



Effects of Various Concentrations of *Lactobacillus plantarum* and Storage of Chicken Sausage Fermentation on Total Microbes and Lactic Acid Bacteria

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ABSTRAK

Penelitian bertujuan untuk mengkaji pengaruh perlakuan kombinasi bakteri *L. Plantarum* dan lama penyimpanan terhadap total mikroba dan bakteri asam laktat sosis ayam fermentasi. Materi penelitian yang digunakan adalah kultur *L. Plantarum* dan daging dari ayam pedaging 2,5 kg bagian dada. Metode penelitian adalah eksperimental menggunakan Rancangan Acak Lengkap (RAL) Pola factorial 3x3 dan dilakukan pengulangan 3 kali ulangan. Perlakuan yang digunakan yaitu konsentrasi *L. Plantarum* TW 14 (5%,10%,15%) dan lama penyimpanan (0 jam, 12 jam,24 jam) dilakukan dengan 3 kali ulangan. Hasil penelitian menunjukkan bahwa perlakuan interaksi dan konsentrasi berpengaruh tidak nyata terhadap total mikroba. Perlakuan konsentrasi total bakteri asam laktat berpengaruh nyata ($P<0,05$) menggunakan konsentrasi *L. Plantarum* terhadap total bakteri asam laktat. Interaksi dihasilkan dari respon linear pada garis $Y = 7,05267 - 0,366586 \cdot \text{penyimpanan}$. Penyimpanan tidak berpengaruh nyata terhadap total bakteri asam laktat. Total mikroba yang diperoleh rata-rata berkisar $15,453 \pm 0,19$ $10 \log \text{CFU/cm}^2$ sampai $17,457 \pm 0,17 \log \text{CFU/cm}^2$ sedangkan total bakteri asam laktat diperoleh hasil $15,565 \log \text{CFU/cm}^2$ sampai $18,709 \log \text{CFU/cm}^2$. Kesimpulan dari penelitian ini adalah pemberian *L. Plantarum* terhadap total mikroba tidak berpengaruh nyata, sedangkan pemberian *L. Plantarum* terhadap total bakteri asam laktat dengan pemberian konsentrasi 5%,10%,15%) berpengaruh nyata terhadap sosis ayam fermentasi, sedangkan lama penyimpanan (0 jam, 12 jam, 24 jam) tidak memberikan pengaruh nyata terhadap sosis. Lama penyimpanan yang mengandung bakteri asam laktat paling banyak di 24 jam.

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ABSTRACT

The research aims to examine the effects of combined treatment with *L. plantarum* bacteria and storage time on total microbes and lactic acid bacteria in fermented chicken sausages. The research material consisted of *L. plantarum* cultures and meat from 2.5 kg of broiler breast chickens. The experimental research method used a completely randomised design (CRD). This study employed a completely randomized 3x3 factorial pattern with three replications. The treatments included *L. plantarum* TW 14 concentrations (5%, 10%, 15%) and storage times (0 hours, 12 hours, 24 hours). The results indicated that the interaction and concentration treatments did not significantly affect total microbial counts. However, the concentration treatment for total lactic acid bacteria had a significant effect ($P < 0.05$) on the *L. plantarum* concentration of total lactic acid bacteria. The

interaction results demonstrated a linear response on the line Y, which was $7.05267 - 0.366586$, while storage time had no significant effect on total lactic acid bacteria. The average total microbes obtained ranged from $15,453 \pm 0.19 \log \text{CFU/cm}^2$ to $17,457 \pm 0.17 \log \text{CFU/cm}^2$, while the total results for lactic acid bacteria ranged from $15,565 \text{ bc } \log \text{CFU/cm}^2$ to $18,709 \text{ a } \log \text{CFU/cm}^2$. This research concludes that administering *L. plantarum* does not significantly affect total microbes. In contrast, applying *L. plantarum* for total lactic acid bacteria at 5%, 10%, and 15% concentration substantially affects fermented chicken sausage. In contrast, storage time (0 hours, 12 hours, 24 hours) did not notably affect the fermented chicken sausage, even though 24 hours of storage produced the highest concentration of lactic acid bacteria in fermented chicken sausage.

INTRODUCTION

Food consists of proteins, carbohydrates, vitamins, fats, and minerals. One of the foodstuffs that is high in protein is meat. Meat is a good but perishable source of protein, essential fatty acids, minerals, and vitamins. Technological advancements in the current era of globalisation, which continue to progress, have led to changes in people's lifestyles. They tend to prefer food that is convenient, economical, and quick. Processed food products such as sausages, meatballs, and nuggets serve as alternatives for society to meet its food needs today. Sausage is a processed meat product that can be consumed by people of all ages and is well-liked by the public. Meat is an important food source for human needs because it produces the most significant amount of protein compared to other agricultural products.

Meat is a popular source of animal protein, with consumption steadily rising each year. However, meat's shelf life is relatively

short, prompting various preservation and processing methods to extend it. Processed meat products provide added value, including nuggets, meatballs, beef jerky, corned beef, shredded meat, and sausages.

Chicken meat is one of the most favoured choices among people, as evidenced by data on chicken meat consumption. In 2015, chicken meat consumption was 54.7 tons, which increased to 55.9 tonnes, 61.7 tonnes, 63 tonnes, and 64.3 tonnes in 2016, 2017, 2018, and 2019 (Kemeneterian Pertanian RI, 2023). The protein levels in chicken meat are 18.71%, tempeh protein 18.44%, and tofu protein 13.84% (Sundari *et al.*, 2015). Chicken meat is a favourable option for processing into sausage. The sausage manufacturing process involves grinding, mixing meat with spices, filling it into casings, steaming, and storing (Rumandor *et al.*, 2018). Fermented sausage is a type of fermented meat that consumers favour for its flavour and texture.

The flavour of fermented sausage is a significant aspect, resulting from the metabolic processes of carbohydrates, proteins, amino acids, fats, and fatty acids. Consumers favour fermented products for their distinctive fermentation flavour properties, unique taste, and rich nutrients. Most long-established sausages undergo natural fermentation, making them susceptible to pathogen contamination (Fadhlorrohan *et al.*, 2024).

Fermentation is proven to be a significant method for extending the shelf life and enhancing the nutritional profile of sausage (Wang *et al.*, 2022). This process involves microorganisms crucial for optimising quality during fermentation (Bao *et al.*, 2018). Numerous studies have utilised various microorganisms as starter cultures in producing fermented sausages, with *Lactobacillus plantarum* being the most widely used and effective (Luan *et al.*, 2021). Furthermore, research has demonstrated improvements in the reduction of pathogenic bacteria (Muhammad *et al.*, 2019), decreased oxidative rancidity of fermented sausage through its in vitro antioxidant activity (Luan *et al.*, 2021), and enhancement of the physicochemical, microbial, and sensory properties due to the use of *L. Plantarum* as starter cultures (Arief *et al.*, 2014). Studies have consistently reported the beneficial impacts of *L. Plantarum* across various sausage types, including beef sausages (Meristica *et al.*, 2020) and lamb sausages (Sulaiman *et al.*, 2016). However, Indonesia, being a country abundant in broiler chicken (Nugroho, 2020), and still has limited information regarding the role of *L. plantarum*

in chicken meat-based fermented sausage, commonly referred to as Salami (Yörük & Güner, 2017).

Salami is a raw sausage fermented with lactic acid bacteria to make the product last longer and improve taste, resulting in a rough texture, brownish colour, and a garlic flavour from herbs and spices. One way to enhance the aroma and flavour of sausages is by applying the fermentation process during the sausage-making process. The fermentation process will break down the complex compounds in the sausage into simpler components, resulting in a more distinctive aroma and flavour than other sausages (Barus *et al.*, 2017). When making this sausage, we can detect the presence and number of microbes in a food ingredient, one of which is this fermented product, so that the quality and quality level of the food ingredient can also be known. The parameter used in this research is the total number of microbial and Lactic acid bacteria. One method for calculating the number of microbes in a food ingredient is to measure the number of cells present using the total plate count.

METHOD

Tools are used include one pot, three jars, 1500 ml glass beaker, two thermometers, one autoclave, 216 test tubes, 144 Petri dishes, 3 Bunsen burners, one colony counter, one micropipette, one stove, one incubator, label paper, umbrella paper, aluminium foil, plastic triangle and thread. Materials used include *L. plantarum* TW 14, 2.5 kg of chicken breast for total three roll plastic sleeve, tapioca flour 250g, table salt six teaspoons (30 g, medium garlic 15

cloves, pepper powder six spoons, egg whites nine eggs MRS Broth 19.1, MRSA (De Man Rogosa Sharpe Agar) 89.28 g skim milk solution 30g, physiological NaCl 12.24 g.

In the initial stage, procure 2.5 kg of chicken breast meat. Subsequently, incorporate a selection of spices and blend the mixture until a homogeneous consistency is achieved. Prepare the sausage casings, after which the prepared mixture will be inserted into the casing and secured tightly. The sausage should then undergo steaming for 20 minutes. Once it has cooled, it is to be extricated from the casing and immersed in a *Lactobacillus plantarum* substrate at concentrations of 5, 10, and 15, relative to a mass of 80 grams of sausage, for 24 hours. Finally, the product will be placed into sterile plastic containers, appropriately packaged, and stored at ambient temperature for intervals of 0, 12, and 24 hours.

The calculation of total microbes can be calculated using the following formula:

$$\sum \text{colonies} = \sum \text{number of colonies} \times \frac{1}{\text{dilution factors}}$$

Dilution factor = Dilution x dissolved in test tube (grown) % with a storage time of 24 hours.

Colony counts are carried out using the standard plate count, which counts from the cup and selects the number of colonies in the sample suitable for application (Azizah & Soesetyaningsih, 2020). In the present study, the colony's pH was not evaluated; the analysis was confined solely to the total microbial count and the aggregate of lactic acid bacteria.

The research design used in this study was a Completely Randomized Design (CRD) with a 3x3 factorial pattern with 3 replications, namely the concentration factor of

Lactobacillus plantarum (K) and storage duration (L). Factor K is divided into 3, namely: $K_1 = 5\%$, $K_2 = 10\%$, and $K_3 = 15\%$. Factor L is divided into three, namely: $L_0 = 0$ jam, $L_1 = 12$ jam, and $L_2 = 24$ hours. So that the treatment is obtained as follows:

K_1L_0 = Sausage + *Lactobacillus plantarum* 5 % with a storage time of 0 hours

K_1L_1 = Sausage + *Lactobacillus plantarum* 5 % with a storage time 12 hours

K_1L_2 = Sausage + *Lactobacillus plantarum* 5 % with a storage time 24 hours

K_2L_0 = Sausage *Lactobacillus plantarum* 10 % with a storage time of 0 hours

K_2L_1 = Sausage *Lactobacillus plantarum* 10 % with a storage time of 12 hours

K_2L_2 = Sausage + *Lactobacillus plantarum* 10 % with a storage time of 24 hours

K_3L_0 = Sausage + *Lactobacillus plantarum* 15 % with a storage time of 0 hours

K_3L_1 = Sausage + *Lactobacillus plantarum* 15 % with a storage time of 12 hours

K_3L_2 = Sausage + *Lactobacillus plantarum* 15 % with a storage time of 24 hours.

The experimental conditions in this study involved the preparation of fermented sausages, each with a uniform weight of 80 grams, and inoculated with bacterial concentrations of 5%, 10%, and 15%. These concentrations refer to the proportion of bacterial inoculum relative to the weight of the sausage. For example, a 5% concentration corresponds to 4 grams of bacterial culture per 80 grams of sausage. The samples were then subjected to storage at room temperature (non-refrigerated) for 0, 12, and 24 hours. Each experimental condition was performed in triplicate, using sausages of

consistent weight (80 grams) to ensure reproducibility and accuracy of the results.

The data obtained were tabulated using SPSS with the Completely Randomized Design (CRD) method, and continued with the orthogonal polynomial test (Oliveira *et al.*, 2019). The formula for the complete random design equation is:

Y_{ijk} : The result of observation at the level of factor K level ke-i, factor L level ke-j and repeat ke-k

μ : Mean Value

α_i : Effect of treatment of factor K to α_i

β_j : Effect of treatment factor r L to β_j

$(\alpha\beta)_{ij}$: The interaction effect of the treatment of factor K and factor L

ε_{ijk} : Effect of randomisation on treatment combination to $i j$ replication to k ,

i : The number treatment of factor K ($i = 1, 2, 3$)

j : Number of treatments of factor L ($j = 1, 2, 3$)

k : Number of replicates (1, 2, 3)

RESULTS AND DISCUSSION

Effect of Various Concentrations of *Lactobacillus plantarum* Bacteria Against Total Microbial CFU/cm²

The results of the total microbial test for fermented chicken sausage with various concentrations of *L. plantarum*, according to Table 1, show that the lowest total microbes were obtained in the 5% *L. plantarum* concentration, 16.30 ± 0.74 log CFU/cm², and the highest total microbes were obtained from the treatment with a concentration of 15% with 17.09 ± 0.37 log CFU/cm².

Traditional fermentation generally involves microbes present in the raw materials when making sausages, so various microbes grow according to environmental changes. In this way, unwanted microbes can increase, causing failure in the fermentation process. Microbes are the cause of product damage. Food ingredients, but the use of *L. Plantarum* did not have a significant effect ($P > 0.05$) on the total fermented chicken sausage microbes seen from the use levels of 5%, 10%, and 15%.

The results in Table 1 suggest that the quality of fermented food is poor or good and often contaminated by pathogenic microbes and putrefactive bacteria at the start of processing, resulting in microbial contamination. The shelf life of the product is increased, but it is short. Microbes generally involved in food fermentation are bacteria, yeast, and mould (Kusuma *et al.*, 2017). The antimicrobial on *L. plantarum* was found not to work optimally because of the low supplemental and moisture content, which allowed more microbes to grow on meat; therefore, the greater the concentration, the greater the antimicrobial inhibition zone formed.

The basic principle of fermentation is activating certain microbial activities to change the properties of ingredients, producing useful fermented products. Several factors influence fermentation, including microorganisms, substrate (medium), pH (acidity), temperature, oxygen, and water activity (Carballo, 2021).

Effect of Storage Time on Fermented Chicken Sausage with Various Concentrations of *Lactobacillus Plantarum* Bacteria Against Total Microbial CFU/cm²

The results of the total microbial test for fermented chicken sausage with adding *L. plantarum* concentration on storage time obtained an average of 15.45 ± 0.19 log CFU/cm² to 17.45 ± 0.17 log CFU/cm². Based on the results above, the interaction of *L. plantarum* TW 14 with different storage times did not have a significant effect ($P > 0.05$) on the total microbial value of fermented chicken sausage.

Table 1 also shows that at a concentration of 5% *L. plantarum* TW 14, total microbes decreased at 0 hours of storage. The relationship of *L. plantarum* TW14 with storage time for total microbes cannot be separated from the fermentation process.

Salami is a raw sausage fermented using acid bacteria, such as lactobacilli, to make it more durable. This process can improve the product's taste by imparting a characteristic rough texture, brownish colour, and garlic and spice flavour (Sultana *et al.*, 2020).

The bacteria used for the fermentation process are acid-forming bacteria, including yoghurt starter with a combination of *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, and *Lactobacillus acidophilus* (Hendarto *et al.*, 2019). One test for pathogenic

microbial contamination is an essential indicator of the quality of processed meat suitable for consumption. Meat contamination can occur during carcass cleavage, cooling, freezing, refreshing frozen meat, carcass cutting, manufacturing processed meat products, preservation, packaging, storage, and marketing (Dewi *et al.*, 2016).

Meat is a suitable medium for the growth and prevalence of microorganisms (Dewi *et al.*, 2016). The microbiological quality of meat is determined by contamination of the Enterobacteriaceae group of bacteria, including *E. coli*, *Staphylococcus aureus*, *Salmonella sp.*, and *Campylobacter sp.* *E. Coli* bacteria inhabit the large intestine in animals (Ramadhani *et al.*, 2020).

Hygienic handling with the best sanitation system can overcome or reduce this contamination. The existence of pathogenic microbes in meat is possible due to its high nutritional content. Microbes usually involved in food fermentation are bacteria, yeast, and moulds (Arini, 2017). The basic principle of fermentation is to activate certain microbial activities to change the nature of the ingredients, resulting in useful fermentation products (Nursita *et al.*, 2022).

Table 1. Average Calculation of Total Microbial CFU/cm² for Treatment Combination of *L. plantarum* TW14 with Extended Storage for Fermented Chicken Sausage

Time (hours)	Concentration <i>L. Plantarum</i> TW14 (%)			Average	Significance
	5	10	15		
0	16.84±0.65	15.81±0.90	17.15±0.25	16.60	NS
12	16.60±0.61	16.21±0.47	16.68±0.48	16.52	NS
24	15.45±0.19	16.09±0.67	17.45±0.17	16.33	NS
Average	16.30±0.74	16.02±0.46	17.09±0.37		

Note: Non-Significant ($P > 0.05$)

According to Kurnianto, during the fermentation process, the sausage's pH value will decrease from 4.2 to 5.2 (Kurnianto & Munarko, 2022). This decrease is generally caused by the breakdown of carbohydrates into acidic lactic acid.

In line with this opinion, according to Table 1 above, traditional fermentation generally involves microbes present in the raw materials when making sausages. Hence, various types of microbes grow according to environmental changes. In some cases, unwanted microbes can develop, causing failure in the fermentation process. Microbes are the cause of damage to food products. Still, the use of *L. plantarum* did not have a significant effect on the total microbes of fermented chicken sausage in the use of 5%, 10%, and 15% concentrations and in the storage of fermented chicken sausage for 0 hours, 12 hours, and 24 hours.

Salami *et al.* (2024) state that the increase in lactic acid bacteria indicates the occurrence of fermentation or reproduction of lactic acid bacteria. Then a decline occurs, which means the condition of the bacteria is in the stable phase, which is the phase of the end of the activity of lactic acid bacteria.

The above results suggest that the quality of fermented foods is already poor and easily contaminated by pathogenic microbes and spoilage bacteria at the beginning of processing, resulting in microbial contamination. Following Rahmi *et al.* (2021) good handling and sanitation are needed to maintain food products; the longer they are in the open air, the lower their freshness. Products that easily and quickly

decay (highly perishable food) require fast, clean, careful, and cold handling to maintain quality.

Effect Of Various Concentrations of *L. plantarum* Bacteria and Storage Time for Sausages Fermented Chicken on Total Lactic Acid Bacteria

The absence of a statistically significant effect ($P>0.05$) on total microbial counts following the application of varying concentrations of *Lactobacillus plantarum* may be attributed to multiple factors (Table 2). One plausible explanation lies in the indigenous microbial populations naturally inhabiting the raw chicken meat. These autochthonous microorganisms may have competed with the inoculated *L. plantarum*, diminishing its ability to dominate and suppress the microbial community. Spontaneous fermentation, as often occurs in traditionally processed meat products, facilitates the proliferation of beneficial and spoilage-associated microbes (Petrovic *et al.*, 2025), which may obscure the specific influence of the starter culture on total microbial dynamics.

Based on the results of the variance analysis and the data above, it can be seen that there is an interaction between *L. plantarum* and total lactic acid bacteria in fermented chicken sausage. From Table 2, the total bacterial results are obtained. The lowest lactic acid was obtained in the treatment with a total concentration of 10% *L. plantarum* TW14 at a storage time of 12 hours, namely 15.56%, and the highest total lactic acid bacteria was obtained in treatment concentration 15% *L.*

plantarum TW14 for a storage period of 24 hours, which is equal to 18.709%.

Table 2 also shows that at a concentration of 5% *L. plantarum* TW14, occurring for a long time, storage had no significant effect ($P > 0.05$) on the total lactic acid bacteria of chicken sausage fermentation with a storage period of 0 hours, 12 hours, and 24 hours. Moreover, Suboptimal fermentation conditions, including inadequate water activity, unfavourable pH, and limited nutrient availability, could have further attenuated the antimicrobial efficacy of *L. plantarum*. In such environments, the bacterium may not have reached sufficient metabolic activity to exert measurable inhibitory effects on competing microbial taxa. This study's relatively short storage duration (0, 12, and 24 hours) may not have provided adequate time for differential microbial growth to manifest distinctly across treatments.

Furthermore, Table 2 shows no significant difference; a 10% *L. plantarum* TW14 concentration decreased total lactic acid bacteria in fermented chicken sausage during long storage (0 hours, 12 hours, and 24 hours). However, a 15% *L. Plantarum* TW14 concentration increased total lactic acid bacteria fermentation in chicken sausage.

The results of the administration effect (5%, 10%, 15%) at a storage period of 0 hours revealed that 10% yielded 16.199 log CFU/cm², while 10% concentrations showed 17.335 log CFU/cm² and 17.949 log CFU/cm². The concentration of *Lactobacillus plantarum*

and the duration of storage can significantly influence the total number of lactic acid bacteria (LAB) through a dynamic interaction between microbial competition and environmental adaptation. Higher initial concentrations of *L. plantarum* introduce more viable cells, accelerating colonisation and enhancing the production of antimicrobial compounds such as lactic acid and bacteriocins (Ayivi *et al.*, 2020). Over time, extended storage allows these bacteria to metabolise nutrients and lower the pH (Addis, 2015), creating conditions that further support LAB proliferation while inhibiting spoilage microbes. Therefore, increased concentration coupled with longer fermentation duration provides a synergistic effect, promoting optimal growth and activity of LAB in fermented meat products.

The greater the concentration of *L. plantarum*, the greater the lactate produced because the nutrient medium is sufficient for the growth and development of acid bacteria in fermented chicken sausage. The relationship of *L. plantarum* TW14 with the old-age storage of total lactic acid bacteria cannot be separated from the fermentation process. Bacterial activity is thought to slow down lactic acid so that acid production decreases. Several factors, including critical nutrition for the bacteria and unsuitable environmental conditions, cause the decline in the activity of lactic acid bacteria.

The decreased total in lactic acid during ripening is due to fermentation caused by less material being converted into acid.

Table 2. Combination of *L. plantarum* TW 14 Treatment with Storage Time for Sausages Fermented Chicken

Time (hpurs)	Concentration <i>L. plantarum</i> TW 14 (%)			Average
	5	10	15	
0	16.199 ^{a,b,c}	17.335 ^{b,c}	17.949 ^{b,c}	17.161 ^{b,c}
12	16.154 ^{a,b,c}	15.565 ^{b,c}	16.808 ^{a,b}	16.175 ^{a,b}
24	18.125 ^{a,b,c}	17.772 ^c	18.709 ^a	18.202 ^a
Average	16.826 ^p	16.890 ^q	17.822 ^p	

Note: Numbers followed by the same superscript (a, b, and c) and (p and q) do not show any real difference

According to [Setiawan *et al.* \(2013\)](#) LAB growth is influenced, among other things, by the initial number of lactic acid bacteria and competing microorganisms, fermentation temperature, and salt concentration. This factor can hinder the growth of increasing the shelf life of meat salami.

Furthermore, Table 2 demonstrates that a longer storage duration is associated with increased total lactic acid bacteria (LAB) concentration in sausages. Although no statistically significant interaction was observed between LAB concentration and storage time on total microbial load, it is plausible that a longer storage duration could enhance the selective proliferation of *L. plantarum*, especially at higher inoculation levels. At low concentrations, the competitive advantage of LAB may be limited. In contrast, at higher concentrations, LAB could begin to exert bacteriostatic or bactericidal effects more prominently, which aligns with the findings of [Baquero & Levi \(2020\)](#), but only after a sufficient incubation period. Therefore, the combined effects of higher LAB concentration and extended storage may exhibit a delayed synergistic interaction, which was not fully captured within the temporal constraints of this study.

This observation aligns with findings by [Utami *et al.* \(2015\)](#), which indicates that LAB populations tend to rise during the fermentation process. As suggested by [Dewi *et al.* \(2016\)](#), it is reasonable to hypothesise that during fermentation, microorganisms metabolise the nutrients in the sausages, including the meat.. Consequently, the combined effects of enhanced *L. plantarum* concentration and extended storage time may be an effective strategy for augmenting total LAB levels.

Additionally, according to [Nisa & Krisna Wardani \(2016\)](#), the process of increasing the nutritional content of sausages is done through fermentation. This fermentation process adds *Lactobacillus plantarum* bacteria, which are homofermentative and resistant to acidic conditions. Lactic acid bacteria are known to improve food safety and quality due to their antagonistic activity against spoilage microorganisms and other microbial pathogens ([da Costa *et al.*, 2018](#)). LAB relies on its ability to inhibit pathogen growth by secreting antibacterial substances such as lactic acid and bacteriocins ([Agüero *et al.*, 2020](#)).

Adding *Lactobacillus plantarum* to sausage products converts glucose into lactic acid, activating and multiplying lactic acid bacteria, thus maintaining the quality of the

sausage (Salami *et al.*, 2024). Higher total acid decreases the optimal pH. Lactic acid bacteria break down glucose into pyruvate, which reduces NADH₂ to lactic acid. As lactic acid increases, H⁺ is added and pH decreases (Sultana *et al.*, 2020). We regrettably did not observe any biological changes besides the chemical observation. Moreover, to our knowledge, no existing studies report biological changes in fermented sausages. Nevertheless, no physical changes were noted during the experimental period. Thus, it is plausible that the fermentation process and the short storage duration did not induce any biological changes in the sausages.

According to Khoiriyah & Ardiningsih (2014) lactic acid bacteria produce antimicrobial compounds, including organic acids, hydrogen peroxide, and bacteriocins. The addition of *L. plantarum* causes the diameter of the inhibition zone to be formed, which can be the diameter of the clear zone around the well, showing the bactericidal properties of killing bacteria, and the pseudohyphal zone diameter, which shows the bacteriostatic properties inhibiting microbial growth (Yulinery & Nurhidayat, 2015).

Adding a starter is expected to produce sausage fermentation with better physical and organoleptic qualities, and the results show that the fermented sausage does not contain *Salmonella sp.* Bacterial activity, the lactic acid in sausages that are still good, can produce organic compounds, bacteriocins, and antimicrobials that effectively inhibit the growth of pathogenic bacteria. For example, the

H₂O₂ produced by *L. plantarum* can hinder the development of *Salmonella sp.*

CONCLUSIONS AND RECOMMENDATIONS

There was a real interaction between the concentration of *L. plantarum* TW14 bacteria and the total lactic acid bacteria in fermented chicken sausage. At the concentration of *L. plantarum* TW14 bacteria with the addition of 5%, 10%, 15%, and storage time of 0, 12, and 24 hours, there is no significant effect on total microbes in fermented chicken sausage. At storage times of 0, 12, and 24 hours, there is no effect on the total lactic acid bacteria in fermented chicken sausage. Still, the concentrations of 5%, 10%, and 15% had a real impact on fermented chicken sausage. Fermented chicken sausage using *L. plantarum* made at a concentration of 15% produces the most total Lactic Acid Bacteria in chicken sausage fermentation. In further research, the concentration of *L. plantarum* can also be increased so that the total number of lactic acid bacteria produced will be greater, improving the quality of fermented chicken sausage.

CONTRIBUTION STATEMENT

All authors contributed to conceptualising the research design. Citra Kusuma (CK) and Elda Frediana Rety Kartika (EFRK) collaborated on data curation, while Aan Andri Yano (AAY) assisted with formal analysis and methodology. Then, EFRK and Awin Pinasthika (AP) contributed to validating and investigating research data. Nella Mutia Arwin (NMA) and Satria Budi Kusuma (SBK) wrote the original draft, which AAY

subsequently reviewed. Finally, all authors approved the final manuscript.

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