Physicochemical and Microbiological Properties of Fermented Lamb Sausages using Probiotic *Lactobacillus plantarum* IIA-2C12 as Starter Culture

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Abstract

The research aims to evaluate physicochemical and microbiological properties of fermented lamb sausages elaborated with probiotic *Lactobacillus plantarum* IIA-2C12 isolated from Indonesian local beef. The result showed that the addition probiotic *L. plantarum* IIA-2C12 (9 log cfu/ml) produced better quality of fermented lamb sausages than control (without probiotic). Physicochemical analysis showed that fermented lamb sausage with *L. plantarum* IIA-2C12 has a considerably soft texture and the final pH value and aw of fermented lamb sausage were 4.13 and 0.88, respectively. Additionally, the presence of *L. plantarum* IIA-2C12 significantly reduced the fat content (6.39% wb) and increased the protein content (19.26 %wb). Microbiological analysis displayed high population of lactic acid bacteria (9 log cfu/g) on fermented lamb sausage with *L. plantarum* IIA-2C12 with low population of *Escherichia coli* (1 log cfu/g) and none of *Salmonella* spp was detected. Preference test revealed that fermented lamb sausage with *L. plantarum* IIA-2C12 is the most preferable product.

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1. Introduction

The wide variety of fermented sausages in the world is a consequence of variation in raw materials, traditional habits of people at different regions and different manufacturing processes [1, 2, 3]. Indonesian fermented sausage, Uruutan, is a Balinese traditional fermented sausage, which is made of lean pork and fat as raw material miced with spices, sugar dan salt. Despite its popularity in Bali, urutan is less popular in the other place due to the lack of pork as main raw material. As Indonesian people have restriction to pork due to their religion, urutan is unable to be widely accepted in Indonesia. Hence, development of fermented sausage using other type of meats as raw material is unavoidable. Lamb is very common in Indonesia and much more acceptable than pork. Lamb is preferable for daily consumption in Indonesia, in which it is treated with certain processing such as roasting and making to be ‘satay’. Given its popularity and preferability, the usage of lamb as a main raw material in fermented sausage is promising to address the limitation of urutan and therefore interested to be deeply studied. We had successfully isolated a promising Indonesia probiotic Lactobacillus plantarum IIA-2C12 from Indonesian local beef Peranakan Ongole cattle. Previous studies revealed that this strain displayed probiotic characteristics including immunomodulator [4], antimicrobial activities against pathogenic bacteria [5], anti-diarrhea [6] and produced bacteriocins [7]. L. plantarum IIA-2C12 had been proved to prevent diarrhea caused by EPEC and repaired the hematology condition of diarrheal suspected rats [6] and can be applied as probiotic in yoghurt [4]. A simple and effective strategy for production of functional meat products is to incorporate functional ingredients such as probiotics [8]. Usually Lactobacillus species are dominant in sausages, such as Lactobacillus sakei, Lactobacillus curvatus and/or Lactobacillus plantarum. Other lactobacilli that may be found at minor levels include Lactobacillus pentosus, Lactobacillus paracasei, Lactobacillus casei and Lactobacillus alimentarius [9]. In this research probiotic L. plantarum IIA-2C12 was used as a starter for lamb sausage fermentation. Physicochemical and microbiological properties were discussed in comparison to spontaneous lamb sausage fermentation (in the absence of L. plantarum IIA-2C12).

2. Material and methods

2.1. Preparation of fermented lamb sausages

Lamb and a cocktail containing 2.5 % (w/v) NaCl, 5% (w/v) sucrose, 2.5% (w/v) ginger, 2.5% (w/v) pepper were mixed and filled to edible casings, resulting in 10 cm long and 5 cm diameter of fresh sausages. The first group sausage was kept ripened without the addition of probiotic L. plantarum IIA-2C12 (control), while the second group of sausage was treated by addition of L. plantarum IIA-2C12 as a starter culture prior to ripening/fermentation. Starter culture (2% with 10⁹ cfu/ml population of L. plantarum IIA-2C12) was added into first mixing. The mixed ingredients was ripened as following: the first stage consisted of one day drying at temperature of 25°C, then were cold smoked for 3 days with about 4 hours for each. The total ripening periods was 4 days at 27°C [10].

2.2. Examination of fermented lamb sausage

The pH value was determined by pH meter (Hanna Instrument, USA), Water activity (a_w) was analyzed by a_w meter (Novasina). Titratable acid was counted by titration methods. The moisture, ash, crude and fat contents were determined by Proximate analysis [11]. Carbohydrate content was calculated by difference analysis. For microbiological analysis of the fermented sausages, 25 g of each sample were homogenized into 225 mL of Buffered Peptone Water (BPW, Oxid). Further decimal solution was made and plate count analysis carried out on duplicated agar plates. The total population of the following bacteria were counted (a) lactic acid bacteria on MRS agar (Oxoid) incubated at 37°C for 48 hrs; (b) Staphylococcus aureus on Baird Parker medium (Oxoid) with addition of yolk tellurite emulsion (Oxoid), incubated at 37 °C for 24–48 hrs, (c) Escherichia coli on Eosin Methylene Blue agar (Oxoid) incubated at 37°C for 24-48 hrs and (d) Salmonella spp was qualitatively determined on serial media, including XLD agar (Oxoid) incubated at 37°C for 48 hrs. All analysis was performed according to BAM (2002). The products were evaluated with the criterion sensory analysis by a group of panels consisting of 75 semi-trained panelist using a preference test.
2.3. Statistical analysis

A completely randomized design was used in this experiment. For physicochemical and microbiological parameters, means and standard of errors from triplicate experiments were calculated and them the significance of the difference between two means was tested with T-test. For sensory parameter, rank sums of data from sensory analysis were tested for significance of the differences by the Kruskall Wallis test [12].

3. Result and Discussion

Table 1 shows the physicochemical and microbiological properties of fermented lamb sausages. The pH value of the fermented sausage with L. plantarum IIA-2C12 was significantly lower compared to that of control (p<0.05). It is plausible since the presence of L. plantarum IIA-2C12 drives the fermentation process and may enhance the production of acidic compounds during fermentation. This result is supported by the higher content of lactic acid, as a main acidic compound produced by LAB, on the fermented sausage with L. plantarum IIA-2C12 than of control (p<0.05) Both pH value and lactic acid content are implications of sugar metabolism by LAB, in which the sugar is converted into lactic acid, thus lowering pH values [2,13,14]. Noteworthy, the final pH value of the sausage was 4.13-4.39, typical of low acidity sausage and this was the result of the classical trend of LAB growth in the fermented sausage. It is interesting to be noted that the population of LAB increased during fermentation. Total LAB in the fresh lamb was 4 log 10 cfu/g and increased up to 6.43 – 9.32 log 10 cfu/g in the final products. L. plantarum IIA-2C12 displayed a growth curve with maximal logarithmic phase achieved within 12 hours of incubation time, in deMan Rogosa Sharp broth media incubated at 37°C (Fig. 1a). The pH value also decreased as increasing titratable acid increased during fermentation in MRS broth media (Fig. 1b).

The a_w values of all products were lower (0.88) compared to that of fresh lamb (0.91). It might be caused by the cold smoking treatment during the fermentation (Table 1). This value was also considerably lower compared with another fermented sausage, Hot Hungarian Salami. NSW Food Authority (2009) reported that Hot Hungarian salami had a standard a_w value of 0.90. In term of safety, lower a_w value is considerably better for food safety as it may inhibit pathogenic bacteria to grow in it. Fermented lamb sausage with L. plantarum IIA-2C12 displayed soft texture which might be include by proteolytic enzyme from L. plantarum IIA-2C12 that subsequently increased crude protein of the product.

Table 1. Physicochemical and microbiological properties of fermented lamb sausages (n=3)

|                         | Control (without probiotic) | With probiotic L. plantarum IIA-2C12 |
|-------------------------|-----------------------------|--------------------------------------|
| **Physicochemical properties** |                             |                                      |
| pH                      | 4.39 ± 0.01^a               | 4.13 ± 0.02^b                        |
| a_w                     | 0.88 ± 0.01                 | 0.88 ± 0.00                          |
| Lactic acid (%)         | 2.03 ± 0.14^a               | 3.03 ± 0.05^b                        |
| Texture (hardness) (kg/cm^2) | 0.42 ± 0.01^a                | 0.36 ± 0.02^b                        |
| Moisture (%wb)          | 65.11 ± 0.14                | 65.97 ± 0.17                         |
| Ash content (%wb)       | 1.86 ± 0.14                 | 1.94 ± 0.17                          |
| Fat content (% wb)      | 7.25 ± 0.18^a               | 6.39 ± 0.16^a                        |
| Crude protein (%wb)     | 18.87 ± 0.29^a              | 19.26 ± 0.24^a                       |
| Carbohydrate (%wb)      | 6.91 ± 0.26                 | 6.44 ± 0.20                          |
| **Microbiological properties** |                             |                                      |
| Total lactic acid bacteria (LAB) (log cfu/g) | 6.43 ± 0.03^a                      | 9.32 ± 0.03^b                      |
| Total E.coli (log cfu/g) | 3.58 ± 0.03^a               | 1.72 ± 0.02^a                        |
| Salmonella sp negative  |                            |                                      |

Data represents means ± standard error from 3 samples

^a,bValues in the same row followed by a superscript alphabet shows significance differences (P<0.05)
cfu/g = colony forming unit per gram
Meanwhile the hydrophilic process by *L. plantarum* IIA-2C12 might play an important roles on decreasing of the fat content in the product. The moisture contents of the fermented lamb sausages (65.11-65.97%) were similar to those described by other authors in other sausages [2,15,16,17]. During the drying period, the moisture content decreased as a result of moisture loss during cold-smoking.

Microbiological properties showed that the addition of probiotic *L. plantarum* IIA-2C12 decreased population of *E. coli* and increased the total lactic acid bacteria of fermented lamb sausages, compared to that of control (without probiotic) (p<0.05). Culture starters may increase the reduction in *E. coli* in fermented sausages. By using fermentation temperatures and conditions that are optimal for growth and acid production, a rapid decrease in pH is achieved and thereby an inhibition of any pathogenic *E. coli* present [18]. The presence of probiotic *L. plantarum* IIA-2C12 completely abolished the growing of *Salmonella* spp in the product. By contrast, *Salmonella* spp was detected on the product fermented without *L. plantarum* IIA-2C12 thus it is considerably unsafe to be consumed (Table 1.) The low aw and low pH value of fermented lamb sausage might promote inhibition of *E. coli* and *Salmonella* to grow in the product. Indeed, *L. plantarum* IIA-2C12 has been reported to display antimicrobial activity against pathogenic bacteria [5,7]. Accordingly, fermented lamb sausages with addition *L. plantarum* IIA-2C12 is considerably safe to be consumed based on GSO standard.

The results of the consumer preference test are presented in Table 2. In respect to aroma, color and texture, fermented lamb sausage with the addition of probiotic *L. plantarum* IIA-2C12 was more preferable than those of control (p<0.05). However, future improvement is needed to increase the acceptability of its taste. The panelists considerably do not like the overall taste of fermented lamb sausage with probiotic which might be due to the sourness in its taste. Sour taste is to a certain level commonly unacceptable by Indonesia people.

![Graph](image)

*Fig. 1 (A) growth curve of *L. plantarum* in MRS broth media and (B) the pH value (open square) and titratable acid was/TAR (filled square) produced of *L. plantarum* IIA-2C12 during fermentation in MRS broth media.*

| Table 2. Consumer acceptability of lamb fermented sausage (n=75) |
|---------------------------------------------------------------|
| **Control (without probiotic)** | **With probiotic *L. plantarum* IIA-2C12** |
| Aroma | 2.90± 0.01 *a* | 3.13± 0.06 *b* |
| Color | 2.97± 0.06 *a* | 3.20± 0.02 *b* |
| Texture | 2.93± 0.02 *a* | 3.17± 0.01 *b* |
| Taste | n.a. | 2.53± 0.01 |

Data represents means ± standard error from 75 samples

*Values in the same row followed by a common letter differ significantly (P<0.05)
nd= not analysis because sample contained *Salmonella*

preference range (1-5) : 1= very dislike, 5= very like
4. Conclusion

The results obtained in this study indicate that probiotic L. plantarum IIA-2C12 can be used as starter culture of fermented lamb sausage. The physicochemical, microbiology and sensory properties of fermented lamb sausages with the addition of L. plantarum IIA-2C12 (population 8 log cfu/g) were better than that of control (without probiotic).

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