

Article

# In Vitro Protein Quality of Goat Milk Yogurt Enriched with Mung Bean Extract

Hardi Firmansyah<sup>1\*</sup>, Risti Rosmiati<sup>2</sup>, Esi Emilia<sup>3</sup>, Zulfa Nur Hanifa<sup>4</sup>, Kanaya Yori Damanik<sup>5</sup>, Iza Ayu Saufani<sup>6</sup>

- <sup>1</sup> Nutrition Study Program, Universitas Negeri Medan; [hardigizi@unimed.ac.id](mailto:hardigizi@unimed.ac.id)  
<sup>2</sup> Nutrition Study Program, Universitas Negeri Medan; [ristirosmiati@unimed.ac.id](mailto:ristirosmiati@unimed.ac.id)  
<sup>3</sup> Nutrition Study Program, Universitas Negeri Medan; [esiemilia@unimed.ac.id](mailto:esiemilia@unimed.ac.id)  
<sup>4</sup> Nutrition Study Program, Universitas Negeri Medan; [zulfanurh@unimed.ac.id](mailto:zulfanurh@unimed.ac.id)  
<sup>5</sup> Nutrition Study Program, Universitas Negeri Medan; [kanayayori@unimed.ac.id](mailto:kanayayori@unimed.ac.id)  
<sup>6</sup> Nutrition Study Program, Universitas Negeri Medan; [saufani@unimed.ac.id](mailto:saufani@unimed.ac.id)  
\* Correspondence: [hardigizi@unimed.ac.id](mailto:hardigizi@unimed.ac.id)

## Abstract

**Background:** Protein is essential for tissue synthesis and immune regulation, with goat milk known for its high protein quality. However, goat milk yogurt may lack a complete essential amino acid profile, leading to fortification strategies for improved nutrition. **Objectives:** This study aims to compare the amino acid composition and in vitro protein quality between goat milk yogurt and goat milk yogurt enriched with mung bean extract. **Methods:** Key protein quality indicators, including Total Sulfur Amino Acids (TSAA), Total Aromatic Amino Acids (TA<sub>r</sub>AA), Total Amino Acids (TAA), and Total Essential Amino Acids (TEAA), were calculated. Ratios such as TEAA/TAA, cysteine/TSAA, and leucine/isoleucine were determined. Protein quality was assessed with Predicted Biological Value (P-BV), Predicted Protein Efficiency Ratio (P-PER), Chemical Score, Amino Acid Score (AAS), and Essential Amino Acid Index (EAAI). **Results:** The amino acid composition of both yogurt samples differed significantly ( $p < 0.05$ ). The addition of mung bean extract increased certain amino acids, such as histidine, cystine, and glycine, but reduced several essential amino acids, like lysine, leucine, and methionine. Mung bean fortification increased TEAA from 14.89 g to 16.10 g, improved the TEAA/TAA ratio, and enhanced protein quality indices, including P-PER and chemical score. **Conclusions:** Mung bean extract significantly enhanced the essential amino acid content and protein quality in goat milk yogurt.

**Keywords:** Essential amino acids, In Vitro protein quality, Goat milk yogurt, Mung bean extract.

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## 1. Introduction

Protein is a fundamental macronutrient essential for numerous physiological processes, including tissue synthesis, enzyme production, and immune system regulation. Adequate intake of high-quality protein is critical for growth, development, and overall health, particularly among vulnerable populations such as children, the elderly, and individuals with dietary restrictions or lactose intolerance<sup>1,2</sup>. Globally, protein insufficiency remains a public health concern, with deficiencies in essential amino acids contributing to protein-energy malnutrition and suboptimal growth outcomes<sup>3</sup>. Dairy

products, particularly goat milk and its derivatives, are widely recognized as high-quality protein sources due to their favorable amino acid composition, bioactive compounds, and high digestibility<sup>4</sup>. Goat milk differs from cow's milk in its protein and fat matrix, enhancing digestibility and tolerance, and making it a suitable dietary option for lactose-intolerant populations<sup>5,6</sup>. Despite these benefits, conventional goat milk yogurt may not provide a complete essential amino acid profile, underscoring the need for nutritional optimization strategies<sup>7</sup>.

Enrichment of dairy products with plant-derived proteins has emerged as a promising approach to enhance protein quality, leveraging complementary amino acid profiles without compromising functional or sensory characteristics<sup>8</sup>. Mung bean (*Vigna radiata*) is particularly suitable for this purpose due to its high protein content, balanced amino acid composition, and associated bioactive compounds<sup>9-11</sup>. Prior studies have demonstrated that mung bean extract can improve physicochemical and sensory properties of goat milk yogurt while potentially enhancing overall protein quality<sup>12,13</sup>. Moreover, mung bean components contribute antioxidant activity, bioactive peptides, and other health-promoting compounds, which further supports their use as a functional fortifier in dairy matrices<sup>14-16</sup>. Together, these attributes indicate that mung bean extract is a suitable enrichment for goat milk yogurt, providing an opportunity to study amino acid complementation and functional improvements *in vitro*.

*In vitro* protein quality assessment is a key tool for evaluating plant-dairy fortification, as it provides insights into digestibility, peptide release, and amino acid availability under simulated gastrointestinal conditions<sup>2</sup>. The digestibility of mung bean proteins is influenced by processing steps such as pre-boiling, which can affect amino acid release and nutrient availability<sup>17</sup>. Studies of legume-enriched dairy products indicate that plant proteins can modulate proteolysis, peptide formation, and amino acid supply, providing a basis for anticipating differences between fortified and unfortified goat milk yogurts<sup>11,18</sup>. Evaluating these outcomes using metrics such as chemical score, essential amino acid index (EAAI), and predicted protein efficiency ratio (P-PER) enables a comprehensive understanding of how fortification affects protein quality *in vitro*<sup>1,2</sup>.

This study is guided by two core hypotheses: first, that the amino acid composition of goat milk yogurt enriched with mung bean extract will differ from that of plain goat milk yogurt due to additional amino acids and peptides provided by the extract; second, that *in vitro* protein quality, reflected in digestibility and peptide release, will be improved or maintained in the enriched yogurt owing to the complementary amino acid patterns and digestibility of mung bean proteins. Prior research supports this expectation, showing enhanced protein content, digestibility, and functional properties when yogurts are fortified with legume proteins, including mung bean, without adversely affecting sensory characteristics<sup>12-14,19,20</sup>. The unique protein and fatty acid composition of goat milk yogurt provides an ideal platform to evaluate interactions with mung bean extract and to assess whether enrichment can optimize protein quality while preserving organoleptic and microbiological properties<sup>12</sup>.

Accordingly, the present study aims to compare two yogurt variants, plain goat milk yogurt and goat milk yogurt enriched with mung bean extract, focusing on amino acid composition and *in vitro* protein quality. Protein quality will be assessed using chemical score, EAAI, and predicted PER, providing a detailed evaluation of whether mung bean extract fortification enhances essential amino acid content and digestibility. By integrating amino acid profiling, digestibility, and *in vitro* protein quality measures with considerations of functional and sensory characteristics, this research provides evidence for the development of nutritionally enhanced dairy products and offers a framework for optimizing plant-dairy fortification strategies in functional yogurt development.

## 2. Materials and Methods

### 2.1 Study Design and Setting

This study employs a comparative experimental design to evaluate and compare the protein quality of two types of yogurt: goat milk yogurt and goat milk yogurt enriched with mung bean extract. Both yogurt variants will be produced and analyzed under controlled laboratory conditions to ensure consistency and reliability of the results. The study was conducted from May to July 2025. Yogurt production was carried out at the Nutrition Laboratory of Universitas Negeri Medan, while amino acid analysis was performed at Saraswanti Indo Genetech Laboratory in Bogor.

### 2.2 Sample Preparation

The mung beans were soaked in water for 9 hours at a 1:2 ratio, then ground with water at a 1:3 ratio. The mixture was filtered using sterilized cloth and stored in an airtight container to prevent contamination. For the yogurt production process, the glass container underwent pasteurization for 15 minutes. The goat milk used in this study was obtained from local dairy farms in North Sumatra, Indonesia, and was pasteurized to ensure safety and consistency in the production process. The goat milk and mung bean extract were combined in ratios of 100:0 and 85:15, with 10% sugar incorporated into each mixture. The pasteurization was conducted at 70°C for 15 minutes. After the mixing and pasteurization process, 7.5% of the total volume was inoculated with lactic acid bacteria (LAB) using a yogurt starter culture (Biokul® plain yogurt, PT. Diamond Cold Storage, Indonesia), which contains *Lactobacillus acidophilus* and *Bifidobacterium* species. The yogurt samples were incubated for 4 hours, followed by fermentation for 24 hours at room temperature in a closed container. Once fermentation was complete, the yogurt was stored in a refrigerator to stop the fermentation process and preserve product quality<sup>12</sup>. All ingredients used in this study, except for the goat milk, were obtained from traditional markets in North Sumatra, Indonesia, ensuring the use of locally available materials.

### 2.3 Amino Acids and Protein Quality Determination

The amino acid composition, except tryptophan, was analyzed using Ultra High Performance Liquid Chromatography (UHPLC). A homogeneous sample weighing 0.1 g was mixed with 5 mL of 6N hydrochloric acid in a sample tube. The mixture was vortexed for 5 minutes, followed by hydrolysis at 110°C for 22 hours. After resting, the mixture was transferred to a 50 mL volumetric flask, filtered using 0.45 µm filter papers, and diluted with aqua bidest. For analysis, 500 µL of filtrate, 40 µL of amino acid standard, and 460 µL of aqua bidest were added to a vial and mixed. The solution was then mixed with 40 µL AABA and 920 µL of aqua bidest. Next, 70 µL of AccQ Tag Ultra borate buffer, 10 mL of sample extracts or standard amino acid, and 20 µL of AccQ Tag Ultra reagent were added and mixed. After vortexing for 1 minute, the mixture was placed in a heating block at 55°C for 10 minutes. Following the incubation, 1 µL of the sample or standard amino acid was injected into the Waters Acquity UPLC System. All analyses were performed using a Waters Acquity UPLC H-Class and H-Class Bio Amino Acid (AAA) system, equipped with a PhotoDiode Array (PDA) detector. Data acquisition and management were done using the Waters Empower™ software. The analysis used a C18 column (100 mm × 2.1 mm, 1.7 µm particle size) and a gradient mobile phase consisting of 5% AccQ-Tag Ultra Eluent A and AccQ-Tag Ultra Eluent B. The flow rate was set to 0.70 mL/min, and the injection volume for both the samples and standards was 1.0 µL. The column temperature was set to 49°C, with the peaks detected at 260 nm<sup>21</sup>.

The tryptophan content was analyzed using High-Performance Liquid Chromatography (HPLC). A standard solution of tryptophan was prepared by dissolving 25 mg of L-tryptophan in 10 mL of distilled water. After sonicating for 15 minutes, the solution was diluted to a final volume of 25 mL with distilled water. One milliliter of the stock solution was further diluted with distilled water to make individual standard solutions with eight different concentration levels. For the sample preparation, 0.1 g of the sample was weighed into a sealed flask. To this, 10 mL of deaerated 4.2M NaOH was

added, followed by 3 drops of 1-octanol, and the mixture was vortexed immediately. The flask was then placed in an oven at 110°C for 20 hours. After cooling, the solution was transferred into a 50 mL beaker, and the flask was rinsed with 1 mL of 0.2 M pH 4.25 sodium citrate buffer three times. The solution was neutralized using 3.5 mL of hydrochloric acid and stirred vigorously. The pH was adjusted to  $4.25 \pm 0.05$ , and the solution was transferred into a 50 mL volumetric flask and made up to volume with distilled water. After mixing thoroughly, the solution was poured into a centrifuge tube and centrifuged at  $1150 \times g$  for 20 minutes. The supernatant was filtered through a glass filter paper, followed by a  $0.45 \mu\text{m}$  membrane filter (GHP). Finally, all standard and sample solutions were injected into the HPLC system for analysis<sup>21</sup>.

To calculate the protein quality indicators such as Total Sulphuric Amino Acid (TSAA), Total Aromatic Amino Acid (TArAA), Total Amino Acid (TAA), and Total Essential Amino Acid (TEAA), the amino acid composition of the yogurt samples is first determined. The individual amino acids in both samples are quantified, and their concentrations are reported in milligrams per 100 grams of the sample. The TSAA is obtained by summing the concentrations of sulfur-containing amino acids, specifically methionine and cysteine. In the case of TArAA, it is calculated by adding the concentrations of the aromatic amino acids, which include phenylalanine, tyrosine, and tryptophan. TAA represents the total content of all amino acids present in the yogurt sample, including both essential and non-essential amino acids, and is calculated by adding the individual concentrations of all amino acids detected in the sample. TEAA, on the other hand, refers to the total amount of essential amino acids, which are those that the body cannot synthesize and must be obtained through the diet. The concentrations of all essential amino acids, including lysine, leucine, valine, phenylalanine, threonine, methionine, tryptophan, histidine, isoleucine, and tyrosine, are summed to obtain the TEAA value<sup>2</sup>.

The percentage of Total Essential Amino Acid (TEAA) to Total Amino Acid (TAA) ratio (%TEAA/TAA) is calculated by dividing the total amount of essential amino acids (TEAA) by the total amount of amino acids (TAA), and then multiplying by 100 to express the result as a percentage. This ratio reflects the proportion of essential amino acids in the overall amino acid profile. Similarly, the percentage of cysteine to total sulfur amino acids (%Cys/TSAA) is determined by dividing the total amount of cysteine (Cys) by the total sulfur amino acids (TSAA), and then multiplying by 100. This ratio indicates the relative abundance of cysteine within the pool of sulfur-containing amino acids, which includes both cysteine and methionine. The %Leucine/Isoleucine ratio is calculated by comparing the relative amounts of the branched-chain amino acids (BCAAs) leucine and isoleucine in a protein sample. The ratio is determined by dividing the amount of leucine by the amount of isoleucine, and the result was expressed as a percentage<sup>2</sup>.

The Predicted Biological Value (P-BV) is determined using the following regression formula:  $P\text{-BV} = 10^{2.15} \times q^{0.141} \text{Lysine} \times q^{0.60} \text{Phenylalanine+Tyrosine} \times q^{0.77} \text{Methionine+Lyssine} \times q^{2.14} \text{Threonine} \times q^{0.21} \text{Tryptophan}$ . Where,  $q = a_1 \text{ sample} / a_1 \text{ reference}$  for  $a_1 \text{ sample} \leq a_1 \text{ reference}$  or  $q = a_1 \text{ reference} / a_1 \text{ sample}$  for  $a_1 \text{ reference} \leq a_1 \text{ sample}$ ;  $a_1 = \text{mg of the amino acid per g of total essential amino acid}$ . The Predicted Protein Efficiency Ratio (P-PER) is calculated using the following formula:  $P\text{-PER} = -0.468 + 0.454 (\text{Leucine}) - 0.105 (\text{Tyrosine})$ <sup>21,22</sup>.

The Chemical Score was calculated by determining the ratio of the limiting essential amino acid in the food protein relative to the essential amino acid content in whole cooked egg protein. This calculation involves identifying the limiting essential amino acid in the yogurt and comparing it to the amino acid profile of cooked egg protein (which is considered a high-quality reference protein). The Amino Acid Score was calculated by comparing the amount of each amino acid in the test protein (goat milk yogurt and the fortified yogurt) to the reference protein's amino acid profile. This is applied to each amino acid present in the samples. The EAAI (Essential Amino Acid Index) is the next

metric to be determined, using the formula, where aa = A/E ratio in the product, AA = A/E ratio of 1–3 years old children requirement<sup>2,21</sup>.

$$EAAI = \sqrt[n]{\frac{aa_1}{AA_1} \times \frac{aa_2}{AA_2} \times \dots \times \frac{aa_n}{AA_n}}$$

### 2.4 Data Analysis

Data analysis was performed using an independent t-test to compare the means of amino acids composition and protein quality between plain goat milk yogurt and goat milk yogurt enriched with mung bean extract. A p-value less than 0.05 indicated a statistically significant difference. Results are reported with mean and standard deviation.

## 3. Results

### 3.1. Amino Acids Composition

Table 1 presents the amino acid composition of goat milk yogurt and goat milk yogurt enriched with mung bean extract. The enrichment significantly influenced the amino acid profile of the product (p < 0.05). In general, the addition of mung bean extract resulted in lower concentrations of most essential amino acids compared to plain goat milk yogurt, including lysine, leucine, phenylalanine, threonine, and methionine, indicating a possible dilution effect or alteration in the protein matrix during fortification. However, some amino acids such as tryptophan, histidine, cystine, alanine, arginine, and glycine were found in significantly higher amounts in the fortified yogurt, reflecting the amino acid contribution from mung bean proteins. Notably, histidine and cystine, which were absent or minimal in the control yogurt, appeared in measurable concentrations after fortification.

**Table 1.** Amino acids composition of goat milk yogurt and goat milk yogurt enrich with mungbean extract<sup>1</sup>.

Amino Acid Type	Goat Milk Yogurt	Goat Milk Yogurt + Mung Bean Extract
Lysine	155.74 ± 1.59 <sup>a</sup>	125.66 ± 0.22 <sup>b</sup>
Leucine	117.84 ± 0.01 <sup>a</sup>	113.54 ± 0.77 <sup>b</sup>
Phenylalanine	86.51 ± 0.64 <sup>a</sup>	50.14 ± 0.30 <sup>b</sup>
Valine	75.43 ± 0.47 <sup>a</sup>	82.30 ± 0.17 <sup>b</sup>
Isoleucine	57.72 ± 0.05 <sup>a</sup>	59.17 ± 0.35 <sup>b</sup>
Threonine	62.39 ± 0.46 <sup>a</sup>	51.47 ± 0.04 <sup>b</sup>
Methionine	11.03 ± 0.02 <sup>a</sup>	9.92 ± 0.29 <sup>b</sup>
Tryptophan	11.63 ± 0.01 <sup>a</sup>	12.57 ± 0.01 <sup>b</sup>
Tyrosine	51.26 ± 0.27 <sup>a</sup>	22.98 ± 0.02 <sup>b</sup>
Histidine	0.00 ± 0.00 <sup>a</sup>	26.05 ± 0.24 <sup>b</sup>
Cystine	3.87 ± 0.05 <sup>a</sup>	6.18 ± 0.19 <sup>b</sup>
Glutamate	355.43 ± 0.13 <sup>a</sup>	311.35 ± 0.20 <sup>b</sup>
Aspartate	132.62 ± 0.60 <sup>a</sup>	128.48 ± 0.05 <sup>b</sup>
Proline	150.34 ± 0.28 <sup>a</sup>	119.33 ± 0.12 <sup>b</sup>
Serine	74.89 ± 0.05 <sup>a</sup>	61.10 ± 0.04 <sup>b</sup>
Alanine	49.48 ± 0.20 <sup>a</sup>	53.63 ± 0.01 <sup>b</sup>
Arginine	39.14 ± 0.01 <sup>a</sup>	41.00 ± 0.13 <sup>b</sup>
Glycine	26.45 ± 0.31 <sup>a</sup>	30.03 ± 0.01 <sup>b</sup>

<sup>1</sup> Data are presented as mean ± standard deviation. Different superscript letters within the same row denote statistically significant differences at the 0.05 level.

Among nonessential amino acids, glutamate was the most abundant in both samples, showing a significant difference. Aspartate and proline were also significantly higher in goat milk yogurt. Serine and alanine were significantly lower than in the fortified yogurt. The levels of arginine and glycine were also significantly higher in goat milk yogurt enriched with mung bean extract. Overall, these results indicate that the incorporation of mung bean extract modifies the amino acid composition of goat milk yogurt, providing additional amino acids while maintaining a balanced profile that may enhance the product’s nutritional diversity.

### 3.2. Protein Quality

Table 2 presents the protein quality indicators of goat milk yogurt and goat milk yogurt enriched with mung bean extract. The data show that the enrichment of goat milk yogurt with mung bean extract caused notable changes in several protein quality parameters. The total sulfur amino acids (TSAA) and total aromatic amino acids (TArAA) were higher in goat milk yogurt compared with the fortified yogurt, indicating lower concentrations of these amino acid groups after mung bean enrichment. Similarly, the total amino acid (TAA) content slightly decreased from 43.33 in goat milk yogurt to 42.91 in the fortified sample. In contrast, the total essential amino acids (TEAA) increased from 14.89 in goat milk yogurt to 16.10 in the fortified yogurt. The ratio of essential amino acids to total amino acids (%TEAA/TAA) also rose markedly from 25.96 to 38.41, reflecting a higher proportion of essential amino acids in the total amino acid pool.

**Table 2.** Protein quality of goat milk yogurt and goat milk yogurt enrich with mungbean extract<sup>1</sup>.

Protein Quality Indicators	Goat Milk Yogurt	Goat Milk Yogurt + Mung Bean Extract
Total Sulphuric Amino Acid (TSAA)	1461.75 ± 1.24 <sup>a</sup>	1304.92 ± 2.79 <sup>b</sup>
Total Aromatic Amino Acid (TarAA)	633.39 ± 0.33 <sup>a</sup>	559.99 ± 2.60 <sup>b</sup>
Total Amino Acid (TAA)	43.33 ± 0.06 <sup>a</sup>	42.91 ± 0.11 <sup>b</sup>
Total Essential Amino Acid (TEAA)	14.89 ± 0.08 <sup>a</sup>	16.10 ± 0.48 <sup>b</sup>
%TEAA/TAA	25.96 ± 0.23 <sup>a</sup>	38.41 ± 0.05 <sup>b</sup>
%Cys/TSAA	137.76 ± 0.37 <sup>a</sup>	73.12 ± 0.32 <sup>b</sup>
%Leucine/Isoleucine Ratio	103.17 ± 143.02 <sup>a</sup>	96.96 ± 134.41 <sup>a</sup>
Predicted Biological Value (P-BV)	4.78 ± 0.01 <sup>a</sup>	4.87 ± 0.04 <sup>b</sup>
Predicted Protein Efficiency Ratio (P-PER)	26.89 ± 0.43 <sup>a</sup>	36.15 ± 0.79 <sup>b</sup>
Chemical Score	35.04 ± 0.18 <sup>a</sup> (SAA) <sup>2</sup>	37.89 ± 1.14 <sup>b</sup> (SAA) <sup>2</sup>
Amino Acid Score	55.16 ± 0.28 <sup>a</sup>	59.64 ± 1.79 <sup>b</sup>
Essential Amino Acid Index (EAAI)	0.84 ± 0.02 <sup>a</sup>	0.90 ± 0.01 <sup>b</sup>

<sup>1</sup> Data are presented as mean ± standard deviation. Different superscript letters within the same row denote statistically significant differences at the 0.05 level.

<sup>2</sup> Sulphuric amino acid (SAA) as limiting essential amino acid

The ratio of cysteine to total sulfur amino acids (%Cys/TSAA) was higher in goat milk yogurt than in the mung bean-fortified yogurt, while the leucine/isoleucine ratio showed no significant difference between the two products. Regarding calculated indices of protein quality, the predicted biological value (P-BV) increased slightly from 4.78 in goat milk yogurt to 4.87 in the fortified yogurt. A more pronounced difference was observed in the predicted protein efficiency ratio (P-PER), which increased from 26.89 to 36.15 after mung bean enrichment. The chemical score also improved from 35.04 in goat milk yogurt to 37.89 in the fortified variant, consistent with the increase in amino acid score. The essential amino acid index (EAAI) followed the same pattern. Overall, these results indicate that goat milk yogurt enriched with mung bean extract exhibited lower total sulfur and aromatic amino acid contents but higher values in essential amino acid-related parameters and calculated indices such as P-PER, chemical score, amino acid score, and EAAI. These differences collectively demonstrate measurable alterations in the protein quality profile following mung bean extract enrichment, with several parameters showing statistically significant differences at  $\alpha = 0.05$ .

## 4. Discussion

Fortifying goat milk yogurt with mung bean extract produced a clear, statistically significant shift ( $p < 0.05$ ) in the amino-acid profile relative to the plain yogurt, indicating compositional remodeling rather than minor fluctuation. This pattern is consistent with reports that adding legume proteins to dairy matrices introduces a distinct plant-derived amino-acid signature and, at the same time, can dilute amino acids that are characteristically dense in milk proteins (e.g., lysine and certain branched-chain amino acids)<sup>11</sup>. The present formulation choice follows earlier work demonstrating the technical

feasibility of mung-bean-enriched goat yogurt and its measurable effects on composition<sup>12</sup>.

Across EAAs, lysine, leucine, phenylalanine, threonine, and methionine were lower in the fortified product than in the plain yogurt. This directional change matches what is expected when a legume protein, with a different indispensable amino-acid distribution, is blended with dairy. The relative abundance of some milk-rich EAAs is reduced in the composite matrix<sup>23</sup>. The sulfur amino acids (SAAs) provide a notable example. Legumes typically supply less methionine (and variable cysteine) per unit protein than animal sources; consequently, methionine decreased after fortification, a result aligned with foundational descriptions of SAA biosynthesis and food distribution<sup>24</sup>. Classic compositional surveys of legume seeds similarly document lower SAA density than in dairy proteins, reinforcing the likelihood of a dilution effect for methionine in the blend<sup>25</sup>. In contrast, valine and isoleucine were higher in the fortified yogurt, indicating that not all EAAs move in the same direction and that mung bean can contribute materially to specific BCAA pools depending on the preparation used<sup>26</sup>.

Fortification increased tryptophan and histidine and introduced measurable cystine where it was negligible in the control. Mung bean ingredients are reported to contain aromatic amino acids (including tryptophan) and imidazole-containing histidine, both of which can appear at notable levels depending on cultivar and processing<sup>11,27</sup>. The rise in cystine (the disulfide-linked dimer of cysteine) is also compatible with legume storage-protein composition, where cysteine/cystine residues are present even when methionine is relatively limiting. Together, these shifts illustrate that the plant signature does not uniformly depress EAAs; instead, it redistributes them—lowering some dairy-dominant EAAs (e.g., lysine, methionine) while elevating residues for which mung bean is a comparatively stronger source (e.g., histidine, tryptophan, cystine)<sup>11</sup>.

For nonessential amino acids (NEAAs), glutamate remained the most abundant in both products, followed by proline and aspartate, which is typical for fermented dairy matrices. Nevertheless, glutamate and proline decreased with fortification, whereas alanine, arginine, and glycine increased. Such divergence is consistent with legume–dairy protein interactions and processing-driven proteolysis, which can shift the measurable free-amino-acid pool during fermentation and ingredient integration<sup>28</sup>. Studies of mung-bean sprouting, fermentation, and fractionation similarly show enrichment of alanine and glycine and broader NEAA remodeling as proteins are modified and peptides released<sup>27–29</sup>. Mung bean reviews that catalogue bioactive peptides and small amino-acid-rich fractions further support this influx of specific NEAAs into fortified foods<sup>30</sup>.

The SAA balance observed here, a drop in methionine with a rise in cystine, matches the general legume pattern and highlights a key consideration when blending plant and dairy proteins<sup>31</sup>. From a protein-quality standpoint, such blends are routinely evaluated through amino-acid complementarity and Digestible Indispensable Amino Acid Score (DIAAS)-based frameworks, where strategic mixing can compensate for limiting amino acids in one source with strengths in another. Although the composition data, rather than digestible scores, are presented, the observed trend—where some dairy-rich essential amino acids (EAAs) decreased and several mung bean-derived residues increased—aligns exactly with the pattern described in complementarity models for pulse-containing foods<sup>11,32</sup>.

Finally, the magnitude and direction of individual amino-acid shifts likely reflect not only intrinsic compositional differences between goat milk and mung bean proteins but also the processing history of the mung bean extract (e.g., sprouting or fermentation) and the yogurt fermentation itself, both of which can modulate peptide release and free-amino-acid levels<sup>11</sup>. The overall pattern observed is consistent with previous findings that mung-bean enrichment alters the physicochemical characteristics and composition of goat yogurt systems<sup>12</sup>, as well as with broader evidence highlighting the variability of legume-derived amino acids and their sensitivity to processing<sup>30</sup>.

The analysis of protein quality in goat milk yogurt and goat milk yogurt enriched with mung bean extract, shows notable differences in several protein quality indicators. The data reveal significant changes in amino acid composition, protein efficiency ratios, and biological value following the fortification with mung bean extract. These findings align with the broader understanding that legume-based fortification can shift both the amino acid composition and protein quality metrics in dairy matrices<sup>33</sup>.

The total sulfur amino acids (TSAA), which include methionine and cysteine, were significantly higher in plain goat milk yogurt compared to the mung bean-fortified yogurt. This difference suggests that the incorporation of mung bean extract, which is generally lower in sulfur amino acids, dilutes the sulfur amino acid content in the final product<sup>34</sup>. This is consistent with the literature, which highlights the limiting levels of methionine and cysteine in legume proteins<sup>35</sup>, making it difficult for the fortified yogurt to maintain the same TSAA levels as the unfortified product.

A similar trend was observed for total aromatic amino acids (TAArAA), where plain goat milk yogurt contained a higher amount than the fortified yogurt. This reduction further reinforces the idea that legume fortification can alter the aromatic amino acid profile, which may influence both nutritional quality and sensory properties<sup>36</sup>. Aromatic amino acids, including phenylalanine, tyrosine, and tryptophan, are important for both flavor development and metabolic processes, and their decrease upon fortification with mung bean extract may impact these characteristics<sup>37</sup>.

While the total amino acid (TAA) content showed a slight decrease from 43.33 in plain yogurt to 42.91 in the mung bean-fortified yogurt, total essential amino acids (TEAA) significantly increased from 14.89 in the plain yogurt to 16.10 in the fortified yogurt. This increase in TEAA is particularly relevant as it reflects a higher proportion of essential amino acids (EAAs) in the total amino acid pool, which is a key indicator of improved protein quality<sup>2</sup>. Essential amino acids, which cannot be synthesized by the body, must be obtained from the diet, and their increased availability in the fortified yogurt is a favorable outcome for nutritional optimization. The ratio of essential amino acids to total amino acids (%TEAA/TAA) was notably higher in the mung bean-fortified yogurt compared to the plain yogurt. This increase supports the hypothesis that mung bean enrichment bolstered the proportion of essential amino acids in the yogurt matrix, thus enhancing the nutritional quality of the product<sup>30</sup>.

The Predicted Biological Value (P-BV) increased slightly from 4.78 in goat milk yogurt to 4.87 in the mung bean-fortified yogurt, which suggests that the protein quality of the fortified yogurt may have been marginally improved. The Predicted Protein Efficiency Ratio (P-PER) saw a more substantial rise, from 26.89 in plain yogurt to 36.15 in the fortified yogurt. P-PER is an indicator of protein utilization efficiency, and the increase in P-PER suggests that the protein in the mung bean-fortified yogurt is more efficiently utilized by the body, likely due to the higher concentration of essential amino acids<sup>2</sup>.

The chemical score, which reflects the limiting amino acids in a protein source, improved from 35.04 in the plain yogurt to 37.89 in the fortified yogurt. This increase indicates that the fortification with mung bean extract enhanced the essential amino acid profile, making it closer to the reference standard. Similarly, the amino acid score showed an increase from 55.16 in goat milk yogurt to 59.64 in the mung bean-fortified yogurt, further supporting the positive impact of the fortification on protein quality<sup>1</sup>. Finally, the Essential Amino Acid Index (EAAI) was also higher in the mung bean-fortified yogurt than in the plain yogurt. EAAI is an integrated measure of protein quality based on the relative amounts of essential amino acids, and its increase reflects a more favorable essential amino acid profile in the fortified yogurt, which contributes to improved protein quality and overall nutritional value<sup>37</sup>.

Mung bean enrichment significantly altered the protein quality profile of goat milk yogurt, with notable increases in the essential amino acids and improvements in various protein quality indices such as P-PER, chemical score, and EAAI. These changes indicate

that mung bean fortification can be an effective strategy to improve the nutritional value of dairy products, particularly by enhancing essential amino acid provision. However, the reduction in total sulfur amino acids (TSAA) and aromatic amino acids (TArAA) highlights the importance of balancing amino acid contributions when fortifying dairy products with plant-based proteins.

## 5. Conclusions

Mung bean extract enrichment in goat milk yogurt significantly enhanced the essential amino acid content and improved protein quality indices, including the predicted protein efficiency ratio (P-PER) and chemical score. Despite reductions in sulfur and aromatic amino acids, the fortification resulted in a more balanced amino acid profile, offering nutritional benefits in terms of protein quality.

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## 7. Conflicts of Interest

The authors declare no conflict of interest.

## REFERENCES

- Mariotti F. Plant Protein, Animal Protein, and Protein Quality. In: Vegetarian and Plant-Based Diets in Health and Disease Prevention. Elsevier; 2017. p. 621–42.
- Sá AGA, House JD. Nutritional properties: protein quality assessment parameters and methods. In: Functionality of Plant Proteins. Elsevier; 2024. p. 253–73.
- Jiang W, Zhao Y, Wu X, Du Y, Zhou W. Health inequalities of global protein-energy malnutrition from 1990 to 2019 and forecast prevalence for 2044: data from the Global Burden of Disease Study 2019. Public Health [Internet]. 2023;225:102–9. Available from: <https://www.sciencedirect.com/science/article/pii/S0033350623003712>
- Chandra D, Rathaur A, Yadav AK, Shraddha Ms. Nutritional and Nutraceutical Properties of Goat Milk for Human Health: A Review. Indian Journal of Dairy Science. 2022;1–10.
- Stergiadis S, Nørskov NP, Purup S, Givens I, Lee MRF. Comparative Nutrient Profiling of Retail Goat and Cow Milk. Nutrients. 2019 Sep 24;11(10):2282.
- Lima MJ, Teixeira-Lemos E, Oliveira J, Teixeira-Lemos LP, Monteiro A, Costa JMC da. Nutritional and Health Profile of Goat Products: Focus on Health Benefits of Goat Milk. 2018;
- Gürsel A, Gürsoy A, Anlı EA, Ozturkoglu-Budak S, Aydemir S, Özkaya FD. Role of Milk Protein-based Products in Some Quality Attributes of Goat Milk Yogurt. J Dairy Sci. 2016;99(4):2694–703.
- Baskar N, Varadharajan S, Rameshbabu M, Ayyasamy S, Velusamy S. Development of Plant-Based Yogurt. Foods and Raw Materials. 2022;274–82.
- Makenova AA, Mussayeva S. Study of the Effect of the Fermentation Process on the Biochemical Characteristics of *Vigna Radiata*. The Journal of Almaty Technological University. 2024;146(4):55–61.
- Ullah R, Ullah Z, Al-Deyab SS, Adnan M, Tariq A. Nutritional assessment and antioxidant activities of different varieties of *Vigna radiata*. The Scientific World Journal. 2014;2014(1):871753.
- Zhu Y, Sun S, Fitzgerald RJ. Mung Bean Proteins and Peptides: Nutritional, Functional and Bioactive Properties. Food Nutr Res. 2018;62(0).
- Firmansyah H, Rosmiati R, Saufani IA, Hanifa ZN. Physicochemical, Microbiological and Sensory Characteristics of Goat Milk (*Capra Aegagrus Hircus*) Yogurt With Mungbean (*Vigna Radiata*) Extract Enrichment. Amerta Nutrition. 2025;9(2):266–77.
- Elwahsh N, Othman EK, El-Sayed MI. Impact of Mung Bean (*Vigna Radiata* L.) Flour on the Physicochemical, Antioxidant, Microbiological, Textural, and Organoleptic Properties of Functional Greek-Style Yogurt. ACS Food Science & Technology. 2025;5(6):2480–8.

14. Winarsi H, Agustia FC, Ramadhan GR, Zaki I, Putri WAK, Sulistyoning AR, et al. Polyphenol rich mung bean (*Vigna radiata*) yogurt for obesity prevention. *Food Res.* 2021 Mar 7;5(2):136–43.
15. Zhao J, Wu X, Song X, Lin H, Yin J, Nie S, et al. Nutrients, Extractable Bound Phenolic Compositions and Their Antioxidant Properties in Different Varieties of Mung Beans. 2023;
16. Feng Q, Niu Z, Zhang S, Wang L, Shen Q, Zheng Y, et al. Mung Bean Protein as an Emerging Source of Plant Protein: A Review on Production Methods, Functional Properties, Modifications and Its Potential Applications. *J Sci Food Agric.* 2023;104(5):2561–73.
17. Prachansuwan A, Kriengsinyos W, Judprasong K, Kovitvadhi A, Chundang P. Effect of Different Pre-Boiling Treatment on in Vitro Protein and Amino Acid Digestibility of Mung Beans [*Vigna Radiata* (L.) Wilczek]. *Malays J Nutr.* 2020;25(3):361–75.
18. Zahir A, Zhuang S, Xin R, Huang J, Hamdard E, Dong M. Antioxidant and ACE-Inhibitory Activity of Common Bean Whey Fortified Yoghurt With Assessed by in Vitro Static Gastrointestinal Digestion. *International Journal of Agricultural Science and Food Technology.* 2020;6(1):011–21.
19. Okfrianti Y, Lestari PA, Yunita Y. The Effect of Feeding Red Beans in Yoghurt on Organoleptic Acceptance and Protein Levels. *Agritepa Jurnal Ilmu Dan Teknologi Pertanian.* 2024;11(2):307–20.
20. Kusumawati E, Koro S, Bana T. Pengaruh Substitusi Yoghurt dan Tepung Kacang Hijau (*Phaseolus Radiatus*) terhadap Penilaian Organoleptik, Kandungan Protein dan Kalsium Puding Silky sebagai Makanan Tambahan Alternatif untuk Anak Stunting. *Jurnal Sains Dan Teknologi Pangan.* 2020;5(3).
21. Kusharto CM, Rosmiati R, Marta EO, Palupi E. Amino acid composition and protein quality of instant liquid food based on Catfish (*Clarias gariepinus*) and Kelor (*Moringa oleifera*) flour. *IOP Conf Ser Earth Environ Sci.* 2018 Nov 27;196:012033.
22. Adeyeye EI. Amino acid composition of three species of Nigerian fish: *Clarias anguillaris*, *Oreochromis niloticus* and *Cynoglossus senegalensis*. *Food Chem* [Internet]. 2009;113(1):43–6. Available from: <http://dx.doi.org/10.1016/j.foodchem.2008.07.007>
23. Liu J, Klebach M, Visser M, Hofman Z. Amino Acid Availability of a Dairy and Vegetable Protein Blend Compared to Single Casein, Whey, Soy, and Pea Proteins: A Double-Blind, Cross-Over Trial. *Nutrients.* 2019 Nov 1;11(11):2613.
24. Gorissen SHM, Crombag JJR, Senden JMG, Waterval WAH, Bierau J, Verdijk LB, et al. Protein content and amino acid composition of commercially available plant-based protein isolates. *Amino Acids.* 2018 Dec 30;50(12):1685–95.
25. Hertzler SR, Lieblein-Boff JC, Weiler M, Allgeier C. Plant Proteins: Assessing Their Nutritional Quality and Effects on Health and Physical Function. *Nutrients.* 2020 Nov 30;12(12):3704.
26. Kim C, Jeon S, Jo YJ, Ha J. Amino acids and BCAA composition of Mungbean (*Vigna radiata* L.) seeds and sprouts for plant-based protein applications. *Sci Rep.* 2025 Aug 12;15(1):29590.
27. Yanti Y, Violina V, Putri CE, Lay BW. Branched Chain Amino Acid Content and Antioxidant Activity of Mung Bean Tempeh Powder for Developing Oral Nutrition Supplements. *Foods.* 2023;12(14):2789.
28. Yen NTH, Huong NTM, Hoa PN, Hung P V. Incorporation of Germinated Mung Bean Flour With Rice Flour to Enhance Physical, Nutritional and Sensory Quality of Gluten-free Cookies. *Int J Food Sci Technol.* 2022;58(1):423–31.
29. Aryashad M, Sadeghi A, Nouri M, Ebrahimi M, Kashaninejad M, Aalami M. Use of Fermented Sprouted Mung Bean (*Vigna Radiata*) Containing Protective Starter Culture LAB to Produce Clean-label Fortified Wheat Bread. *Int J Food Sci Technol.* 2022;58(6):3310–20.
30. Hou D, Yousaf L, Xue Y, Hu J, Wu J, Hu X, et al. Mung Bean (*Vigna Radiata* L.): Bioactive Polyphenols, Polysaccharides, Peptides, and Health Benefits. *Nutrients.* 2019;11(6):1238.
31. Merzlov S, Tsebro A, Rol N, Nadtochii V, Kachan A. Comparative Analysis of the Amino Acid Composition of Beverages Based on Plant Raw Materials and Cow's Milk. *Scientific Horizons.* 2022;25(10).
32. Han I, Na S. Health-promoting Effect of Tofus Prepared With Mung Bean and Soybean. *Cereal Chem.* 2021;98(6):1175–82.
33. Ülger TG, Tunçer E, SARICA E, Kutlubağ EN, Altuğ G, Bahadır M. Investigation of Nutritional Profile, Sensory Properties and Antioxidant Potential of Legume-Based Yoghurt Analogues. *Food and Health.* 2025;11(3):258–67.
34. Wongsiri S, Ohshima T, Duangmal K. Chemical Composition, Amino Acid Profile and Antioxidant Activities of Germinated Mung Beans (*Vigna Radiata*). *J Food Process Preserv.* 2015;39(6):1956–64.

35. Zhang X, Zhang Z, Shen A, Zhang T, Jiang L, El-Seedi H, et al. Legumes as an alternative protein source in plant-based foods: Applications, challenges, and strategies. *Curr Res Food Sci.* 2024;9:100876.
36. Qadir R, Wan Mohamad Nasir WNA, Azmi AB, Fatima S, Mehmood N, Meor Hussin AS. An insight into plant-based yogurts: Physicochemical, organoleptic properties and functional food aspects. *Journal of Food Composition and Analysis.* 2025 Jul;143:107578.
37. Dhakal D, Younas T, Bhusal RP, Devkota L, Henry CJ, Dhital S. Design rules of plant-based yoghurt-mimic: Formulation, functionality, sensory profile and nutritional value. *Food Hydrocoll.* 2023 Sep;142:108786.