

## Application of bacteriocin as a preservative for IPB final stock of pre-cooked (ungkep) chicken stored at room and cold temperatures

<sup>1</sup>Fauziah, S.A., <sup>2,\*</sup>Arief, I.I., <sup>2</sup>Ulupi, N. and <sup>2</sup>Sumantri, C.

<sup>1</sup>Magister Program in Animal Science and Production Technology, Faculty of Animal Science, Bogor Agricultural University (IPB University), Agathis Street, Campus IPB Darmaga, Bogor 16680, Indonesia

<sup>2</sup>Department of Animal Production and Technology, Faculty of Animal Science, Bogor Agricultural University (IPB University), Agathis Street, Campus IPB Darmaga, Bogor 16680, Indonesia

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### Abstract

IPB final stock (FS) ungkep chicken is a recently introduced local breed with significantly better growth and carcass performance compared to other local chicken. However, the carcass is vulnerable to bacterial spoilage during storage. To address this problem, a local bacteriocin known as plantaricin IIA-1A5 was identified in an Indonesian probiotic called *Lactobacillus plantarum* IIA-1A5. Bacteriocin has shown great potential as a natural preservative agent for extending the shelf life of meat, but its application has yet to be tested in chicken carcasses. Therefore, this study aimed to evaluate the antibacterial effectiveness of bacteriocin on the quality of IPB FS ungkep chicken stored at room or cold temperature. A complete randomized design was used with Duncan's new multiple range test for total plate count (TPC) and physicochemical analysis, while organoleptic data were assessed with the Kruskal-Wallis test, followed by multiple comparison tests. The results showed that the chicken stored at room temperature had increased TPC with higher storage time but there was no growth of *Escherichia coli*. The pH value, dry matter, moisture, and ash content were also significantly affected by the storage time. For chicken stored at cold temperature, the TPC was significantly affected by the storage time, but there was no growth of *Escherichia coli*. In addition, the pH value and ash content were also significantly affected. Based on the results, the shelf-life of IPB FS ungkep chicken can be extended by adding bacteriocin and storing it for 24 hrs at room temperature or 28 days at cold temperature.

## 1. Introduction

The main product of broiler livestock is carcasses, defined by BSNI (2009) as part of the chicken body separated from the head, neck, lungs, kidneys, legs, feathers, and offal following a halal slaughter process CAC/GL 24-1997. The chicken used in this study was from the IPB final stock (FS) strain developed by IPB University and recognized by the Indonesian Ministry of Agriculture based on Decree No. 693/KPTS/PK.230/M/9/2019. This breed is a product of the food processing industry from the RISPRO-LPDP Program (2019-2021). According to Ulupi *et al.* (2018), the percentage of IPB chicken carcasses is high, reaching approximately 73%.

Carcasses are products that have the potential to cause physical, chemical, and biological damage, but can be processed to enhance taste, such as through the preparation of ungkep chicken. Despite the processing, ungkep carcasses remain a perishable product (Sangadji

*et al.*, 2019). Some of the factors causing damage include aspects of processing, storage, and shelf life. According to Katiyo *et al.* (2020), chicken carcasses stored in the refrigerator can only last 3 days at 4°C with good quality. Sangadji *et al.* (2019) found that damage to chicken carcasses is usually caused by poor handling, providing opportunities for the growth of putrefactive microbes, which decrease the quality and shelf life.

*Lactobacillus plantarum* IIA-1A5, an Indonesian lactic acid bacteria (LAB)-based probiotic, has been earlier reported to produce bacteriocin, namely Plantaricin IIA-1A5. Bacteriocin was found to inhibit Gram-negative and positive pathogenic bacteria with high thermal stability (>80°C). Moreover, it is safe for consumption due to its sensitivity against proteolytic digestive enzymes (Arief *et al.*, 2015; Afyah *et al.*, 2015). Nurraifah *et al.* (2021) reported that bacteriocin is suitable for extending the shelf-life of products by

\*Corresponding author.

Email: [isnafia@apps.ipb.ac.id](mailto:isnafia@apps.ipb.ac.id)

inhibiting the growth of pathogenic bacteria. Plantaricin IIA-1A5 showed great effectiveness for meatball and sausage preservation (Kia *et al.*, 2016). However, this has never been tested for the preservation of chicken meat, particularly the unkep breed. Based on the experiment of Siswara *et al.* (2019), Bacteriocin has great potential as a preservative, with application based on physical, chemical, microbiological, and organoleptic tests showing that chicken meat had better quality than without bacteriocin. This leads to the hypothesis that plantaricin IIA-1A5 might be workable for unkep chicken, but this remains to be experimentally confirmed. Therefore, this study aimed to evaluate the antibacterial effectiveness of bacteriocin on the quality of IPB FS unkep chicken stored at room or cold temperature.

## 2. Materials and methods

### 2.1 Sample preparation

IPB FS chicken carcasses were provided by CV. Citra Lestari Farm, Bekasi, Indonesia. The carcasses used for microbiological, physicochemical, and organoleptic analysis were from the breast part. This study used two different storage locations namely room temperature at 20-30°C for 24 hrs in an open-air container, and cold temperature at 4°C for 35 days in a refrigerator (Display Cooler Expo-37FC produced by GEA company, China). The number of carcasses used in the analysis was 60 pre-cooked (unkep), 30 each for room and cold temperature storage.

### 2.2 Production of plantaricin IIA-1A5 by *Lactobacillus plantarum* IIA-1A5

The growth medium used was Whey, inoculated with *L. plantarum* IIA-1A5 of  $> 8 \log$  CFU/mL at 10% (v/v). Centrifugation was performed (10,000×g, 20 mins, 4°C) in a Centrifuge Micro 200 R (Hettich, Germany) to collect the supernatant. Subsequently, 2% sterile maltodextrin was added to the supernatant, followed by freeze-drying at 55°C with a pressure of 0.2 MBAR using a Lyovapor L-200 produced by Buchi, Switzerland. The final product was designated plantaricin (bacteriocin) which was dissolved in an EDTA solution before application.

### 2.3 Determination of bacteriocin activity

Bacteriocin activity was calculated according to the method described by Arifin *et al.* (2021). The antibacterial activity of bacteriocin was tested in Muller Hinton Agar (MHA) medium at 37°C for 24-48 hrs. The incubation process used a NuAire Autoflow IR Direct Heat CO<sub>2</sub> Incubator NU-5500. For the testing, the pathogenic bacteria of *Escherichia coli* and

*Staphylococcus aureus* were used. The antibacterial activity of bacteriocin was observed through the formation of a clear zone around the paper disc, which was measured using a caliper.

### 2.4 Microbial analysis

The result of microbial analysis was calculated according to the formula from the Bacteriological Analytical Manual (U.S. Food and Drug Administration (US FDA), 2001). The method entailed using 1 mL suspension of the sample and dilutions of 10<sup>-1</sup> to 10<sup>-3</sup> inoculated into a duplicate petri dish. Total Plate Count (TPC) was conducted using the Plate Count Agar (PCA) medium with the pour plate method, incubated at 37°C for 36-48 hrs. *Escherichia coli* was detected using an Eosin Methylene Blue Agar (EMBA) medium at 37°C for 24-36 hrs. All Petri dishes were incubated upside down. *Escherichia coli* was detected by the production of a metallic green color by the colony.

### 2.4 Physicochemical analysis

Physicochemical analysis parameters included pH, a<sub>w</sub>, dry matter, water content, and ash content. Measurement of the pH value was carried out with a pH meter, PH5F Ionix (Kosim *et al.* 2015), and the a<sub>w</sub> value was obtained using an a<sub>w</sub> meter, Novasina msl (Salejda *et al.* 2014). The dry matter was assessed by reducing the percentage of water content (Reiling 2011) measured using an oven produced by Memmert, Germany at a temperature of 105°C for 18 hrs. The ash content was determined through the furnace produced by Nabertherm, Germany, for combustion at 650°C for 5 hrs (Association of Analytical Collaboration (AOAC) International, 2005).

The dry matter content was calculated using the formula according to Reiling (2011):

$$\text{Dry Matter (\% DM)} = 100 - \text{Water Content (\% WC)}$$

The water and ash content were calculated based on the formula of AOAC International (2005):

$$\text{Water Content} = (W1 - W2) / W \times 100\%$$

Where W = Sample Weight (g), W1 = Weight (Sample + Porcelain Crucible) before drying (g) and W2 = Weight (Sample + Porcelain Crucible) after drying (g)

$$\text{Ash Content (\%)} = [\text{Ash weight (g)}] / [\text{Sample Weight (g)}] \times 100\%$$

### 2.5 Organoleptic test

Organoleptic properties tested included a hedonic test [score scale of 1-5; scale 1 (strongly dislike), 2 (dislike), 3 (somewhat like), 4 (like), and 5 (very like)]

and hedonic quality test (color, aroma, and texture). The assessment scale for color is 1 (very yellow), 2 (yellow), 3 (slightly brown), 4 (brown), and 5 (very brown). The scale for aroma is 1 (strongly scented rancid), 2 (rancid scented), 3 (slightly rancid scented), 4 (non-rancid scent), and 5 (predominantly aroma spices and non-rancid scent). The scale for texture was 1 (very slimy), 2 (slimy), 3 (slightly slimy), 4 (not slimy), and 5 (very slimy). The organoleptic test used 40 semi-trained panelists as replications according to BSNI (2006). Each panelist received five samples, each stored at room and cold temperatures.

## 2.6 Data analysis

The study used a complete randomized design (CRD) with two treatments (cold and room temperature storage), while the parameters observed included microbiological, physicochemical, and organoleptic aspects. The data from the CRD method for TPC and physicochemical analysis were analyzed by variance analysis (ANOVA) with a confidence level of 95%, followed by further testing using Duncan's new multiple range test (DMRT) (Gaspersz, 1991). Furthermore, data for the organoleptic test were considered non-parametric and analyzed by the Kruskal-Wallis (Steel and Torrie, 1997), followed by multiple comparison tests. The statistical software used in this study was IBM SPSS Statistics 26.0 version.

## 3. Results and discussion

### 3.1 Determination of bacteriocin activity

Bacteriocin is a natural preservative with antibacterial properties that inhibit bacterial growth, and its addition to unkep chicken carcass was proven with detection zone inhibition using *E. coli* and *S. aureus* bacteria. The inhibition zone diameter for *E. coli* ATCC 11229 was  $26.61 \pm 0.94$  mm, while that of *S. aureus* ATCC 6538 was  $24.55 \pm 0.88$  mm. The diameter of the inhibition zone showed the strongest inhibition of bacterial growth. This is in line with Davis and Stout (1971), who classified antibacterial inhibition into four categories based on inhibition zone diameter: very strong ( $>21$  mm), strong (11-20 mm), medium (6-10 mm), and weak ( $< 5$  mm).

The main role of bacteriocin is to explore its ability

to inhibit bacteria. According to Siswara *et al.* (2019), the main activity of antibacterial agents is to inhibit the activity of pathogens, which can lower the speed of decay and prolong shelf life. Similar to Soenarno *et al.* (2020), bacteriocins have antimicrobial characteristics that can extend the shelf-life of food products.

This study showed that bacteria can increase the shelf life of products, making them more valuable in terms of nutritional content and economic value. Moreover, bacteriocins serve not only as antibacterial agents. According to Meristica *et al.* (2020), bacteriocins not only inhibit the growth of pathogenic microorganisms but also suppress the growth of other harmful microorganisms capable of producing toxins.

### 3.2 Room temperature storage treatments

#### 3.2.1 Microbial analysis

The microbial analysis results included the TPC and the presence of *E. coli* as presented in Table 1. For TPC and *E. coli* analysis showed that the population growth of pathogenic bacteria in the product was safe for consumption ( $<6.0$  log CFU/g for TPC and  $<2.0$  log CFU/g for *E. coli*), based on predetermined standards (BSNI, 2009). Ginting *et al.* (2014) reported that the total number of microbes also increased with an increase in storage length. Based on the results, despite the increase in total microbes, the microbes produced were still within the safe limits of microbial consumption. This is influenced by using bacteriocins to maintain the quality of food products. Arief *et al.* (2015) attributed this to the ability of plantaricin IIA-1A5 (bacteriocin) to inhibit the growth of pathogenic bacteria.

Statistical analysis showed that TPC had a significant value as the shelf life increased. The increase in the significant value ( $P < 0.05$ ) started after 12 hrs of storage. The increase in microbial growth was exponential, and the growth curve increased sharply. This increase is influenced by environmental factors such as temperature, light and humidity (Suriawiria, 2011). Nevertheless, this increase in bacterial count was still within the safe limits ( $<6.0$  log CFU/g).

In addition, *E. coli* was not detected as the shelf life increased. Adiyoga *et al.* (2022) showed that bacteriocins have probiotic characteristics that can produce activity against *E. coli*. Substantiated by Kia *et*

Table 1. Microbiological quality of chicken meat stored at room temperature.

Parameter	Treatments				
	0 hr	6 hrs	12 hrs	18 hrs	24 hrs
Total plate count (log CFU/g)	$1.64 \pm 0.18^a$	$3.91 \pm 0.35^{ab}$	$5.73 \pm 0.04^b$	$5.22 \pm 0.69^b$	$5.95 \pm 0.03^b$
<i>E. coli</i> (log CFU/g)	$0.76 \pm 0.43$	$0.16 \pm 0.32$	Negative	Negative	Negative

Values are presented as mean $\pm$ SD. Values with different superscripts in the same row are statistically significantly different ( $p < 0.05$ ) using DMRT.

al. (2016), the addition of bacteriocin to the product effectively preserved the product quality for 24 hrs after contamination with pathogenic bacteria. The product had a low contamination value under 1 log CFU/g at 0 hr dan 6 hrs of storage. Suriawiria (2011) stated that this could be caused by microbial growth during the stationary and dead phases. In this phase, the microbial curvature stagnated and decreased significantly until it was no longer microbially detectable. The decrease in microbial growth was caused by the reduced nutrient sources, and the microbes entered the death phase.

### 3.2.2 Physicochemical analysis

Physicochemical analysis included  $a_w$ , pH, dry matter, water, and ash content values, as presented in Table 2. Based on these results, the addition of bacteriocin had a significant effect as a preservative during storage at room temperature for 24 hrs. However,  $a_w$  values decreased as storage time increased. As stated by Setiarto *et al.* (2018),  $a_w$  is directly proportional to the water content of a product.

Statistical analysis showed that pH, dry matter, water, and ash content significantly increased ( $P < 0.05$ ) as shelf life increased. The pH value increased with longer storage time, and a significantly different value was obtained after 24 hrs of storage. The increase in pH caused by the bacteria growth phase occurs in the decay phase of the product (Suradi, 2012).

Furthermore, the water content decreased with longer storage time and showed an inverse relationship with dry matter content. The lower the water content for a long storage time, the higher the dry matter content. This is in accordance with Effendi (2009), who reported that a decrease in the moisture content is influenced by adjustments to the storage environment of the product. The decrease in the product's moisture content can be caused by the evaporation of the water contained in the product into the air during storage. The evaporation process continues until the water equilibrium is reached, that is when the vapor pressure in the chicken meat is balanced with that of the surrounding environment. The vapor pressure of chicken meat is balanced by that of the

surrounding environment. The loss of water during evaporation leads to an increase in dry matter content. Another significant value was the ash content, which fluctuated from 0 to 24 hrs but remained below the maximum limit set by the Ministry of Health, Republic of Indonesia (2017), that is 12.1%.

### 3.2.3 Organoleptic test

The results of the organoleptic hedonic test (Figure 1) on the color parameter showed a significant effect on storage at 12 and 24 hrs, whereas the results of the aroma parameter showed a significant effect on storage at 6 and 24 hrs. The texture parameter showed a significant effect on storage at 24 hrs, and the general appearance parameter did not show a significant effect during storage at room temperature. Overall, the results of the hedonic organoleptic test of unkep chicken samples with plantarisin IIA-1A5 showed a decreasing level of preference with increasing storage time. The principle of the hedonic test in organoleptic analysis is the degree of liking or disliking of the panelists for the parameters being assessed (Qamariah *et al.* 2022).

The results of the organoleptic hedonic quality test (Figure 1) on the color parameter showed a significant effect on the color of the sample. Plantarisin IIA-1A5 on the color parameter significantly affected meat color. 6, 18, and 24 hrs of storage, as meat stored at room temperature changes color owing to direct interaction with oxygen (Narty *et al.* 2019). The aroma parameters showed a significant effect from 12 to 24 hrs of storage,

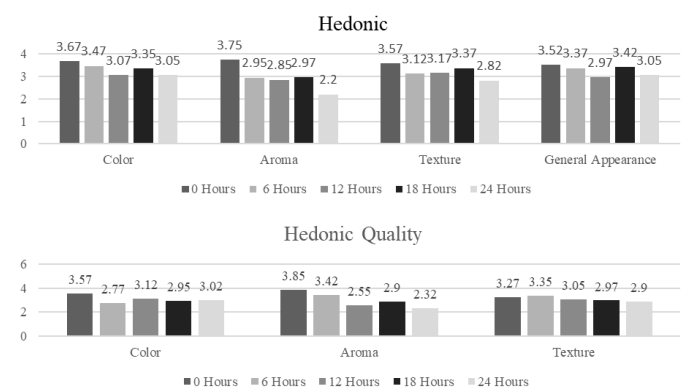


Figure 1. Organoleptic properties of chicken meat stored at room temperature.

Table 2. Physicochemical quality of chicken meats stored at room temperature.

Parameter	Treatments				
	0 hr	6 hrs	12 hrs	18 hrs	24 hrs
$a_w$	0.83±0.02	0.83±0.01	0.83±0.01	0.83±0.01	0.82±0.01
pH	6.07±0.18 <sup>a</sup>	6.27±0.09 <sup>a</sup>	6.25±0.06 <sup>a</sup>	6.45±0.10 <sup>a</sup>	6.96±0.07 <sup>b</sup>
Water content (%)	64.54±1.16 <sup>b</sup>	58.52±2.58 <sup>ab</sup>	56.25±1.74 <sup>ab</sup>	53.59±1.44 <sup>a</sup>	51.21±0.89 <sup>a</sup>
Dry matter (%)	35.46±1.16 <sup>a</sup>	41.48±2.58 <sup>ab</sup>	43.75±1.74 <sup>ab</sup>	46.41±1.44 <sup>b</sup>	48.79±0.89 <sup>b</sup>
Ash content	4.94±0.15 <sup>a</sup>	5.11±0.71 <sup>a</sup>	4.86±0.27 <sup>a</sup>	5.88±0.04 <sup>b</sup>	5.82±0.08 <sup>b</sup>

Values are presented as mean±SD. Values with different superscripts in the same row are statistically significantly different ( $p < 0.05$ ) using DMRT.

because the longer the storage period, the lower the color of the meat. This is because a longer shelf life can reduce the fat content of products, resulting in rancidity. product, which can produce rancidity or rancid aromas (Bhaskara *et al.*, 2021).

Based on the results and statistical analysis, storage at room temperature for 24 hrs can significantly accelerate organoleptic damage to food products and result in a dislike score by panelists. Lukman *et al.* (2024) reported that the addition of bacteriocin did not enhance panelist preference in the organoleptic test of color, aroma, and texture.

### 3.3 Cold temperature storage treatment

#### 3.3.1 Microbial analysis

The microbial analysis results included the TPC and the presence of *E. coli*, as presented in Table 3. TPC statistical analysis showed a highly significant effect ( $P < 0.01$ ). The TPC value decreased by the 7th day of storage and increased significantly with the length of storage. According to Suriawiria (2011), on the microbial growth curve, there are nutritional conditions that can be continuously supplied, so that the continuity of growth in the number of microbes will occur, although the longer it leads to a decrease in the number of microbes. The TPC values were below the safe limit for microbial contamination. According to the BSNI, the maximum limit of TPC is  $1 \times 10^6$  CFU/g (BSNI, 2009).

The results were negative for *E. coli* bacteria. This proved that the addition of bacteriocins inhibited the growth of the microorganisms. Arief *et al.* (2013) showed that bacteriocins can inhibit the growth of *E. coli* and other bacteria. Rahayu *et al.* (2018) reported that *E. coli* is an indicator of sanitation and hygiene during product processing. Proper processing and storage at

controlled temperatures can inhibit microbial growth and minimize *E. coli* growth. Controlled storage has been proven to inhibit bacterial growth, as shown by Edi and Rahmah (2018), who reported that storage in cold refrigerator temperatures can inhibit bacterial growth because it reduces the metabolic activity of bacterial cells.

#### 3.3.2 Physicochemical analysis

Physicochemical analysis included  $a_w$ , pH, dry matter, water, and ash content values, as presented in Table 4. The results showed that the addition of bacteriocin had a significant preservative effect ( $P < 0.05$ ) on the pH and ash content during storage at cold temperatures for 35 days. The addition of herbs capable of supporting a long shelf life and storage at cold temperatures can decrease the pH value. According to Wala *et al.* (2016), an example of a suitable herb is turmeric, which contains ascorbic acid capable of counteracting the increase in pH value through osmosis to extend shelf-life.

Ash content increased significantly ( $P < 0.05$ ) with the duration of cold storage. This finding is consistent with the results of Faizah and Sri (2020), who reported a tendency for the ash content to increase with prolonged storage. According to Sholeha and Amertaningtyas (2024), ash content refers to the inorganic residue remaining after a substance is heated to temperatures between  $500^\circ\text{C}$  and  $800^\circ\text{C}$ . This analytical method is based on the principle that organic compounds decompose into water and carbon dioxide during combustion, whereas inorganic materials remain intact. The ash content in meat serves as an indicator of its mineral composition. The increase in ash content in this study had a beneficial effect on the quality of cold-stored

Table 3. Microbiological quality of chicken meats stored at cold/refrigerated temperature.

Parameter	Treatments				
	Day 0	Day 7	Day 14	Day 28	Day 35
Total Plate Count (log CFU/g)**	2.04±0.27 <sup>b</sup>	1.52±0.15 <sup>a</sup>	1.95±0.18 <sup>b</sup>	2.28±0.10 <sup>b</sup>	2.26±0.21 <sup>b</sup>
<i>E. coli</i> (log CFU/g)	Negative	Negative	Negative	Negative	Negative

Values are presented as mean±SD. Values with different superscripts in the same row are statistically significantly different ( $p < 0.05$ ) using DMRT.

Table 4. Physicochemical quality of chicken meats stored at cold/refrigerated temperature.

Parameter	Treatments				
	Day 0	Day 7	Day 14	Day 28	Day 35
$a_w$	0.87±0.00	0.87±0.01	0.86±0.02	0.85±0.02	0.87±0.01
pH	6.17±0.34 <sup>c</sup>	5.83±0.04 <sup>a</sup>	6.01±0.07 <sup>bc</sup>	5.95±0.05 <sup>ab</sup>	6.11±0.14 <sup>bc</sup>
Water content (%)	61.24±1.04	62.36±1.13	64.48±2.39	61.94±0.53	64.78±0.54
Dry matter (%)	38.76±1.04	37.64±1.13	35.52±2.39	38.06±0.53	35.22±0.54
Ash content	4.91±0.13 <sup>a</sup>	4.89±0.44 <sup>a</sup>	4.90±0.13 <sup>a</sup>	5.08±0.71 <sup>a</sup>	5.57±0.07 <sup>b</sup>

Values are presented as mean±SD. Values with different superscripts in the same row are statistically significantly different ( $p < 0.05$ ) using DMRT.

ungkep chicken meat, as it remained at a safe level of ash content of <12.1% (Ministry of Health, Republic of Indonesia, 2017).

The results for  $a_w$ , water content, and dry matter showed no significant changes during 35 days of storage at cold temperatures. This observation correlated with the TPC values obtained. The relatively insignificant increase in TPC during the storage period suggests successful inhibition of microbial growth through the addition of bacteriocin as a natural preservative in ungkep chickens. This is in line with the findings of Arief et al. (2014), who found that food products stored at low temperatures can produce a slower rate of microbial inactivation. When the microbial population is inhibited, the food quality is extended. Kia et al. (2016) reported that controlled  $A_w$  values would restrict the growth of microorganisms because the amount of water to be used in their metabolism is limited. The  $a_w$  value is important for extending the shelf life of a product.

### 3.4 Organoleptic test

Figure 2 shows the results of the organoleptic tests obtained at storage times of 0, 7, 14, 28, and 35 days. The results of the hedonic organoleptic test for all parameters showed changes in the level of panelist preference on day 35 of storage. The level of panelist preference is a perception that is given and not bound, which is in accordance with Katiyo et al. (2020) that the perception of panelists is subjective. Therefore, the level of determination of the assessment refers to the circumstances, feelings, and tastes of each panelist during the test. The results of hedonic organoleptic testing on all parameters indicated that the panelists rather liked the ungkep chicken sample, which was

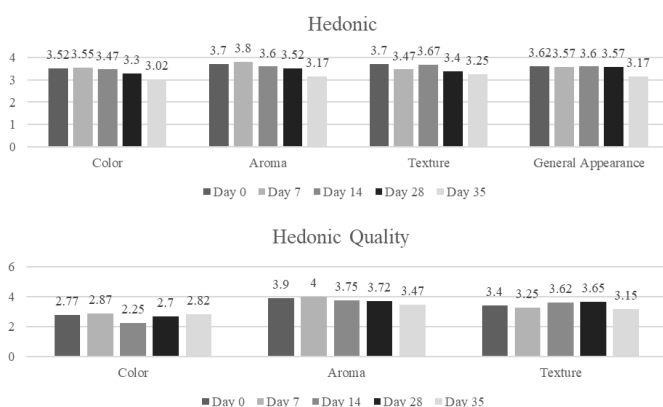


Figure 2. Organoleptic properties of chicken meat stored at cold/refrigerated temperature.

assessed by the panelists.

The results of the organoleptic hedonic quality tests on color, aroma, and texture parameters did not show any significant changes during cold storage, as shown in Figure 2. However, the aroma and texture parameters of

the 35th day storage sample had the lowest values compared to the other storage times. This is in accordance with the results of Katiyo et al. (2020), who found that increasing the storage time increased the intensity of unwanted aroma and texture changes in the material.

According to Katiyo et al. (2020), the longer the products are stored, the higher the undesirable scent and the change in product texture. Panelist perspectives are subjective and require the level of determination to be based on the conditions, experiences, and preferences of each panelist at the time of testing. Arief et al. (2014) state that panelists have a neutral preference for products. Therefore, improvement of panelists' preferences in the future is required to obtain more precise results.

## 4. Conclusion

In conclusion, bacteriocin application to IPB FS pre-cooked (ungkep) chicken stored at room or cold temperature reduced TPC and inhibited the growth of *Escherichia coli* bacteria. Differences between the characteristics of physicochemical traits and the microbiological quality of chicken were associated with storage conditions. The 24 hrs storage at room temperature and 28 days at cold temperature with bacteriocin addition extended the shelf life and maintained the quality of IPB FS pre-cooked (ungkep) chicken.

## Conflict of interest

The authors declare no conflict of interest.

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