Effect addition *Lactobacillus pentosus* and juice carrot on nutrition quality of fermented chicken sausage

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**Abstract.** The study aimed to effect the addition of *Lactobacillus pentosus* and juice carrot on the nutrition quality of the fermented chicken sausage. The research method was the experimental method by using a randomized block design (RBD) 3x3 factorial pattern with three replications as a group. Factor A is concentration of lactic acid bacteria, 0% (A1), 10% (A2), 20% (A3) and Factor B is the concentration of carrot, 0% (B1), 10% (B2), 20% (B3). The nutrition of composition measured was water content, protein content, fat content, and pH of fermented chicken sausages. The results showed no significant interaction (P> 0.05) on water content, protein content, fat content, and pH of fermented chicken sausage. Still, lactic acid bacteria concentration has a highly significant effect (P <0.01) on pH. The fermented chicken sausage contains water 63.27% - 66.47%, protein 15.40% - 17.20%, fat 6.89% - 7.55%, and pH 4.47-4.86. The results of fermented chicken sausage research are in accordance with Indonesian nutritional value standards.

1. **Introduction**

Meat is the main ingredient for making sausages, while the additives are fillers, binders, and other permitted seasonings. The meat commonly used in sausage processing comes from beef, chicken, and goat, but the three types of meat with high protein content at affordable prices is chicken meat with a protein content of 20-23% [1]. Fermented sausage (salami) is a fermented product of processed meat using lactic acid bacterial cultures. Lactic acid bacterial cultures convert carbohydrates into lactic acid [2]. The use of lactic acid bacteria in fermented sausage products aims to improve food safety. Lactic acid bacteria found in fermented sausages act as protection and biopreservation in improving food safety in these products [3].

Lactic acid bacteria produce various types of antibacterial compounds. Gram positive bacteria and gram negative bacteria can be inhibited by antibacterial compounds found in lactic acid bacteria. One of them is *Lactobacillus fermentum* L23, which can inhibit *Escherechia coli* and *Staphylococcus aereus* and *Listeria monocytogenes* [4][5][6][7]. Various antibacterial substances produced by probiotic bacteria are organic acids, hydrogen peroxide, diacetyl, and bacteriocins [8].

Lactic acid bacteria used to ferment milk also produces antimicrobial substances that act as natural antibiotics against pathogenic bacteria, increase digestibility is lactose sensitive, help maintain digestive health prevent various diseases, individuals and increase the safe storage time the product [9]. Lactic acid bacteria produces organic acids, primary metabolites, and lowers the pH of the
environment to 3 to 4.5 during the fermentation process so that it can kill other bacteria that live in the pH range 6 to 8. Compounds resulting from LAB metabolism such as hydroperoxide ($\text{H}_2\text{O}_2$), amino acids, diacetyl, $\text{CO}_2$ and bacteriocins [10]. The addition of bacteriocins in food is additive and biopreservative because it can bacteriostatic of pathogenic bacteria.

In this study, *Lactobacillus pentosus* was used, which is the result of isolation from Batusangkar. The probiotic properties contained in these bacteria are resistant to acidic pH, resistant to bile salts, and can inhibit the growth of pathogenic bacteria such as *Escherichia coli*, *Staphylococcus aureus*, and *Listeria monocytogenes*. Bacteria are added in the processing of fermented sausages to diversify processed meat products that have a distinctive taste and are beneficial for health.

To improve the functional properties of this fermented sausage, carrots are added. Carrots (*Daucus carota L.*) are a type of root vegetable and are known to the public as a source of vitamin A. Carrots can also function as antioxidants because they contain carotenoid compounds, especially β-carotene, which is a precursor to vitamin A or provitamin [11]. The hard cell walls in raw carrots make the body less than 25% beta-carotene into vitamin A. The development of fermented sausage products is the addition of antioxidants, one of which is the addition of carrots which can be used as a source of antioxidants.

2. Materials and methods

2.1. The material of the research

The ingredients used were sausages with the addition of lactic acid concentration and 30 grams of carrots. The materials used to see the nutritional value of fermented chicken sausage are H2SO4, 30% NaOH, distilled water, methyl red indicator, 0.1 N NaOH, spirits, benzene, phenolphthalein (pp). The equipment used in this study were label paper, porcelain plates, pH meter, electric oven, analytical scales, Kjeldahl flasks, funnels, distillation flasks, beaker glasses, Erlenmeyer, fume hoods, Bunsen, hyacinth pipettes, volumetric flasks, a set of Soxhlet tools, bunsen, grease paper, and aluminum foil.

2.2. Methods of the research

The method used was a 3x3 factorial randomized block design (RBD) with three replications. Where Factor A is the concentration of lactic acid bacteria, namely 0% (A1), 10% (A2) and 20% (A3), while Factor B is the concentration of carrots, namely 0% (B1), 10% (B2) and 20% (B3). The parameters observed were water content, protein content, fat content, and pH.

3. Results and discussion

3.1. The moisture content of fermented chicken sausage

The moisture content value of fermented chicken sausage in the combination of the addition of lactic acid bacteria and the concentration of adding carrots to fermented chicken sausage showed no interaction with the moisture content of fermented chicken sausage (analyzed using SPSS version 22 for windows software). Table 1 below shows the average protein content of fermented chicken sausage in each treatment.

| Concentration of Lactic Acid Bacteria (Factor A) | Concentration of Juice Carrot (Factor B) | Average |
|-----------------------------------------------|----------------------------------------|---------|
| A1                                            | 64,92                                   | 66,16   |
| A2                                            | 67,09                                   | 62,14   |
| A3                                            | 67,40                                   | 65,28   |
| Average                                       | 66,47                                   | 64,53   |
The combination with the addition of lactic acid bacteria and carrots showed no interaction with the moisture content of fermented chicken sausage. This shows that the addition of lactic acid bacteria up to 20% and carrots up to 20% has the same activity during the fermentation period so that the resulting lactic acid is relatively the same so that it does not affect the moisture content of the resulting fermented chicken sausage.

Table 1 shows the average moisture content of fermented chicken sausage by giving LAB concentrations (Factor A) 0%, 10%, and 20% ranging from 63.27% - 66.16%. In Factor A with different LAB concentrations, it was found that there was no significant effect (P > 0.05) on the percentage of water content. This is because the range of LAB concentrations added is not much different. So that the activity of lactic acid bacteria has no effect on water content. The lowest water content was found in the addition of 10% (A2) lactic acid bacteria concentration, with an average of 63.27%. The highest water content was found in the addition of 0% (A1) lactic acid bacteria concentration treatment, with an average of 66.16%.

For fermented chicken sausage with the addition of juice carrot (Factor B) 0%, 10%, and 20% concentrations ranged from 63.77% - 66.47%. The addition of juice carrot concentration (Factor B) did not have a significant effect (P > 0.05) on the moisture content of fermented chicken sausages. This is because the concentration ranges of carrots are not much different. The high and low water content can also be affected by the high fiber in carrots, the higher the fiber content, the higher the water content. Large polymer size, complex structure and many hydroxyl groups so that it is able to absorb large amounts of water are the causes of high water absorption in food fiber [12].

The research of fermented lamb sausage with Lactobacillus plantarum IIA-2C12 obtained a water content of 65.11%. This shows that the research results of fermented chicken sausage that have been carried out have a higher water content [13], and the results of research lamb with Lactobacillus plantarum IIA-2C12 starter produced a water content of 60.75% and sausages with Lactobacillus acidophilus IIA-2B4 starter produced a moisture content of 59.06% [14]. However, these results do not exceed the standard limit according to SNI concerning Quality Requirements for Meat Sausage, which is a maximum of 67%. This shows that fermented chicken sausage has nutrition value by the Indonesia nutrition standards [15].

3.2. The protein content of fermented chicken sausage
The value of protein content of fermented chicken sausage in the combination of the addition of lactic acid bacteria and the concentration of adding carrots to fermented chicken sausage showed no interaction with the protein content of fermented chicken sausage (analyzed using SPSS version 22 for windows software). Table 2 below the average protein content of fermented chicken sausage in each treatment.

| Concentration of Lactic Acid Bacteria (Factor A) | Concentration of Juice Carrot (Factor B) | Average |
|-------------------------------------------------|----------------------------------------|--------|
| B1                                              | B2                                     | B3     |
| A1                                              | 15.69                                  | 14.98  | 15.54  | 15.40  |
| A2                                              | 15.45                                  | 17.93  | 18.21  | 17.20  |
| A3                                              | 16.53                                  | 16.19  | 16.25  | 16.32  |
| Average                                         | 15.89                                  | 16.37  | 16.67  |        |

The combination with the addition of lactic acid bacteria and carrots showed no interaction with the protein content of fermented chicken sausage. This shows that the same activity during the fermentation period so that the resulting lactic acid is relatively the same as the addition of lactic acid bacteria up to 20% and carrots up to 20% so that it does not affect the protein content of the resulting fermented chicken sausage.
Table 2 shows the average fermented chicken sausage protein levels by giving LAB concentrations (Factor A) 0%, 10%, and 20% ranging from 15.40% - 17.20%. The average protein content of fermented chicken sausage had no significant effect (P> 0.05) on the addition of LAB with different concentrations (Factor A). This is because the control sausages also contain natural microbes that can donate protein. By the opinion, which states that if the acid produced is high enough by lactic acid bacteria, the proteolytic microbes will experience inhibition. The lactic acid bacteria protein has a higher digestibility because the constituent particles are more simple, namely amino acids [16].

For fermented chicken sausage with the addition of juice carrot (Factor B) 0%, 10%, and 20% concentrations ranged from 15.89% - 16.67%. The addition of juice carrot concentration (Factor B) did not have a significant effect (P> 0.05) on fermented chicken sausage protein content. This is due to the occurrence of protein denaturation during processing. The opinion states that denatured proteins result in the breaking of hydrogen bonds, hydrophobic interactions, salt bonds, and the opening of molecular folds. Besides that, another factor is the leaching process [17].

In this study, it was shown that the protein of fermented chicken sausage with the addition of the concentration of lactic acid bacteria (Factor A) and the addition of the concentration of carrots (Factor B) had protein content above 15% (Table 2). This result of the fermented chicken sausage has nutrition value by the Indonesia nutrition standards, with the minimum protein content limit for sausages is 13% [15].

### 3.3. Fat content of fermented chicken sausage

The value of fat content in fermented chicken sausage in the combination of the addition of the concentration of lactic acid bacteria and the concentration of adding carrots to fermented chicken sausage showed no interaction with the fat content of fermented chicken sausages (analyzed using SPSS version 22 for windows software). Table 3 below shows the average protein content of fermented chicken sausage in each treatment.

| Concentration of Lactic Acid Bacteria (Factor A) | Concentration of Juice Carrot (Factor B) | Average |
|-----------------------------------------------|-----------------------------------------|---------|
| A1                                            | 8.23, 7.23, 7.17                         | 7.54    |
| A2                                            | 7.40, 6.95, 7.83                         | 7.39    |
| A3                                            | 7.02, 6.39, 7.72                         | 6.38    |
| Average                                       | 7.55, 6.89, 6.91                         |         |

The combination with the addition of lactic acid bacteria and carrots showed no interaction with the fat content of fermented chicken sausage. This shows that the addition of lactic acid bacteria up to 20% and carrots up to 20% has the same activity during the fermentation period so that the resulting lactic acid is relatively the same so that it does not affect the resulting sausage fat content.

Table 3 shows the average fat content of sausages by giving LAB (Factor A) concentrations of 0%, 10%, and 20% ranging from 6.38% - 7.55%. The addition of LAB concentration (Factor A) did not have a significant effect (P> 0.05) on the fat content of fermented chicken sausage. This is because the increase in lactic acid bacteria concentration up to 20% slightly affects the fat breakdown activity. This is by the opinion which states that lactic acid bacteria have secondary lipolytic activity, which can break down fat into simpler chemical compounds [18]. Lipolytic activity is controlled by the lipase enzyme owned by lactic acid bacteria to free fatty acids [19]. The results showed that the fat content in the addition of the concentration of lactic acid bacteria (Factor A) tended to decrease because lactic acid bacteria could reduce fat in the meat. This research states that giving probiotic lactic acid bacteria can reduce the fat content of meat by up to 0.06% [20].
In sausages with the addition of juice carrot concentration (Factor B) 0%, 10%, and 20% ranged from 6.89% - 7.55%. The addition of juice carrot concentration (Factor B) did not have a significant effect (P> 0.05) on the fat content of sausages. This is due to the increased antioxidant activity with increasing carrots, preventing fat oxidation, thereby maintaining fat quality. The increase in the concentration of antioxidants added to the inhibition of fat oxidation increases, with the increase in inhibition, the antioxidant activity also increases to inhibit fat oxidation [21].

In this study, it was shown that the fat content of fermented chicken sausage with the addition of the concentration of lactic acid bacteria (Factor A) and the addition of the concentration of juice carrot (Factor B) had fat content above 6% (Table 3). This result of the fermented chicken sausage has nutrition value by the Indonesia nutrition standards, with the maximum fat content limit for sausages is 20% [15].

3.4. pH value of fermented chicken sausage

The pH value of fermented chicken sausage in the combination of the addition of the concentration of lactic acid bacteria and the concentration of adding carrots to fermented chicken sausage showed no interaction with the pH value of fermented chicken sausage (analyzed using SPSS version 22 for windows software). Table 4 below shows the results of further data testing using the DMRT test at the 5% level for each factor.

| Concentration of Lactic Acid Bacteria (Factor A) | Concentration of Juice Carrot (Factor B) | Average |
|-----------------------------------------------|-----------------------------------------|---------|
|                                               | B1           | B2     | B3     |                 |
| A1                                             | 4.87         | 4.83   | 4.87   | 4.86\(^a\)       |
| A2                                             | 4.63         | 4.53   | 4.53   | 4.56\(^b\)       |
| A3                                             | 4.57         | 4.43   | 4.40   | 4.47\(^c\)       |
| Average                                        | 4.69         | 4.60   | 4.60   |                 |

The numbers followed by different letters indicate a highly significant difference (P<0.01).

Combining the concentration of lactic acid bacteria and carrots showed no interaction with the pH value of fermented chicken sausage. Table 4 shows the average pH value of fermented chicken sausage by giving LAB concentrations (Factor A) 0%, 10%, and 20% ranging from 4.47 - 4.86. The treatment of addition of LAB concentration (Factor A) had a very significant effect (P <0.01) on the pH value of fermented chicken sausage, namely the highest was the LAB concentration of 0% (A1) with an average pH value of 4.86 and the lowest was the concentration administration. LAB 20% (A3) with an average pH value of 4.47. This is because the more lactic acid is formed, the pH value will decrease. The opinion states that the value of acidity and pH has a close relationship with increasing metabolic activity so that lactic acid production increases while the pH value decreases [22]. Lactic acid produced during the fermentation process can increase the taste and increase the acidity or decrease the pH [23].

For fermented chicken sausage with the addition of juice carrot (Factor B) 0%, 10%, and 20% concentrations ranged from 4.60 - 4.69. The addition of juice carrot concentration (Factor B) did not have a significant effect (P> 0.05) on the pH value of fermented chicken sausage. This is because sausages' pH value is influenced by the pH value of normal meat, which undergoes processing factors and other ingredients. By the opinion processing factors and other ingredients added, namely: filler, binder, and spices, affect the pH value. Meat has the ultimate pH value between 5.3 and 5.8 [24]. That treatment during the meat processing process can change the pH value. The milling process will cause damage to the protein bonds of the meat, which will make it easier to change the position of H + and OH- ions when cooking, and this isoelectric point change causes the pH to change [25].
4. Conclusion

From the research results, it is known that there is no interaction between the addition of the concentration of lactic acid bacteria and the concentration of juice carrot on water content, protein content, fat content, and pH of fermented chicken sausage. Water content ranges from 63.27% - 66.47%, protein 15.40% - 17.20%, fat 6.89% - 7.55%, and pH 4.47-4.86. The result is that the fermented chicken sausage has nutrition value by the Indonesia nutrition standards (SNI, 2015). Therefore, it is suggested that further research is made by making fermented chicken sausage using Lactobacillus pentosus starter and juice carrot with different concentrations.

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