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Use of Produced Cell -Free Supernatant Antibacterial Produced by *Pediococcus pentosaceus* BAF715 as Biopreservative of Buffalo Meatballs at Cold Temperatures

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ABSTRACT

Meatballs are processed meat products with high nutritional value and are classified as easily damaged food products. Therefore, preservatives are necessary to maintain the shelf life of meatball products. Natural preservatives that can be used are antibacterial compounds derived from lactic acid bacteria. This study aims to determine the antibacterial use of the cell-free supernatant produced by *Pediococcus pentosaceus* BAF715 as a biopreservative for buffalo meatballs when stored at cold temperatures (± 5°C). Buffalo meatballs are soaked in cell-free antibacterial supernatant for 30 minutes, then stored at cold temperature (± 5°C) for 0, 3, 6, 9, and 12 days as treatment. The results showed that long storage of meatballs soaked in cell-free antibacterial supernatant can reduce total bacteria, *Staphylococcus aureus* and *Escherichia coli*. Long storage can maintain the pH value and percentage of free water. Total bacteria and *Staphylococcus aureus* at nine days of storage and still within the requirements of the Indonesian National Standard (SNI 3818: 2014) regarding the quality of meatballs, at 12 days of storage, it has exceeded the threshold, namely 5.99 cfu/log10, while the control meatballs (without soaking) only lasted for three days, namely 7.99 cfu/log10. It was concluded that antibacterial cell-free supernatants were produced. *Pediococcus pentosaceus* BAF715 can maintain the microbiological and physical quality of meatball buffalo meat for up to 9 days of storage at cold temperatures (± 5°C).

Keywords: antibacterial, biopreservative, meatballs, *Pediococcus pentosaceus*, cell-free supernatant

INTRODUCTION

Meatballs are processed meat products popular in Indonesia, such as fast food. Meatball products have pretty high nutritional value and are very sensitive to contamination by spoilage bacteria due to the physical and chemical properties of the product (Hastaoglu et al., 2016), so they are easily damaged, and the shelf life does not last long.

Meatball products are made in a round shape from a mixture of at least 50% meat (BSN, 2014) with other ingredients, such as flour, salt, ice, sodium tripolyphosphate (STPP) and spices (Putri, 2009). This product can be processed using preservatives to maintain quality and extend shelf life. For example, borax and nitrite are often used to preserve meat and meatballs (Arslan et al., 2008; Kia et al., 2016). Because these preservatives can inhibit the growth of microorganisms, food ingredients can be preserved for a more extended period (Normah et al., 1984; Sindelar and Milkowski, 2011). However, the impact of using borax is risky for health due to consuming food products containing borax (See et al., 2010). Therefore, alternative natural preservatives can be an option to ensure the safety and quality of meatballs.

The use of bacteriocins produced by lactic acid bacteria (LAB) has been proven to have antibacterial properties that can inhibit pathogenic bacteria and extend the shelf life of food products (Savadogo et al., 2006; Hata et al., 2010). From a safety aspect, bacteriocins are classified as safe because they can be degraded by proteolytic enzymes in the digestive tract (Cleveland et al., 2001; Arief et al., 2015). Use of the antibacterial plantaricin IIA-1A5 produced from *Lactobacillus plants* IIA-1A5 (Kia et al., 2016), nisin and natamycin (Hastaoglu et al. 2016, 2017) can reduce the total population of bacteria, *S. Aureus* and *E. coli* and can extend the shelf life of meatball products.

Concerning the potential antibacterial compounds produced from lactic acid bacteria, cell-free antibacterial supernatants produced by lactic acid bacteria *Pediococcus pentosaceus,* it is possible that BAF715 isolated from fermented fish products (bekasam) can be used as a natural preservative (biopreservative) for processed meat products such as meatballs (Afriani et al., 2018). Soaking the meatballs in a cell-free supernatant antibacterial solution allows the organic acids in the solution to diffuse and ionize, ultimately destroying the bacterial cell nuclei, especially psychrophilic bacteria that can survive cold

temperatures. The antibacterial potential of cell-free supernatant as a natural preservative for buffalo meatball products stored at cold temperatures (±5°C) is still unknown. Using antibacterial cell-free supernatant from Pediococcus pentosaceus BAF715 as a natural preservative is assumed to maintain buffalo meatballs' microbiological and physical quality during storage at cold temperatures (± 5°C). This research aimed to determine the effectiveness of using the antibacterial cell-free supernatant produced*Pediococcus pentosaceus* BAF715 as a biopreservative for buffalo meatballs when stored at cold temperatures (± 5°C).

MATERIALS AND METHODS

Materials and tools

The material used in this research is bacteria *Pediococcus pentosaceus* BAF715 (Afriani, 2018), buffalo meat obtained from the Angso Duo primary market, an ingredient for making meatballs. Bacterial growth media are MRS agar and MRS broth (oxoid)*, Eosyn methylen blue agar* (EMBA, Merck), mannitol salt agar (MSA, Merck), *Nutrient* agar (NA, Merck)*buffer peptone water*, alcohol, spirits, standard solutions pH 7 and 4 and*Aquadest* sterile.

Equipment used: cup s*petri*, glassware, *incubato*r, *laminar air flow*, *vortex*, pH meter, Bunsen lamp, micropipette and 1 ml tip, *refrigerator*, *filter millipore* size 0.22 µm, Whatman filter paper no—41 and *hot plate ironer*.

Production of Cell-Free Antibacterial Supernatant

Lactic acid bacteria *Pediococcus pentosaceus* BAF715 was grown in liquid De Man Rogosa Sharpe medium (MRS broth) plus yeast extract at a concentration of 10^8 sel/mL incubated at 37.5°C for 20 hours, then centrifuged at 5000 rpm for 20 minutes. Filtration uses a millipore filter membrane measuring 0.22 µm to separate sediment from liquid. The fluid obtained is called cell-free supernatant (SBS), which contains antibacterial compounds. Cell-free antibacterial supernatant production method modified from the procedure of Arief et al. (2017)

Making Buffalo Meatballs

400 gr of fresh buffalo meat was used. The composition of the ingredients used is based on the weight of buffalo meat, namely tapioca flour (10%), salt (3%), sodium tripolyphosphate (0.3%), ice cubes (35%), pepper (0.5%), and garlic (0.5%). Meat is ground using a *food processor* until evenly mixed, and at the same time, tapioca flour, pepper, garlic, and half the ice are added until a homogeneous dough is formed. Then, put it in the refrigerator at a temperature of ± 5°C for 10-15 minutes. After cooling, the meatball mixture is formed into balls (diameter ± 2 cm) manually using a spoon, then placed in hot water (± 80°C) until the meatballs float for ± 15 minutes, then remove the meatballs and drain.

Preserving Meatballs with Cell-Free Antibacterial Supernatant

Cell-free supernatant (SBS) was added to a beaker glass, a 100 ml sterile glass, and buffalo meatballs were put in the *beaker glass* and soaked for 30 minutes (Arief et al., 2017), then the treatment meatballs were removed. The control meatballs (without immersion in the supernatant) were stored in sterile polyethene plastic at a cold temperature (± 5°C) for 12 days. Observations were made on days 0, 3, 6, 9, and 12.

Cell-Free Supernatant Antibacterial Activity Test

Antibacterial activity testing used a modified paper disc diffusion method, according to Dhiman et al. (2011). *E. Coli* ATTC 25922 and *S. Aureus* ATTC 25923 are the test bacteria used. Antibacterial activity can be determined by pouring 0.1 ml of test bacteria into MHA (Muller Hinton Agar) media (20 ml) in a cup of *Petri*. Soak *paper disc* on the cup *petri* sterile (Oxoid, United Kingdom) in 50 µL supernatant for one day. After the media hardens, place a sterile paper disc with a diameter of 6 mm. Cup *petri* was incubated at 37°C for 24 hours. The inhibition zone formed around the paper disc was measured based on the diameter of the transparent area by averaging the measurements of several places.

Microbiological Analysis of Meatballs (AOAC, 2005)

Microbiological analysis of meatballs includes Total bacteria, *S. Aureus*, and *E. coli*. Measurement of total microbes by taking 5 g samples of control meatballs and treatment meatballs that have been crushed, placing them in a diluent solution (0.1% peptone), diluting to 10^{-5} and at a dilution of 10^{-4}, and 10^{-5}, taken 1 ml

added to the cup *petri* containing 20 ml NA media and homogenized by forming the number 8. After the media freezes, it is incubated at 37°C for ± 24 hours in an inverted position. Bacteria that grow form white colonies. The number of viable colonies ranges from 25-250 colonies.

Total measurement *Staphylococcus aureus* and *Escherichia coli* by placing 5 g samples of buffalo meatballs (control meatballs and treatment meatballs) in a sterile diluent solution (0.1% peptone), the dilution level used is 10^{-1}, 10^{-2}, and 10^{-3}. Mannitol salt agar (MSA) media for bacterial growth *Staphylococcus aureus*, and media *Eosyn Methylene Blue Agar* (EMBA) for bacterial growth *Escherichia coli,*1 ml of sample from 3 levels of dilution was added to a petri dish containing 20 ml of media and homogenized to form figure 8. After the media had frozen, it was incubated at 37°C for ± 24 hours in an inverted position. Colony *Staphylococcus aureus* grows black and is surrounded by yellow, and *Escherichia coli* looks greenish in bright lights or sunlight.

Physical Analysis of Meatballs

pH value

Measuring the pH of buffalo meat meatballs using a Corning Meter. Before use, the pH meter was calibrated with a standard solution (pH 4 and 7), then 5 g of buffalo meatballs were crushed with a blender and dissolved in 50 ml of distilled water. The pH meter electrode was inserted into the meatball solution, and the pH value was measured.

Water holding capacity

Water holding capacity was measured using the press method according to Hamm's instructions (Swatland 1984 in Soeparno, 2015), namely placing 0.3 g of sample between 2 Whatman number 41 filter papers and placing it between two glass plates then pressing a weight weighing 35 kg for 5 minutes. The area of the wet area can be determined by dividing the difference between the outer ring and the inner ring by 100. The amount of water that comes out of the meatballs is calculated using the formula:

$$mgH_2O = \frac{\text{Wet area } (cm^2\ mgH)_2O}{0{,}0948} - 8{,}0$$

The formula can determine the percentage of free water:

$$\text{Free Water Percentage (\%)} = \frac{mgH_2O}{300\ mg} \times 100\ \%$$

Eber test

The Eber test is to determine the level of rottenness of buffalo meatballs. Eber's reagent consists of concentrated HCl, 96% alcohol and ether in a ratio of 1:3:1. Samples of control meatballs and treatment meatballs that have been pierced with a sterile toothpick are placed in a test tube containing Eber's reagent. The test tube is closed by sticking a sterile cork at the top or base of a toothpick to cover the entire tube to prevent evaporation of the Eber's reagent. Observe whether or not steam or white clouds form, which is NH_4Cl gas, on the tube wall. Eber test results are declared hostile (-) if no white clouds form on the tube wall, positive 1 (+) = a little white cloud forms (after 10 minutes), positive 2 (++) = quite a lot of white clouds form (within 5-10 minutes), and positive 3 (+++) = lots of white clouds are formed (in < 5 minutes) (Fransisca et al., 2018).

Data analysis

The data collected was analyzed using analysis of variance based on a completely randomized design (CRD) with five treatments and four replications—Duncan's test determined treatment differences (Steel and Torrie 1995).

RESULTS AND DISCUSSION

Table 1 shows the antibacterial activity of the cell-free supernatant that produced *Pediococcus pentosaceus* BAF715 against bacteria *Escherichia coli* and *Staphylococcus aureus*. The diameters of the inhibition zones formed were 11.00 ± 0.2 and 10.50 ± 0.1 mm, respectively. Sihombing et al. (2015) state high antibacterial activity with an inhibition zone of >10.00 mm in diameter, moderate antibacterial activity with an inhibition zone of 5.00-10.00 mm in diameter, low antibacterial activity with an inhibition zone of <5.00 mm and no antibacterial activity no inhibition zone.

Table 1. Inhibitory zones of produced cell-free supernatants*Pediococcus pentosaceus* BAF715 against test bacteria (mm)

No.	Test Bacteria	Inhibition
1.	*E. coli* ATTC 25922	11.00 ± 0.2
2.	*S. Aureus* ATTC 25923	10.50 ± 0.1

Cell-free antibacterial supernatants were produced by *Pediococcus pentosaceus* BAF175,

which shows high antibacterial activity against gram-negative and positive bacteria. The research results of Arief et al. (2012) show that plantaricin IIA-1A5 from *L. Plantarum* shows high antibacterial activity against *E. coli* and *S. Aureus* by forming an inhibition zone of 10.25 - 10.39 mm for each bacteria. The research results of Soenarno et al. (2020) show that plantarisin IIA-1A5 has moderate antibacterial activity against *Salmonella* 38 and *Shigella* A33 with a barrier zone of 7.58 and 9.10 mm. According to Nurraifah (2019), the use of a bacteriocin concentration of 6.25% of *L. plantarum* IIA-1A5 shows moderate antibacterial activity against *Staphylococcus aureus* with an inhibition zone of 9.29 ± 1.33 mm.

Microbiological Quality

Observations on the microbiological quality of control and treated meatballs include total bacteria, S aureus, and *E. coli*. The results of measurements and statistical analysis of the microbiological quality of buffalo meatballs are presented in Table 2.

Table 2. Microbiological quality of buffalo meatballs when stored at cold temperatures (±5 °C)

Parameter		Storage Time (days)				
		0	3	6	9	12
Total bacteria (cfu/log^{10})	Control	1.87±0.47^{d}	4.88±0.09^{c}	6.11±3.39^{b}	7.39±0.16^{a}	7.99±0.33^{a}
	Supernatant	0.14±0.38^{d}	3.88±0.09^{c}	4.33±0.13^{b}	4.41±0.49^{b}	5.99±0.34^{a}
S. Aureus (cfu/log^{10})	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(cfu/log^{10})	Control	1.10±0.30^{c}	1.49±0.50^{c}	1.93±0.11^{c}	3.56±0.05^{b}	5.56±0.17^{a}
	Supernatant	0.00	0.00	0.00	0.00	0.00

Note: Superscripts with different lowercase letters on the same line indicate significantly different (P<0.05).

Total Bacteria

In Table 2 can see the long cold temperature storage (±5°C) in meatballs without soaking; the cell-free supernatant (control) showed a significant effect (P<0.05) after three days of storage on the total bacteria of control buffalo meatballs during storage between 1.87 ± 0.47 (log cfu/g) - 7.99 ± 0.33 (log cfu/g). Fresh buffalo meatballs (0 days) have shown growing colonies during storage at cold temperatures (± 5^{O}C); the number of colonies increased after three days and did not increase until 12 days of storage. The total bacteria in meatballs without soaking the cell-free supernatant (control) after three days of storage has exceeded the National Standard for Meatball Products (SNI 3818: 2014) (National Standardization Agency, 2014).

Cold storage time (±5°C) buffalo meatballs soaked in supernatant showed a significant increase (P<0.05) in total bacteria. Total bacteria at 0 days of storage was 0.14 ± 0.38, 3 days was 3.88 ± 0.09, 6 days amounted to 4.33 ± 0.13, 9 days amounted to 4.14 ±0.49 and 12 days is 5.99±0.34 (log cfu/g). The results of this study are not much different from Arief et al. (2012); total bacteria in beef meatballs with the addition of bacteriocin from *L. Plantarum* IIA-1A5 is 0 days of storage of 3.65 ± 0.25, 3 days of 4.40 ± 0.00 and 6 days was 4.39 ± 0.00 (log cfu/g). Pato et al. (2022) research results, use of bacteriocins from *Pediococcus pentosaceus* Strain 2397 at a concentration of 0.60% showed the lowest total microbes, namely 0.95 x 10^2 CFU/g for nine days of storage at freezing temperature.

Total bacteria in buffalo meatballs soaked in supernatant during cold storage (± 5°C) are still within the threshold according to (SNI 3818: 2014) (National Standardization Agency, 2014), namely 1x10^5 colony/g. Buffalo meatballs stored for up to 9 days are still in good condition. These results have proven that the antibacterial supernatant is cell-free. *Pediococcus pentosaceus* BAF715 is effective in controlling the growth of buffalo meatball bacteria. The antibacterial action of lactic acid bacteria disrupts cell membranes, affects intracellular biomolecules, and inhibits essential cellular processes. According to Hua Z. et al. (2021), the action of lactic acid from *Leuconostoc mesenteroides* QZ1178 inhibits pathogenic bacteria *Gallibacterium anatis* by inducing cell damage, membrane disruption and cytoplasmic leakage leading to cell lysis.

Total *Staphylococcus aureus.*

Storage time for control meatballs at cold temperature (± 5°C) shows a significant influence (P<0.05) on the total *Staphylococcus aureus***.** Total range *Staphylococcus aureus* buffalo meatballs between 2.75 ± 0.27 (log cfu/g) arrived at 7.13± 0.46 (log cfu/g).

Cold storage time (5°C) of the meatballs soaked in supernatant showed no significant effect (P>0.05) on the total *Staphylococcus aureus.* The results of this study show that the cell-free antibacterial supernatant contains high levels of lactic acid, thereby inhibiting bacteria *St. aureus Pediococcus pentosaceus* BAF715 is a homofermentative lactic acid bacteria that produces 85% lactic acid from its fermentation results. The lactic acid produced is antibacterial and is bacteriostatic against bacteria *Staphylococcus aureus*. Table 2 shows that control meatballs and meatballs soaked in cell-free supernatant showed the presence of colonies *Staphylococcus aureus,* which is growing, possibly due to sanitation and hygiene standards found to be spread through the hands of food workers in the food and restaurant industry (Kadariya et al. 2014). Hand hygiene, washing and disinfection are prerequisites for hygiene management in the food industry to reduce the risk of foodborne infections (WHO, 2011). During storage at cold temperatures, colonies increased up to 12 days. Total *Staphylococcus aureus* control meatballs during storage exceed the Indonesian National Standard for meatball products (SNI 3818:2014) (Badan National Standardization, 2014).

Cold storage time (± 5°C) In meatballs soaked in cell-free supernatant, there was no significant effect (P>0.05) on total bacteria *S. Aureus*. During growth storage, *S aureus* can be inhibited due to storage at cold temperatures (± 5°C) and the inhibitory effectiveness of antibacterial compounds from *Pediococcus pentosaceus* BAF715. The antibacterial activity of *Pediococcus pentosaceus* BAF715 can inhibit the growth of *S. Aureus.* It is characterized by forming a clear zone of 10.50 ± 0.1 mm (Table 1) and the antibacterial produced in organic acids. Organic acids can cause the cytoplasm of pathogenic bacterial cells to become acidic and inhibit transmembrane potential and substrate transport (Alokami et al., 2000). According to Yang (2022), Lacidophilin activity from *Lactobacillus pentosus* disrupts bacterial cytomembranes *S. Aureus*, increases permeability, inhibits phosphorus metabolism, alters proteins and induces oxidative damage, inhibiting bacterial growth. According to Cao J. et al. (2019), the antibacterial lactobionic acid (LBA) damages the cell walls of *S. Aureus* and membrane integrity, causing leakage of cellular contents, inhibiting protein synthesis and cell death. According to Pato et al. (2020), the antibacterial compounds produced by P. *Pentosaceus* 2397 have been shown to have an antibacterial activity that can inhibit growth *S. Aureus*.

Total *S. Aureus* cell-free supernatant of buffalo meatball meatballs during cold storage (± $5^{°C}$) 1.05 ±0.27 log cfu/g to 1.73 ± 0.46 log cfu/g. Total bacteria *S. Aureus* research results within the range of BSN quality standards (2014) (SNI 3818:2014), i.e. total *S. Aureus* for meatball products, the maximum is 1.0 x 10^2 colony/g. Antibacterial cell-free supernatant from *P. Pentosaceus* BAF715 is bacteriostatic and can inhibit bacterial growth in *S. Aureus*. According to Hafsan (2014), antibacterial compounds are chemical or biological compounds that can inhibit the growth and activity of microbes.

Total *Escherichia coli*

Cold storage time (5°C) for the control buffalo meatballs showed a significant effect (P<0.05) on the total *Escherichia coli***.** Total range *Escherichia coli* buffalo meatballs between 1.1 ± 0.30 (log cfu/g) arrived at 4.56±0.05 (log cfu/g). The total Escherichia coli obtained exceeds the National Standard for Meatball Products (SNI 01-3818-1995) (National Standardization Agency, 1995), with a maximum of 3 MPN/g.

Storage at a cold temperature (± 5°C) in the soaked buffalo meatballs, the supernatant was not found *E. coli*. Antibacterial activity of the free supernatant *Pediococcus pentosaceus* BAF715 and cold temperature (± 5°C) can inhibit *E. coli*. Bacteria *E. coli*. It grows well at mesophilic (moderate temperatures), so cold storage (5°C) causes stunted growth. According to Kusumawati (2000), the inhibition of microbial growth is due to the synergistic action between antimicrobial activity and storage temperature.

Antibacterial cell-free supernatant from*Pediococcus pentosaceus* BAF715 is bactericidal against bacteria *E. coli*. These antibacterials have cationic properties and kill target cells by disrupting membrane-potential and cellular solute leakage, which ultimately causes cell death (Diep et al., 2009). Research results from Enyun Ma et al. (2023), antibacterial from *Lactobacillus reuteri* disrupt cell membranes of *E. coli*, affecting biomolecules and changing enzyme levels that cause cell death.

Physical quality of meatballs

Observations on the physical quality of control and treated meatballs included pH value,

water holding capacity (WHC) and Eber's test. The results of measurement and statistical analysis of the physical quality of meatballs are shown in Table 3.

Table 3. Quality Physical characteristics of buffalo meatballs when stored at cold temperatures (5°C)

Parameter		Storage Time (days)				
		0	3	6	9	12
pH	Control	6.3 ± 0.1[a]	6.4±0.10 [a]	6.8±0.20 [b]	6.8±0.10 [b]	6.9±0.10 b
	Supernatant	4.60±0.10[c]	5.10±0.15[b]	5.31±0.21[b]	5.40±0.20[b]	5.85±0.10[a]
% Free Water	Control	26.97±1.05[e]	31.58±0.57[d]	36.93±0.63[c]	39.20±0.78[b]	40.53±1.0[a]
	Supernatant	24.52±0.5[c]	26.58±5.4 [b]	26.09±0.8 [b]	27.96±2.0 [b]	30.68±3.6 [a]
Uji Eber	Control	-	-	+	++	+++
	Supernatant	-	-	-	+	++

Note: Superscripts with different lowercase letters on the same line indicate significantly different (P<0.05).
Negative (-) = no white clouds around the meatballs, positive 1 (+) = a little white cloud formed (10 minutes of observation), positive 2 (++) = quite a lot of white cloud formed (5-10 minutes), positive 3 (+++) = lots of white clouds are formed (< 5 minutes).

pH value

Table 3 shows that control buffalo meatballs during cold storage (± 5°C) increased pH until day 6; the storage pH value on day 6 was no different from days 9 and 12. The pH value of meatballs ranged from 6.7 ± 0.1 to 6.0±0.10. Increase in pH value during cold storage (± 5$^{\circ C}$) because psychrophilic bacteria's growth is ongoing. The pH value dramatically influences the shelf life of processed meat products. According to Suradi (2012), the pH value increases during cold storage due to enzyme activity and the decomposition of chemical compounds, such as proteins that produce essential compounds such as indole, cans, and cadaverine. At specific 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 buffalo meatballs soaked in supernatant increased until day 12; the pH value of buffalo meatballs ranged from 4.60 ± 0.10 to 5.85 ± 0.10. The pH value of buffalo meatballs is influenced by the antibacterial pH value of the cell-free supernatant, namely 4.3. During the soaking process, the lactic acid in the supernatant seeps into the buffalo meatballs, resulting in the buffalo meatballs being acidic. Lactic acid is the primary metabolite produced by*Pediococcus pentosaceus* BAF715, an antibacterial agent against spoilage bacteria and pathogens. The results of the study by Adeniyi et al. (2006) showed that *P. pentasaceus and P. acidilactici give L. plantarum* classified as homofermentative lactic acid bacteria, where the antimicrobial substance in the form of lactic acid contains more than 85% of the results of carbohydrate fermentation.

Storing buffalo meatballs at cold temperatures (± 5°C) until the 9th day produces intense antibacterial inhibitory activity. After nine days of storage, namely 12 days, the antibacterial inhibitory activity becomes weaker. This is because the activity of psychrophilic bacteria continues to break down the food substances in the meatballs, increasing the pH.

Water Binding Power

Water binding capacity is calculated based on the percentage of mgH_2O. Storage time for buffalo meatballs at cold temperatures (± 5°C) has a significant effect (P<0.05) on water binding capacity. The water binding capacity values range between 26.97 ± 1.05 – 40.53±1.05. The length of storage shows that the value of water binding capacity (% mg H_2O) is increasing, which shows that the meat's ability to bind water is decreasing. According to Ismail et al. (2016), the percentage of mgH_2, the higher the O, the lower the ability of the meat to hold water.

The water binding capacity of buffalo meatballs soaked in supernatant did not increase at 3, 6 and 9 days of storage and increased at 12 days of storage. The water binding capacity values range from 24.52±0.5 to 30.68 ± 3.6. The resulting cell-free antibacterial supernatant *Pediococcus pentosaceus* BAF715 can maintain air contained in meatballs to maintain the protein content. The resulting pH value influences the binding capacity of meatball water. The pH value of the meatballs did not differ until nine days of storage, causing the water binding capacity of the meatballs to not increase until nine days of storage. According to Soeparno (2015), the pH

value of meatballs does not change, so the water binding capacity does not change because the ability of meat protein to bind water is influenced by pH. Myofibril meat protein is the substance responsible for binding meat water (Arief et al., 2012)

Eber test

The results of Eber's test showed that the control buffalo meatballs, after six days of storage, had started to rot, as indicated by the presence of steam or white clouds on the tube walls around the buffalo meatballs (+). This rot is synonymous with the activity of putrefactive bacteria, characterized by the formation of foul-smelling compounds such as ammonia H2S, indole and amine, which result from protein breakdown by microorganisms (Suradi, 2012).

The buffalo meatballs soaked in supernatant on day 9 of storage had not experienced any changes marked negative (-). Cell-free antibacterial supernatants were produced by *Pediococcus pentosaceus* BAF715, which can maintain the quality of meatballs for up to 9 days of storage. This condition is related to the meatballs' low pH value and high water binding capacity, which indicates that they can still maintain their physical quality during storage at a temperature of (± 5°C).

CONCLUSIONS

Cell-free antibacterial supernatants produced by *pentosaceus* BAF715 can maintain the microbiological and physical quality of meatball buffalo meat for up to 9 days of storage at cold temperatures(± 5°C). Cell-free antibacterial supernatants were produced by *pediococcus pentosaceus* BAF715, which can be recommended as *bio preservative* beef meatballs.

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