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Value Addition of Beef Sausage by using *Lactiplantibacillus plantarum* IIA-1A5 Isolated from Meat

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ABSTRACT

Phytic acid, identified as an antinutrient in food materials especially in the soybean included jack bean, poses a challenge to nutritional absorption. Previous investigations have presented the promising functional attributes of lactic acid bacteria, such as *Lactiplantibacillus plantarum* IIA-1A5, isolated from Indonesian local beef, has ability to produce phytase enzyme that can reduce pythic acid level in jack bean. Therefore, this study aimed to explore the functional properties of *L. plantarum* IIA-1A5 and the utilization of jack bean, in the production of fermented sausage to develop a prototype of probiotic sausage with beef as an ingredient. The results of the combination of beef and jack bean flour showed no significant differences in physicochemical characteristics and nutritional content between fermented sausages with or without the addition of *L. plantarum* IIA-1A5. The bacteria inhibited the growth of *S. aureus* and produced phytase enzymes at concentrations ranging from 0.003 to 0.064mg. Furthermore, the highest phytase activity from *L. plantarum* IIA-1A5 was found at 123 mU/mL. This study showed that the use *L. plantarum* IIA-1A5, in the fermented beef sausage combined with jack bean flour (*Canavalia ensiformis*), had the potential to develop functional food products.

Key words: Fermented sausage, Jack bean, *Lactiplantibacillus plantarum* IIA-1A5.

INTRODUCTION

Fermented sausage is a processed meat product with significant potential for development as a functional food. As a high-quality source of animal protein, beef serves as a key ingredient with broad consumer appeal. Additionally, the innovation of fermented sausage products can be further enhanced by incorporating local ingredients, such as jack bean (*Canavalia ensiformis*) flour, as a plant-based protein source. This approach not only supports food diversification but also increases the economic value of the product.

Beef is rich in essential nutrients, particularly high-quality proteins that meet human dietary needs. Furthermore, the importance of beef production also extends to the veterinary field, especially in ensuring animal health and welfare, which directly influences the quality of meat products (Nkosi et al. 2023). Veterinary studies have highlighted the role of animal health management in optimizing the yield and quality of beef products (Rzabayev et al. 2024). Thus, the quality of fermented sausages is closely linked to the veterinary aspects of livestock production.

On the other hand, jack bean flour contains a substantial protein content of up to 26.83% (Ramli et al. 2021), making it a promising raw material in sausage production. However, the use of jack beans is limited due to the presence of antinutritional compounds such as phytic

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acid. Phytic acid is known to reduce the bioavailability of essential minerals like Cu, Zn, Ca, Mg and Fe by forming insoluble complexes during digestion (Bloot et al. 2021).

Therefore, appropriate processing methods, such as fermentation using lactic acid bacteria (LAB), offer a solution to reduce antinutrient levels.

Fermentation with LAB, particularly the strain *Lactiplantibacillus plantarum* IIA-1A5, has been shown to improve the quality of fermented sausage. This strain produces phytase enzymes capable of hydrolyzing phytic acid into compounds more readily absorbed by the body. Phytase, an enzyme naturally produced by microorganisms such as bacteria, yeast, and fungi, has long been utilized in the food industry to enhance the nutritional value of food products (Afify et al. 2012). Previous studies have demonstrated that *L. plantarum* IIA-1A5, isolated from Peranakan Ongole beef, exhibits high phytase activity and the ability to inhibit pathogenic microbes such as *Staphylococcus aureus* (Arief et al. 2015).

In veterinary science, the role of LAB has been extensively studied, especially in the prevention of zoonotic pathogens during meat processing. LAB has been reported to inhibit *Escherichia coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* (Sukmawinata et al. 2025), which are critical concerns in food safety. Moreover, probiotic LAB strains can also enhance gut health in livestock, thereby improving feed efficiency and meat quality (Zhang et al. 2019; Smolentsev et al. 2025). These findings underscore the importance of integrating veterinary knowledge into the development of safe and functional meat products.

The combination of beef and jack bean flour in fermented sausage production presents a promising opportunity to create functional foods. In addition to increasing protein content, utilizing local ingredients such as jack bean flour supports national food diversification initiatives. Moreover, the fermentation process with LAB, such as *L. plantarum* IIA-1A5, optimizes the functional value of fermented sausage by reducing phytic acid levels and enhancing the product's nutritional quality. This study aims to explore the potential of combining beef and jack bean flour, fermented with *L. plantarum* IIA-1A5, to produce a high-quality functional fermented sausage suitable for modern functional food markets.

MATERIALS AND METHODS

Materials

Jack beans (*C. ensiformis*) were obtained from East Java, Indonesia; while the phytase assay kit was procured from Megazyme, Ireland. Probiotic strains isolated from Indonesian local beef, namely *L. plantarum* IIA-1A5 (Arief et al. 2015) were acquired from the Department of Animal Production and Technology Laboratory, Faculty of Animal Science, IPB University- Indonesia. The bacteria were stored at GenBank (OR473281.1).

Qualitative identification of phytase enzyme from *L. plantarum* IIA-1A5

Probiotic strains (*L. plantarum* IIA-1A5), isolated from Indonesian local beef (Arief et al. 2015) were inoculated in De Man, Rogosa, and Sharpe Broth (MRSB) medium followed by incubation at 37°C for 24 hours. After transmissions, approximately 10% (vol/vol) of activated culture was transferred to 10mL aliquots of MRSB (Oxoid, USA) and incubated at 37°C for 48 hours. The identification and purification of phytase enzyme included several steps, such as ammonium sulfate precipitation (Sigma-Aldrich, USA), protein testing, SDS-PAGE analysis (Bio-Rad, USA) and calculation of phytase activity. The entire purification process was conducted at 4°C. After 2 days of phytase growth from *L. plantarum* IIA-1A5, the culture was centrifugated at 24,000xg for 30min. The supernatant was collected and gradually precipitated with solid ammonium sulfate at saturations of 0-20, 20-40, 40-60 and 60-80% (vol/vol). Each precipitation step comprised centrifugation at 24,000xg for 20min. The resulting pellet was dissolved in 50mM sodium acetate buffer at pH 5.5, transferred to dialysis bags, and dialyzed with dialysis buffer (10mM pH 5.5 sodium acetate) (Demir et al. 2017). Protein concentration was measured following the Bradford method with Coomassie Brilliant Blue G-250 (Thermo Fisher Scientific, USA), using bovine serum albumin as the standard (Bradford 1976). SDS-PAGE analysis was conducted after enzyme purification, and purity was assessed using the (Laemmli 1970) with 3-8% batch SDS-PAGE. The gel was stained using the silver staining method and photographed post-staining.

Production of jack bean flour (*C. ensiformis*)

The production of jack bean flour (*C. ensiformis*) was based on the modified method of (Ariyantoro et al. 2016). A total of 5kg of the bean seeds was soaked for 72 hours, with water changes every 6 hours, followed by peeling and cleaning. Subsequently, the seeds were dried in an oven at 55-60°C for 7 hours, ground into flour, and sieved using an 80-mesh sieve.

Formulation of probiotic fermented sausages

The culture of *L. plantarum* IIA-1A5 was refreshed following the method of Arief et al. (2015) and then re-cultured in sterilized milk. Autoclaved milk at 115°C for 3min was inoculated at 37°C for 24hrs, yielding a starter culture for the production of probiotic fermented sausages. A total of 8kg of beef was utilized in the process.

Testing the quality of probiotic fermented sausages

Samples of fermented sausages, combining beef and jack bean, were tested for quality, including pH, water activity (aw), total titratable acidity (TTA), moisture content, fat, protein, carbohydrates, ash, total lactic acid bacteria (LAB), total plate count (TPC), pathogenic bacteria (*E. coli*, *S. aureus*, and *Salmonella*), amino and fatty acid analysis, as well as flavor analysis. The water content, fat, protein, carbohydrates, ash, and pH were determined using methods specified by Association of Official Analytical Chemistry (2005). The aw value was measured using an aw meter, while TTA was determined using the Association of Official Analytical Chemistry (2007). TPC and total LAB were conducted using the pour plate method outlined by Fardiaz (1993). Pathogenic bacteria were counted following the procedures described by Arief et al. (2016).

High-Performance Liquid Chromatography (HPLC) was used for amino acid analysis (Agilent Tech, USA), following the HPLC Laboratory protocol at IPB. The

procedure started with sample preparation, which comprised adding an orthophthalaldehyde (OPA) reagent to the injector. This reagent reacted with primary amino acid in a basic environment containing mercaptoethanol, forming a fluorescent compound detectable using a fluorescence detector. The time required for the sample to elute from the column is termed retention time.

Fatty acid composition of sausages was analyzed according to Yang et al. (2019) and methylation was conducted through transesterification. Injector and detector temperatures were set at 250 and 300°C, respectively, with helium as the carrier gas at a flow rate of 1.0mL/min. Results were expressed as mg FFA/g extracted lipid, with all tests performed in triplicate. Volatile compounds were analyzed following (Arief et al. 2015), using solid-phase microextraction gas chromatography-mass spectrometry (Shimadzu, Japan). Bottles containing 1g of sample were preincubated at 50°C for 5min. Volatile compounds were extracted from the headspace by fiber for 20min while stirring at 50°C. Elution was conducted with a temperature increase from 40 to 280°C at a rate of 7.5°C/min and an additional 3min hold at the end. A 4-methyl-2-pentanol internal standard at 200ppb was used, and quantities were expressed as ppb in an equivalent internal standard. The results were adjusted for sample dry matter.

Statistical Analysis

The data collected in this study were primary data. All measurements were carried out in triplicate, and all results were reported as the mean and standard deviations. One-way analysis of variance (ANOVA) maintaining a=0.05 was used for all results. The Tukey's test was used to compare differences between mean values of individual samples (Gaspersz 1991). Analysis of phytase activity was done by using Mega-CalcTM software tool followed by an independent T test (Kim 2015).

RESULTS AND DISCUSSION

The physicochemical and microbiological analysis of fermented sausages combining beef and jack bean flour

An analysis of the physical, chemical, and microbiological properties of fermented sausages, with or without the addition of *L. plantarum* IIA-1A5, is presented in Table 1. Physical analyses included pH value, water activity (aw), and total titratable acidity. The pH values of fermented sausages, regardless of the presence of the bacteria, showed no significant difference (P>0.05), ranging from 4.76 to 5.06. This is because a natural fermentation process occurs in cases without *L. plantarum* IIA-1A5. According to Yang et al. (2019), LAB species, including *L. plantarum*, survive in pure beef. Statistically (P>0.05) determined aw values ranging from 0.84 to 0.83 had no significant influence on sausages without or with the addition of the bacteria. In line with previous studies, the aw value in fermented sausages containing rosemary extract was > 0.8 < 1 (Bowser et al. 2014). The value is crucial for assessing moisture content in food materials and its relation to product preservation and shelf life. Furthermore, total titratable acidity values ranging from 0.24 to 0.35% showed no significant differences (P>0.05) and are influenced by 2 days fermentation process. The results of total titratable acidity are inversely related to the pH value produced. However, this study does not show any differences and the pH value does not decrease. The fermentation process in fermented sausages affects the amount of organic acids, primarily lactic acid, produced through the addition of *L. plantarum* IIA-1A5. These acids are formed by breaking down sugars in the medium (Colombo et al. 2018). Therefore, the combination of jack bean and beef, with or without the addition of *L. plantarum* IIA-1A5, does not significantly affect the physicochemical properties of fermented sausages.

Table 1: Physicochemical and microbiological testing of the combination of jack bean flour and beef for making fermented sausages

Variables	Fermented sausage without *L. plantarum* IIA-1A5	Fermented sausage with *L. plantarum* IIA-1A5
pH	5.06±0.16	4.76±0.05
a_w	0.84±0.02	0.83±0.02
TAT (%)	0.35±0.04	0.29±0.02
Water content (%)	49.64±1.97	46.26±2.39
Ash content (%)	3.71±0.19	3.86±0.19
Protein content (%)	25.44±0.48	26.53±1.05
Fat content (%)	4.11±1.15	5.65±1.12
Carbohydrate content (%)	17.08±0.03	17.68±0.03
Escherichia coli	nd	nd
Salmonella	nd	nd
S. aureus (log CFU/g)	3.13±0.4[a]	1.50±0.04[b]
TPC (log CFU/g)	9.60±0.14[a]	8.83±0.07[b]
LAB (log CFU/g)	8.90±0.04	9.00±0.16

Note: nd = not detected; Values (mean±SD) bearing different letters in the same row indicate significant (P<0.05) differences.

Chemical analysis conducted in this study included proximate analysis, evaluating the moisture, ash, protein, fat, and carbohydrate content in fermented sausages, both without or with the addition of *L. plantarum* IIA-1A5. The results of proximate testing showed no significant difference (P>0.05). Moisture content in the fermented sausages adhered to the standards outlined in Badan Standardisasi Nasional (2015), which specified a maximum water content of 67%. However, the ash content does not significantly differ (P>0.05) but exceeds the SNI requirements for sausages. This is in line with the study conducted by Nie et al. (2014), suggesting that LAB can produce lactic acid, contributing to an increase in ash content. Furthermore, protein content in the fermented sausages also does not show a significant difference (P>0.05). This result complies with SNI regulations, which specify a minimum protein content of 13% in sausages. Fat content in the fermented sausages ranged from 4.11 to 5.65%, while the carbohydrate content fell between 17.08 and 17.68%. The carbohydrate content exceeded the SNI requirement, which specified a maximum of 8% due to the addition of jack bean flour during production.

Microbiological analysis conducted in this study aimed to detect the presence of pathogenic bacteria, including *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella*, as well as total plate count (TPC) and total LAB in fermented sausages without or with the addition of *L. plantarum* IIA-1A5. The analysis of the growth of pathogenic bacteria *E. coli* and *Salmonella* showed no significant difference (P>0.05). *E. coli* can originate from the human digestive system and the environment, and the presence in food products reflects unhygienic production processes as well as sanitation. Additionally, *Salmonella* bacteria are a significant cause of outbreaks in Europe and the United States. They can contaminate meat and meat

products by colonizing the digestive tracts of livestock. According to the study by (Potočnjak et al. 2017; Meristica et al. 2020), *L. plantarum* strains can inhibit the growth of *Salmonella enterica serovar* Typhimurium, with no significant difference observed among *Salmonella* strains. Microbiological analysis showed a significant difference ($P<0.05$) in the presence of *S. aureus* bacteria, which were at 3.13 log CFU/g and 1A5 1.50 log CFU/g, in fermented sausages without and with the addition of *L. plantarum*, respectively. This is due to the ability of *L. plantarum* to inhibit pathogenic bacteria such as *E. coli*, *S. aureus*, and of *Salmonella enterica serovar* Typhimurium. *S. aureus* is a pathogenic bacterium capable of causing infections. According to Sihombing et al. (2015), the population of *S. aureus* in meat treated with plantaricin is lower than the national standard (Badan Standar Nasional 2008). *Lactobacillus* bacteria can produce antimicrobial substances and bacteriocins that are bactericidal against the growth and resistance of pathogenic microbes. Based on testing results, the TPC in sausages with and without the addition of *L. plantarum* IIA–IA5 were 8.83 log CFU/g and 9.60 log CFU/g. According to Soenarno et al. (2020), the bacterium *L. plantarum* IIA–1A5 has antimicrobial properties equivalent to ampicillin and penicillin antibiotics against *E. coli*, as well as nisin against *S. aureus*. The populations of LAB generated did not significantly differ ($P>0.05$) between the 2 treatments due to equal fermentation time. The total values without and with the addition of *L. plantarum* IIA-1A5 were 8.90 log CFU/g and 9.00 CFU/g. LAB tends to increase with longer fermentation times, but other factors such as the initial population, competing microorganisms, fermentation temperature, and salt concentration can also influence growth. Additionally, the presence of naturally occurring LAB in beef, such as *L. plantarum*, explains the lack of significant differences between the 2 treatments.

Analysis of amino acids and fatty acids in fermented sausages combining beef and jack bean flour

Amino acids are formed as a result of proteolysis activity during the fermentation process by LAB. Analysis conducted in fermented sausages with or without the addition of *L. plantarum* IIA-1A5 yielded 18 types, categorized into 9 essential and 9 non-essential amino acids (Table 2). Essential types include threonine, valine, methionine, isoleucine, leucine, phenylalanine, histidine, lysine, and tryptophan. Non-essential types comprised aspartic acid, serine, glutamic acid, proline, glycine, alanine, cystine, tyrosine, and arginine. The amino acid glutamate was observed to be the dominant component in fermented sausages, regardless of the treatment. This is in line with the findings of the previous studies (Lorenzo and Franco 2012; Sulaiman et al. 2016; Ikonic et al. 2019). Amino acids contributed to the taste of the fermented product and the activity of proteases in the meat or the addition of LAB led to an increase in free amino acids (Xu et al. 2008; Nie et al. 2014; Candogan et al. 2009). Based on statistical analysis, no significant difference ($P>0.05$) was observed between sausages with or without the addition of *L. plantarum* IIA-1A5, except for tryptophan, which showed a significant effect ($P<0.05$). Tryptophan is an essential amino acid for humans because it is required and not synthesized within the human body. Tryptophan is higher in sausages supplemented with *L. plantarum* IIA-1A5. This amino acid type, derived from plants, is necessary for *in vivo* protein biosynthesis (Mendel 2018). However, only 1% of available tryptophan is used for protein synthesis, with 99% used as precursors for tryptamine, melatonin and serotonin (Jeong et al. 2021). Markus (2008) also noted that tryptophan consumption can increase brain 5-HT levels and decrease cortisol levels. Other research has indicated that *Lactobacilli* strains have been shown to catabolize tryptophan in the stomach and ileum of rats (Zelante et al. 2013). Some Lactobacillus species have also been detected to have the ability to metabolize tryptophan into indole and its derivatives (Jeong et al. 2021). Additionally, tryptophan is necessary for protein biosynthesis in plants (Friedman 2018). Further studies were needed to detect the genes synthesized by *L. plantarum* IIA-1A5.

Table 2: Amino acid contents of fermented sausage combination of beef and jack beans

Amino acid (% w/w)	Fermented sausage with/without *L. plantarum* IIA-1A5 (%)	
	Without	With
Essential Amino Acids		
Lysine	1.63±0.01	1.8±0.06
Leucine	2±0.01	1.99±0.03
Methionine	0.37±0.02	0.31±0.01
Threonine	1.05±0.01	1.07±0.01
Phenylalanine	1.34±0.03	1.33±0.03
Valine	1.27±0.01	1.24±0.01
Histidine	0.93±0.01	0.96±0.02
Triptophan	0.01±0.01a	0.08±0.01b
Non-Essential Amino Acids		
Alanine	1.39±0.01	1.45±0.08
Glycine	1.11±0.01	1.13±0.06
Proline	0.93±0.01	0.95±0.01
Aspartic Acid	2.56±0.08	2.53±0.01
Cysteine	0.1±0.01	0.16±0.06
Serine	0.97±0.01	0.97±0.01
Tyrosine	0.57±0.02	0.66±0.03
Glutamic Acid	4.05±0.05	4.03±0.06
Arginine	1.29±0.05	1.3±0.03
Total amino acids	23.57±0.02	23.95±0.03
Essential Amino Acids	10.6±0.02	10.77±0.02
Non-Essential Amino Acids	12.97±0.03	13.18±0.03

Different letters on the same row indicate significant differences ($P<0.05$).

Fatty acids play essential roles in pharmaceutical formulations aimed at preventing as well as enhancing health. Furthermore, its profile in fermented products can vary depending on various factors, including the type of microorganisms, raw materials, and fermentation conditions (Vergallo 2020). Free fatty acids produced contribute to the formation of the flavor of fermented sausages. Excessive oxidation can lead to increased acidity levels, changes in texture and color, the growth of spoilage microorganisms, and a decrease in the quality of fermented sausages (Falowo et al. 2014). Analysis of the free fatty acid profile identified 33 types in fermented sausages, with or without the addition of *L. plantarum* IIA-1A5, as shown in Table 3. These can be divided into 16 saturated and 17 unsaturated categories (9 monounsaturated fatty acids and 8 polyunsaturated fatty acids). The 16 saturated fatty acids include butyric acid (C4:0), caproic acid (C6:0), caprylic acid (C8:0), capric acid (C10:0), undecanoic acid (C11:0), lauric acid (C12:0), tridecanoic acid (C13:0), myristic acid (C14:0), pentadecanoic acid (C15:0), palmitic acid (C16:0),

heptadecanoic acid (C:17), stearic acid (C18:0), arachidic acid (C20:0), heneicosanoic acid (C21:0), behenic acid (C22:0), and lignoceric acid (C24:0). Meanwhile, the 17 unsaturated types comprised of 9 monounsaturated fatty acids, namely myristoleic acid (C14:1), cis-10-pentadecanoic acid (C15:1), palmitoleic acid (C16:1), cis-10-heptadecanoic acid (C17:1), elaidic acid (C18:1n9t), oleic acid (C18:1n9c), cis-11-eicosenoic acid (C20:1), erucic acid methyl ester (C22:1n9), and nervonic acid (C24:1). The remaining 8 polyunsaturated fatty acids were linolelaidic acid (C18:2n9t), linoleic acid (C18:2n6c), γ-linolenic acid (C18:3n6), linolenic acid (C18:3n3), cis-11.14-eicosedienoic acid (C20:2), cis-11.14.17-eicosatrienoic acid methyl ester (C20:3n3), arachidonic acid (C20:4n6), and cis-4.7.10.13.16.19-docosahexaenoic acid (C22:6n3). A study conducted by Simopoulos (2016) and Pires et al. (2018) showed that variations in fatty acid composition were influenced by several factors, such as species, feed availability and quality, heat usage, seasonal changes and environmental salinity.

The most dominant saturated fatty acid profile in both samples of fermented sausages, with or without the addition of *L. plantarum* IIA-1A5, includes myristic acid (C14:0), palmitic acid (C16:0) and lignoceric acid (C24:0). Previous study has stated that common fatty acids discovered in vegetable oils were caprylic acid, lauric acid, myristic acid, palmitic acid, stearic acid, palmitoleic acid, oleic acid, linoleic acid and linolenic acid (Rahman et al. 2022). The most dominant monounsaturated type identified in both treatments was palmitoleic acid (C16:1). Palmitic acid falls within the saturated category and is commonly discovered in various foods such as meat and dairy products (50-60%), cocoa butter (26%), as well as olive oil (8-20%) (Innis 2006).

Lipids, which consist of various types of fatty acids, perform a range of functions. One major group is essential fatty acids, which includes polyunsaturated fatty acids such as linoleic acid (omega-6) and linolenic acid (omega-3) (Osendarp 2011; Adriani and Wirjatmadi 2016)). Linoleic and linolenic acids are produced at higher levels in fermented sausages with the addition of *L. plantarum* IIA-1A5, although the statistical analysis shows this difference is not significant. Essential fatty acids crucial for growth and development, especially in children, include linoleic acid, linolenic acid, arachidonic acid (ARA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA) (Djuricic and Calder 2021; Clinic 2022).

Omega-3 fatty acids are necessary for brain and eye development and have benefits in maintaining overall health (Djuricic and Calder 2021). Generally, omega-3 fatty acids are known for their anti-inflammatory properties and can help combat cardiovascular diseases (CVD), including hypotriglyceridemia. According to Siriwardhana et al. (2012), omega-3 fatty acids also possess antihypertensive, anti-arthritis, antioxidant, anticancer, anti-aging and antidepressant properties. Meanwhile, omega-6 fatty acids play an important role in brain and liver function and support normal growth and development (Castle and Paula 2010).

Based on statistical results, the types of fatty acids produced showed no significant differences in the samples of fermented sausages, with exceptions of heneicosanoic acid (C21:0) and polyunsaturated fatty acid cis-11.14.17-eicosatrienoic acid methyl ester (C20:3n3), which were higher in sausages with the addition of *L. plantarum* IIA-1A5. This is because the omega-3 counterparts were subjected to slower oxidation. According to Deveci et al. (2023), *L. plantarum* can metabolize proteins into bioactive peptides with antioxidant properties. The fermentation process using LAB produces peptide compounds that protect fatty acids from lipid peroxidation disturbances, resulting in a higher amount of C20:3n3 in sausages with the addition of *L. plantarum* IIA-1A5.

Analysis of the flavor of fermented sausages made from a combination of beef and jack bean flour

Volatile compounds are produced through protein hydrolysis, amino acid breakdown, and lipid oxidation. During the maturation of fermented sausages, several volatile compounds can be formed in significant quantities (Li et al. 2023). This study identified the various classes, including organic acids, alcohols, aldehydes, esters, hydrocarbons, ketones, sulfur compounds, and other components, as listed in Fig. 1. Analysis of fermented sausages made from a combination of beef and jack bean flour signified that alcohols and hydrocarbons were the most prevalent. Furthermore, the aroma components

Table 3: Fatty acids contents of fermented sausage combination of beef and jack beans

Fatty acids Profile	Fermented sausage with/ without *L. plantarum* IIA-1A5 (%)	
	Without	With
Saturated fatty acid		
Butyric acid C4:0	0.1±0.05	0.18±0.14
Caproic acid C6:0	0.11±0.03	0.08±0.001
Caprylic acid C8:0	0.08±0.05	0.05±0.001
Capric acid C10:0	0.06±0.01	0.06±0.02
Undecanoic acid C11:0	0.04±0.02	0±0
Lauric acid C12:0	2.21±0.07	2.26±0.01
Tridecanoic acid C13:0	0.46±0.08	0.33±0.03
Myristic acid C14:0	14.26±0.34	14.72±2.58
Pentadecanoic acid C15:0	1.06±0.11	0.82±0.09
Palmitic acid C16:0	19.93±0.41	20.93±3.44
Heptadecanoic acid C17:0	0.77±0.57	0.05±0.01
Stearic acid C18:0	0.37±0.16	0.21±0.05
Archidic acid C20:0	0.67±0.37	0.94±0.26
Heneicosanoic acid C21:0	0.01±0.005[a]	0.05±0.01[b]
Behenic acid C22:0	0.11±0.02	0.13±0.06
Lignoceric acid C24:0	6.58±0.47	5.91±0.16
Unsaturated fatty acid		
Monounsaturated fatty acid (MUFA)		
Myristoleic acid C14:1	2.36±0.09	2.23±0.31
Cis-10-Pentadecanoic acid C15:1	0.67±0.10	0.58±0.005
Palmitoleic acid C16:1	14.68±1.47	14.99±1.90
Cis-10-Heptadecanoic acid C17:1	1.06±0.01	0.96±0.04
Elaidic acid C18:1n9t	0.07±0.05	0.02±0.01
Oleic acid C18:1n9c	1.43±0.12	1.12±0.19
Cis-11-Eicosinoic acid C20:1	0.05±0.02	0.03±0.01
Erucic Acid Methyl Ester C22:1n9	0.02±0.01	0.02±0.01
Nervonic acid C24:1	6.11±0.01	0±0.03
Polyunsaturated fatty acid (PUFA)		
Linolelaidic acid C18:2n9t	0.65±0.08	0.49±0.01
Linoleic acid C18:2n6c	0.17±0.12	0.23±0.01
γ-Linolenic acid C18:3n6	0.12±0.05	0.07±0.01
Linolenic acid C18:3n3	0.05±0.01	0.06±0.01
Cis-11.14-Eicosedienoic acid C20:2	0.05±0.01	0.03±0.01
Cis-11.14.17-Eicosatrienoic Acid Methyl Ester C20:3n3	0.16±0.01[a]	0.21±0.03[b]
Arachidonic acid C20:4n6	1.49±0.01	1.66±0.01
Cis-4.7.10.13.16.19-Docosahexaenoic acid C22:6n3	0.91±0.22	1.1±0.27

Values (mean±SD) bearing different letters in the same row indicate significant (P<0.05) differences.

produced presented the identification of 156 volatile compounds in both treatments. These compound components can be categorized into several groups, comprising 4 organic acids, 34 alcohols, 8 aldehydes, 7 esters, 57 hydrocarbons, 22 ketones, 3 sulfur compounds, and 21 other components, as listed in Table 4.

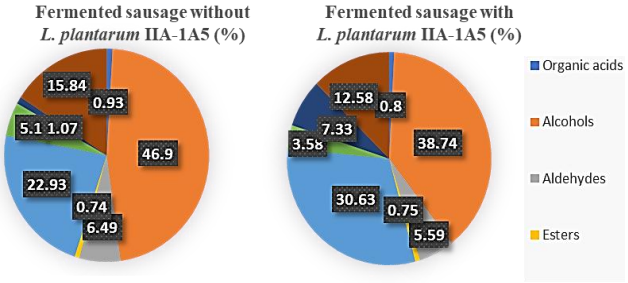

Fig. 1: Percentage of volatile compound groups for fermented sausage combination of beef and jack bean flour.

Organic acids produced showed no difference between fermented sausages without or with the addition of *L. plantarum* IIA-1A5. The types identified include acetic acid, butanoic acid, n-caprylic acid, and propanoic acid. Alcohol is formed through the process of lipid degradation and oxidation (Zhao et al. 2021). In this study, a total of 34 types were detected in all fermented sausages.

Aldehydes, known as odorous compounds with low taste threshold, play an important role in fermented meat products (Zhang et al. 2019). The contents, such as 3-Hydroxy-4-methylbenzaldehyde were not detected in sausages with the addition of *L. plantarum* IIA-1A5, while 3-furaldehyde, acetaldehyde, benzeneacetaldehyde, furfural, nonanal, tetradecanal, and β-Cyclocitral were detected in all treatments. Nonanal, contributing the aroma of green grass and fat to fermented meat, is derived from the oxidation of linoleic acid and linolenic acid (Wang et al. 2022). Additionally, its compounds are an important type of aldehydes (Chen et al. 2015).

Esters play a vital role in sausage aroma, providing a fruity scent that helps mitigate the potential for a rancid aroma in the product (Stahnke 1994). Additionally, the addition of nitrite and LAB influences the formation of ethyl ester (Stahnke 1995).

Volatile compounds from the hydrocarbon group were mostly discovered in both treatments. Several volatile compounds from the hydrocarbon group were formed exclusively in fermented sausages with the addition of *L. plantarum* IIA-1A5, including 1-Acetoxy-2-butanone, 1-Allyl-2-isopropyldisulfane, 1S-α-Pinene, 2-Nonanone, Acetophenone, Cyclene, and α-Phellandrene. Meanwhile, those formed in the other treatment were Cyclooctene, Dodecane, Limonene, p-Cymene, α-Cubebene and β-Terpinen.

Volatile compounds from the ketone group were commonly discovered in β-Terpinen, 2-cyclopenten-1-one, 3-methyl-, furfural, 5-methyl-, and thiophene, 2-propyl-. Ketones also have a low taste threshold value and can impart fruit, rose, floral, as well as tea aromas to fermented sausages (Wang et al. 2022). In this study, 22 of the types were detected in all fermented sausages.

The formation of volatile compounds containing sulfur, namely diallyl disulphide, was most commonly discovered in fermented sausages with the addition of *L. plantarum* IIA-1A5. Other aroma components were formed more in fermented sausages without the addition of *L. plantarum* IIA-1A5. Based on the PCA test, the plot results in Fig. 2 show that fermented sausages without the

Table 4: Volatile compounds of fermented sausage combination of beef and jack bean.

Compounds	Fermented sausage without *L. plantarum* IIA-1A5	Fermented sausage with *L. plantarum* IIA-1A5	Code
	Peak area (x 10^7)		
Total Organic Acids	21.98	19.12	-
Acetic Acid	12.88	10.57	48
Butanoic Acid	6.87	7.36	60
n-Caprylic Acid	0.64	1.19	104
Propanoic Acid	1.59	0	121
Total Alcohols	1114.74	921.97	-
2,3,4-Trimethyl-1-pentanol	0	2.41	11
2-Ethylhexanol	0	4.57	26
2-Furanmethanol	40.01	26.57	27
3,4-Xylenol	5.26	3.54	34
3,5-Xylenol	1.44	0.96	36
3-test-Butylphenol	0.55	0.55	40
4-Ethylbenzyl alcohol	0.84	0	41
4-Formylphenol	4.17	3.72	42
4-Mercaptophenol	5.89	4.09	43
4-Vinylguaiacol	5.39	3.63	45
Acetylcarbinol	2.13	0	49
cis-Sabinol	2.56	3.55	63
cis-Thujane-4-ol	7.18	5.98	64
Ethanol	7.77	2.63	79
Eugenol	7.13	5.98	81
Isocreosol	7.54	5.35	88
Isoeugenol	1.27	0.74	89
Isohomogenol	3.07	2.71	90
Linalool	32.58	34.36	93
m-Cresol	11.54	7.54	94
m-Cymen-8-ol	4.11	3.90	95

Mequinol	4.53	4.26	96
Methyleugenol	17.04	16.58	100
m-Ethylphenol	3.37	2.86	101
o-Ethylphenol	3.14	2.68	107
o-Guaiacol	260.12	188.38	108
o-Xylenol	2.82	1.72	109
Phenol	477.83	376.47	114
Phenylethyl alcohol	5.31	5.99	118
p-Propylguaiacol	4.73	2.97	120
Terpinen-4-ol	153.92	168.97	126
trans-4-Thujanol	5.65	5.18	130
trans-Isoeugenol	8.63	5.16	131
α-Terpineol	17.22	17.97	141
Total Aldehydes	154.32	133.08	-
3-Furaldehyde	2.35	2.13	38
3-Hydroxy-4-methylbenzaldehyde	0.85	0	39
Acetaldehyde	70.81	67.97	47
Benzeneacetaldehyde	9.40	4.14	57
Furfural	60.83	50.63	82
Nonanal	4.82	4.66	105
Tetradecanal	1.11	1.20	127
β-Cyclocitral	4.15	2.35	145
Total Esters	17.68	17.87	-
2-Furfuryl acetate	3.26	6.55	29
2-Furfuryl butyrate	1.69	0	30
Bornyl acetate	5.35	5.15	59
Ethyl caprylate	0	3.02	80
Methyl caprylate	2.56	0	97
Methyl p-anisate	4.07	3.15	98
Methyl valerate	0.75	0	99
Total Hydrocarbons	544.76	728.6	-
(+)-4-Carene	8.44	23.05	1
.psi.-Cumohydroquinone	5.70	3.63	2
.psi.-Limonene	8.16	3.18	3
1,3,8-p-Menthatriene	8.63	2.95	4
1-Acetoxy-2-butanone	0	2.17	5
1-Allyl-2-isopropyldisulfane	0	2.25	6
1-Phenyl-1H-indene	1.78	2.28	8
1R-α-Pinene	6.66	11.21	9
1S-α-Pinene	0	8.62	10
2,3-Dimethoxytoluene	3.01	4.58	12
2-Carene	21.33	7.49	16
2-Methylenebornane	4.79	5.85	31
2-Nonanone	0	7.15	32
3-Carene	32.89	14.90	37
4-Methyl-5H-furan-2-one	2.36	1.30	44
Acetophenone	0	8.93	50
Allo-Ocimene	7.35	2.19	52
Bicyclo(3.2.1)octane	1.91	2.76	58
Butyrolactone	7.30	5.85	61
Caryophyllene	127.54	234.72	62
cis-β-Farnesene	8.23	1.70	65
Copaene	6.56	9.08	66
Cyclooctene	2.63	0	68
Cyclene	0	14.26	69
Cyclotene	11.90	9.38	71
D-Limonene	14.56	41.64	75
Dodecane	11.90	0	76
Humulene	8.47	14.03	80
Isobutenylbenzene	9.34	5.56	84
Isocaryophyllene	3.70	17.68	86
Limonene	28.40	0	87
Naphthalene	4.84	4.55	92
o-Cymene	19.20	41.26	103
p-Cymene	16.68	0	106
Sabinene	9.14	1.22	112
trans-α-Bergamotene	1.76	1.78	124
Undecane	3.44	0.85	132
α-Cubebene	4.20	0	134
α-Guaiene	4.08	6.17	135

α-Indanone	3.19	2.14	136
α-Phellandrene	0	29.07	137
α-Pinene	2.58	4.15	138
α-Selinene	2.92	1.81	139
α-Terpinene	5.50	28.78	140
α-Thujene	17.39	7.12	143
β-Bisabolene	3.58	9.38	144
β-Elemene	2.37	3.28	146
β-Ocimene	1.59	1.30	147
B-Phellandrene	16.54	17.54	148
β-Pinene	8.51	11.96	149
β-Selinene	1.64	2.38	150
β-Terpinen	22.50	0	151
β-Thujene	12.51	13.98	152
γ-Gurjunene	2.21	2.92	153
γ-Terpinen	18.15	54.46	154
δ-Cadinene	2.32	4.46	155
δ-EIemene	4.38	9.65	156
Total Ketones	121.09	85.12	-
2-Acetylfuran	17.16	14.31	15
2-Cyclopenten-1-one, 2,3,4-trimethyl-	0.32	0	17
2-Cyclopenten-1-one	0	2.43	18
2-Cyclopenten-1-one, 2,3-dimethyl-	4.65	4.77	19
2-Cyclopenten-1-one, 2-methyl-	6.96	5.40	20
2-Cyclopenten-1-one, 3,4,5-trimethyl-	0	0.24	21
2-Cyclopenten-1-one, 3,4-dimethyl-	3.88	3.35	22
2-Cyclopenten-1-one, 3-ethyl-	5.09	4.48	23
2-Cyclopenten-1-one,3-ethyl-2-hydroxy-	4.27	2.94	24
2-Cyclopenten-1-one, 3-methyl-	13.98	11.64	25
2-Furanone, 2,5-dihydro-3,5-dimethyl	6.17	0	28
2-Pentenal, 2-methyl-	4.51	4.14	33
3,5-Octadien-2-one	5.61	5.40	35
Acridine, 9-methyl-	5.9	2.77	51
Benzene, (2-methoxyethyl)-	4.96	0	55
Cyclopropane, octyl-	1.29	0	70
Decane, 3,6-dimethyl-	3.68	0.32	72
Furfural, 5-methyl-	18.57	11.96	83
Indole, 5-methyl-2-phenyl-	1.43	0.90	85
Phenol, 2,5-dimethyl-	3.23	2.26	116
Phenol, 4-methoxy-3-methyl-	6.37	7.35	117
Pyrazine, trimethyl-	1.47	0.46	122
Pyrazole, 1-ethyl-3,5-dimethyl-	1.59	0	123
Total Sulphur	25.39	174.46	-
1-Butyne, 1,1'-thiobis-	2.29	2.05	7
Diallyl disulphide	8.57	157.77	73
Thiophene, 2-propyl-	14.53	14.64	128
Total Other Compounds	376.5	299.1	-
2,4-Dimethylanisole	0.50	0	13
2-Acetyl-5-methylfuran	3.90	1.52	14
5-Ethylfurfural	0	1.33	46
Anisole	3.58	3.01	53
Anisole, o-fluoro-	4.80	3.91	54
Benzene, 1,2-dimethoxy-	6.53	6.08	56
Creosol	122.23	78.18	67
Diallyl sulfide 2,4-Dimethylanisole	0	15.10	74
Elemicin	3.27	3.14	77
Estragole	0	1.27	78
Isosafrole	2.75	2.96	91
Myristicin	79.29	68.62	102
p-Cresol	14.98	8.96	110
p-Cymen-8-ol	1.90	1.59	111
p-Ethylguaiacol	68.58	41.06	113
p-Cymene-2,5-diol	1.13	0	115
p-Menth-2-en-1-ol, trans	2.21	3.12	119
Safrole	57.24	59.26	125
Toluene, 3,4,5-trimethoxy-	0.95	0	129
α-Terpinolen	2.66	0	142
Total Volatile Compounds	2377.18	2379.93	-

addition of *L. plantarum* IIA-1A5 had a different aroma profile. The total PC-1 and PC-2 of 98% indicates that the data was normally distributed and the PCA model was acceptable (Utama et al. 2022; Chen et al. 2023).

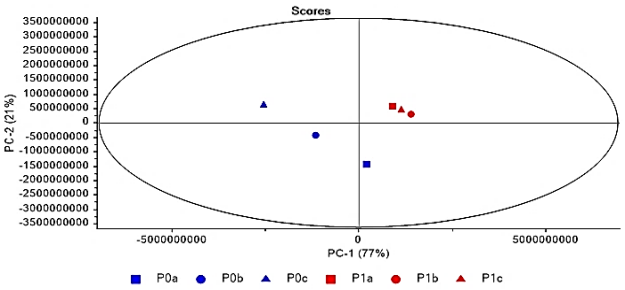

Fig. 2: PCA plot of fermented sausage samples without *L. plantarum* IIA-1A5 (P0) and with *L. plantarum* IIA-1A5 (P1).

Based on Fig. 3, the most volatile compounds were more dominantly produced in fermented sausages without the addition of *L. plantarum* IIA-1A5. Meanwhile, caryophyllene and diallyl disulphide are more dominantly formed in fermented sausages with the addition of *L. plantarum* IIA-1A5. Caryophyllenes have been identified as the main volatile compounds produced in significant quantities in spice and food crops. These compounds can provide a distinctive aroma and taste to fermented sausages. However, based on the sensory evaluation conducted by Lukman et al. (2023) through hedonic testing of fermented sausages with the addition of jack bean flour, it was indicated that the addition of 5% *L. plantarum* IIA-1A5 did not result in significant differences in color, aroma, taste, and texture. Caryophyllene components can provide spice characteristics and aromatic ingredients that enhance the taste profile, while diallyl disulphide provides rich garlic characteristics and produces the desired flavor profile in the product. The diallyl disulphide compound was formed from the metabolism of sulfur amino acids such as cysteine and methionine (Smit et al. 2000). Caryophyllene and diallyl disulphide were also identified in the study of dos Santos (Cruxen et al. 2018) on fermented sausages produced from goat meat. In conclusion, the formation of volatile compounds in this study was different from other previous reports due to the addition of jack bean flour (*C. ensiformis*).

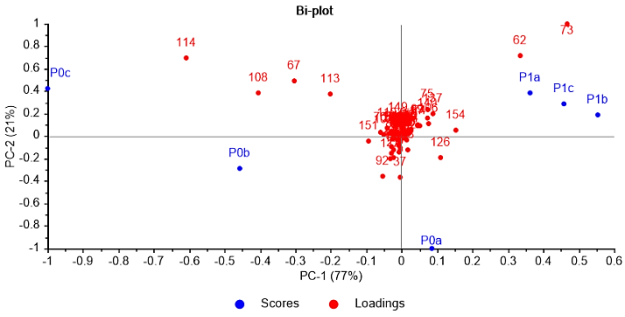

Fig. 3: PCA biplot of fermented sausage samples without *L. plantarum* IIA-1A5 (P0) and with *L. plantarum* IIA-1A5 (P1).

Bradford assay and SDS-PAGE analysis of L. plantarum IIA-1A5

The protein concentration based on the Bradford assay produced by *L. plantarum* IIA-1A5 differs from the results of previous studies. According to Demir et al. (2017), Boukhris et al. (2015) and Parhamfar et al. (2015), specific activities were reported as 227.73 EU/mg (*Weissella halotolerans*), 27 EU/mg (*Bacillus amyloliquefaciens* US573) and 71.5 EU/mg (*Geobacillus stearothermophilus*). This variation could be contingent upon the purity of the enzyme from each bacterial strain. The concentration results of the phytase enzyme protein from *L. plantarum* IIA-1A5 obtained through ammonium sulfate precipitation samples ranged from 0.014 to 0.322 mg/mL, as shown in Table 5. The highest total protein content, measuring 0.056mg, was observed in the sample containing 60% ammonium sulfate, while the lowest at 0.003mg, was identified in the sample with 80% ammonium sulfate addition. These results differed from the phytase activity produced by *L. plantarum* IIA-1A5. Phytase activity testing from *L. plantarum* IIA-1A5 with the addition of 20-80% ammonium sulfate is presented in Fig. 4. The highest activity was recorded at 20% ammonium sulfate, registering 123 mU/mL, while activity levels of 100 mU/mL were observed at 40-60% ammonium sulfate.

Table 5: Concentration of phytase enzyme protein from *L. plantarum* IIA-1A5

Treatment	Protein (mg/mL)	Volume (mL)	Total Protein (mg)
Addition of 20% $(NH_4)_2SO_4$	0.209	0.2	0.042
Addition of 40% $(NH_4)_2SO_4$	0.083	0.2	0.017
Addition of 60% $(NH_4)_2SO_4$	0.282	0.2	0.056
Addition of 80% $(NH_4)_2SO_4$	0.014	0.2	0.003

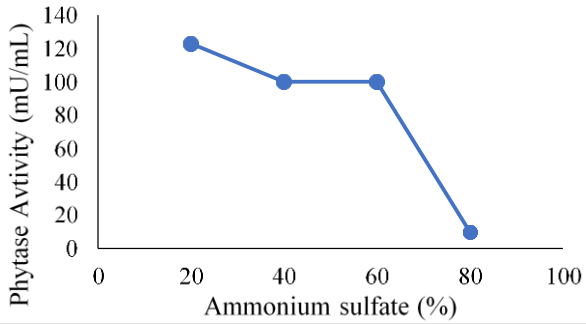

Fig. 4: Phytase activity of *L. plantarum* IIA-1A5 resulting from precipitation with ammonium sulfate.

The molecular weight of the *L. plantarum* IIA-1A5 enzyme was determined using SDS-PAGE gel electrophoresis, as shown in Fig. 5. Furthermore, the bands shown after the ammonium sulfate precipitation step and stained with Silver stain were located at the 87-98 kDa position. This result differed from previous studies (Escobin-Mopera et al. 2012; Boukhris et al. 2015; Parhamfar et al. 2015; Demir et al. 2017) where the molecular weights of phytase were reported as 41.52kDa (*Weissella halotolerans*), 42kDa (*Bacillus amyloliquefaciens* US573), 45kDa (*Klebsiella pneumoniae* 9-3B) and 28 kDa (*Geobacillus stearothermophilus*). These differences could be attributed to variations in methods and LAB used in the previous studies. As well known, the purification of enzymes is a challenging process. Enzymes are susceptible to changes

in conditions influenced by factors such as the substrate molecule acted upon by the enzyme, the product formed after the reaction, or other precursor parameters for the enzymatic reaction. The molecular activity is influenced by several factors, including enzyme concentration, substrate concentration, temperature, pH, allosteric effects, ionic strength, as well as the presence of inhibitors and activators (Demir et al. 2017).

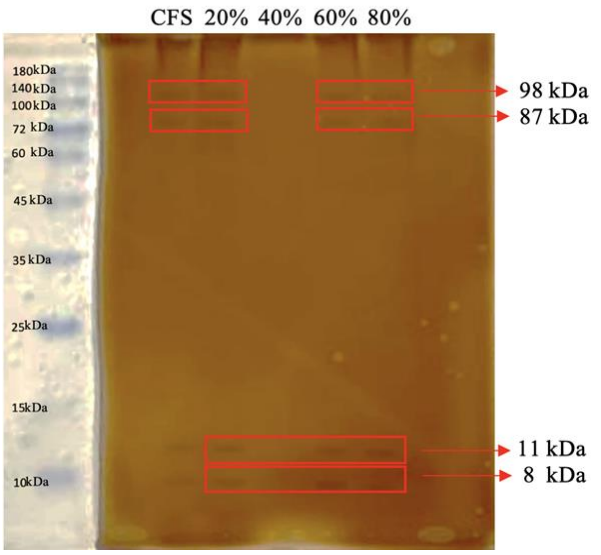

Fig. 5: Phytase enzyme electrophoresis gel from *L. plantarum* IIA-1A5 (CFS: Cell-free supernatant; 20%; 40%; 60; 80% ammonium sulfate).

Conclusion

In conclusion, the utilization of *L. plantarum* IIA-1A5 and jack bean flour (*C. ensiformis*) in fermented sausages inhibited the growth of pathogenic bacteria such as *Staphylococcus aureus*. Analysis of amino acids showed higher levels of tryptophan in fermented sausages with the addition of *L. plantarum* IIA-1A5. It is important to note that saturated fatty acid C21:0 and polyunsaturated fatty acid C20:3n3 were more abundant in fermented sausages with the addition of *L. plantarum* IIA-1A5. The aroma components produced consisted of 156 volatile compounds identified in both treatments. The total concentration of phytase enzyme produced ranged from 0.003 to 0.064mg, with the highest activity obtained in 20% ammonium sulfate ($(NH_4)_2SO_4$) precipitation at 123mU/mL. Therefore, the use of *L. plantarum* IIA-1A5 and jack bean flour (*C. ensiformis*) had the potential to develop functional food products.

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Author's Contribution: Conceptualization: IIA. Investigation: GLML, IIA, CB. Writing – original draft: GLML. Writing – review, and editing: GLML, IIA, IKGW, CB, and ZA. Data curation and Validation: GLML, IIA, IKGW, CB and ZA. Project administration and Supervision: IIA.

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