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PHYSICOCHEMICAL, MICROBIOLOGICAL, AND ORGANOLEPTIC PROPERTIES OF FERMENTED LAMB SAUSAGE ENHANCED BY JACK BEAN FORTIFICATION

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Abstract
Processing lamb meat into fermented sausages can reduce the risk of spoilage and extend shelf life. Fermented sausages are commonly made using lactic acid bacteria (LAB), resulting in a product that is acidic and less firm. Therefore, it is necessary to add jack bean flour as a binding agent. This study aims to analyze physicochemical, microbiological and organoleptic characteristics of premium IPB lamb fermented sausages with varying proportions of jack bean flour (0% and 30%). The analyses included pH, water activity (a_w), total acid, water, ash, fat, crude protein, carbohydrate, total LAB, E. coli, S. aureus, texture profile analysis (TPA), and sensory evaluation. The incorporation of jack bean flour into lamb meat fermented sausages can elevate the pH, springiness, chewiness, crude protein, and carbohydrate content of the fermented sausages. Furthermore, the addition of jack bean flour may reduce the total acid, a_w, gumminess, water content, and bacteria (E. coli and S. aureus). The sensory aspects such as color in fermented sausage with addition of jack bean flour were preferred by the panelists. The addition of jack bean flour increased the total unsaturated fatty acids and essential amino acids in fermented sausages. The incorporation of jack bean flour aligns with the Indonesian National Standards for Meat Sausages 3820–2015 concerning moisture, fat, and protein content. This study suggests that incorporating lamb fermented sausage with 30% jack bean flour could result in significant benefits, including increased nutrition, enhanced sensory quality, improved texture, and extended storage life for fresh lamb products.

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Introduction

Meat is a crucial component in meeting nutritional needs due to its complete and balanced proteins, essential amino acids, as well as various minerals and vitamins [1]. Lamb meat is one of the most widely consumed meats in Indonesia. The average protein content in sheep meat is 9.65%, fat content is 20.59%, water holding capacity (WHC) is 24.6% and cholesterol content is 19.2 mg/100 g of meat [2]. The high water and nutrient content in lamb meat make it susceptible to quality deterioration or spoilage (*perishable food*) [3]. The deterioration of quality in lamb meat can be caused by physical, chemical, and biological contaminants [4]. The processing of animal-origin food ingredients can suppress or inhibit the growth of bacteria in food products, extending the shelf life of meat, preventing spoilage, enhancing digestibility, and diversifying processed meat products. Among processed lamb meat products are fermented sausages. Fermented sausages are food products obtained from a mixture of meat, fat, spices, or seasonings, with or without the addition of lactic acid bacteria (LAB) as starter cultures, which are then stuffed into sausage casings [5].

The acid content produced by LAB can inhibit the growth of pathogenic bacteria and food spoilage bacteria, making LAB a group of beneficial bacteria that meet the GRAS (Generally Recognized as Safe) status, which means that they are safe for humans and can be applied as probiotic agents [6]. Lactic acid bacteria (LAB) as a source of probiotics have functional properties such as antihypertensive, antimicrobial, antidiabetic, antioxidant, and anticancer effects [7,8,9]. Additionally, according to Beltrán-Barrientos et al. [10], probiotics can also boost the immune system and inhibit the activity of cholesterol-forming enzymes, thereby reducing cholesterol levels in the body. Other functional properties include hypocholesterolemic effects and the production of bioactive peptides. In the production of fermented sausages, LAB play a crucial role in converting carbohydrates into lactic acid. One widely used and commercially available culture is *Lactobacillus plantarum* [11].

Typically, fermented sausages are made using only LAB, resulting in a less solid texture and an acidic taste [12]. These sausages can be enhanced by incorporating binding agents such as jack bean flour. In Indonesia, jack bean yields are approximately 3.9–4.6 tons per hectare, compared to soybeans at 1.7–2.6 tons per hectare [13]. Jack beans contain 27.4% protein, 66.1% carbohydrates, and 2.9% fat, being a good source of carbon and nitrogen for bacterial growth [14]. They can be processed into flour for using in various products including cakes, cookies, crackers, nuggets, tempeh, and tofu, and can also be used in fermented foods such as soy sauce and tauco. However, the use of jack bean flour in sausages is limited due to toxic substances, such as cyanide, phytic acid, tannins, saponins, and oxalates, which can cause undesirable tastes and toxicity [15]. These levels can be reduced through soaking, peeling, boiling, cooking, and fermentation. Recommended phytic acid consumption is 25 mg/100 g [16]. Using jack beans as a binding agent in fermented sausages can reduce the dependence on imported tapioca and soy flour in Indonesia. This study aims to analyze physicochemical, microbiological and organoleptic characteristics of premium IPB lamb fermented sausages with varying proportions of jack bean flour (0% and 30%).

Materials and Methods

Materials

Premium IPB lamb meat was obtained from farms in Banjarnegara, Central Java. Premium IPB lambs were slaughtered at PT Pramana Pangan Utama, IPB University. The entire process in the research complied with the "Institutional Animal Care and Use Committee (IACUC)" issued by IPB University (approval ID: 118–2018 IPB). Jack beans (*Canavalia ensiformis*) were obtained from East Java. The probiotic strain *Lactiplantibacillus plantarum subsp. plantarum* strain IIA-1A5 was taken from the collection of the Laboratory of Animal Product Technology, IPB University.

Production of jack bean flour

Five kilograms of jack bean seeds were soaked in water for 72 hours with water changes every 12 hours, then peeled and cleaned. The seeds were dried in an oven at 60 °C for 7 hours. Once thoroughly dried, the jack beans were finely ground and sifted through an 80-mesh screen [17].

`Preparation of starter culture

The *Lactiplantibacillus plantarum subsp. plantarum* strain IIA-1A5 culture was refreshed by incubating it in 9 mL of de Man, Rogosa and Sharpe Broth (MRS Broth) medium at 37 °C for 24 hours until turbidity indicated adaptation. This refreshed culture was then inoculated at 2% into the sterile 10% skim milk solution and incubated at 37 °C for 24 hours to form the mother culture. Subsequently, intermediate and working cultures were produced through successive steps. The initial population of the working culture was determined by inoculating it onto de Man, Rogosa and Sharpe Agar (MRS Agar) medium with a suitable culture having over 10^8 CFU mL^{-1} [18].

Production of fermented sausages

The production of fermented sausages began with standardizing 80% of meat, separating whole meat from meat containing fat. The standardized meat underwent grinding, freezing, and mixing with seasoning ingredients including salt (2%), sugar (0.5%), garlic powder (1.5%), pepper (0.5%), nutmeg (0.3%), jack bean flour (0% and 30%), and lactic acid bacteria (5%). The mixture was then filled into sausage casings and conditioned at 27 °C for 24 hours. After resting, the sausages underwent cold smoking for 5 hours over 2 days at 28–30 °C. Finally, the fermented sausages were ready for testing [19].

Analysis of physicochemical characteristics

The physicochemical analysis of fermented sausage samples encompassed the measurement of pH [20], water activity (a_w) [20], total acid [21], water content [20], ash content [20], fat content [20], crude protein content [20], carbohydrate content [20], total amino acids [22], total fatty acids [23], and texture profile analysis (TPA) [24].

Analysis of microbiological characteristics

The microbiological analysis of fermented sausages involved assessing total LAB, *E. coli*, and *S. aureus*. Samples of 25 g fermented sausages were diluted in the Buffered Peptone Water solution to achieve dilutions ranging from 10^1 to 10^8. Colony counting was conducted by inoculating dilutions onto specific agar media for each bacteria type: de Man, Rogosa and Sharpe Agar for LAB, Eosin Methylene Blue Agar for *E. coli*, and Baird Parker Agar Base for *S. aureus*. The dishes were then incubated at 37 °C for 48 hours [25].

Analysis of organoleptic characteristics

Organoleptic testing of fermented sausages utilized both hedonic and hedonic quality tests. Variables assessed included color, taste, aroma, and texture with 35 panelists rating each on a scale from 1 to 4 [26].

Data Analysis

The research data were analyzed using T-test and Kruskal-Wallis test for sensory analysis. The applied treatments were as follows:

P0: Fermented lamb sausage and *Lactobacillus plantarum* IIA-1A5

P1: Fermented lamb sausage and *Lactobacillus plantarum* IIA-1A5 with the addition of 30% jack bean flour.

Results and discussion

In this study, samples of fermented sausages underwent characteristic analyses, including determination of pH, water activity (a_w), total acid, water content, ash content, fat content, crude protein content, carbohydrate content, texture analysis, total LAB analysis, analysis of *Escherichia coli* and *Staphylococcus aureus*. The results of these tests are presented in Table 1.

Tabel 1. Characteristic of fermented sausage

Parameter	Treatment		Description
	Control	With jack bean flour	
Physicochemical			
pH	4.50 ± 0.02[a]	5.42 ± 0.02[b]	—
Total acid (%)	1.70 ± 0.06[a]	1.24 ± 0.05[b]	—
a_w	0.85 ± 0.00[a]	0.82 ± 0.00[b]	—
Springiness (mm)	1.17 ± 0.17a	1.87 ± 1.25[b]	—
Cohesiveness	0.86 ± 0.06	0.78 ± 0.05	—
Gumminess (N)	2211.76 ± 140.38[a]	1998.50 ± 142.55[b]	—
Chewiness (N)	3959.93 ± 516.17[a]	5527.45 ± 552.46[b]	—
Proximate composition			
Water content (%)	62.52 ± 1.14[a]	47.27 ± 1.24[b]	Max. 67
Ash content (%)	4.14 ± 0.05	4.28 ± 0.09	Max. 3
Fat content (%)	5.07 ± 0.70	5.26 ± 1.18	Max. 20
Crude protein content (%)	26.11 ± 0.27[a]	27.32 ± 0.82[b]	Min. 13
Carbohydrate (%)	2.16 ± 0.75[a]	15.87 ± 1.45[b]	Max. 8
Microbiology			
Lactic acid bacteria (log CFU/g)	7.09 ± 0.09	7.01 ± 0.06	Dough
	7.20 ± 0.09	7.12 ± 0.01	Conditioning
	8.14 ± 0.02	8.13 ± 0.01	Smoking 1
	8.19 ± 0.04	8.16 ± 0.01	Fermented Sausage
Escherichia coli (log CFU/g)	1.73 ± 0.41[a]	0.87 ± 0.10[b]	Dough
	—	—	Fermented Sausage
Staphylococcus aureus (log CFU/g)	1.20 ± 0.09[a]	0.91 ± 0.01[b]	Dough
	—	—	Fermented Sausage

Note: Different letters in the same row indicate significant differences (P < 0.05).

The pH values of fermented sausages with the addition of jack bean flour were higher compared to those without the addition. This can be attributed to the fact that jack bean flour has a neutral pH of 7 [27]. The addition of jack bean flour is expected to make sausages more palatable to consumers due to its less acidic taste. A decrease in pH can be attributed to the growth of LAB that produces lactic acid [19]. Total acid value of fermented sausages with the addition of jack bean flour was lower compared to those without jack bean flour. The addition of jack bean flour can increase the pH in the fermented sausage product, thereby reducing the total acid. Total acid is influenced by the fermentation process in the product, occurring over three days. Total acid values are inversely proportional to the pH values produced; as pH decreases, total acidity increases [19].

The a_w value of fermented sausages with the addition of jack bean flour was lower compared to those without jack bean flour. The hygroscopic nature of jack bean flour allows it to absorb water effectively, reducing free water content [28]. This property depends on protein quality and the presence of polar amino acids [29]. Adding jack bean flour to fermented sausages increases the polar amino acid composition, lowering their water activity [29]. Lower a_w values significantly enhance food safety as they can inhibit the growth of pathogenic bacteria within the product [19].

Springiness and chewiness increase in fermented sausages with jack bean flour due to its high amylopectin content [30]. The elevated amylopectin contributes to elasticity, while glutamic acid in jack beans enhances water absorption, resulting in a denser texture [31]. This aligns with studies indicating that high amylopectin content produces denser and more elastic products [32]. Gumminess decreases in sausages with jack bean flour due to its water-absorbing properties [31]. The addition of jack bean flour did not affect cohesiveness of sausages.

The addition of jack bean flour decreases the water content in fermented sausages due to its hygroscopic properties, as it can effectively absorb water [28]. Glutamic acid, the predominant amino acid in jack bean flour, contributes to this water absorption [31]. Consequently, sausages made with jack bean flour have a denser texture. The inclusion of jack bean flour did not affect the ash and fat content of the sausages. However, it enhanced both the protein and carbohydrate content, suggesting improved nutritional value. Jack bean flour is rich in protein and has a low glycemic index, making it a promising functional food ingredient [33,34,35].

The addition of jack bean flour to fermented sausages did not significantly affect the total LAB count, as shown in Table 1. However, sausages without jack bean flour tended to have slightly higher LAB counts, possibly due to variations in sausage weight and smoking process conditions. LAB thrive within a pH range of 3.5–10.0 and temperatures of 5–45 °C [36]. Fermented sausages with jack bean flour exhibited lower levels of *E. coli* and *S. aureus* bacteria, likely due to antimicrobial compounds present in jack bean flour, such as flavonoids [37]. Additionally, LAB starter cultures can contribute to reducing *E. coli* populations due

to their antibacterial properties [19]. For example, *L. plantarum* IIA-1A5 has been reported to possess antibacterial properties against pathogenic bacteria, including *E. coli, S. aureus* [38] and *Salmonella typhimurium* [39].

Table 2. Fatty acid characteristics of fermented sausage

Parameter	Treatment (% w/w)	
	Control	With jack bean flour
Total saturated fatty acids (SFAs)		
Butyric acid C4:0	2.71	0.07
Caproic acid C6:0	0.02	2.19
Caprylic acid C8:0	0.05	0.01
Capric acid C10:0	0.09	0.07
Lauric acid C12:0	0.07	0.07
Myristic acid C14:0	1.23	1.18
Pentadecanoic acid C15:0	0.35	0.35
Palmitic acid C16:0	11.36	11.00
Heptadecanoic acid C17:0	0.71	0.69
Stearic acid C18:0	7.46	7.15
Total poly-unsaturated fatty acids (PUFAs)		
Linolenic acid C18:3n3	0.08	0.76
Linolelaidic acid C18:2n9t	0.16	0.19
Total mono-unsaturated fatty acids (MUFAs)		
Myristoleic acid C14:1	0.05	0.05
Palmitoleic acid C16:1	1.02	1.09
Oleic acid C18:1n9c	19.13	21.07
Elaidic acid C18:1n9t	1.86	1.95
Total fatty acids	46.36 ± 11.44	47.90 ± 11.88
Total saturated fatty acids (SFAs)	24.05 ± 1.09	22.78 ± 0.90
Total unsaturated fatty acids (UFAs)	22.84 ± 7.58	25.11 ± 8.30
Total poly-unsaturated fatty acids (PUFAs)	0.24 ± 0.05	0.95 ± 0.40
Total mono-unsaturated fatty acids (MUFAs)	22.06 ± 9.10	24.16 ± 10.05
Ratio of total SFAs/UFAs	1.05	0.90

The content of total fatty acids, saturated fatty acids, and unsaturated fatty acids in premium lamb meat is 73.35%, 39.64%, and 30.37%, respectively. The content of unsaturated fatty acids, such as oleic acid, palmitoleic acid, and linoleic acid, is 24.26%, 1.58%, and 2.37% [40]. Table 2 shows that adding jack bean flour reduces butyric acid and increases caproic acid levels in fermented sausages due to the fermentation process converting butyric acid into other compounds [41]. According to Kinteki at al. [42], increased caproic acid levels are a byproduct of proteins and amino acids produced by LAB. Sridhar and Sharma [43] state that jack beans contain omega-6 fatty acids, including linoleic acid, linolenic acid, cis-11,14-eicosadienoic acid, and arachidic acid, and omega-3 fatty acids such as timnodonic acid, docosahexaenoic acid, and docosapentaenoic acid. Therefore, adding jack bean flour can increase the linolenic acid and oleic acid content in fermented sausages, enhance unsaturated fatty acids, and reduce the SFAs/UFAs ratio. A lower SFAs/UFAs ratio improves texture and flavor stability, making the sausages softer with a more complex taste. Overall, the fatty acid profile of fermented sausages with jack bean flour is better than that of control sausages, offering health benefits such as reduced cardiovascular disease risk and enhanced product flavor [44].

Table 3. Amino acid characteristics of fermented sausage

Parameter	Treatment (%w/w)	
	Control	With jack bean flour
Essential amino acids		
Lysine	1.80	1.77
Leucine	1.69	1.78
Isoleucine	0.92	0.98
Methionine	0.44	0.36
Threonine	0.90	0.98
Phenylalanine	1.43	1.42
Valine	0.99	1.03
Histidine	0.89	1.21
Non-essential amino acids		
Alanine	1.33	1.19
Aspartate	1.96	2.11
Glutamate	3.59	3.42
Arginine	1.05	1.14
Glycine	1.26	0.97
Tyrosine	0.62	0.62
Serine	0.79	0.90
Total amino acids	19.67 ± 4.64	19.87 ± 4.69
Essential amino acids	9.06 ± 0.46	9.53 ± 0.47
Non-essential amino acids	10.60 ± 1.01	10.35 ± 0.97

Table 4. Chemical score of amino acids

Essential amino acids	Treatment	
	Control	With jack bean flour
Isoleucine	57.50	61.25
Leucine	34.50	36.32
Lysine	59.50	58.51
Methionine + cysteine	35.92	29.40
Phenylalanine+ tyrosine	56.94	56.66
Threonine	56.25	61.25
Tryptophan	—	—
Valine	39.60	41.20

Table 3 shows that the essential amino acid content in fermented lamb sausages with the addition of jack bean flour is higher compared to fermented lamb sausages without jack bean flour. According to Kanetro et al. [45], jack bean flour contains amino acids such as aspartic acid (2.41%), glutamic acid (2.10%), asparagine (0.71%), histidine and L-serine (1.49%), threonine (2.21%), glutamine (1.01%), arginine (1.41%), tyrosine (0.94%), lysine (3.00%), alanine (1.01%), glycine (1.01%), tryptophan and methionine (0.94%), valine (1.00%), phenylalanine (1.97%), isoleucine (1.01%), and leucine (1.09%). The histidine and serine content in jack bean flour also increases the histidine and serine content in fermented sausages with the addition of jack bean flour. Products with high essential amino acid content are considered better because essential amino acids cannot be produced by the body and must be obtained

from dietary sources. High levels of essential amino acids are beneficial for health as they are crucial for growth, repair, and maintenance of body tissues [46]. Table 4 displays the chemical score of amino acid values. Further examination revealed that all samples lack tryptophan. Additionally, sausages with added jack bean flour showed higher scores for isoleucine, threonine, and valine. These elevated scores indicate sufficient or even excess levels of these essential amino acids, crucial for various body functions. Essential amino acids, indispensable for human health, must be obtained through diet. Hence, higher chemical scores for these amino acids signify a superior amino acid profile in supporting overall body functions [47].

Table 5. Organoleptic characteristics in fermented sausage

Parameter	Treatment	
	Control	With jack bean flour
Hedonic test		
Color	2.25 ± 0.44[a]	2.00 ± 0.48[b]
Taste	2.25 ± 0.57[a]	2.86 ± 0.79[b]
Aroma	1.84 ± 0.52	1.95 ± 0.57
Texture	2.02 ± 0.55[a]	2.34 ± 0.64[b]
Hedonic quality test		
Color	1.07 ± 0.25[a]	2.02 ± 0.34[b]
Taste	2.68 ± 0.47[a]	2.40 ± 0.58[b]
Aroma	2.75 ± 0.44[a]	2.97 ± 0.55b
Texture	2.20 ± 0.41[a]	1.43 ± 0.50[b]

Note: Different letters in the same row indicate significant differences (P < 0.05). Hedonic scale: 1 (like very much), 2 (like), 3 (dislike), and 4 (dislike very much). Hedonic quality scale: Color: 1 (dark brown), 2 (light brown), 3 (pink), 4 (dark red); Taste: 1 (not sour), 2 (slightly sour), 3 (sour), 4 (very sour); Aroma: 1 (not smoky aroma), 2 (slightly smoky aroma), 3 (smoky aroma), 4 (very smoky aroma); Texture: 1 (not chewy), 2 (somewhat chewy), 3 (chewy), 4 (very chewy)

Table 5 shows the organoleptic characteristics of fermented sausages without and with the addition of jack bean flour. The addition of jack bean flour significantly impacted both the hedonic testing and hedonic quality evaluation of fermented sausages. It altered color, taste, aroma, and texture aspects in both tests. Fermented sausages with jack bean flour had a light brown color, slightly acidic taste, and non-chewy texture, but were less preferred overall. This could be due to the bitter aftertaste from HCN in jack bean flour [29,48] and its hygroscopic properties [28], leading to drier and less chewy sausages. However, they exhibited a stronger smoky aroma, likely enhanced by the Maillard reaction during jack bean flour production [49]. According to Daun [50], the brown color is caused by carbonyl compounds such as acetol, glycolaldehyde, and methylglyoxal in the smoke. These changes are influenced by factors such as smoking process and sausage positioning during smoking [51]. Overall, panelists preferred sausages without jack bean flour for their acidic taste, somewhat chewy texture, and favorable aroma.

Conclusion

Adding jack bean flour to premium IPB fermented lamb sausages enhanced various attributes such as pH, springiness, chewiness, crude protein, and carbohydrate content, total unsaturated fatty acids, and essential amino acids. It also lowered total acid value, water activity, gumminess, water content, and the counts of *E. coli* and *S. aureus* bacteria. These sausages meet the Indonesian National Standard for Meat Sausages 3820–2015 regarding water, fat, and crude protein content. The sensory aspects such as color in fermented sausages with the addition of jack bean flour were preferred by the panelists. Fermented sausages with the addition of jack bean flour can be considered a superior choice in terms of nutritional quality and food safety compared to fermented sausages without the addition of jack bean flour.

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