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RESEARCH ARTICLE
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Development of Functional Fermented Dairy Goat Milk Using Indigenous Probiotic *Lactiplantibacillus plantarum* subsp. *plantarum* strain IIA-1A5

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ABSTRACT

This study evaluated the physicochemical, microbiological, and functional properties of fermented goat milk from three breeds: Peranakan Etawa (PE), Saanen, and Sapera, using the indigenous probiotic *Lactiplantibacillus plantarum* subsp. *plantarum* IIA-1A5. Nine milk samples (three per breed) were fermented at 37°C for 16 h until pH 4.5 and then stored at 4°C. A randomized block design with three treatments, three replications, and a duplicate per analysis was applied. Evaluations included proximate composition, pH, titratable acidity, water activity, amino acid and fatty acid profiles (via HPLC and GC), antioxidant activity (DPPH assay), and enumeration of lactic acid bacteria (LAB). Fermentation significantly ($P<0.05$) enhanced amino acid content, antioxidant activity, and LAB population in all breeds. Fermented milk from PE goat showed superior attributes, including higher fat (6.18%), total amino acids (3.72%), antioxidant capacity (132.89mg EVC/g), and LAB count (8.60log CFU/g). These findings suggest that PE milk provides a more suitable medium for probiotic proliferation and bioactive compound synthesis compared to Saanen and Sapera milk. Overall, PE goat milk fermented with *Lactiplantibacillus plantarum* subsp. *plantarum* IIA-1A5 demonstrates superior nutritional and functional qualities, indicating its strong potential as a base for functional probiotic dairy products.

Keywords: Fermented goat milk, *Lactiplantibacillus plantarum* subsp. *plantarum* IIA-1A5; Antioxidant activity, Functional food.

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INTRODUCTION

Fermented milk is valued as a functional food for providing nutrients and health-promoting microbes (Yerlikaya, 2023; Pascariu et al., 2025). Goat's milk is ideal for fermentation due to its high digestibility, unique fatty acid profile, and superior nutritional content compared to cow's milk (Liao et al., 2024; Salhi et al., 2025). Its smaller fat globules, lower αs1-casein, and higher medium-chain fatty acids enhance digestibility and reduce allergenicity, while bioactive compounds, vitamins, and minerals support health, particularly for those with lactose intolerance or cow's milk protein sensitivity (Nayik et al., 2022; Santos et al., 2023). In Indonesia, major dairy goat breeds include Peranakan Etawa (PE), Saanen, and Sapera, totaling 5.71 million heads, mostly in Java (Statistic Center of Indonesia/ Central Bureau of Statistics, 2024). The Peranakan Etawah (PE) goat, commonly referred to as PE, is a crossbreed between the indigenous Indonesian Kacang goat and the Etawah or Jamnapari goat from India (Sujarwanta et al., 2024). The Saanen goat is a well-known dairy breed originating from the Saanen Valley in Switzerland (Europe) and has been widely distributed to many countries, including Indonesia (Stojiljković et al., 2025). Saanen are renowned for their high milk yield and relatively mild flavor (Šlyžius et al., 2023). Peranakan Etawa goats, a crossbreed of local Kacang goats and Jamnapari goats, are valued for their adaptability and milk yield (Subagyo et al., 2025). Sapera goats, which are crossbreeds of Saanen and Etawa, combine high milk productivity with adaptability to tropical climates (Sumarmono, 2022). These breed-specific differences in milk composition, particularly in protein, fat,

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and mineral contents, are believed to influence fermentation behavior and the resulting functional quality of the fermented product (Amalfitano et al., 2020; Li et al., 2024).

Probiotic fermentation further enhances functionality, *Lactiplantibacillus plantarum* subsp. *plantarum* IIA-1A5, an indigenous Indonesian strain from Peranakan Ongole beef, tolerates low pH and exhibits immune-modulating and antidiarrheal properties (Arief et al., 2015; Sihombing et al., 2015). Its use in fermented milk improves microbial balance and delivers anticancer, hypocholesterolemic, and antihypertensive effects (Arief et al., 2023; Adiyoga et al., 2024). Despite known antioxidant, antimicrobial, and cholesterol-lowering benefits of goat milk (Yang et al., 2023), studies on breed-specific fermentation with local probiotics are scarce, highlighting the need to evaluate PE, Saanen, and Sapera milk with *Lactiplantibacillus plantarum* subsp. *plantarum* IIA-1A5 to optimize functional dairy products in Indonesia. Therefore, this study aimed to evaluate the physicochemical, microbiological, and functional characteristics of fermented goat milk from three breeds: Peranakan Etawa (PE), Saanen, and Sapera, using the indigenous probiotic *Lactiplantibacillus plantarum* subsp. *plantarum* IIA-1A5. It was hypothesized that variations in milk composition among different goat breeds could affect probiotic growth, amino acid patterns, and antioxidant properties following fermentation with *Lactiplantibacillus plantarum* subsp. *plantarum* IIA-1A5. Exploring these differences may help optimize the use of local goat milk in producing functional probiotic dairy products, thereby promoting nutritional innovation and sustainable livestock development in Indonesia.

MATERIALS & METHODS

Preparation of Starter Culture

The Indonesian probiotics of *Lactiplantibacillus plantarum* subsp. *plantarum* strain IIA-1A5 was also used, which was from our own collection culture (Arief et al., 2015). The *Lactiplantibacillus plantarum* subsp. *plantarum* strain IIA-1A5 culture was refreshed in 9mL de Man Rogosa Sharp broth (Oxoid) at 37°C for 24h until turbid, then inoculated (2%) into sterile 10% skim milk and incubated to form the mother culture. Intermediate and working cultures were prepared in succession, and the working culture was confirmed to have > 10^8 CFU/mL when grown on de Man Rogosa Sharp Agar medium (Arief et al., 2023).

Production of Fermented Milk

Goat's milk from three breeds, Peranakan Etawa (PE), Saanen, and Sapera, was used as the raw material. The production of fermented milk followed the methods of Afiyah et al. (2022) with modifications: goat milk was heated at 85°C for 30 min, cooled to 40–45°C, and fermented with *Lactiplantibacillus plantarum* subsp. *plantarum* IIA-1A5. Fermentation occurred at 37°C for 16h until pH ~4.5, after which samples were stored at 1–5°C for analysis.

Physicochemical Characteristic Measurement

The physicochemical properties of fermented milk were analyzed, including pH, water activity (aw), total acidity, moisture, ash, fat, crude protein, and carbohydrate contents, following AOAC (2012).

Antioxidant Activity

Radical scavenging activity was measured using the DPPH method described by Juandini et al. (2023). Briefly, 250µL of the sample was mixed with 3mL of 60µM DPPH in ethanol, and the absorbance was read at 517nm until stable. A control used 250µL of distilled water instead of the sample.

Fatty Acid Analysis

The fatty acid composition of the sausage was analyzed following the AOAC (2012) method. Fatty acids were converted into methyl esters through transesterification for detection by gas chromatography. The injector and detector were set at 250°C and 300°C, with helium as the carrier gas at 1mL/min. Results were expressed as mg FFA/g of extracted lipid, and all analyses were conducted in triplicate.

Amino Acid Analysis

Amino acids were analyzed by HPLC at the Integrated Laboratory, IPB University. Samples were treated with ortho-phthalaldehyde (OPA) reagent, which forms fluorescent derivatives with primary amino acids under alkaline conditions, and retention time was used for identification (modification from Hajrawati et al., 2025).

Data Analysis

A randomized block design with three treatments, three replications, and duplicate were used for each treatment. Data are presented as mean ± standard deviation, and differences among treatments were analyzed by ANOVA, with Tukey's post hoc test applied for multiple comparisons when $P<0.05$.

RESULTS AND DISCUSSION

Physicochemical Characteristics

The pH values of fermented goat milk across the three breeds were consistent (4.32–4.35), indicating uniform fermentation by *Lactiplantibacillus plantarum* subsp. *plantarum* IIA-1A5 through lactose conversion into lactic acid (Table 1). This stability was confirmed by titratable acidity (TTA), which remained within 0.61–0.63%, reflecting reliable bacterial metabolism and adaptation to differences in lactose and buffering capacity (Arief et al., 2023). In contrast, water activity (aw) varied, being lower in PE milk (0.75) than in Saanen and Sapera (0.77–0.78), due to its higher fat content (6.18%) that reduced free water availability. The lower water activity (aw) observed in PE milk (0.75) is beneficial, as reduced free water limits the growth of spoilage microorganisms and enhances product storage stability. The pH of fermented goat milk was consistent across breeds (4.32–4.35), reflecting uniform lactose fermentation by *Lactiplantibacillus plantarum* IIA-1A5 (Table 1). Titratable acidity (0.61–0.63%) confirmed stable bacterial metabolism (Arief et al., 2023). Water activity differed, being lower in PE milk (0.75) due to higher fat content (6.18%), which limits contaminant growth and enhances storage stability.

Table 1: Physicochemical, antioxidant, and microbiological properties of fermented goat milk

Parameters	Goat Breeds		
	Saanen	Sapera	PE
pH	4.35±0.02	4.34±0.01	4.32±0.02
Titratable acid /TTA (%)	0.62±0.62	0.61±0.00	0.63±0.00
a_w	0.78±0.00[b]	0.77±0.00[b]	0.75±0.05[a]
DPPH Inhibition (%)	83.68±0.18[b]	83.03±0.02[c]	85.62±0.06[a]
Antioxidant Capacity (mg EVC g^{-1})	129.89±0.29[b]	128.88±0.03[c]	132.89±0.10[a]
Water content (%)	87.19±1.05	86.21±2.82	86.40±2.13
Ash content (%)	0.81±0.00[a]	0.71±0.01[b]	0.78±0.04[a]
Fat content (%)	2.86±0.15[b]	1.17±0.40[c]	6.18±0.09[a]
Crude content (%)	4.32±0.01	4.42±0.15	4.24±0.17
Lactic acid bacteria (log CFU/g)	8.47±0.09	8.57±0.02	8.60±0.04

Values (mean+SD) bearing different superscripts in the same row differ significantly (P<0.05).

Moisture content (86.21–87.19%) and crude protein (3.53–3.69%) were comparable among breeds, suggesting that fermentation with *L. plantarum* IIA-1A5 did not significantly alter the total solids. The strain likely hydrolysed only part of the protein into peptides and amino acids without reducing total protein levels, as well as microbes in kefir grain that can ferment milk (Setyawardani et al., 2020). Fat content was highest in PE milk (5.42%), consistent with the characteristics of local breeds containing fatter, while ash values (0.75–0.81%) indicated stable mineral composition. Overall, proximate results demonstrate that fermentation maintained the nutritional integrity of milk from all breeds.

Antioxidant Activity

Fermented PE milk exhibited the highest DPPH radical-scavenging activity, followed by Sapera and Saanen (Table 1). Fermentation by lactic acid bacteria enhances antioxidant capacity through the release of bioactive peptides with proton-donating residues such as histidine, tryptophan, and tyrosine (Hanum et al., 2022). The higher antioxidant potential of PE milk may reflect its greater protein availability for peptide generation, emphasizing that milk composition strongly influences the formation of antioxidant compounds. These results are consistent with the findings of Maleki et al. (2025) and Liu et al. (2024), who reported that *Lactiplantibacillus plantarum* and *Lactobacillus fermentum* enhance antioxidant activity in fermented milk.

Total Lactic Acid Bacteria (LAB)

As shown in Table 1, fermented goat milk contained 8.47–8.60log CFU/g LAB, with the highest counts in Peranakan Etawa, followed by Sapera and Saanen. This indicates that *Lactiplantibacillus plantarum* IIA-1A5 grew well in all milks, with the higher protein and fat in PE milk supporting optimal proliferation, consistent with nutrient-dependent LAB growth (Juandini et al., 2024). All counts exceeded the probiotic threshold (≥6 log CFU/g), confirming the potential of fermented goat milk to support intestinal microflora balance and immune function (Adiyoga et al., 2022).

Fatty Acids Composition

As shown in Table 2, fat content varied among breeds, highest in Sapera, followed by PE and Saanen, reflecting genetic influence. Saturated fatty acids dominated, mainly palmitic and stearic, with Sapera showing higher butyric acid, while PE had the highest caprylic and capric acids, linked to gut health and biofunctionality. Monounsaturated fatty acids (MUFA) remained stable, dominated by oleic acid, whereas polyunsaturated fatty acids (PUFA) were low, with linoleic and linolenic acids contributing essential roles (Nayik et al., 2022). Overall, fatty acid composition differed significantly (P<0.05), lower in Saanen than Sapera and PE, confirming genetic impact and highlighting potential for selection and functional product development (Pérez et al., 2020). Goat milk contains a high proportion of MUFA and PUFA, which are beneficial for therapeutic purposes. In addition, fermented milk produces numerous bioactive peptides and simple bioactive lipids with high bioavailability (Zhang et al., 2022).

Table 2: Fatty acids (mg/g) in different types of goat milk

Parameters (mg/g)	Goat Breeds		
	Saanen	Sapera	PE
Fat content	2.32	3.515	2.895
Saturated Fatty Acids (SFA)			
Butyric acid (C4:0)	0.785[b]	0.875[a]	0.785[b]
Caproic acid (C6:0)	1.16	1.405	1.555
Caprylic acid (C8:0)	1.35[b]	1.675[ab]	2.205[a]
Capric acid (C10:0)	4.17[b]	4.99[ab]	7.005[a]
Lauric acid (C12:0)	1.415	1.62	2.19
Myristic acid (C14:0)	3.815	4.145	5.54
Pentadecanoic acid (C15:0)	0.49	0.49	0.465
Palmitic acid (C16:0)	15.475	16.63	17.125
Heptadecanoic acid (C17:0)	0.50	0.49	0.485
Stearic acid (C18:0)	16.025	16.80	14.755
Arachidic acid (C20:0)	0.15	0.22	0.145
Behenic acid (C22:0	0.065	0.06	0.035
Monounsaturated Fatty Acids (MUFA)			
Palmitoleic acid (C16:1)	0.46	0.49	0.57
Elaidic acid (C18:1n9t, trans)	5.85	7.7	5.745
Oleic acid (C18:1n9c, cis)	18.06	18.555	18.42
Polyunsaturated Fatty Acids (PUFA)			
Linolelaidic acid (C18:2n9t, trans)	0.185	0.215	0.235
Linoleic acid (C18:2n6c, cis, ω-6)	2.685	3.23	2.31
Linolenic acid (C18:3n3, ω-3	0.105	0.155	0.09
Total	72.745[b]	79.76[a]	79.65[ab]

Values (mean+SD) bearing different superscripts in the same row differ significantly (P<0.05).

Amino Acids Composition

The amino acid composition of fermented goat milk differed by breed (Table 3), with PE having the highest total (3.72%), followed by Sapera (2.99%) and Saanen (2.27%), confirming PE's superior protein quality. Histidine was dominant (0.56–0.90%), while PE and Sapera had higher methionine and arginine, supporting protein synthesis, immunity, and cardiovascular health. Other essential amino acids, including lysine, leucine, isoleucine, valine, and phenylalanine, were richer in PE, with Sapera intermediate and Saanen lowest, and fermentation further increased availability via proteolysis, producing free amino acids and bioactive peptides (Darmawati et al., 2024). Chen et al. (2024) also demonstrated that fermentation of goat milk by *L. plantarum* 69 produces bioactive peptides through a proteolytic mechanism.

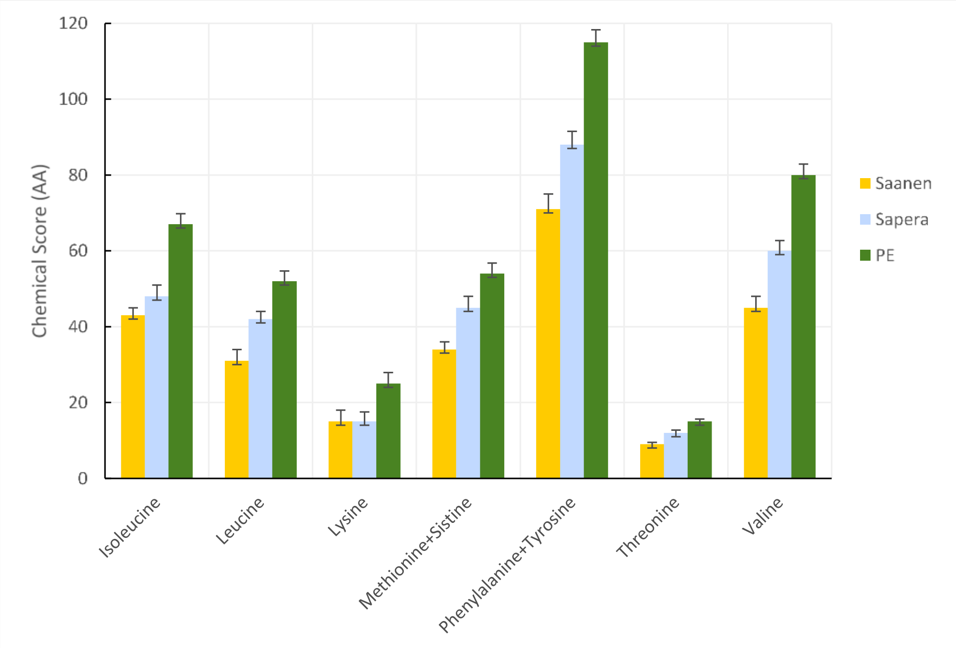

Fig. 1: Comparison of amino acid chemical scores in fermented goat milk from Saanen, Sapera, and Peranakan Etawa breeds.

Table 3: Amino acids (%w/w) in various types of goat milk fermentation

Parameter	Goat Type (%w/w)		
	Saanen	Sapera	PE
Essential Amino Acids			
Lysine	0.15	0.15	0.24
Leucine	0.17	0.23	0.29
Isoleucine	0.09	0.10	0.14
Methionine	0.24	0.32	0.38
Threonine	0.08	0.11	0.14
Phenylalanine	0.12	0.16	0.20
Valin	0.09	0.12	0.16
Histidine	0.56	0.76	0.90
Non-Essential Amino Acids			
Alanine	0.07	0.08	0.11
Aspartate	0.17	0.24	0.30
Glutamate	0.12	0.15	0.19
Arginine	0.16	0.21	0.25
Glycine	0.05	0.06	0.08
Tyrosine	0.12	0.14	0.19
Serin	0.13	0.17	0.21
Total Amino Acids	2.27	2.99	3.72
Essential Amino Acids	1.50	1.95	2.45
Non-Essential Amino Acids	0.82	1.05	1.33

Chemical Scores of Amino Acids

Fig. 1 shows breed-dependent differences in amino acid quality, with PE milk having the highest scores, especially for phenylalanine + tyrosine (115) and branched-chain amino acids that support metabolism (Li et al., 2024). Threonine and lysine were the first limiting amino acids, while methionine and cystine were moderate but again highest in PE, reflecting their antioxidant role (Hashim et al., 2025). Overall, PE provided the most favorable profile, Sapera intermediate, and Saanen the lowest, confirming genetic influence on protein quality (Amalfitano et al., 2020).

Conclusion

This study demonstrated that fermenting goat milk from Peranakan Etawa (PE), Saanen, and Sapera with *Lactiplantibacillus plantarum* subsp. *plantarum* IIA-1A5 improved its physicochemical, microbiological, and functional qualities. Among the breeds, PE milk showed the best performance, with higher fat, richer amino acid content, stronger antioxidant activity, and greater lactic acid bacteria counts, supporting better probiotic growth and nutritional value. Fermentation also promoted bioactive peptide release, while breed-specific differences in fatty acid and amino acid profiles influenced overall quality, highlighting PE milk as the most promising raw material for functional probiotic dairy products.

DECLARATIONS

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Data Availability: The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Ethics Statement: This study was approved by the Animal Ethics Committee, Faculty of Veterinary Medicine, IPB University (Approval No. 380/KEH/SKE/IX/2025), and conducted in accordance with its ethical guidelines.

Author's Contribution: The first author designed and conducted the research, performed data collection and analysis, and wrote the manuscript. The co-authors assisted in research coordination, provided intellectual input, and supported manuscript revision. All authors read and approved the final version of the manuscript.

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