Effect of adding bacteriocin from *Lactobacillus pentosus* strain MIL 195 on the quality of chicken sausage as an alternative natural preservative

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Abstract. One of the preservatives often used in processed meat, such as sausages, is nitrite; however, bacteriocins produced by lactic acid bacteria (LAB) offer a natural concentration of bacteriocin from *Lactobacillus pentosus* st MIL 195 to use as a natural preservative for chicken sausages. A completely randomized design was employed with five bacteriocin concentrations (0.0%, 0.2%, 0.3%, 0.4%, and 0.5% v/w) and three replications. Parameters analyzed included water content, ash, fat, protein, total LAB count, and sensory characteristics. The results indicated that 0.4% bacteriocin provided the most effective preservation. After nine days, these sausages met SNI-01-3820-2015 quality standards, exhibiting 57.52% water content, 2.54% ash, 15.40% protein, 5.55% fat, and a total microbial count of 5.23 log cfu.g-1. Sensory analysis revealed a grayish-white color, a slightly chicken aroma, a slightly meaty taste, and a chewy texture. Importantly, consumer preference was highest for sausages treated with 0.4% bacteriocin. Bacteriocins can inhibit the growth of spoilage bacteria; therefore, the effect can extend the shelf life of food goods and improve food security.

1 Introduction

Agricultural natural resources, both vegetable and animal, support the development of the food processing industry in Indonesia. Many processed food products have been produced and have changed for the better. One processed food product in great demand is processed animal food made from meat, such as sausage.

Sausages are meat products that contain fillers and binders such as vegetable flour or starch, seasonings, and other food ingredients. These components are subsequently filled into sausage casings [1]. Sausages are generally made from meats such as chicken, fish, beef, and rabbit [2]. Additionally, meat can enhance flavor and nutritional value by combining it with various food ingredients, such as *Merang* mushroom (*Volvariella volvacea*). The *Merang* mushroom is a vegetable known for its substantial fiber content of 4% w/w-1. The *Merang* mushroom possesses a unique texture, flavor, and comprehensive nutritional profile. *Merang*

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mushrooms have sufficient nutritional value to serve as primary ingredients and supplements in producing processed food items.

Sausages made from beef or chicken have a relatively short shelf life. The decrease in the shelf life of sausages can be influenced by inappropriate storage techniques, such as storing at room temperature. Decreased shelf life can be prevented by storing sausages at low temperatures in the refrigerator. According to United States Department of Agriculture recommendations, fresh sausages stored in the refrigerator at 4ºC have a shelf life of 1–2 days, while sausages that are smoked first have a shelf life of about 6–7 days, and semi-dry sausages have a shelf life of about three weeks [3]. Efforts can be made to extend the shelf life of sausages, one of which is by adding preservatives to the sausage. One preservative that is often used is nitrite or nitrate. Nitrite and nitrate are food additives extensively studied in meats. Their levels are contingent upon the particular product qualities, and no research is currently available regarding these distinctions [4].

The continuous use of nitrite is not suitable for the health of the human body. Regulation of the Minister of Health No. 033 of 2012 and Regulation of the Head of BPOM RI No. 36 of 2013 concerning the maximum use of nitrite preservatives in processed meat products is 30 mg.kg-1. Excessive use of nitrite harms the body. Using nitrates and nitrites in processed meat products plays a vital role in preservation, but these compounds have potential carcinogenic effects [5]. Nitrites can combine with the amino and amide groups found in animal proteins, creating carcinogenic nitrosamines [5]. Chronic poisoning occurs when nitrites are used repeatedly over a long period. One way to reduce nitrite as a preservative is to use natural preservatives such as bacteriocins [6].

Bacteriocins are antimicrobial proteins produced by bacteria that can kill or inhibit the growth of other microorganisms [7]. Bacteriocins can be utilized as natural preservatives in food products that are safe for consumption, such as meat [8,9] and fish [10]sausage. Other potential uses of LAB bacteriocin include guaranteeing the safety of fruit goods because its peptides have antibacterial properties against various spoilage and harmful microorganisms associated with these items [5]. The substances contained in bacteriocins are proteins that can be degraded by proteolytic enzymes [8]. Bacteriocin used as a preservative, can be obtained from lactic acid bacteria (LAB) isolated from various sources such as palm sap [11], whey [12], and solid waste of soymilk production [13]. Some LABs that produce bacteriocins are *Lactobacillus pentosus* [14], *L. plantarum* [15], and other microorganisms such as *Pectobacterium carotovorum* subsp. carotovorum [16].

Bacteriocins are used as a natural preservative to replace the use of nitrites in sausages, which can suppress the growth of pathogenic bacteria and extend the shelf life of sausages [17]. Using 0.3% bacteriocin as a natural preservative can maintain the quality of fish meatballs in frozen storage for 30 days [18]. Research conducted by [19] determined that the application of 0.3% bacteriocin derived from *L. plantarum* 2C12 effectively suppressed the proliferation of *Escherichia coli, Staphylococcus aureus,* and *Salmonella typhimurium* in meatballs while preserving their original flavor. This research aimed to determine the best concentration of bacteriocin from *L. pentosus*, which is used as a natural preservative for sausages and meets the Indonesian standard for sausage quality SNI 01-3820-2015 [20].

2 Materials and methods

2.1 Materials, media, and chemicals

Lactobacillus pentosus strain MIL 195 isolated from okara [21]. Sausage ingredients: chicken breast, straw mushrooms, tapioca, garlic powder, ground pepper, ground nutmeg, cooking oil, table salt, ice water, granulated sugar, and sausage casings (Edible film).

Analytical materials: *de-Man Rogosa Sharpe-broth* (MRS-B) (Merck), N-hexane, selenium reagent, methyl red indicator, HCL 0.1 N, NaCl 0.85%, H2SO4 98%, NaOH 40%, NaOH 0.1 N, alcohol 95 %, and distilled water.

2.2 Methods

A completely randomized design with five treatments and three replications was used in this study. The treatments were the crude bacteriocin concentrations, namely: A0 = bacteriocin 0.0%, A1 = bacteriocin 0.2% (v/w), A2 = bacteriocin 0.3% (v/w), A3 = bacteriocin 0.4% (v/w), and A4 = bacteriocin 0.5% (v/w).

2.2.1 Activation and propagation of Lactobacillus pentosus strain MIL 195

Lactobacillus pentosus strain MIL 195 was activated by taking 1 ml inoculated into a test tube containing 5 ml MRS-B medium [22]. Furthermore, it was incubated for 24-48 hours at 37ºC.

2.2.2 Production of crude bacteriocins

The bacteriocin production process refers to Rossi et al. [15]. The production process was carried out for three days. The first day was activated by adding 1 ml of *L. pentosus* strain MIL 195 into 5 ml of MRS-B media and incubating for 24 hours at 37°C in a water bath shaker at 100 rpm. The second day of the bacteriocin production process was activated by adding 1 ml of the first-day culture into 5 ml of MRS-B (duplo, tubes A and B) and then incubated for 24 hours at 37°C in a water bath shaker at 100 rpm. Adding 1 ml of the second-day culture from tubes A and B to 100 ml of MRS-B media started the third day of the bacteriocin production process. The tubes were then incubated at 37°C with a water bath shaker at 100 rpm for 24 hours. Cultures activated for three days were centrifuged at 4°C at 14,000 rpm for 30 minutes. The culture centrifuged was then filtered through 0.20 µm Millipore filter paper. To make the crude bacteriocin neutral, 0.1 N NaOH was added until the pH reached 6.

2.2.3 Sausage making

The preparation and formulation of sausage ingredients refers to Rossi et al. [15] with modifications. Chicken and straw mushrooms are cleaned of dirt and cut into small pieces so they are easy to grind. After that, add ice water and grind using a food processor until smooth. According to the treatment, ground spices and preservatives are added during the crushing process. The sausage dough is then put into the casing and tied with thread. Sausages are steamed at 80°C for ±45 minutes or until they expand. The sausages were then cooled and stored for six days at 4°C. Observations were made on sausages on days 0, 3, and 6.

2.2.4 Sensory assessment

The sausage was evaluated using sensory assessment, which involved descriptive and hedonic assessments. A descriptive test was conducted by 30 semi-trained panelists from the Agricultural Product Technology Study Program students at the Faculty of Agriculture, Riau University. The hedonic test was carried out by 80 untrained panelists affiliated with the Faculty of Agriculture at Riau University. Sausage samples were maintained for six days at varying bacteriocin concentrations and displayed in a glass container. Every sample was assigned three random numbers. A sensory evaluation form was supplied, containing test numbers for evaluating the attributes of sausage such as color, scent, flavor, texture, and overall preference on a scale ranging from 1 to 5, representing enormously dislike to highly like.

2.2.5 Statistic analysis

The variables examined in this study were the content of moisture, ash, fat, and protein and the total number of sausage plates at each storage time (0, 3, and 6 days). Sensory assessment of sausages was only carried out on sausages stored for six days. The collected data was evaluated using analysis of variance (ANOVA). Suppose the F value was equal to or greater than the F table. In that case, the analysis will proceed with Duncan's Multiple Range Test at a significance level of 5% using SPSS version 16 to identify variations among the treatments.

3 Result and discussion

3.1 Moisture content

Table 1. showed that the water content of sausages in all treatments at each observation time (day 0, day 3, and day 6) was not significantly different (P>0.05). This study's average water content of sausages was 57.2− 57.7%. This result was caused by the concentration of bacteriocin added to the sausage dough being relatively small, namely 0−0.5%. The same results were also obtained by Aritonang et al. [9], who found that adding a small amount of bacteriocin did not affect the sausage's water content. This result was because the bacteriocin used as a solution during the cooking process also increases the water content of the bacteriocin.

Bacteriocins that were incorporated into the sausage dough function as preservatives so that the decomposition of nutrients by bacteria in the sausage does not occur. The inhibited activity of these decomposing bacteria causes the water content in the sausage not to bind, so the water content is relatively the same in each treatment.

Ash content showed the total amount of mineral content contained in the material. Table 1 showed increased sausage ash content with increasing bacteriocin concentrations at each observation time. The average ash content produced on each observation was on day 0 (2.11−3.31%), day 3 (2.19−2.37%), and day 6 (2.25−2.60%). The increase in ash content in sausage products was caused by the crude bacteriocin used in this study, which may still contain MRS-B media. This MRS-B medium was used to produce bacteriocins, and minerals might still be carried away during the filtering process, contributing to increased ash content in the resulting sausage product.

Table 1. The chemical composition of sausage uses different bacteriocin concentrations and stored at a temperature of 4°C.

Bacteriocins Concentration	**Storage time**		
	0 day	**3 days**	**6 days**
Moisture (%)			
A0= 0.0% (v/w)	57.57	57.56	57.55
A1= 0.1% (v/w)	57.55	57.55	57.54
A2= 0.3% (v/w)	57.55	57.54	57.53
A3= 0.4% (v/w)	57.54	57.53	57.52
A4= 0.5% (v/w)	57.53	57.53	57.52
Ash (%)			

A0= 0.0% (v/w)	2.11[a]	2.19[a]	2.31[a]
A1= 0.1% (v/w)	2.16[a]	2.23[a]	2.37[a]
A2= 0.3% (v/w)	2.27[b]	2.27[b]	2.58[b]
A3= 0.4% (v/w)	2.28[b]	2.32[b]	2.54[b]
A4= 0.5% (v/w)	2.31[b]	2.37[b]	2.72[c]
Fat (%)			
A0= 0.0% (v/w)	5.15[a]	5.08[a]	5.07[a]
A1= 0.1% (v/w)	5.27[b]	5.33[b]	5.42[b]
A2= 0.3% (v/w)	5.38[c]	5.39[bc]	5.50[bc]
A3= 0.4% (v/w)	5.38[c]	5.45[bc]	5.55[bc]
A4= 0.5% (v/w)	5.41[c]	5.49[c]	5.62[c]
Protein (%)			
A0= 0.0% (v/w)	14.13[a]	14.30[a]	14.47[a]
A1= 0.1% (v/w)	14.31[ab]	14.37[ab]	14.62[ab]
A2= 0.3% (v/w)	14.38[b]	14.48[b]	14.77[b]
A3= 0.4% (v/w)	15.31[c]	15.35[c]	15.40[c]
A4= 0.5% (v/w)	15.38[c]	15.39[c]	15.49[c]

Note: Numbers followed by different lowercase superscripts in the same column indicate significant differences (P<0.05)

The average fat content of sausages in all treatments at each observation time ranged from 5.06–5.62%. The fat content of sausages on day 0 ranged from 5.15−5.41%. Sausage fat content on day 3 ranged from 5.08−5.49%. Sausage fat content on day 6 ranged from 5.07–5.62%. It was shown that using crude bacteriocin in sausages significantly increased the fat content of sausages compared to the treatment without bacteriocin. The higher the concentration of bacteriocin added, the higher the fat content. Because the bacteriocin added to the sausage was crude and still contained MRS-B, MRS-B is used as a growth medium for LAB during the production process of crude bacteriocin. MRS-B media contains several constituent ingredients, including meat extract containing fat, contributing to the increase in sausage fat content. The fat content of the sausages produced in this study tends to be lower than the maximum fat determination [20], which was 25%. The primary raw material used in making this sausage was lean chicken breast meat mixed with Merang mushrooms, which have a low-fat content.

Sausage protein content (Table 1) showed that the higher the concentration of bacteriocin added to the sausage product, the higher the protein content at each storage period. The use of bacteriocins in sausages significantly increased the protein content of sausages compared to without bacteriocins. This increase in protein content was due to the crude bacteriocin produced by *L. pentosus* strain MIL 195, a protein/peptide compound with small molecules [23]. Peptides are one of the molecular structures that form proteins.

3.2 Total plate count

Total plate count (TPC) was an indicator of heterotrophic microbes, including bacteria, molds, and yeasts. Table 2 showed that using bacteriocin in sausages significantly (P<0.05) decreased the TPC of sausages compared to those without bacteriocin. These results indicated that bacteriocin worked as a preservative.

Table 2. Average sausage total palate account content during storage at 4°C.

Bacteriocins Concentration	Total palate account CFU/g per day		
	0 day	3 days	6 days
A0= 0.0% (v/w)	$5.47x10^{4\ b}$	$5.48\ x10^{4\ b}$	$5.56\ x10^{4\ b}$
A1= 0.1% (v/w)	$5.35x10^{4\ a}$	$5.31\ x10^{4\ a}$	$5.30\ x10^{4\ a}$
A2= 0.3% (v/w)	$5.35\ x10^{4\ a}$	$5.30\ x10^{4\ a}$	$5.25\ x10^{4\ a}$
A3= 0.4% (v/w)	$5.32\ x10^{4\ a}$	$5.25\ x10^{4\ a}$	$5.23\ x10^{4\ a}$
A4= 0.5% (v/w)	$5.29\ x10^{3\ a}$	$5.23\ x10^{4\ a}$	$5.20\ x10^{4\ a}$

Note: Numbers followed by different lowercase superscripts in the same column indicate significant differences (P<0.05).

Bacteriocins destroy the cell membrane's permeability of decomposing bacteria or pathogens. This membrane permeability will disrupt the function of the cytoplasmic membrane. This disturbed permeability will damage or inhibit the growth of decomposing or pathogenic bacterial cells and can even cause death. For sausages without adding bacteriocins (control), the longer the storage time, the higher the TPC number obtained. It was concluded that storing food at a temperature of 4ºC only inhibits the growth of bacteria that cause spoilage and spoilage. It does not kill all microorganisms but only inhibits their growth. The TPC in this study with the addition of 0.5% bacteriocin to sausages stored for six days (5.20 x 10^4 CFU/mg equivalent to 4.71 log CFU/g) was lower than the research [24], who added supernatant bacteriocin from *L. plantarum* IBL-2 to fresh ground meat and stored it for six days. The low TPC in this study was due to the bacteriocin being applied to sausages, which were made through a steaming process. Several studies have shown a decrease in TPC with the use of bacteriocin for sausage [9,19] and fish meatballs [18].

3.3 Sensory assessment

Generally, consumers judge product quality and acceptability based on their appearance. This study did not find a significant effect (P>0.05) of using different concentrations of bacteriocins in sausages on descriptive sensory characteristics. However, the overall hedonic acceptability of sausages was significantly influenced (P<0.05) by the concentration of bacteriocins used (Table 3).

The descriptive panelists assessed the sausage color-averaged 3.00–3.23 (grayish white). The grayish-white color was caused by the rough bacteriocin produced by *L. pentosus*, which has a brownish-yellow color. The brownish-yellow color of the bacteriocin did not appear in the color of the sausage because the amount of bacteriocin used in the sausage is relatively small (0–0.5% v/w). The color of the sausage produced was greatly influenced by the color of the meat used and was related to the myoglobin content in the chicken meat. The average sausage aroma was 3.43–3.73 (slightly chicken aromatic to chicken aromatic). This aroma is caused by the crude bacteriocin produced not being aromatic, so if applied to sausages, it did not affect the product's aroma.

Table 3. The descriptive and hedonic sensory assessment of sausage on the 6th day of observation.

Treatment	**Descriptive**				**Hedonic**
	Color	**Aroma**	**Flavor**	**Texture**	**Overall Assessment**
A0 (Control)	3.23^a	3.73^a	3.83^a	3.70^a	3.40^a
A1 (0,2%)	3.13^a	3.53^a	3.56^a	3.70^a	3.77^b

A2 (0,3%)	3.16^a	3.46^a	3.46^a	3.56^a	3.82^b
A4 (0,4%)	3.00^a	3.50^a	3.43^a	3.66^a	3.87^b
A5 (0,5)	3.03^a	3.43^a	3.50^a	3.60^a	3.95^b

Note: Numbers followed by different lowercase superscripts in the same column indicate significant differences (P<0.05). **Descriptive Range**: **Color**: 1(very grey), 2(grey), 3(grayish white), 4(white), 5 (very white); **Aroma**:1 (not very chicken flavored), 2((not chicken flavored), 3(slightly chicken), 4(scented to chicken-scented). **Flavor:** 1(very tasteless chicken meat), 2(tasteless chicken meat), 3**(**somewhat chicken flavored), 4(chicken flavored), 5(really taste like chicken)**; Texture:** 1(very hard), 2(hard), 3(a bit chewy), 4(chewy), 5(very chewy)**.** Overall Assessment of **Hedonic Range**: 1(very dislike), 2(dislike), 3(little like), 4(like), 5(really like).

Taste was one of the sensory quality characteristics that attracts consumers' attention. The descriptive panelists' assessment of the taste of sausage was relatively the same (P>0.05), namely an average ranging between 3.43‒3.83 (somewhat chicken taste to chicken taste). The aspects assessed in the texture criteria are the roughness and smoothness of the product produced. The descriptive panelist's assessment of sausage texture showed that variations in bacteriocin concentration had no significant effect on sausage texture, with an average value of 3.56‒3.70 (chewy texture). The chewy texture of the sausage was due to the emulsification process between water, fat, and protein as a binder or emulsifier. The ability of fillers to bind water played a role in forming a stable emulsion because the starch contained in fillers can gelatinize or absorb water so that the starch granules swell. However, it cannot return to its original condition. The bacteriocin added to the sausage did not change. The sensory characteristics of a product result from the interaction of complex physicochemical, biochemical, and microbiological processes and play a role in forming and balancing chemical compounds in the product that affect the product's color, aroma, texture, and overall appearance [25,26].

The bacteriocin concentration had a significant influence (P<0.05) on the level of panellists' preference for the sausage produced (Table 3). The panelists preferred sausages with added bacteriocins (A2, A3, A4, and A5) to sausages without bacteriocins (A1=control). Bacteriocin was a protein compound [27], so sausages with the addition of bacteriocin become denser and have a smoother texture, and the aroma does not change. These results indicate that bacteriocin from Lactobacillus pentosus MIL 195 can be used as a sausage preservative because it does not change the sensory assessment of sausages.

4 Conclusions

Bacteriocin from *L. pentosus* strain MIL 195 can be used as a preservative for chicken sausage with a concentration of 0.4% (v/b) during six-day storage, resulting in sausage quality by SNI-01-3820-2015, namely moisture content of 57.52%, ash content of 2.54%, protein content of 15.40%, fat content of 5.55%, and total plate number of 5.23 log cfu.g-1. The overall hedonic assessment of 0.4% treated chicken sausage was also preferred by panelists with a score of 3.87 (like), with sensory analysis descriptively on the color of chicken sausage had an assessment score of 3.00 (grayish white), aroma with an assessment score of 3.50 (slightly scented chicken), flavor with an assessment score of 3.43 (slightly meaty), texture with an assessment score of 3.66 (chewy).

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