Microbial qualities of rabbit meat fermented with
*Lactobacillus plantarum*

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Abstract. Rabbit meat had been known as the high protein content of meat. Protein was derived from peptides that it was a bioactive component and potential for human health. The bioactive component of meat could be obtained by proteolysis. Fermentation was one of the methods for meat proteolysis. *Lactobacillus (L.) plantarum* is one of the microorganisms for meat fermentation. This study aimed to determine the effect of adding *L. plantarum* and fermentation time of rabbit meat on Total Plate Count (TPC), Acid Lactic Bacteria (LAB) total, Titratable acidity and pH. The method was an experimental using factorial randomized block design. The first factor was concentration of *L. plantarum* (0%, 6%, 8% and 10%) and the second factor was fermentation time of meat (12 hours, 18 hours and 24 hours). The results showed that *L. plantarum* concentration had no effect on LAB total and TPC, while fermentation time had a significant effect (P<0.01) on TPC and LAB total (P<0.05). *L. plantarum* concentration and fermentation time had a significant effect (P<0.01) on titratable acidity, and on pH (P<0.05). As conclusion, the best rabbit meat fermented with *L. plantarum* concentration was 10% and the fermentation time was 18 hours.

1. Introduction

Meat is one of good protein sources that deliver all the amino acids to human body needs. Nutritional content and food safety are factors which can be used to determine the quality of meat. Rabbit meat has good nutritional content which is rich in protein and low in fat. Rabbit meat has a protein content of 20.80%, 10.20% fat, 67.90% water content, energy 7.30 MH/Kg, and cholesterol concentration of 50 mg/Kg [1]. The protein meat content is composed of peptides which are bioactive components. Bioactive component of meat protein is not active in protein, it needs a process to release the component (proteolysis), and one of ways is by the fermentation process [2].

Fermentation is a process of chemical change that overhauled organic subtraction using the help of enzymes produced by microorganisms [3]. Meat protein is generally degraded by the action of endogenous enzymes katepsin B, D, H and L, but from the type of lactic acid bacteria are also able to produce proteolytic enzymes during the fermentation process. Proteolytic enzymes help degrade protein by decreasing the pH value of meat and result in increased proteolytic activity that already exists in meat [2]. Microorganisms are often used in the process of meat fermentation are lactic acid bacteria from the types of *Lactobacillus, Micrococcus*, and *Staphylococcus* [3].

*L. plantarum* is a bacterium that is often used in the fermentation process, because the adaptation ability in higher fermentation temperatures with other fermentation bacteria. Food fermentation using *L. plantarum* will be homo-fermentative so that it does not produce gas, and only produces lactic acid
as the main product of fermentation [4]. Fermentation of rabbit meat using *L. plantarum* will produce lactic acid as a result of metabolism. Increasing the amount of lactic acid will have an impact on the pH of the meat as well as the microbiological state of the meat. Microbiological state of meat can be determined by calculating the total microorganisms and total lactic acid bacteria in meat. So far, *L. plantarum* concentration and duration of fermentation have not been well known in regard to produce the best quality fermented rabbit meat. Therefore, this study was expected to find the *L. plantarum* and fermentation time to produce best-fermented rabbit meat in terms of Total Plate Count (TPC), Total Lactic Acid Bacteria, Lactic Acid Content and pH.

## 2. Materials and methods

### 2.1. Samples collection and preparation

Rabbit meat was obtained from the fresh meat of Male New Zealand White rabbits aged 3-4 months. The study was an experimental that arranged using a factorial Randomized Block Design (RBD). Samples of 250 gr rabbit meat were prepared, then grinded and put into an air-tight jar. *L. plantarum* was added according to the treatment (0%, 6%, 8% and 10% of the fresh meat weight) then covered tightly for the fermentation process according to handling treatment (12 hours, 18 hours and 24 hours). Each treatment was repeated three times. The data obtained were analysed using Variant Analysis and continued with Duncan’s Multiple Range Test.

### 2.2. Assessment of variables

**2.2.1. Total Plate Count (TPC).** Method in SNI 2897 in this study was occupied in testing the number of plate count. The procedure of testing were (1) a sample was weighed at 25 g then put it into Erlenmeyer; (2) a 225 mL of 0.1% BWP solution was added and homogenized (This was a 10-1 dilution solution), (3) a 1 mL of 10-1 dilution suspense was taken and mixed into a 9 mL BWP solution to obtain a 10-1 dilution for further to achieve a 10-8 dilution (4), then a 1 mL of suspension was introduced from the last 3 dilutions (10-6, 10-7 and 10-8) into the petri dishes, then added with 20 mL of Plate Count Agar (PCA) and both front and back was released using number 8 and (5) incubated at temperature 34-36°C for 24 hours by setting the cup upside down.

**2.2.2. Total of lactic acid bacteria.** In counting the number of Lactic Acid Bacteria, a combination of SNI 2897 and Harrigan [5] were used. The testing procedure was similar with the SNI procedure but the difference was the bacterial growth media. Total Lactic Acid bacteria used Man Rogosa Sharpe Agar (MRS Agar) (MRS Agar 68.2 g: 1 L Aquadest) for Lactic Acid Bacteria media growth.

**2.2.3. Total of titratable acidity.** The acidity testing in fermented rabbit meat was a titration method based on AOAC [6]. The procedure was: (1) a sample of 5 g was taken then crushed, then (2) put in 100 mL measuring flask and added 50 mL aquadest, stirred. (3) The diluted sample was added with 3 drops of 1% phenolphthalein, then (4) titrated using 0.1 N NaOH solution until pink colour arise. (5) The total titrated acid was assumed to be total lactic acid and calculated by the following formulation.

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\text{Total acid (\%) = \frac{m}{0.1 \text{ N NaOH} \times 0.009 \times 100}}
\]

**2.2.4 pH.** Meat pH was carried out using a pH meter and based on SNI 06-6989.11-2004. The following procedure was (1) preparation a sample of 5 g then put in a beaker and added as much as 50 mL aquadest, Stirring until mixed, the electrode was dried with a tissue and (3) dipped the electrode into the test sample to the pH meter. (4) Note the reading of the scale or number on the display of the pH meter.
3. Results and discussion

3.1 Total Plate Count (TPC)
TPC was a method for calculating the number of all microbes present in a product by counting the number of colonies in the growth media. Based on the analysis of variance, there was no interaction in the L. plantarum concentration and the fermentation time factors. The difference in L. plantarum concentration was small, so it did not have a significant effect on the total plate count, but at the time of fermentation factor showed the significant effect (P <0.01) on TPC. The following TPC data were based on the required concentration of L. plantarum factor as shown in table 1 and fermentation time factor in table 2.

Table 1. TPC of rabbit meat based on L. plantarum concentration

| Concentration (%) | Average (10^7 cfu/gr) |
|-------------------|-----------------------|
| 0                 | 98.56                 |
| 6                 | 100.78                |
| 8                 | 102.33                |
| 10                | 104.00                |

Table 2. TPC of rabbit meat based on fermentation time

| Fermentation Time (Hour) | Average (10^7 cfu/gr) |
|--------------------------|-----------------------|
| 12                       | 89.00^a               |
| 18                       | 154.67^b              |
| 24                       | 60.58^a               |

^a Superscripts in the same column differ significantly (P<0.01)

The number of microbes as shown in table 1 are mostly found in the treatment with the addition of 10%. It is higher when compared to the SNI standard 2009 (aerobic and mesophyl 1x 10^6 cfu/g). The high TPC found was due to a control fermentation process. The high bacterial population of L. plantarum in the fermentation of rabbit meat might be caused by high protein content. Proteins are composed by C, H, O, and N compounds, in which needed for the growth of living things including bacteria. An appropriate environment including the availability of adequate nutrition would increase bacterial productivity which can be described as biomass output per unit time of fermentation [8].

The fermentation time of rabbit meat based as shown in table 2 shows that 12 and 24 hours treatments had the same quality, while the 18 hours fermentation had a higher TPC, indicates the best quality. The results of TPC were lower when compared with L. plantarum fermentation in white egg; 5.884 ± 0.157 x 10^10 cfu/mL [11]. The difference in the number of microbes is determined by the fermentation time [12]. Several studies had showed the fermentation time which affected the microbial population, including L. plantarum bacteria in the Bekasam fish, increasing the number of bacteria from 10^5 to 10^8 cfu/g for 6 days of fermentation [11].

3.2 Total of Lactic Acid Bacteria (TLAB)
The addition of L. plantarum and the different fermentation times did not show any interaction on the total of lactic acid bacteria, this was caused by only the fermentation time that had a significant effect (P<0.05) on the LAB total, but also the difference in concentration of L. plantarum adding was small. TLAB research data based on L. plantarum concentration factor can be seen in the table 3, and fermentation time factor can be seen in the table 4.
Total of Lactic Acid Bacteria as shown in table 3 are from 0% to 10%. The increase in TLAB was due to the controlled fermentation temperature of 35-37°C. It was supported the growth of lactic acid bacteria, especially *L. plantarum* bacteria. Based on the temperature level, microbes can be divided into three groups, namely psychophilic microbes (microbes grew at the temperatures ranging from 0-30°C); mesophilic microbes (microbes grew at the temperatures ranging from 30-60°C) and thermophilic microbes (microbes grew at the temperatures around 40-80°C) [12]. *Lactobacillus plantarum* was a type of mesophilic microbial, it could have optimum activity at temperatures ranging from 30-40°C [13]. The final pH, Total Soluble Solid and lactic acid content of 7-days fermented meat samples as affected by starter culture and fermentation temperature on fermentation of carbohydrates [14].

Based on the table 4 shows that 18 hours fermentation time had the best effect on the LAB Total; 117.33 x 10⁷ cfu/gr. It is generally believed that the minimum concentration of living probiotic microorganisms (*L. plantarum* strain) in the fermented food/product at the time of consumption should be at least 10⁷ cfu/mL or g, to achieve the proposed health benefits [15]. *Lactobacillus plantarum* live with a temperature range of 5-53°C and at pH conditions of 4.5 to 6.5, the optimum temperature usually range from 30-40°C [9]. *L. plantarum* T3, *L. plantarum* UA3, *L. plantarum* AA2, and *L. plantarum* AA11 had the log phase occurred for 8 hours (0–8 hours of incubation), except for isolates *L. plan trum* T32 (0–10 hours of incubation) and T13 (0–20 hours incubation) which had a longer log phase. *L. plantarum* UA3 was the fast log phase (0–8 hours of incubation) and stationary phase (8–17 hours of incubation), the number of isolate cells also the highest, which was 9.74 log CFU/mL. *L. plantarum* AA2, *L. plantarum* AA11, *L. plantarum* T13 showed the stationary growth that still continued until end of incubation for 24 hours [20].

### Table 3. TLAB of rabbit meat based on *L. plantarum* concentration

| Concentration | Average (10⁷ cfu/gr) |
|---------------|---------------------|
| 0%           | 65.56               |
| 6%           | 82.11               |
| 8%           | 92.44               |
| 10%          | 104.33              |

### Table 4. TLAB of rabbit meat based on fermentation time

| Fermentation Time | Average (10⁷ cfu/gr) |
|-------------------|---------------------|
| 12 Hours          | 79.83<sup>ab</sup>  |
| 18 Hours          | 117.33<sup>b</sup>  |
| 24 Hours          | 61.17<sup>a</sup>   |

<sup>Superscripts in the same column differ significantly (P<0.05)</sup>

3.3 **Total of Titratable Acidity (TTA)**

*Lactobacillus plantarum* concentration and fermentation time had significant effect (P<0.01) on titratable acidity however there was no interaction was found. Research data based on *L. plantarum* concentration can be seen in the table 5, while fermentation time are shown in the table 6.

### Table 5. TTA of rabbit meat based on *L. plantarum* concentration

| Concentration | Average (%) |
|---------------|-------------|
| 0%           | 0.68<sup>a</sup> |
| 6%           | 0.73<sup>b</sup> |
| 8%           | 0.81<sup>c</sup> |
| 10%          | 0.88<sup>d</sup> |

<sup>Superscripts in the same column differ significantly (P<0.01)</sup>
Table 6. TTA of rabbit meat based on fermentation time

| Fermentation Time | Average |
|-------------------|---------|
| 12 Hours          | 0.72$^a$|
| 18 Hours          | 0.78$^b$|
| 24 Hours          | 0.82$^c$|

$^a$Superscripts in the same column differ significantly (P<0.01)

TTA from 0% to 10% concentration had increased, because the *Lactobacillus* bacteria was a bacterium with the main metabolite results in the form of lactic acid. The more of lactic acid bacteria in a product could be produce the more of lactic acid. Lactic acid production of *Lactobacillus plantarum* was highest when compared to *Pediococcus acidilactici, L. brevis*, and *Ln. mesenteroides* [16]. *Lactobacillus plantarum* was one of the lactic acid-producing bacteria with a tendency to live in anaerobic conditions, and the result of fermentation was only lactic acid [4]. The results of the study were higher when compared with the results of lactic acid of fermented egg albumen by *L. plantarum* 0.077-0.167 [9]. Rabbit meat had a high protein so Lactic acid content was produced from protein. *L. plantarum* was a proteolytic bacterium that could convert protein compounds into simpler compounds such as lactic acid [17].

Fermentation time from 12 hours to 24 hours showed increased TTA. The optimum proliferation temperature (37°C, which was close to 35°C) of *L. plantarum*, and the optimum time for LAB activity (>12 hours), *L. plantarum* would produce more lactic acid, which would lead to a reduction in pH, increased TTA, and higher acidity in the sourdough [18]. A similar study using *L. plantarum* bacteria in the bekasam fish experienced an increase in total titrated acid of 0.02% to 0.18% for 9 days of fermentation [13]. The difference in the percentage of total acid every food was determined by the ability of microbes to break down the constituent components of the food. Utilization of *L. plantarum* could increase acidity by 1.5 to 2.0% in the substrate [17].

3.4 pH

The concentration of *L. plantarum* and fermentation time did not provide any interaction. However, each factor had a significant effect (P <0.05) on the pH value of rabbit meat. The pH value of rabbit meat based on increasing *L. plantarum* level and fermentation time, can be seen at table 7 and 8, respectively.

Table 7. pH of rabbit meat based on *L. plantarum* concentration

| Concentration | Average |
|---------------|---------|
| 0%            | 5.28$^b$|
| 6%            | 4.85$^{ab}$|
| 8%            | 4.63$^a$|
| 10%           | 4.58$^a$|

Table 8. pH of rabbit meat based on fermentation time

| Fermentation Time | Mean |
|-------------------|------|
| 12 Hours          | 5.16$^b$|
| 18 Hours          | 4.73$^{ab}$|
| 24 Hours          | 4.62$^a$|

$^{ab}$Superscripts in the same column differ significantly (P<0.05)

The results showed that the more increasing concentration of *L. plantarum*, could decreased the pH value of rabbit meat, as well as the longer of fermentation time, could decreased the pH value of rabbit meat (table 7 and 8). The pH values of fermented rabbit meat were ranged from 4.58-5.28. In the general situation, the microbes could grow in pH values range of 3-6. Most of microbes are affected
by the optimum pH value, which caused the optimum growth. Based on pH minimum requirement livings, microbes were divided into three groups. Those were acidophilic microbes (microbes that grew at a pH ranging from 2.0 to 5.0); mesophilic microbes (microbes that grew at pH ranges from 5.5 to 8.0) and alcaliphilic microbes (microbes that grew at pH ranges from 8.4 to 9.5) [14]. Lactobacillus plantarum was a mesophilic microbe type, because it lived at pH value ranging from 4.5 to 6.5 [13]. The pH value of rabbit meat with the addition of 6% L. plantarum, and rabbit meat without the addition of L. plantarum mostly had the same quality.

The fermentation time of rabbit meat at 18 hours, resulted the same quality of pH value of the rabbit meat. The fermentation time was very influential on the pH value of rabbit meat. This was the same result as egg albumen fermentation using L. plantarum. The decreased of pH value at the fermentation time showed significant differences between 18 hours, 24 hours and 30 hours, with the results 7.689±0.035; 6.434±0.501; 6.353±0.65 respectively. Although there was no significant differences between 24-hour fermented egg albumen and 30-hour fermentation [9]. The utilization of L. plantarum bacteria in fermented fish could reduce of pH value from 6.15 to 4.41 after 9 days of fermentation [13]. The pH value of rabbit meat with 24-hour fermentation time was higher when compared to the pH value of Dark Firm Dry (DFD) beef fermentation using 2% L. plantarum and 24-hour fermentation time was 4.56 [19]. L. plantarum was able to modeled complex compounds into the simpler compounds, with the end result was lactic acid. The production of lactic acid could cause a lower pH value on the substrate, and causing an acidic atmosphere [17].

4. Conclusion
Based on the results of the study, it was found that the TPC and LAB Total of rabbit meat was mostly found at 10% concentration of L. plantarum and 18 hours fermentation time, TTA was 10% concentration of L. plantarum and 24 hours fermentation time, and pH value was 6% concentration of L. plantarum and 18 hours fermentation time. It can be concluded that the concentration of L. plantarum is 10% and 18 hours fermentation time to produce the best quality of fermented rabbit meat in terms of TPC, LAB Total, Titratable Acidity and pH Value. The results of this study are preliminary study to know bioactive peptide compound from rabbit meat.

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