

Characteristics of Beef Sausage Mixed with Modified Bekasam Meat Fermented by *Lactobacillus plantarum*

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ABSTRACT

Bekasam is a fermented food from Indonesia that potentially improves the quality of sausage when incorporated in the ingredients. This research was aimed to study the chemical, microbial, and sensory characteristics of sausage made of various concentrations of beef and bekasam meat. The chemical, microbial and sensory characteristics of sausage were investigated using an experimental method in a completely randomized design with 5 treatments, namely T1 (100% beef); T2 (75% beef + 25% bekasam meat); T3 (50% beef + 50% bekasam meat); T4 (25% beef + 75% bekasam meat); and T5 (100% bekasam meat). Data analysis of chemical and microbial characteristics was subject to ANOVA and post-hoc Duncan test, while sensory characteristics were analyzed using Kruskal-Wallis test and The Mann-Whitney test. The results showed that the addition of bekasam meat into the sausage mixture significantly ($p < 0.05$) decreased fat content and sensory characteristics (color, flavor, texture, and overall acceptability). Meanwhile, it increased total bacteria, *E. coli* and *S. aureus* inhibition in the sausage. In conclusion, bekasam meat fermented with *L. plantarum* showed probiotic potentials and could modify beef sausage production.

Keywords: *Lactobacillus plantarum*; fat content; microbial inhibition; sensory; sensory

INTRODUCTION

Sausage is a common food across the world. The commonly approved sausage should be chewy, free of synthetic preservatives and coloring agents, and safe for consumption. However, the common meat-based sausage contains a high cholesterol level, which is harmful to human health (Rahardjo, 2003). Incorporating bekasam meat into a sausage mixture is one of the efforts to suppress the high cholesterol content. Also, it diversifies the currently available functional foods.

Bekasam is a popular traditional fish-based fermentation product in several regions in Indonesia, especially East Java, Kalimantan, and Sumatera. Bekasam is made by mixing fish, rice, and salt and fermenting/incubating the mixture in anaerobic conditions. Bekasam can also be made from beef to obtain a higher protein content around 22% (Ahmad *et al.*, 2018), which is relatively similar to fish, i.e. 18% (Jag *et al.*, 2018).

The traditionally-made bekasam still lacks uniformity, especially the quality; therefore, incorporating lactic acid bacteria in bekasam fermentation would improve the product. *Lactobacillus plantarum* is a lactic acid bacteria that is naturally available in beef and capable of producing extracellular protease enzyme. Also,

it contains a high affinity and bacteriocin. Bacteriocin produced by LAB is a compound organic acid, such as lactic and acetic acids, and it is safe for consumption (Farinde *et al.*, 2010). Bacteriocin produced during fermentation would also inhibit the growth of pathogens or other harmful microorganisms.

Lactobacillus plantarum has shown a high antibacterial activity towards pathogenic bacteria, both gram-negative and positive bacteria (Zafarullah *et al.*, 2019). Arief *et al.*, (2014) reported that the addition of probiotic *L. plantarum* IIA-2C12 would increase the physicochemical, microbial, and sensory properties of fermented mutton sausage. Moreover, the antibacterial substrates from *L. plantarum* 1 BS22 and *L. plantarum* 1 BL12 would affect the physical and microbial qualities of meat (Afriani *et al.*, 2017). Incorporating *L. plantarum* into the fermented sausage would improve the taste and aroma as well as inhibiting the growth of pathogenic bacteria compared to the addition of commercial bacteria (Ba *et al.*, 2017). Research on the beef sausage made from fermented bekasam meat is still limited; therefore, the present study investigating the potency of fermented bekasam meat as a functional food by examining the chemical, microbial, and sensory activity.

MATERIALS AND METHODS

This experiment used beef from Agro Meat Shop in Bandung and the probiotic culture *L. plantarum* isolates were obtained from the Academic Leadership Grant (ALG) B 1.13. The *Staphylococcus aureus* and *Escherichia coli* were obtained from the Central Laboratory of Padjadjaran University, Jatinangor, Indonesia.

Preparation of Culture Starter

Lactobacillus plantarum isolates were cultured in an MRSB medium at 37°C for 48 hours (Mamta *et al.*, 2017).

Bekasam Preparation

Bekasam was modified by using beef as the main ingredient. Samples of 300 g beef shank were washed and cut into 3 mm pieces and salted (10% total weight). The beef cutlets were put into a sealed sterile container, added with 300 g rice and *Lactobacillus plantarum* starter 20%, then incubated at 37°C for 3 days (Zummah & Wikandari, 2013).

Bekasam Sausage

The production of bekasam sausage followed a method by Hidayat *et al.* (2018) with a slight modification. Beef and bekasam were ground in a food processor and added with 12.5 g ice cube. The ground meat was mixed with 1.5 g sugar, 6 g pepper, 1.5 g nutmeg, 1 g margarine, 7.5 g skim milk, 4.5 g garlic, and 25 g tapioca flour. The dough was ground and mixed thoroughly until homogenous, put into a sausage casing and weighed. The raw sausage was smoked for 8 hours at ±45°C. Then, the cooked sausage was examined for chemical, microbial, and sensory quality. The formula of sausage modified with bekasam meat is presented in Table 1.

Chemical Analysis

The moisture, protein, and fat contents were determined using an analysis of variance (AOAC, 2005).

Microbial Analysis

Total bacteria. Total bacteria was measured using the total plate count (TPC) method. TPC indicates the total aerobic bacteria in 1 mL of the sample compared to the standard. The calculation was done by pour plate method when visible colonies were at 25-250. The total bacteria was calculated by the following formula (Maturin & Peeler, 2001):

$$N = \sum c / [(1 \times n_1) + (0.1 \times n_2)] \times d$$

where N was total colonies/mL of sample, $\sum c$ was total colonies on all plate (25-250), n_1 was total visible colonies on the first dilution, n_2 was total visible colonies on the second dilution, and d was firstly calculated dilution/total plate with 25-250 colonies.

Bacteria inhibition. The pathogenic bacteria contamination in this research included *Staphylococcus aureus* (gram-positive bacteria) and *Escherichia coli* (gram-negative bacteria). *Staphylococcus aureus* and *Escherichia coli* isolates were cultured in a slant nutrient agar for 18 hours. The indicator bacteria were inoculated in 10 mL nutrient broth and incubated in a shaking water bath at 37°C for 18 hours. Then, 20 μ L bacteria were incorporated into 20 mL liquid Mueller Hinton Agar (MHA) media at 40°C. The inoculated liquid MHA media was poured into a sterile petri dish and bacteria inhibition was performed with an agar well diffusion. As reported by Davis & Stout (1971), bacteria inhibition is indicated from a clear zone around the well, and the diameter of the clear zone is called the inhibitory zone.

Sensory quality. The sensory quality (color, aroma, flavor, texture and overall acceptability) of the sausage

Table 1. Sausage formulation for all treatments

Ingredients	Treatments (g)				
	T1	T2	T3	T4	T5
Beef	250	187.5	125	62.5	0
Bekasam	0	62.5	125	187.5	250
Ice cube 5%*	12.5	12.5	12.5	12.5	12.5
Tapioca flour 10%*	25	25	25	25	25
Skim milk 3%*	7.5	7.5	7.5	7.5	7.5
Margarine 3%*	7.5	7.5	7.5	7.5	7.5
Seasoning**					
Salt 2%	6	6	6	6	6
Sugar 0,5%	1.5	1.5	1.5	1.5	1.5
Garlic powder 1.5%	4.5	4.5	4.5	4.5	4.5
Pepper 0.5%	1.5	1.5	1.5	1.5	1.5
Nutmeg 0.3%	1	1	1	1	1
Total dough	317	317	317	317	317

Notes: T1= 100% beef; T2= 75% beef + 25% bekasam meat; T3= 50% beef + 50% bekasam meat; T4= 25% beef + 75% bekasam meat; T5= 100% bekasam meat. *weight ingredients of beef; ** total weight of ingredients from the dough (meat, ice cubes, tapioca flour, skim milk and margarine).

was examined by 20 semi-trained students of the Faculty of Animal Science, Padjajaran University using a hedonic scale (1= dislike very much, 2= dislike, 3= dislike slightly, 4= like slightly, 5= like, 6= like very much, 7= like extremely) (Ba *et al.*, 2017).

Data Analysis

The experiment was conducted in a completely randomized design with 5 treatments, namely T1 (100% beef); T2 (75% beef + 25% bekasam meat); T3 (50% beef + 50% bekasam meat); T4 (25% beef + 75% bekasam meat); and T5 (100% bekasam meat). Each treatment was replicated 4 times, and the obtained data were subject to an analysis of variance (ANOVA) followed by Duncan's test to determine differences. The sensory data were analyzed using the non-parametric Kruskal-Wallis test. Also, the Mann-Whitney test was used to differentiate across the means (significance $p < 0.05$).

RESULTS

Chemical Quality of the Sausage

The chemical analysis (moisture, protein, and fat content) of the sausage is presented in Table 2. The result showed that incorporating bekasam meat by 25%,

50%, 75%, and 100% did not affect the moisture and protein content of the sausage compared to control (100% beef). However, the fat content significantly decreased ($p < 0.05$) with the addition of bekasam meat.

Microbial Quality of the Sausage

Total bacteria and bacterial inhibition of the sausage are presented in Table 3. The bacterial inhibition was specifically examined for *E. coli* and *S. aureus*. Total bacteria and bacterial inhibition significantly increased with the addition of bekasam meat ($p < 0.05$). However, there was no inhibitory zone in sausage mixed with 0% and 25% bekasam meat (Figure 1).

Sensory Quality of the Sausage

The sensory characteristics of sausage added with bekasam meat are presented in Table 4. The average score for aroma, flavor, texture, and overall acceptability was higher for sausages without bekasam meat. The mean score of aroma for sausage without bekasam meat was 5, while for sausages that contained 25%, 50%, 75%, and 100% bekasam meat were 5, 4, 5, and 4, respectively. In contrast, there was no difference in the color of sausage added with bekasam meat.

Table 2. Chemical composition of sausage mixed with modified bekasam meat fermented by *Lactobacillus plantarum*

Variables	Treatments				
	T1	T2	T3	T4	T5
Moisture content (%)	61.59±0.60	63.96±2.81	64.10±1.22	61.59±2.58	62.08±1.22
Protein content (%)	14.81±0.14	15.13±0.24	13.93±0.17	12.59±0.27	13.17±0.63
Fat content (%)	3.91±0.34 ^a	2.85±0.36 ^{ab}	2.94±0.50 ^{ab}	1.03±0.42 ^{bc}	0.57±0.21 ^c

Notes: T1= 100% beef, T2= 75% beef + 25% bekasam meat, T3= 50% beef + 50% bekasam meat, T4= 25% beef + 75% bekasam meat, T5= 100% bekasam meat. Means in the same row with different superscript differ significantly ($p < 0.05$).

Table 3. Total bacteria and inhibitory zone of sausage added bekasam meat against *Escherichia coli* and *Staphylococcus aureus*

Variables	Treatments				
	T1	T2	T3	T4	T5
Total bacteria ($\times 10^7$ CFU/g)	1.06±0.03 ^a	2.06±0.03 ^a	1.95±0.05 ^a	11.33±0.04 ^b	21.47±0.05
Inhibitory zones (mm)					
<i>E. coli</i>	0±0 ^a	0±0 ^a	9.57±1.87 ^b	9.67±0.94 ^b	16.07±1.44 ^c
<i>S. aureus</i>	0±0 ^a	0±0 ^a	10.00±0.62 ^b	10.57±1.76 ^b	17.40±1.54 ^c

Notes: T1= 100% beef, T2= 75% beef + 25% bekasam meat, T3= 50% beef + 50% bekasam meat, T4= 25% beef + 75% bekasam meat, T5= 100% bekasam meat. Means in the same row with different superscript differ significantly ($p < 0.05$).

Table 4. Sensory quality of sausage mixed with modified bekasam meat fermented by *Lactobacillus plantarum*

Variables	Treatments				
	T1	T2	T3	T4	T5
Color	5±1.32 ^a	5±1.21 ^a	5±0.69 ^a	4±1.05 ^a	4±1.38 ^a
Aroma	5±1.61 ^a	5±1.16 ^a	4±1.23 ^a	5±1.22 ^{ab}	4±1.27 ^b
Flavor	5±1.38 ^a	4±1.47 ^b	2±1.31 ^{bc}	2±0.87 ^c	3±0.67 ^c
Texture	5±1.41 ^a	5±1.26 ^a	4±1.04 ^b	2±1.12 ^{bc}	2±1.29 ^c
Overall acceptability	5±1.05 ^a	4±0.99 ^b	4±1.08 ^c	3±1.19 ^{cd}	3±0.88 ^d

Notes: T1= 100% beef, T2= 75% beef + 25% bekasam meat, T3= 50% beef + 50% bekasam meat, T4= 25% beef + 75% bekasam meat, T5= 100% bekasam meat. Means in the same row with different superscript differ significantly ($p < 0.05$).

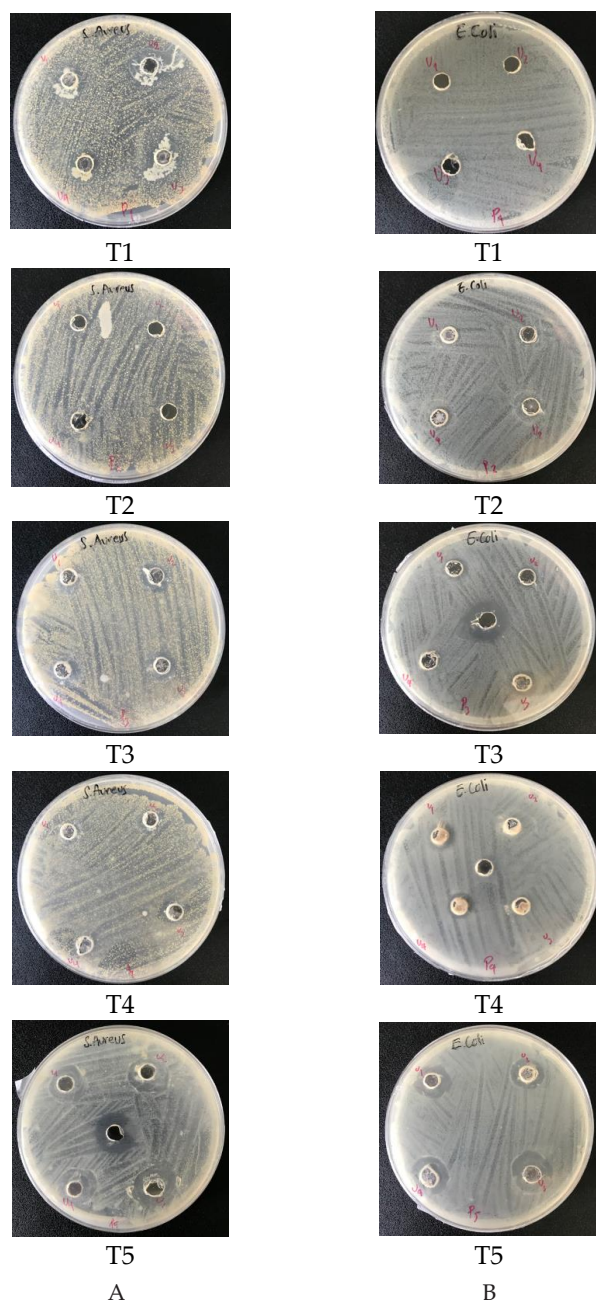


Figure 1. Inhibition zone of sausage of all treatment against bacteria (A) *Staphylococcus aureus*, (B) *Escherichia coli*.

DISCUSSION

Chemical Quality of the Sausage

This study showed that the moisture and protein content of the sausage was not affected ($p > 0.05$) across treatments because the smoking process evaporates moisture in the sausage for 8 h. According to Askill (2017), a similar smoking time in cooking the sausage would result in similar moisture content in the sausage. Also, modifying sausage with bekasam meat did not affect the protein content because the *L. plantarum* in bekasam meat produced proteolytic enzyme. Amadou *et al.* (2011) reported that *L. plantarum* culture produced proteolytic enzymes that could degrade proteins into

simpler proteins. According to Gobbetti *et al.* (1996), some strains of *Lactobacillus* produce protease enzymes that could hydrolyze proteins such as casein or gluten with different activities. Despite the differences in the proteolytic activities of *L. plantarum*, the protein hydrolyzed by protease enzyme is relatively similar (Yusmarini *et al.*, 2010). Regarding sausage quality standards, the Indonesian National Standards (Indonesian National Standards; SNI 01-3820-2015) has determined that the minimum protein and moisture content of beef sausage is 8% and 67%, respectively. Therefore, regardless of the addition of bekasam meat, the sausage produced in this research conforms to the SNI standard quality.

Incorporating 75% and 100% bekasam meat into sausage resulted in a decrease of fat content by 1.03% and 0.57%, respectively, in comparison with the control. It was in line with Arief *et al.* (2014) that the presence of *L. plantarum* significantly reduced the fat content.

In addition, the composition of fat in beef and bekasam meat (in this study) was 7.3% and 2.83%, respectively. The higher fat content in beef was due to different compositions of fat compared to other types of meat; therefore, beef sausage contained higher fat than bekasam meat. In this study, *L. plantarum* as one of LAB played a major part in fat formation. Fat content is related to the lipolytic activity of lactic acid bacteria (LAB) during the fermentation process. Lipolytic activity is controlled by the lipase enzyme produced by lactic acid bacteria. It was in accordance with Emine & Kivanc (2017) that *L. plantarum* had the highest lipolytic activity compared to other lactic acid bacteria.

Microbiological Analysis

Total bacteria. Total plate count is the method to analyze all types of non-specific bacteria in the product. In this study, the addition of bekasam meat significantly increased ($p < 0.05$) the total bacteria of sausage. Furthermore, the more bekasam meat added, the higher the total bacteria in the sausage. It was indicative of lactic acid bacteria growth in the fermented meat. *L. plantarum* has been isolated from various sources, including fermented food across the world, and promoted benefits to human health; accordingly, the products are called potential probiotics (Crowley *et al.*, 2012).

According to Steinkraus (1994) and Fellows (2017), meat is a protein source classified as perishable food that presents pathogenic and non-pathogenic microbes during the fermentation process. The addition of 100% bekasam meat in beef sausage resulted in the highest of total bacteria value ($21.47 \pm 0.05 \times 10^7$ CFU/g) followed by the 75%, 50%, and 25% levels, i.e., $11.33 \pm 0.04 \times 10^7$ CFU/g, $1.95 \pm 0.05 \times 10^7$ CFU/g, and $2.06 \pm 0.03 \times 10^7$ CFU/g, respectively. It was similar to the previous study (Umam *et al.*, 2019), reporting that the total viable count of microorganisms significantly increased in the fermented goat meat dendeng (mutton jerky) by 5.25 to 6.34×10^7 CFU/g.

Inhibitory zone. The inhibition of pathogens depends on the rate of nutrients absorption, the inherent metabolic

velocity, the growth rate, and the excretion of specific inhibitors (Balcázar *et al.*, 2006). The addition of 100% bekasam meat in sausage demonstrated a strong inhibitory effect against *E. coli* and *S. aureus* with an inhibitory zone value of 16.07±1.44 mm and 17.4±1.54 mm, respectively. In contrast, the inhibitory zone was non-existent in sausage with 0% and 25% addition of bekasam meat. Costa *et al.* (2013) reported that the addition of *L. plantarum* to bekasam meat produced a higher lactic acid that would inhibit the growth of *E. coli* and *S. aureus*. Similarly, Botthoulath *et al.* (2018) found an inhibitory activity in fermented pork sausage against *E. coli* DMST 4212 and *S. aureus* DMST 8840, as the pathogen cell counts fell rapidly by approximately 3 log units at 24 h, and further to below detection limit after 36 h of incubation.

The production of bekasam meat using gram-positive lactic acid bacteria (*L. plantarum*) would produce a zone of inhibition to the growth of pathogenic bacteria (Gloria *et al.*, 2012; Wang *et al.*, 2018). According to Arief *et al.* (2014), *L. plantarum* IIA-2C12 produced an antimicrobial compound that inhibits the growth of pathogenic bacteria and safe to be consumed according to the GSO standard. In addition, the smoking process also contributed to the inhibition of bacterial growth. According to Askild *et al.* (2017), the bacteriostatic and fungistatic activity from the smoke would inhibit the growth of pathogenic bacteria, such as *E. coli*, *Staphylococcus* and *Pseudomonas*. The aliphatic acid and phenolic compounds produced during the smoking process could also inhibit the growth of pathogenic bacteria. Research by Dias *et al.* (2015) showed that *L. plantarum* had a bacterial inhibition activity against *E. coli*, *L. monocytogenes*, and *S. typhi*.

L. plantarum is known to produce higher antibacterial compounds against *E. coli*, *B. cereus*, and *L. monocytogenes* (Desniar *et al.*, 2016). The inhibition of *Staphylococcus* bacteria was due to the lactoline compound, which could also inhibit the growth of *S. aureus* (Davidson & Hoover, 1993, Wicher *et al.*, 2020). The plantaricin compounds produced by *L. plantarum* was known to inhibit the growth of *S. aureus*. Another antimicrobial compound produced by lactic acid bacteria that could inhibit the growth of *S. aureus* was hydrogen peroxide (Amro *et al.*, 2018).

Sensory Analysis

The addition of bekasam meat has significantly decreased ($p < 0.05$) the sensory properties, including aroma, flavor, texture, and overall acceptability, except for color. The similar color across treatments was due to the 8-hour smoking process where phenolic compounds darkened the sausage color. It was in line with Junianingsih *et al.* (2014) that the changing color of smoked fish was due to the reaction of phenolic compounds to protein and sugar during smoking. Moreover, the Maillard reaction between amino acids and sugar in meat is known to contribute to the changing color of sausage.

The aroma of sausage modified with bekasam meat was accepted by the panelists. A similar aroma across treatments was due to the burnt coconut shell charcoal

to produce the smoke, while the long smoking process reduced the fat content, which also affected the sausage aroma. This result was confirmed by Widjanarko *et al.* (2003) that the smoking process would initiate the absorption of smoking compounds into the sausage and resulted in an authentic sausage aroma.

The addition of bekasam meat in sausage produced unfavorable/disliked flavor. The panelists disliked the overall flavor of bekasam-fortified sausage with bekasam meat because of the acidic taste. Also, sausage flavor is affected by other variables, such as ingredients, cooking process, and the starter cultures (Ahmad & Amer, 2013). In the texture parameter, the addition of bekasam meat in sausage produced unfavorable texture (too soft). Therefore, future improvement is important to increase the acceptability of the sausage added with bekasam meat.

CONCLUSION

The present study concluded that different levels of bekasam meat in the sausage significantly affected the chemical, microbial, and sensory characteristics, except for water content, protein content, and colors. Bekasam meat fermented using *L. plantarum* showed probiotic potential and could modify beef sausage production. Further studies need to confirm the quality of food functionality.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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