Research Article

Application of Bacteriocin from *Pediococcus pentosaceus* Strain 2397 as Biopreservatives for Fishballs During Cold Storage

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Abstract | Fishballs are perishable foods having complete nutrients is suitable for microbial growth. Bacteriocins are natural biopreservative from lactic acid bacteria. This study is conducted to obtain a proper concentration of bacteriocin to preserve the fishballs and extend their shelf-life without compromising on quality. In a randomized design, five different concentrations of bacteriocins were used. The treatments in this study were the addition of the bacteriocin concentration, namely B1 (0%), B2 (0.15%), B3 (0.3%), B4 (0.45%), and B5 (0.6%). Samples were stored in a cold environment for different durations, such as 0, 3, 6, 9, and 12 days. Data were analyzed statistically using ANOVA, and differences between treatments were analyzed using DNMRT at the 5% level. The result showed that the concentration of bacteriocin significantly affected moisture, ash, and protein contents but did not significantly influence the descriptive test. The best treatment in this study was B5 (the addition of bacteriocin 0.6%), which can maintain the quality of fishballs for up to 12 days at cold temperatures with a moisture content of 73.10%, ash 1.29%, protein 8.35%, and a descriptive test had a surface less smooth, slightly hollow and less bright, odour and taste less specific product and texture solid, compact, and rather springy. Adding 0.60% bacteriocin produced fishballs that could maintain quality according to fishball quality standards for 12 days of storage at cold temperatures.

Keywords | Antimicrobial activity, bacteriocin, *Pediococcus pentosaceus*, natural biopreservative, fishballs, cold temperature

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INTRODUCTION

Meatballs are a traditional processed meat product the community favours and have high economic value. Meatballs are made from ground beef with tapioca flour and spices, with or without adding other food ingredients, shaped into rounds, and cooked (National Agency of Drug and Food Control, 2019). Meats commonly used in making meatballs are beef, buffalo, lamb, goat, chicken, and fish. Fishballs are food products in the form of spheres or other shapes, obtained from a mixture of fish meat or surimi (fish content not less than 40%) and with starch or cereals, and without the addition of permitted food additives. Good fishballs have a pure white colour, compact texture, and are chewy (Indonesian National Standardization, 2017). Catfish is one of the fish meats that can be used as raw material for making fishballs.

Catfish is a freshwater fish that is widely cultivated in Indonesia. Catfish production in 2019 in Indonesia reached 384,883 tons, while in Riau Province, it reached 27,335 tons (Central Bureau of Statistics, 2020). Catfish have thick meat and contain high protein. The catfish yield reaches 40-50% (Dewita *et al*., 2012), and the protein content in catfish meat reaches 17.0% (Mahmud *et al*., 2018). Based on these data, catfish have the potential to be used as a raw material for making fishballs. Sinaga *et al.* (2013) have characterized catfish meatballs with the addition of carrageenan, soy protein isolate, and sodium tripolyphosphate. The results showed that the average water content of catfishballs ranged from 70.14-75.41%, protein content from 3.98-7.13%, and fat content from 1.4 to 2.16%. Based on the nutritional content, catfish meatballs are a food product with relatively high nutrition and properties that are easily damaged due to the activity of microorganisms. Therefore, it is necessary to carry out preservation techniques to extend the shelf life of fishballs.

Preservatives currently widely used by the public in making meatballs are synthetic preservatives such as nitrites. Anand and Sati (2013) stated that nitrites could react with proteins in the body and produce nitrosamines, carcinogenic substances. This fact makes using nitrites as preservatives detrimental to health if consumed. To overcome this, we need alternative natural food preservatives to maintain fishballs' quality and shelf life. One of the natural preservatives that can be used is bacteriocin. Lactic acid bacteria are found in many fermented foods and can be used as food biopreservation agents. Lactic acid bacteria act as a natural preservative by producing antimicrobial metabolites, including organic acids, diacetyl, ethanol, hydrogen peroxide, and bacteriocins (O'Connor *et al.*, 2020). Lactic acid bacteria can grow antimicrobial components, including bacteriocins. Bacteriocins made from lactic acid bacteria can be used as natural biopreservatives to replace synthetic preservatives. Bacteriocins can preserve products because they inhibit the growth of pathogenic and spoilage microbes (Yang *et al.*, 2012).

Bacteriocins are proteins produced by lactic acid bacteria with bactericidal properties and have been widely used in food biopreservatives (Savadogo *et al.*, 2006). Bacteriocins can be made by Lactococcus, Lactobacillus, and Pediococcus bacteria (O'Connor *et al.*, 2020). One species of the genus Pediococcus that has the potential to make bacteriocins is *Pediococcus pentosaceus*. The molecular weight of strain 2397 of *Pediococcus pentosaceus*' bacteriocin is 14.4 kDa (Pato *et al.*, 2022a). It can inhibit the growth of *Staphylococcus aureus* (Pato *et al.*, 2020a), *Listeria monocytogenes* (Pato *et al.*, 2020b), *Escherichia coli* (Pato *et al.*, 2021), and *Erwinia carotovora* (Pato *et al.*, 2020b). Sinaga (2013) utilized bacteriocin with a concentration of 0.3% in beef sausage products. Bacteriocins can extend the shelf life of sausages at cold temperatures until storage on the 6th day. In contrast, control sausages only reach the 3rd day because the growth rate of *Staphylococcus aureus* has exceeded the limit of SNI 3820:2015. Aritonang *et al.* (2020) stated that using 0.9% bacteriocin extended the shelf life of sausages in cold storage for 12 days. Bacteriocin as a natural preservative for meatballs has been used by Arief *et al.* (2012). The addition of bacteriocin with a concentration of 0.3% extended the shelf life of meatballs at 4°C until the 6th day.

In contrast, control on the 3rd day, the number of microbes exceeded the limit of SNI 7266:2017. Using bacteriocin from *Lactobacillus plantarum* 2C12 can inhibit the growth of *Escherichia coli, Staphylococcus aureus*, Salmonella sp, and *Pseudomonas aeruginosa* bacteria. The use of bacteriocins can maintain the nutritional value of the meatballs and does not cause changes in their physical and sensory characteristics. This study aimed to determine the appropriate concentration of bacteriocin from *Pediococcus pentosaceus* Strain 2397 on fishballs' quality and shelf life based on SNI 7266:2017 (Indonesian National Standardization, 2017).

MATERIALS AND METHODS

Raw Materials, Media, Chemicals

Fishballs were mainly made from cultured catfish (*Pangasianodon hypophthalmus*) that weighed 300 to 325 g and measured 25 to 30 cm in length (Figure 1). Other primary raw materials for manufacturing fishballs were tapioca, garlic, table salt, pepper and ice.

Figure 1: Catfish (*Pangasianodon hypophthalmus*)

Activation Of *P. Pentasaceus* Strain 2387

P. pentosaceus strain 2397 isolated from dadih from Bukittingi, West Sumatera, Indonesia (Hosono *et al.*, 1989) was used in this study. Bacterial activation was carried out according to Pato *et al.* (2017). A total of 1 ml of *P. pentosaceus* strain 2397 isolate was inoculated into a test tube containing 5 ml MRSB medium and incubated at 37°C for 24 h. The active culture obtained was marked by a change in the medium to become cloudy.

Bacterial Propagation

Bacterial propagation was carried out according to Pato *et al.* (2017). Propagation of *Pediococcus pentosaceus* strain 2397 was carried out by taking 1 ml of activated bacterial suspension using a micropipette and then inoculating it into a test tube containing MRSB medium. *Pediococcus pentosaceus* strain 2397 was incubated at 37°C for 24 h.

Preparation Of Cell-Free Supernatant

The cell-free supernatant was prepared according to Pato *et al.* (2021). A total of 15 ml of *Pediococcus pentosaceus* culture was inoculated into an Erlenmeyer containing 300 ml MRSB medium, which had been added with 6 ml of yeast extract and incubated at 37⁰C for 48 h. The incubated medium was poured into a 15 ml centrifuge tube and centrifuged at 4500 rpm for 10 min. The separated liquid is called cell-free supernatant, which contains metabolite compounds formed during fermentation.

Bacteriocin Preparation

Proteins from cell free supernatant were separated by ammonium sulfate precipitation (Pato *et al.*, 2021). The cell-free supernatant was added with 70% ammonium sulfate. Briefly, 300 ml supernatant and 143.32 g of ammonium sulfate were added, stirred until dissolved and placed in the refrigerator at 4˚C to precipitate the protein. This mixture was then put in a 15 ml centrifuge tube and centrifuged again to obtain pellets. The pellet formed was dissolved with phosphate buffer (0.1 M, pH 7.0). As much as 1 ml of phosphate buffer was put into a centrifuge tube, stirred using an ose needle, then poured into another tube and allowed to stand for 24 h in the refrigerator at 4˚C to obtain concentrated bacteriocin.

Preparation Of Fishballs

The formula and preparation of fishballs were carried out according to Sinaga *et al.* (2013). Weigh the catfish fillets as much as 200 g, then put them in the chopper with garlic, salt, and pepper. The bacteriocin was added according to treatment (0 g, 0.3 g, 0.6 g, 0.9 g, and 1.2 g v/w catfish fillet). Bacteriocin is mixed into ice water to mix in the dough evenly. All ingredients are ground until smooth. The smooth meatball dough is added to the tapioca little by little while kneading until smooth. The dough is printed into meatballs using a spoon and boiled in boiling water for 10-15 min until the meatballs float. After that, the meatballs are drained and cooled, after which they are packaged using polypropylene plastic containers. Packaged catfish meatballs are then stored in the refrigerator.

Storage Of Fishballs

Arief *et al.* (2012) carried out the storage of fishballs. Fishballs are stored in the refrigerator at a cold temperature (4°C). Storage is carried out for 12 days, and analysis every 3 days. Observations were made on moisture content, ash content, protein content, and sensory analysis descriptively.

Chemical And Microbiological Analysis

Following AOAC recommendations, chemical components, including ash and moisture contents, were evaluated (1999). *Escherichia coli* was quantified using the MPN method following ISO 7251, and the total plate count and amount of *Staphylococcus aureus* were quantified using the surface dish method per AOAC (2005).

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Ethical Approval

The Research Ethics Committee of the Faculty of Nursing, Universitas Riau, Pekanbaru, Indonesia, approved this experimental investigation on sensory analysis (Reference No. 156/UN.19.5.1.8/KEPK.FKp/2021).

Sensory Analysis

According to Setyaningsih *et al.* (2010), a sensory assessment was carried out. The sensory test used is a descriptive test. The sensory evaluation was conducted by 15 semi-trained panellists from students who had passed the Sensory Evaluation course. A descriptive sensory assessment was conducted to determine the panellist's assessment of the fishballs' appearance, aroma, taste, and texture in each treatment sample. The sensory evaluation was conducted by presenting a sample of fishballs as much as one fishball (± 5 g) according to the treatment in a clean container coded with random numbers. The presenter provides mineral water, tissues, sensory test forms, and stationery. Samples stored at cold temperatures on days 3, 6, 9, and 12 were boiled for about 10 min, cooled, and then presented to the panellists. The sensory assessment form refers to the Indonesian National Standardization Agency (Indonesian National Standardization, 2015).

Experimental Design And Data Analysis

This study was conducted in an experimental setting with a completely randomized design. Fishballs were frozen nine days after exposure to five different bacteriocin concentration treatments. Each of the research procedures was used three times. Fishball chemical composition and sensory analysis data were statistically analyzed at the 5% level using the Duncan Multiple Range Test (DMRT) and Analysis of Variance (ANOVA) in SPSS Software Version 23. Data on the microbiological counts of fishballs were gathered and descriptively analyzed.

RESULTS AND DISCUSSION

Table 1 shows fishballs' protein, moisture, and ash contents following DMRT at the 5% level. One of the elements that can impact the shelf life of fishballs is their moisture level. The higher the free moisture content in the food, the faster the food spoils. The variance results showed that adding bacteriocin to fishballs significantly affected the water content of fishballs during cold storage. In general, the water content of fishballs with the addition of bacteriocins increased with increasing concentrations of the added bacteriocins. The increase in the water content of fishballs is thought to be due to bacteriocins with water-binding properties. Negash and Tsehai (2020) stated that bacte

Table 1: The chemical properties of fishballs preserved by addition of various concentrations of bacteriocin during cold storage

Concentration of bacteriocin	**Moisture content (%) on the day**				
	0	**3**	**6**	**9**	**12**
0.0%	69.57[a]	70.78[a]	71.68[a]	71.72[a]	72.31[a]
0.15%	69.60[a]	71.00[a]	71.88[a]	71.94[ab]	72.45[ab]
0.30%	70.20[a]	71.31[a]	72.03[a]	72.06[ab]	72.50[ab]
0.45%	71.25[b]	72.04[b]	72.04[a]	72.27[b]	72.81[bc]
0.60%	72.23[c]	72.30[b]	72.93[b]	72.90[c]	73.10[c]
Concentration of bacteriocin	Ash (%) at day				
	0	3	6	9	12
0.0%	1.12[a]	0.95[a]	0.97[a]	0.97[a]	1.13[a]
0.15%	1.18[ab]	1.13[b]	1.06[b]	1.00[ab]	1.16[ab]
0.30%	1.22[ab]	1.24[b]	1.16[c]	1.10[bc]	1.17[b]
0.45%	1.24[b]	1.27[b]	1.18[c]	1.16[c]	1.25[c]
0.60%	1.46[c]	1.25[b]	1.26[d]	1.23[c]	1.29[d]
Concentration of bacteriocin	Protein (%) at day				
	0	3	6	9	12
0.0%	8.79[a]	9.72[a]	8.39[a]	6.67[a]	5.73[a]
0.15%	9.25[b]	9.61[a]	8.69[a]	6.89[a]	6.02[a]
0.30%	9.74[c]	9.82[ab]	8.83[ab]	7.13[a]	7.67[b]
0.45%	9.87[c]	10.18[bc]	9.49[b]	9.59[b]	8.05[c]
0.60%	10.45[d]	10.25[c]	12.53[c]	9.72[b]	8.35[c]

Means followed by the lowercase letters indicate a significant difference (P < 0.05).

riocins are peptides with amphiphilic properties to bind water. Lombardo *et al.* (2015) stated that amphiphiles are compounds in which one of the groups has hydrophilic properties. This fact resulted in the higher the concentration of bacteriocins added, the more water contained in the fishballs. In line with the research of Arief *et al.* (2012), the moisture content of sausages with the addition of bacteriocin (79.09%) was higher than the control (76.67%). The data in Table 1 shows that the water content of fishballs has increased during cold storage; this is caused by protein degradation during storage. Herlinda *et al.* (2018) stated that the increase in water content during cold storage occurred due to protein degradation into simple compounds such as trimethylamine, ammonia, and water due to enzymatic and microbiological activities, so the water content in the material increased. The increase in water content is in line with the research of Aritonang *et al.* (2020), which stated that the moisture content of sausages stored at 4°C increased during storage, with the moisture content of storage on day 0 ranging from 52.74 to 52.85% and on the 12th day of storage ranging from 53. 09-54.20%. The water content of fishballs during storage does not meet the quality requirements of fishballs based on SNI 7266: 2017, which states that the maximum moisture content is 70%. The addition of bacteriocins can increase the water content of fishballs but can maintain the water content during storage. The water content of food products is affected by the main ingredients, additives, processing, packaging, and storage processes.

Ash is an inorganic residue that results from burning fuel at a high temperature. Ash content provides information on a food's mineral content. The findings demonstrated that the ash content of fishballs during cold storage was significantly impacted by the addition of bacteriocins. In general, the ash content of fishballs increased with the increasing concentration of bacteriocins used. This finding is thought to result from the bacteriocin used being crude bacteriocin. Because the bacteriocin employed is partially purified, ammonium sulfate's sulphur residue can be left behind. Hapsari *et al.* (2021) state that additional purification is required since the protein precipitated with ammonium sulphate salt still includes ammonium sulphate. Wardani and Nindita (2012) stated that dialysis is needed to remove the residual ammonium sulfate salt after protein deposition. Pato *et al.* (2022b) noted that the more bacteriocins added to fishballs, the higher the amount of sulfur, which contributes to an increase in the ash content of fishballs. In line with the study of Fuziawan (2012), the ash content of meatballs with the addition of 0.3% bacteriocin was higher than the control, where the ash content of meatballs with bacteriocin was 5.26%, while the

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ash content of the control was 3.97%. The data in Table 1 shows that the ash content of the fishballs during storage tended not to change because the fishballs were stored in propylene plastic packaging, preventing other minerals from entering the fishballs during storage. Based on Situmorang (2013), storage time did not significantly affect beef sausage ash because the packaging prevents minerals or other contaminants from entering the sausages. The ash content of fishballs meets the quality requirements based on SNI 7266: 2017, which is a maximum of 2.5%.

Protein is one of the essential macromolecules in food—protein is a fuel for energy, building substances, and regulators in the body. The results showed that adding bacteriocin to fishballs significantly affected the protein content of fishballs during cold storage. In general, the protein content of fish balls increased from 5.73% to 8.35% on day 12 along with the increase in bacteriocin concentration. The increase in protein levels was due to the addition of bacteriocins and peptides produced by *Pediococcus pentosaceus* strain 2397. O'Connor *et al.* (2020) stated that bacteriocins are peptides synthesized from bacterial ribosomes. Based on Pato *et al.* (2020), *Pediococcus pentosaceus* strain 2397 is a bacteriocin-producing LAB. Bacteriocins are protein compounds resistant to heat temperatures up to 121°C, resistant to changes in pH, and have antimicrobial activity. The findings of this investigation are consistent with those of Aritonang *et al.* (2020), who found that adding 0.3-0.9% bacteriocins to the sausage treatment raised its protein content from 18.37% in the treatment without bacteriocins to 18.72-19.53%. Table 1 also shows a decrease in protein content during storage. This fact is thought to be due to dissolved protein lost with water during t the thawing process. Prihatiningsih *et al.* (2020) stated that thawing could reduce protein levels due to the hydrophilic nature of the protein, which allows the protein to dissolve in water and come out of the product. Research by Nento and Ibrahim (2017) states that the liquid from the product causes nutrients such as salts, polypeptides, amino acids, and others that dissolve in water to be carried away with the water that comes from the ingredients. The protein content of fishballs generally met the quality requirements for fishballs based on SNI 7266: 2017, namely a minimum of 7. However, fishballs treated B1 and B2 on days 9 and 12 no longer met the requirements.

One of the criteria for fishball quality is the quantity of harmful and spoilage bacteria. The study's findings are shown in Table 2. The higher the bacteriocin concentration, the lower the amount of TPC. The lowest number of microbes was found in fishballs, which were given bacteriocin with a concentration of 0.45 - 0.60%. The longer the storage time at cold temperatures, the higher the TPC count. Using 0.45–0.60%, bacteriocin maintained the TPC amount between 0.38 x 10^5 - 0.71 x 10^5 CFU/g on day 9, which still met meatball quality standards. However, the amount of TPC in the treatments with and without the addition of bacteriocin 0.15-0.30% on day 9 showed the amount of TPC was 0.11 x 10^6 CFU/g, an amount which did not meet the fishball quality standard (maximum 1 x 10^6 CFU/g). On the 12th day, only the treatment with the addition of 0.60% bacteriocin still met the fishball quality requirements. In contrast, the other treatments did not meet the fishball quality requirements because the microbial count had exceeded 1 x 10^6 CFU/g. Fishballs stored at cold temperatures for more than 12 days did not meet the quality requirements in all treatments.

The absence of *S. aureus* growth in all treatments of the catfish meatball samples is most likely due to the appropriate sanitation and hygiene standards during the processing. Table 3 also showed that on day 0, the use of 0.6% bacteriocin inhibited the growth of *E. coli*. This fact is supported by our previous research, which found bacteriocins could inhibit the growth of pathogenic *E. coli in vitro* (Pato *et al.*, 2021). From day 3 to day 12, no further growth of *E. coli* was found. This finding is probably due to the bacteria not growing and dying in cold temperatures. *E. coli* grows well at moderate temperatures, around 36-40°C (Jang *et al.*, 2017).

Appearance assessment aims to determine panellist acceptance of product characteristics, namely surface appearance, odour, taste, and texture. The variance analysis showed that adding bacteriocin did not significantly affect the appearance, aroma, and taste of fishballs but significantly influenced the texture of fishballs (Table 3).

The average score ranges from 6.87-8.07% (the surface is relatively smooth, slightly hollow, and rather bright). Observations on days 0, 3, 6, 9 and 12 showed that the bacteriocin addition treatment was not significantly different between treatments. The control fishballs were not subjected to sensory analysis on the 12th day because they had gone wrong (change in aroma and texture to become soft and runny). Rotten fishballs are caused by the growth of spoilage bacteria whose numbers exceed the quality standard based on SNI 7266: 2017, which is 10^7 log CFU/g. The data in Table 3 shows that adding bacteriocins did not change the appearance of the fishballs. This finding is due to the added bacteriocin, which acts as a preservative and does not change the appearance of the fishballs. Zacharof *et al.* (2013) stated that bacteriocins would not affect sensory quality when added to food. This finding is in line with research conducted by Fuziawan (2012), who noted that adding bacteriocin did not cause changes in the physical and sensory characteristics of the meatballs. The resulting fishballs have a relatively smooth surface, are slightly

Table 2: Microbial counts of fishballs preserved by addition of various concentrations of bacteriocin during cold storage

Concentration of bacteriocin	**Total Plate Count (CFU/g) on day**				
	0	**3**	**6**	**9**	**12**
0.0%	3.17×10^2	1.78×10^4	1.69×10^5	0.11×10^6	1.86×10^6
0.15%	1.43×10^2	1.23×10^4	1.74×10^5	0.95×10^6	1.13×10^6
0.30%	0.53×10^2	3.12×10^3	3.38×10^4	0.12×10^6	1.37×10^6
0.45%	0.37×10^2	6.06×10^3	4.34×10^4	0.71×10^5	0.94×10^6
0.60%	0.20×10^2	5.10×10^3	3.58×10^4	0.38×10^5	1.27×10^5
Concentration of bacteriocin	*Staphylococcus aureus* (CFU/g) on day				
	0	3	6	9	12
0.0%	0	0	0	0	0
0.15%	0	0	0	0	0
0.30%	0	0	0	0	0
0.45%	0	0	0	0	0
0.60%	0	0	0	0	0
Concentration of bacteriocin	*E. coli* (MPN/g) on day				
	0	3	6	9	12
0.0%	14.7	0	0	0	0
0.15%	7.70	0	0	0	0
0.30%	13.0	0	0	0	0
0.45%	10.7	0	0	0	0
0.60%	0	0	0	0	0

Table 3: Sensory evaluation of fishballs preserved by addition of various concentrations of bacteriocin during cold storage

Concentration of bacteriocin	**Appearance at day**				
	0	**3**	**6**	**9**	**12**
0.0	7,13	7,13	7,00	6,87	-*
0.15%	7,53	7,53	7,40	7,00	6,87
0.30%	7,67	7,53	7,40	7,13	7,00
0.45%	8,07	8,07	7,27	7,27	7,27
0.60%	7,93	7,93	7,40	7,27	7,00
Concentration of bacteriocin	Odor at day				
	0	3	6	9	12
0.0%	7.67	7.53	7.40	6.87	-*
0.15%	8.47	7.93	7.67	7.00	6.60
0.30%	8.07	7.80	7.67	7.27	7.00
0.45%	8.33	7.80	7.80	7.53	7.40
0.60%	7.00	8.47	8.47	7.93	7.40
Concentration of bacteriocin	Taste at day				
	0	3	6	9	12
0.0%	7.53	6.73	6.73	6.60	-*
0.15%	7.80	7.27	7.13	7.00	7.00
0.30%	7.53	7.27	7.00	6.73	6.33
0.45%	7.67	7.40	7.40	7.27	6.87
0.60%	8.33	7.67	7.93	7.67	7.27
Concentration of bacteriocin	Texture at day				
	0	3	6	9	12

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0.0%	7.67	7.53	7.40	7.13	-*
0.15%	7.67	7.67	7.53	7.53	6.00[a]
0.30%	7.67	7.67	7.13	7.00	7.27[bc]
0.45%	8.20	7.67	7.53	7.53	7.00[ab]
0.60%	8.33	8.20	8.20	7.93	7.40[c]

Means followed by the lowercase letters indicate a significant difference ($P < 0.05$)
* The sample was damaged, so no sensory analysis was performed

hollow, and are rather bright (Figure 2).

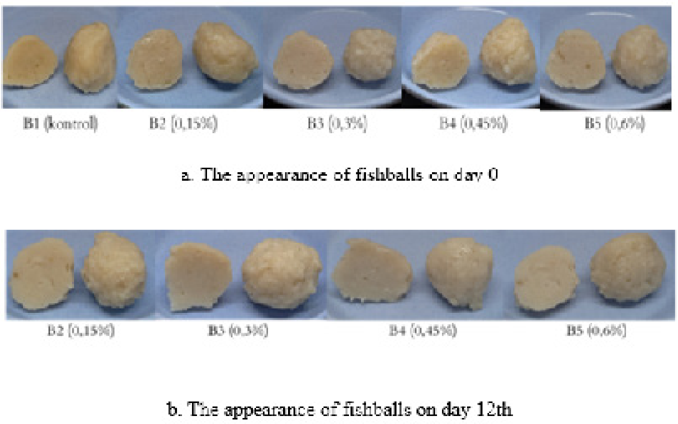

Figure 2: The appearance of fishballs during cold storage

Golge *et al.* (2018) research stated that the use of meatball surfaces is influenced by the fiber content contained in the ingredients. Starch is low in fiber and produces meatballs with a smooth surface. Using catfish and tapioca meat makes meatballs that have a relatively bright appearance. Setyadi *et al.* (2020) stated that white catfish meat produces bright meatballs. Furthermore, based on research by Husain *et al.* (2021) regarding the manufacture of tuna fishballs indicated that tapioca would form a brighter amylopectin paste. Based on the data in Table 7, fishballs meet the requirements of SNI 7266: 2017 with a minimum score of 7 (slightly smooth surface, somewhat hollow, and a little bright).

Aroma is an odour caused by chemical stimuli that the olfactory nerves in the nasal cavity smell. The variance results showed that adding bacteriocins did not significantly influence the aroma of fishballs produced during cold storage. The average score obtained ranges from 6.60-8.47 (rather product-specific to product-specific), which is catfish-scented. Observations on days 0, 3, 6, 9, and 12 showed that the bacteriocin addition treatment was not significantly different between treatments. The control fishballs were not subjected to sensory analysis on the 12th day because they had gone bad (change in aroma and texture to become soft and runny). The addition of bacteriocin did not affect the aroma of the resulting fishballs. This fact is because bacteriocins will not affect sensory quality when added to food. Zacharof *et al.* (2013). In line with research conducted by Fuziawan (2012), bacteriocins did not change the aroma assessment of meatballs. The aroma of the catfish meat used influences the aroma of the meatballs. Setyadi *et al.* (2020) stated that the use of catfish produces a distinctive fishy odour from fish caused by the protein content in fish. The aroma in fishballs arises from the evaporation of volatile compounds in fish meat during processing or cooking, such as mercaptans, skatol, and H_2S (Harmain *et al.*, 2017). In addition, the rather specific aroma of the product is also influenced by the use of spices such as garlic and pepper (Zakaria *et al.*, 2010). In general, fishballs meet the sensory quality requirements of fishballs based on SNI 7266: 2017 with a minimum score of 7 (slightly product-specific aroma).

Taste is one of the impressions received through the senses of taste and is a parameter that determines the level of preference of the panellists for the product produced. Taste is formed from a combination of food ingredients. The variance analysis showed that adding bacteriocins did not significantly affect the taste of fishballs during cold storage. The average score obtained ranged from 6.33-8.33 (rather product-specific to product-specific), namely the taste of catfish. Observations on days 0, 3, 6, 9, and 12 showed that the bacteriocin addition treatment was not significantly different between treatments. The control fishballs were not subjected to sensory analysis on the 12th day because they had rotted (changes in aroma and texture to become soft and watery). The addition of bacteriocin did not change the taste of fishballs. This fact follows the statement of Patrovský *et al.* (2016), who stated that adding bacteriocins did not alter food taste. The taste of catfish meatballs is formed from protein and fat compounds in fish meat. The amino acid components in catfish include glycine, lysine, and arginine, while the fatty acids in catfish are palmitic acid, stearic acid, oleic acid, and arachidonic acid (Yuniarti *et al.*, 2021). Odour is formed due to the heating process, resulting in the interaction of the meat components. The specific taste of fish meatball products is also influenced by adding additional ingredients such as pepper, garlic, and salt (Salanggon *et al.*, 2017). Sensory assessment of the taste of fishballs generally meets the requirements for sensory quality of fishballs based on SNI 7266: 2017 with a minimum score of 7 (taste somewhat specific to the product).

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The texture is one factor that determines a product's acceptance. Texture changes in foodstuffs are caused by loss of water or fat, emulsion formation, carbohydrate hydrolysis, and protein coagulation. The analysis of variance showed that the addition of bacteriocins significantly affected the texture of the fishballs on day 12. In contrast, on days 0, 3, 6, and 9, the effect was insignificant during cold storage. The average score ranged from 6.00-8.33 (dense, compact, slightly rubbery to solid, compact, elastic). Observations on days 0 and 9 showed that the bacteriocin addition treatment was not significantly different between treatments. On the 12th day, the scores for treatment B3 were significantly different from treatments B4 and B5 but not significantly different from treatment B1. The control fishballs were not subjected to sensory analysis on the 12th day because they had gone bad (change in aroma and texture to become soft and runny). The addition of bacteriocin did not affect the texture of the fishballs. This finding follows the statement of Zacharof *et al*. (2013), who stated that bacteriocins would not affect sensory quality when added to food. The texture of the fishballs is influenced by tapioca, protein, and water in the ingredients. Salanggon *et al*. (2017) stated that tapioca, which contains high amylopectin, affects the elasticity of the meatballs. Hydrophilic Proteins in the material will cause the texture to become flexible and compact (Musdalifah & Tanod, 2016). The low water content makes the texture of the meatballs relatively dense (Poernomo *et al*., 2013). Sensory assessment of fishballs' texture generally meets the sensory quality requirements of fishballs based on SNI 7266: 2017 with a minimum score of 7 (dense, somewhat compact, and chewy texture).

CONCLUSIONS

Based on the results of the research that has been done, it can be concluded that the addition of bacteriocin concentrations from *Pediococcus pentosaceus* strain 2397 significantly affected moisture content, ash, and protein contents but did not significantly influence the sensory assessment of appearance, aroma, taste, and texture of fishballs. Treatment B5 (addition of 0.6% bacteriocin) is the selected treatment that meets fishballs' quality (SNI 7266: 2017) with a moisture content of 73.10%, ash of 1.29%, and protein of 8.35%. Descriptive sensory assessment of appearance (slightly smooth surface, somewhat hollow, little bright), aroma and taste (slightly product specific) and texture (solid, compact, somewhat chewy). The addition of 0.6% bacteriocin maintained the quality of fishballs until the 12th day of storage at cold temperatures.

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CONFLICT OF INTEREST

None of the authors has any conflict of interest to declare.

NOVELTY STATEMENT

This study is the first to describe the use of bacteriocin, a naturally preservative for fishballs, as generated from Pediococcus pentosaceus strain 2397, a local lactic acid bacterium from Indonesia.

AUTHORS CONTRIBUTION

Research on the main theme was initiated by UP, who also oversaw various stages of the project. SF carried out the next research stages in this study, designing the experiments and supervising several phases of the work. The main contributors to research and writing articles are UP, YY, and ER. This experiment was planned by SF, UP, and EmR, who also proofread the article. Article writing and editing were done in collaboration with SF and UP.

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