up to 2 days of storage. In addition, microbiologically, the cell-free supernatant antimicrobial from *Pediococcus pentosaceus* BAF715 was able to inhibit the growth of pathogenic bacteria *Escherichia coli*, *Staphylococcus aureus* and *Salmonella Typhimurium* for up to 8 days of storage, in accordance with the Indonesian National Standard (SNI.7266:2014) for fish balls.

**Keywords:** *antimicrobial*, *bio-preservative*, *supernatant,* Fish balls, *P. pentosaceus BAF715*

**INTRODUCTION**

Fishery processing products such as fish balls are popular with all circles and are widely sold in the community. One type of marine fish that is widely used in the manufacture of fish balls is mackerel (*Scomberomorus commersoni*). Mackerel fish balls are preferred because of their delicious (savoury) taste, distinctive aroma and high nutritional content. According to SNI.7266:2014, mackerel fish balls contain 75.06% water, 10.16% protein and 0.86% fat, in which under these conditions mackerel fish balls are a good medium for the growth of spoilage microbes so they are easily damaged (highly perishable). Due to this microbial contamination, fish balls cannot be stored for a long time.

There are efforts to maintain the quality and extend the shelf life of fish balls by providing certain treatments such as using preservatives. Natural preservatives, for example, are the use of lactic acid bacteria. Lactic acid bacteria in their growth period produce antimicrobial compounds such as bacteriocin, hydrogen peroxide and organic acids. The mechanism of antimicrobial compounds (bacteriocins) as bio-preservatives is to form holes in the membrane resulting changes in the membrane potential gradient and the release of intracellular molecules as well as the entry of extracellular substances (environment), which then causes inhibition of cell growth and ultimately results in the death of cells. (Hafsan, 2014). Antimicrobials from lactic acid bacteria are able to eliminate pathogenic microbes and food spoilage, non-toxic, have low levels of inhibition and are classified as GRAS (generally recognized as safe) bacteria, which are microbes that do not interfere with health (Hafsan, 2014; Hata et al., 2010). One of the antimicrobials from lactic acid bacteris is *Pediococcus pentosaceus* BAF715. *Pediococcus pentosaceus* BAF715 isolated from tamarind were identified by 16S rRNA sequencing. These bacteria have antimicrobial activity against pathogenic bacteria such as *E. coli*, *S. aureus*, *Listeria monocytogenes*, *Salmonella sp* and *Proteus* (Afriani, 2018).

Storage using cold temperatures is an effort to maintain quality and extend the shelf life of fish balls. Decomposing and pathogenic bacteria experience optimal growth at room temperature (mesohpile), so that by storing at low temperatures (psychrofile), respiration activity can decrease which then results in inhibition of the growth of these bacteria.

**MATERIALS AND METHODS**

**Supernatant Preparation**

Lactic acid bacteria ***Pediococcus pentosaceus* BAF715** was grown in liquid De Man Rogosa Sharpe medium (MRS broth) with a concentration of 108 cells/mL and incubated at 37,5 0C for 48 hours. Then it was centrifuged at 6000 rpm for 20 minutes. Furthermore, it was filtered using a 0.45 m millipore filter membrane to separate the sediment and liquid. The obtained liquid is called cell-free supernatant (CFS) which contains antimicrobial compounds.

**Mackerel Fish Balls Preparation**

The fish meat used for making fish balls is mackerel (*Scomberomorus commersoni*)as much as 1.5 kg. The composition of the ingredients used is based on the weight of the fish meat, namely tapioca flour (10%), salt (3%), Sodium Tripoly Phosphate (0,3 %), ice cubes (35%), pepper (0,5%), dan garlic (0,5%).

The steps of making fish balls according to the modified Ismail et al (2016) procedure are: firstly, grind fish meat, STTP, salt, and half of the ice with a food processor until all ingredients are evenly distributed. Next, add flour, pepper, garlic, and half of the ice to the mixture and then grind again until it becomes smooth and well mixed. Leave the mixture for 10-15 minutes in the refrigerator. Later, form the mixture into ball shapes or as desired, then put into warm water for ±10 minutes. Lastly, boil fish balls in boiling water until completely cooked (±10-15 minutes) then remove and drain.

**Antimicrobial Activity Test**

Antimicrobial activity testing used modified paper disc diffusion method according to Dhiman et al. (2011). The bacteria used for testing were *E. coli* ATTC 25922*, S. aureus*, ATTC 25923 and *Salmonella typhimurium* ATTC 14028. Determination of antimicrobial activity was done by pouring 0.1 ml of the test bacteria into Muller Hinton Agar medium (20 ml) in a petri dish. Sterile paper discs (Oxoid, United Kingdom) were soaked overnight in 50 μL supernatant. After the medium hardened, sterile paper discs with a diameter of 6 mm were then placed. Petri dish was incubated at 370C for 24 hours. The inhibition zone formed around the paper disc was measured based on the diameter of the clear area by averaging the measurements in several places.

**Soaking Fish balls in Supernatant**

Cell-free supernatant (CFS) was put into a 100 ml sterile glass beaker, then fish balls were put into it and soaked for 30 minutes. Later, those fish balls were removed and then the treatment meatballs (soaked in supernatant) and control fish balls (without soaking in supernatant) were put separately into sterile plastics and stored at cold temperatures (50C) for 8 days. Observations were made on day 0, 2, 4, 6, and 8.

**Physical Analysis of Fish Balls**

Physical analysis of fish balls included pH, water binding capacity (DMA), and Eber test. Measurement of the pH of fish meat was carried out using a Corning pH meter. The pH meter was calibrated with a standard solution (pH 4 and 7), then 5 grams of fish meat was crushed with a blender and dissolved in 45 ml of distilled water, later a pH meter electrode was inserted into the fish ball solution to check its pH. (AOAC, 2005). The measurement of the water-holding capacity was carried out using the press method in accordance with Hamm's instructions (Swatland 1984 in Soeparno, 2015) where 0.3 g of sample was placed between 2 Whatman filter paper number 41. These papers and sample were placed between two glass plates and pressed by a load weighing 35 kg for 5 minutes. The area of wet zone can be determined by the difference between the outer circumference and the inner circumference divided by 100. The amount of water that came out of the fish balls is calculated by using the formula:

Area of wet zone (cm2 mgH ) 2O

mgH 2O = - 8,0

0,0948

The percentage of free water determined by using the formula:

mgH2O

Free Water Percentage (%) = x 100 %

300 mg

The rot test was carried out using the Eber test. Eber reagent consisting of concentrated HCl, 96% alcohol and ether in a 1:3:1 ratio was put into the test tube. Next, samples of fish balls that had been pierced with sterile toothpicks were put into a test tube containing Eber's reagent. The test tube was closed by plugging a sterile cork on the top or base of the toothpick to cover the entire top of the tube to prevent evaporation of Eber's reagent. The presence or absence of the formation of steam or white clouds which are NH4Cl gas on the tube walls was later observed. The results of the Eber test are declared negative (-) if there is no white cloud formed on the tube wall, positive 1 (+) if a little white cloud is formed (after 10 minutes), positive 2 (++) if quite a lot of white clouds formed (within 5-10 minutes), and positive 3 (+++) if a lot of white clouds formed (within <5 minutes) (Fransisca et al , 2018).

**Microbiological Analysis of Fish Balls** (AOAC, 2005)

Microbiological Analysis of Fish Balls included Total Plate Count (TPC), *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* sp. TPC measurement was carried out by taking 5 g samples of control meatballs and treatment meatballs that had been crushed and adding them to a diluent (0.1% peptone) to make a sequence of dilution up to 10-6. On the 10-4, 10-5, and 10-6 dilutions, 1 ml of the solution was taken and added to a petri dish containing 20 ml of NA (Merck) medium and then homogenized to form a figure 8. After the medium froze, it was incubated at 370 C for ±24 hours in an inverted position. Bacteria that had grown formed white colonies. The number of viable colonies ranges from 25-250 colonies. Measurement of total *Staphylococcus aureus, Escherichia coli* and *Salmonella sp* was carried out by taking samples of fish balls (control fish balls and treatment fish balls) as much as 5 grams which later were put into a sterile diluent (peptone 0.1%) with a dilution level of 10-1, 10-2, dan 10-3. Mannitol salt agar (MSA, Merck) medium was used for the growth of *Staphylococcus aureus* bacteria, *Eosyn Methylen Blue Agar* (EMBA, Merck) medium for the growth of *Escherichia coli* bacteria and *Bismuth Sulphite Agar* (BSA, Difco) medium for the growth of *Salmonella sp*. A total of 1 ml of samples from 3 dilution levels were taken to be added to petri dishes containing 20 ml of medium which were then homogenized to form a figure 8. After the medium solidified, the samples were incubated at 370 C for ± 24 hours in an inverted position. *Staphylococcus aureus* colonies that grew were black surrounded by yellow colour, for *Escherichia coli* colonies, they appeared greenish under the bright light or sunlight while *Salmonella sp* colonies looked dark with a metallic appearance and had a brown zone around the colonies.

**Data Analysis**

The collected data were analyzed by analysis of variance (ANOVA) based on a completely randomized design (CRD) with 5 treatments and 4 replications. The difference between treatments was measured by Duncan’s Multiple Range Test (Steel and Torrie 1995).

**RESULTS**

**Antimicrobial Activity of the *Pediococcus pentosaceus* BAF715 Supernatant**

Table 1 shows the antimicrobial activity of the cell-free supernatant of *Pediococcus pentosaceus* BAF175 against E. coli, Staphylococcus aureus and Salmonella thypimurium bacteria. The diameter of the inhibition zone formed against E. Coli, Staphylococcus aureus and Salmonella thypimurium bacteria were 11.0, 10.5 and 10.1 mm, respectively.

|  |  |
| --- | --- |
| **Table 1.** | **The inhibition zone of *Pediococcus pentosaceus* cell-free supernatant against test microbes (mm)** |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| No. | Test Microbes | Average ø | Paper disc ø | Inhibition ø |
| 1. | *E. coli* ATTC 25922 | 17,0 | 6,00 | 11,0 |
| 2. | *S. aureus* ATTC 25923 | 16,5 | 6,00 | 10,5 |
| 3. | *Salmonella* thypimurium ATTC 14028 | 16,1 | 6,00 | 10,1 |

**Physical Quality of Fish Balls**

Measurement of the physical quality of fish balls includes pH value, Water-Holding Capacity (WHC) and Eber test. The results of statistical analysis of the physical quality of fish balls can be seen in Table 2.

|  |  |
| --- | --- |
| **Table 2.** | **Physical Quality of Fish Balls in Cold Storage** |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Treatments | Storage time (h) | | | | |
| 0 | 2 | 4 | 6 | 8 |
| pH | Control | 5,7± 0,1a | 6,0± 0,10 b | 6,2±0,20 b | 6,2±0,10 b | 6,5± 0,10 c |
| Supernatant | 4,70 ±0,10c | 5,03±0,15b | 5,17±0,21b | 5,20±0,20b | 5,96± 0,10a |
| Water-Holding Capacity (%) | Control | 24,52±0,5 e | 25,38± 5,4 b | 26,29±0,8 a | 26,96±2,0 a | 26,68±3,6 a |
| Supernatant | 25,96 ± 2,05a | 26,68±3,57a | 26,09±0,80a | 25,58±5,38a | 28,52±0,54b |
| Uji Eber | Control | - | - | + | ++ | +++ |
| Supernatant | - | - | - | - | + |

Notes: - Superscripts with different lowercase letters on the same line show significant differences (P<0.05).

- Negative (-): No white cloud around the meat, Positive 1 (+): Little white cloud formed (formed after 10 minutes), Positive 2 (++): Quite a lot of white clouds formed (formed in 5-10 minutes), Positive 3 (+++): A lot of white clouds formed (formed in < 5 minutes).

Table 2. shows that fish balls that were not soaked in supernatant (control) during cold storage had an increase in pH. The pH of meatballs at day 0 of storage was significantly different (P < 0.05) with day 2 of storage while at day 2, 4 and 6 of storage there were no significant differences (P>0.05). The significant differences (P < 0.05) can be found between day 2, 4 and 6 of storage and day 8 of storage. The pH values at day 0, 2, 4, 6 and 8 of storage were 5.7; 6.0; 6.2; 6.2 and 6.5, respectively.

Fish balls that were soaked in supernatant during cold storage also experienced an increase in pH. The pH of meatballs at day 0 of storage was significantly different (P>0.05) from day 2 of storage while at day 2, 4 and 6 of storage there were no significant differences (P>0.05). The significant differences (P < 0.05) can also be found between day 2, 4 and 6 of storage and day 8 of storage. The pH of meatballs at day 0, 2, 4, 6 and 8 of storage were 4.7; 5.03; 5.17; 5.20 and 5.96, respectively.

Table 2 displays the water binding capacity of fish balls without supernatant soaking increased significantly (P<0.05) during storage. On the day 0, 2, 4, 6 and 8 of storage the water binding capacity were 24%, 52%; 25.38%; 26.29%; 26.96% and 26.68%, respectively.

The water binding capacity of supernatant-soaked fish balls did not increase at day 0, 2, 4 and 6 of storage with values of 25.96; 26.68; 26.09 and 25.58% respectively, which then increased after day 8 of storage with a value of 28.52%.

The results of the Eber test showed that the fish balls without supernatant soaking on day 0 and 2 of storage have not experienced decay, which is marked by the absence of white clouds on the tube wall around the meatballs after more than 10 minutes of observation. On day 4, 6 and 8 of storage, the fish balls had begun to decay which was marked by the presence of steam or white clouds on the tube wall around each of the fish ball; (+) on observation for 10 minutes, (++) on observation for 5-10 minutes and (+++) on observation for <5 minutes.

**Microbiological Quality**

The microbiological quality of fish balls during storage at cold temperatures, including total microbes, *S. aureus, E. coli* and *Salmonella*, is presented in Table 3.

|  |  |
| --- | --- |
| **Table 3.** | **Microbiological Quality of Fish Meatballs in Cold Storage** |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Treatments | Storage time (h) | | | | |
| 0 | 2 | 4 | 6 | 8 |
| TPC  (log cfu/g) | Control | 1,87±0,47 d | 4,88 ±0,09 c | 5,11 ±3,39 c | 7,39 ±0,16 b | 7,99± 0,33 b |
| Supernatant | 0,14±0,38d | 3,88±0,09c | 4,33±0,13b | 4,41±0,49b | 5,99±0,34a |
| *S.aureus* (log10 cfu/g) | Control | 2,75± 0,27 e | 3,32± 0,70 d | 4,57± 0,12 c | 6,73± 1,50 b | 7,13± 0,46 a |
| Supernatant | 1,05 ±0,27 | 1,68±1,33 | 1,57±0,13 | 1,50±0,16 | 1,73 ± 0,46 |
| *E. coli*  (log cfu/g) | Control | 1,1±0,30 d | 1,89±0,50 d | 3,33±0,11 c | 4.56±0,05 b | 5,56±0,17 a |
| Supernatant | 0,00 f | 0,00 f | 0,00 f | 0,00 f | 0,00f |
| *Salmonella* | Control | negative | negative | negative | negative | negative |
| Supernatant | negative | negative | negative | negative | negative |

Notes: Superscripts with different lowercase letters on the same line show significant differences (P<0,05).

Table 3 show the fish balls without supernatant soaking during cold temperature storage exhibited an increase in total microbes. The value of total microbes on day 0, 2, 4, 6 and 8 of storage were 1.87; 4.88; 5.11; 7.39 and 7.99 (log cfu/g), respectively.

The length of cold temperature storage of the supernatant-soaked fish balls showed an increase in total microbes. The value of total microbes at 0, 2, 4, 6 and 8 days of storage were 0.14; 3.88; 4.33; 4.41 and 5.99 (log cfu/g), respectively.

Table 3 presents the results of fish meatballs without supernatant soaking during cold temperature storage showed no significant increase (P>0.05) in total *Staphylococcus aureus*. Total *Staphylococcus aureus* on day 0, 2, 4, 6 and 8 of storage were 2.75; 3.32; 4.57; 6.73 and 7.13 (log cfu/g), respectively.

Fish balls soaked in supernatant during cold temperatures storage exhibited a significant increase (P < 0.05) in total Staphylococcus aureus. Total *Staphylococcus aureus* on day 0, 2, 4, 6 and 8 of storage were 1.05; 1.68; 1.57; 1.50 and 1.73 (log cfu/g), respectively.

Table 3 indicates that fish balls without cell-free supernatant soaking during cold temperatures storage showed a significant increase (P < 0.05) in total *Escherichia coli*. Total *Escherichia coli* on day 0, 2, 4, 6 and 8 of storage were 1.1; 1.89; 3.33; 4.56 and 5.56 (log cfu/g), respectively.

Fish balls soaked in cell-free supernatant during cold temperatures storage were not found to have the presence of *E. coli*.

In fish balls that were not soaked with supernatant during cold temperatures storage, the presence of *Salmonella* bacteria was not detected (negative).

**DISCUSSION**

**Antimicrobial Activity of The *Pediococcus pentosaceus* BAF715 Supernatant**

The antimicrobial activity of cell-free supernatant of *Pediococcus pentosaceus* in inhibiting the growth of microbes *E. coli*, ATTC 25922, *S. aureus* ATTC 25923 and *Salmonella thypimurium* ATTC 14028 can be seen from the inhibition zone area formed (Table 1). The diameter of the inhibition zone ranged from 10.1 - 11.0 mm. From the results of research by Arief et al. (2012), the antimicrobial activity of bacteriocin from *L. plantarum* 2C12 had an inhibition zone diameter (mm) against pathogenic bacteria such as *Esherichia coli* ATCC 25922 of 11.83 ± 0.83, *Staphylococcus aureus* ATCC 25923 of 10.95 ± 0.09, and *Salmonella thypimurium* ATCC 14028 of 11.28 ± 0.24.

The magnitude of the bacterial inhibition zone is classified into 3 criteria, which are moderate inhibition between 6 - 9 mm, strong inhibition of 10 - 14 mm, and very strong inhibition of 15 - 18 mm (Lade *et al*. 2006). In the results of this study, the inhibition zone formed from antimicrobial activity fits the strong inhibition criterion. Antimicrobial supernatant from *Pediococcus pentosaceus* BAF715 shows strong antibacterial activity in inhibiting the growth of gram-positive and gram-negative bacteria.

**Physical Quality**

The length of storage of fish balls without supernatant soaking increased the pH value along with the increase in bacterial growth. The pH value greatly affects the shelf life of processed fish products. According to Suradi (2012), the increase in pH value during storage at cold temperatures is due to enzyme activity and decomposition of chemical compounds such as proteins that produce alkaline compounds like indole, scatole, and cadaverine. At certain pH values, it can seriously damage microorganism cells due to changes in membrane permeability and ion transport (Kia et al, 2016).

The pH value of supernatant-soaked fish balls during storage was still acidic. The pH of the fish balls during storage ranged from 4.7 to 5.96 where the pH value was influenced by the pH value of the supernatant which was 4.3. During the soaking process, lactic acid contained in the supernatant seeped into the fish balls resulting in acidic fish balls. Lactic acid is the main metabolite produced by *Pediococcus pentosaceus* BAF715 which is antimicrobial against spoilage and pathogenic bacteria. The results of the study by Adeniyi et. al. (2006) showed that *P. pentasaceus*, *P. acidilactici* and *L. plantarum* are classified as homofermentative lactic acid bacteria, where the antimicrobial substance in the form of lactic acid contains more than 85% of the fermentation of carbohydrates.

The water-holding capacity (WHC) can be calculated based on the percentage of mgH2O. The higher the percentage of mgH2O, the lower the water-holding capacity. The length of storage on fish balls without supernatant soaking shows that the water-holding capacity decreases. This is related to the resulting pH where the longer the storage, the pH value of the fish balls increases, thus causing the water-holding capacity of the fish balls to decrease due to the ability of meat protein to bind water is influenced by pH (Soeparno, 2015). Myofibril meat protein is a substance that is responsible for water-holding in meat. (Arief *et al.*, 2012).

In supernatant-soaked fish balls with storage for up to 6 days, the water-holding capacity did not increase, presumably the antimicrobial activity in it was still high so that the meat protein could maintain the water content in fish balls. After 8 days of storage, the activity of supernatant antimicrobial compounds weakened, accompanied by an increase in pH. In this condition, the activity of spoilage bacteria continued to decompose the food substances contained in fish balls.

Fish balls without supernatant soaking in 4 days of storage have begun to show decay characterized by the presence of dew on the tube wall around the fish balls. This decay is identical to the activity of spoilage bacteria characterized by the formation of foul-smelling compounds such as ammonia H2S, indole and amine, which are the result of protein breakdown by microorganisms (Suradi, 2012).

Fish balls soaked in supernatant for up to 8 days of storage have not changed (-). Antimicrobial supernatant from *Pediococcus pentosaceus* BAF715 can maintain the quality of fish balls until 8 days of storage. This condition is related to the low pH value and high water-binding capacity of fish balls, indicating that fish balls are still able to preserve their physical quality during storage at 5 OC.

**Microbiological Quality**

Fresh fish balls (day 0) already showed the presence of growing colonies, then during storage at cold temperatures the number of colonies increased until day 6 and did not increase on day 8 of storage. The total microbes of fish balls without soaking supernatant after the day 4 of storage have exceeded the National Standard for Fish Ball Products (SNI 7266:2014) (National Standardization Agency of Indonesia, 2014).

The results of this study are not much different from Arief et al. (2012), where the total bacteria of beef meatballs with the addition of bacteriocin from L. plantarum IIA-1A5 were 3.65±0.25 log cfu/g for 0 days, 4.40±0.00 log cfu/g for 3 days, and 4.39±0.00 log cfu/g for 6 days of storage. From the results of research by Pato *et al.* (2022), the use of bacteriocin from Pediococcus pentosaceus Strain 2397 at a concentration of 0.60% showed the lowest total microbes of 0.95 x 102 CFU/g for 9 days of storage at freezing temperatures.

The total microbes of fish balls soaked in supernatant during cold storage are still within the threshold according to SNI 7266: 2014 (National Standardization Agency of Indonesia, 2014) which is 1x105 colonies/g. Fish balls stored for up to 6 days are still in good condition. These results have proven that antimicrobial supernatant from *Pediococcus pentosaceus* BAF715 is effective for controlling microbial growth.

Table 3 shows that both the unsoaked fish balls and those soaked in cell-free supernatant have shown the growth of *Staphylococcus aureus*, possibly due to poor sanitation and hygiene standards during the manufacturing process. Pathogenic bacteria such as S. aureus found in fish balls can spread through the hands of workers in the food industry and restaurants (Kadariya et al., 2014). Hand hygiene, washing and disinfection are prerequisites for hygiene management in the food industry to lower the risk of foodborne infections (WHO, 2011). During cold storage the number of colonies increased further up to 8 days. Total Staphylococcus aureus in fish balls during storage exceeded the Indonesian National Standard for Fish Ball Products (SNI 7266:2014) (National Standardization Agency of Indonesia, 2014).

The length of cold storage in supernatant-soaked fish balls did not show a significant increase (P>0.05) in total *S. aureus* bacteria. During storage, the growth of *S. aureus* could be inhibited due to cold storage and the inhibitory effectiveness of antimicrobial compounds from *P. pentosaceus* BAF715. These results are in line with the results of the invitro analysis in Table 1. According to Pato *et al.* (2020), antimicrobial compounds produced by *P. pentosaceus* 2397 have been shown to have antimicrobial activity that can inhibit the growth of *S. aureus*. The range of total *S. aureus* in fish balls during cold storage was 2.13 ± 0.46 cfu/g to 2.75 ± 0.27 cfu/g. Total *S. aureus* bacteria in the results of this study are still below the BSN (2014) quality standard (SNI 7266: 2014) which is a maximum of 1.0 x 102 colonies/g. Antimicrobial supernatant from *P. pentosaceus* BAF715 is bacteriostatic which can inhibit the growth of *S. aureus* bacteria. According to Hafsan (2014), antimicrobial compounds are chemical or biological compounds that can inhibit microbial growth and activity.

The range of total Escherichia coli in fish balls without supernatant soaking was between 1.1 ± 0.30 (log cfu/g) and 4.56 ± 0.05 (log cfu/g). The total Escherichia coli obtained exceeded the National Standard for Fish Ball Products (SNI 7266:2014) (National Standardization Agency of Indonesia, 2014) which is a maximum of 3 MPN/g.

In cold temperature storage of supernatant-soaked fish balls, no *E. coli* was found. The antimicrobial activity of free supernatant from *Pediococcus pentosaceus* BAF715 and cold temperature can inhibit the growth of *E. coli*. *E. coli* bacteria grow optimally at mesophilic temperatures so they are sensitive to cold temperatures. According to Kusumawati (2000), inhibition of microbial growth is due to the synergistic work between antimicrobial activity and storage temperature.

The cell-free supernatant antimicrobial of *Pediococcus pentosaceus* was bactericidal against *E. coli* bacteria. These antimicrobials are cationic and kill target cells by interfering with the membrane-potential and/or making cellular solutes to leak that ultimately put cells to death. (Diep et al., 2009).

*Salmonella* was not detected (-) in both fish balls without soaked in supernatant and fish balls soaked in supernatant during storage at cold temperatures. The negative (-) S*almonella* result in this study is due to the sanitary nature of the preparation of fish balls which was free from *Salmonella*. In addition, the boiling process of the fish balls may also be responsible for the removal of Salmonella from the final product.

Antimicrobial cell-free supernatant from *Pediococcus pentosaceus* BAF715 effectively inhibits *E. coli* and *Salmonella sp.* (Gram-negative). According to the quality standard from BSN (2014) SNI 7266: 2014, good quality fish balls should not be contaminated with *Salmonella sp*. These results indicate that antimicrobial cell-free supernatant from *Pediococcus pentosaceus* can be used as bio-preservatives for fish balls.

**CONCLUSION**

Cell-free supernatant antimicrobial from *Pediococcus pentosaceus* BAF715 can act as a bio-preservative that can maintain the physical quality of fish balls for up to 6 days of storage, while fish balls without supernatant soaking can only last for up to 2 days of cold storage (50C), and the antimicrobial activity of the supernatant can inhibit the growth of pathogenic bacteria such as *S. aureus, E. coli* and *Salmonella sp.*

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