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Investigating the Nutraceutical Potential of Mung Bean Based Hybrid Paneer: A Comprehensive Analysis of Amino Acid Profiling and Antioxidant Capacity

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ABSTRACT

With increasing consumer awareness of health, sustainability, and dietary restrictions, there is a growing need for innovative, plant-based alternatives to conventional dairy products. Traditional paneer production depends on animal milk, which poses environmental challenges and excludes vegan and lactose-intolerant populations. Hence, the present study comprehensively evaluated the amino acid composition and antioxidant potential of novel mung bean (*Vigna radiata* L.) based hybrid paneer formulations, developed as sustainable alternatives to conventional dairy products. Four distinct prototypes were analyzed *i.e.,* T1 (100% mung bean), T2 (70% mung bean/30% buffalo milk), T3 (70% mung bean/30% cow milk) and T4 (50% mung bean/50% soy milk). Using HPLC-based amino acid profiling, we identified nutritionally significant levels of essential amino acids across all formulations, with T1 demonstrating particularly high concentrations of lysine (6.4%) and leucine (8.1%), addressing typical legume protein limitations. Antioxidant assessment revealed superior free radical scavenging capacity (84.2% DPPH inhibition) and total phenolic content (12.4 mg GAE/g) in the pure mung bean formulation, with a progressive 18-32% reduction observed upon dairy incorporation. Notably, T1 achieved a protein digestibility-corrected amino acid score (PDCAAS) of 0.92, confirming its status as a complete plant protein, while dairy-blended variants showed enhanced mineral bioavailability. These findings highlight mung bean's dual functionality as both a high-quality protein source and natural antioxidant reservoir, offering scientifically validated formulation strategies for developing next-generation hybrid dairy products that balance nutritional excellence with functional benefits for health-conscious consumers and specialized dietary applications.

Keywords: Amino acid profiling, Antioxidant capacity, Hybrid paneer, Mung bean, Plant-based dairy.

INTRODUCTION

The nutritional paradigm of modern food science increasingly prioritizes two fundamental metrics *i.e.,* protein quality and antioxidant capacity the key determinants of a food's health-promoting potential. *Vigna radiata* L. (mung bean), an underutilized nutritional powerhouse in Asian agriculture, presents a compelling case study, offering an exceptional dual matrix of plant proteins (25-28% content) and bioactive phytochemicals (Cao *et al,* 2011). Its protein architecture, though rich in lysine (6.4% of total AA), reveals a classic legume limitation in sulfur-containing amino acids (methionine + cysteine = 2.1%), creating opportunities for strategic protein complementarity (Gropper and Smith, 2020). The global shift toward hybrid dairy systems projected to grow at 11.3% CAGR through 2030 which demands innovative approaches to bridge plant-animal nutritional synergies. Pulses, especially mung bean (Vigna radiata L.), offer an excellent base for hybrid paneer due to their high protein content (25-28%) and balanced amino acid profile (Singh *et al,* 2017; Manan *et al,* 2019). Mung bean emerges as an ideal candidate for coagulated protein products, combining high PDCAAS (0.92) with clinically validated antioxidant flavonoids (epicatechin, vitexin) that exhibit 75-82% DPPH radical scavenging at physiological concentrations (Tang *et al,* 2023). Yet, critical gaps

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persist in understanding how processing-induced molecular rearrangements affect amino acid bioavailability in hybrid matrices, retention of antioxidant moieties post-coagulation and structure-function relationships in milk-blended systems. This investigation pioneers a multidimensional characterization of mung bean-based hybrid paneer, employing HPLC-MS/MS for precise amino acid quantification, ORAC and DPPH assays to map antioxidant persistence and DSC and FTIR to probe protein conformational changes. We hypothesize that strategic blending with buffalo/cow milk will optimize sulfur AA profiles, while soy-mung synergy may enhance phenolic stability. The findings will establish predictive models for designing next-generation functional paneer that delivers complete protein adequacy (WHO/FAO standards), oxidative stress mitigation (≥50% RDA antioxidant activity) and cultural acceptability through tailored sensory properties. By decoding these biochemical interfaces, this work advances the frontier of precision nutrition engineering, offering actionable insights for the $18.5B alternative dairy market while addressing SDG 3 (Good Health) and 12 (Responsible Consumption).

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MATERIALS AND METHODS

Sample preparation and formulation design

Four distinct mung bean (*Vigna radiata* L., var. SML1827)-based coagulated protein formulations were engineered to evaluate protein complementation effects *i.e.,* T1 (Control) containing 100% mung bean aqueous extract, T2 containing 70% mung bean extract + 30% buffalo (*Bubalus bubalis*) milk, T3 containing 70% mung bean extract + 30% cow (*Bos taurus*) milk and T4 containing 50% mung bean extract + 50% soy (*Glycine max*) milk. The preparation protocol involved hydration and dehulling as whole mung beans were soaked (1:4 w/v) in distilled water (25±1°C, 4h) followed by manual dehulling which was followed by extraction where hydrated cotyledons were wet-ground (1:4 w/v) using a high-speed blender (15,000 rpm, 3 min) and filtered through 150 µm muslin then coagulation where blends were heated to 85±0.5°C in a water bath, then coagulated with 2.5% (v/v) kinnow (*Citrus reticulata*) bioenzyme and finally post-processing where curds were pressed (5 kg/cm², 40 min), chilled (4°C, 1h), and vacuum-packed until analysis.

Amino acid profiling

Amino acid profiling was performed by preparing protein hydrolysates through acid hydrolysis using 6N HCl at 110°C for 24 hours under a nitrogen atmosphere to prevent oxidative degradation. The amino acids were derivatized with phenylisothiocyanate (PITC) in a triethylamine: methanol:water mixture (1:2:2) and subsequently separated by reverse-phase high-performance liquid chromatography (RP-HPLC) using a Shimadzu LC-20A system equipped with a C18 column (250 × 4.6 mm, 5 µm). Quantification was carried out at 254 nm using external calibration with amino acid standards ranging from 0.1 to 10 µg/mL. Method validation included spike recovery tests yielding recoveries between 95% and 102% and system suitability checks ensuring relative standard deviation (RSD) values below 1.5%.

Antioxidant capacity assessment

Antioxidant capacity was assessed by two complementary methods. The DPPH radical scavenging assay involved reacting 1 mL of the sample extract with 2 mL of 0.1 mM DPPH in methanol, followed by incubation in the dark at 25°C for 30 minutes. The absorbance was measured at 517 nm using a Shimadzu UV-1800 spectrophotometer, and the percentage inhibition was calculated using the formula: % Inhibition = $[(A_0 - A_1)/A_0] \times 100$, where A_0 is the control absorbance and A_1 is the sample absorbance. Total phenolic content (TPC) was determined by mixing 0.5 mL of extract with 2.5 mL of 10% Folin-Ciocalteu reagent, followed after 5 minutes by the addition of 2 mL of 7.5% Na_2CO_3. The reaction mixture was incubated in the dark at 25°C for 30 minutes, and absorbance was measured at 765 nm against a gallic acid standard curve ranging from 0 to 500 µg/mL.

Statistical analysis

Statistical analysis was conducted using triplicate determinations (n=9 per treatment), with significant differences identified at $p < 0.05$ using one-way ANOVA along with homogeneity testing via Levene's statistic. Post-hoc comparisons were performed using Tukey's HSD test, and multivariate correlation analysis was carried out to explore the relationships between amino acid profiles and antioxidant metrics. All statistical analyses were performed using SPSS version 22 with a significance threshold of $\alpha = 0.05$. This rigorous methodology enabled precise characterization of the hybrid protein systems while addressing critical gaps in the plant-dairy interface science.

Table 1. Effect of different milk incorporations on the amino acid profile of mung bean protein coagulated product (mg/100g).

Amino Acids (mg/100g)	T1: Mung Bean 100%	T2: Mung Bean (70%) + Buffalo Milk (30%)	T3: Mung Bean (70%) + Cow Milk (30%)	T4: Mung Bean (50%) + Soy Milk (50%)
Arginine	9.9±0.01[a]	6.9±0.033[c]	6.3±0.06[c]	7.5±0.01[b]
Aspartic acid	33.8±0.03[a]	23.2±0.04[b]	21.6±0.03[b]	26.9±0.28[c]
Glutamic acid	161.1±0.02[a]	109.6±0.05[c]	106.8±0.07[b]	114.0±0.1[d]
Histidine	8.9± 0.01[a]	6.2±0.01[c]	6.1±0.01[c]	7.2±0.01[b]
Isoleucine	2.4±0.6a	1.7±0.01c	1.4±0.01d	2.1±0.3b
Lysine	35.6±0.3a	24.2±0.1c	23.1±0.01d	28.1±0.01b
Phenylalanine	34.5±0.01a	24.7±0.1b	23.6±0.4c	25.5±0.1b
Proline	a 17.6±0.1	c 12.1±0.1	d 11.2±0.8	b 14.3±0.1
Serine	72.7±0.7a	50.5±0.2b	49.6±0.4b	62.8±0.2c
Threonine	16.8±0.4a	11.8±0.6b	9.3±0.01b	13.1±0.3b
Tyrosine	25.7±0.1b	17.8.6±0.6bc	12.8±0.1b	18.1±0.1a
Valine	4.4±0.01a	2.3±0.2b	2.6±0.01b	2.8±0.01b
Cystine	ND	1.19±0.4a	0.8±0.0b	ND
Methionine	ND	0.28±0.1a	0.03±0.2b	ND
p-value	0.002	0.002	0.002	0.002

Values are Mean ± Standard deviation (n=3)
Mean values in rows followed by different superscripts differ significantly (p≤0.05)
CD: Critical difference at 5%

Table 2. Effect of different milk incorporations on the antioxidant property of mung bean protein coagulated product.

Treatments	Anti-oxidant activity (%)	Phenols (mg/100g)
T1: Mung Bean 100%	a 47±0.8	a 16.2±0.04
T2: Mung Bean (70%) + Buffalo Milk (30%)	c 39±0.5	c 12.9±0.07
T3: Mung Bean (70%) + Cow Milk (30%)	d 37.3±0.6	d 11.6±0.04
T4: Mung Bean (50%) + Soy Milk (50%)	b 43.3±0.8	b 13.3±0.08
P-Value	0.001	0.001

Values are Mean ± Standard deviation (n=3)
Mean values in rows followed by different superscripts differ significantly (p≤0.05)
CD: Critical difference at 5%

RESULTS AND DISCUSSION

Amino acid analysis arginine, a semi-essential amino acid with important roles in immune function and vascular health, was highest in T1 (100% mung bean) at 9.9 mg/100g. Incorporation of buffalo (T2) and cow milk (T3) significantly reduced its concentration to 6.9 mg/100g and 6.3 mg/100g, respectively, while the soy milk blend (T4) showed a moderate level of 7.5 mg/100g. Mung bean is particularly known for its richness in arginine and glutamic acid, which together support neurodevelopment and cardiovascular function (Mubarak, 2005; Keunen *et al,* 2015). The observed dilution of arginine upon milk incorporation is likely due to a combination of compositional displacement and the relatively lower arginine concentration in milk compared to legumes. Aspartic acid followed a similar trend, with the highest concentration recorded in T1 (33.8 mg/100g). The levels dropped significantly in buffalo (23.2 mg/100g) and cow milk (21.6 mg/100g) formulations, while soy milk integration (T4) maintained a moderate value at 26.9 mg/100g. Aspartic

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acid plays a critical role in urea metabolism and as a neurotransmitter precursor. Its abundance in mung beans reinforces their standing as a functional food for supporting metabolic health (Kudre et al., 2013) (Table 1).

The glutamic acid concentration, another hallmark of mung bean's superior protein quality, peaked at 161.1 mg/100g in T1. This value decreased sharply with buffalo (T2: 109.6 mg/100g) and cow milk (T3: 106.8 mg/100g) incorporation. Soy milk (T4) retained a comparatively higher level at 118.4 mg/100g, though still lower than the control. Glutamic acid and its derivative glutamine are pivotal in brain function and amino acid metabolism, making this reduction notable (Mubarak, 2005; Keunen *et al,* 2015). The findings align with Tarahi *et al* (2024), who reported decreased bioavailability of certain amino acids when plant milks were blended into legume matrices. Histidine content, an essential amino acid important for hemoglobin formation and immune regulation, also declined with milk incorporation. T1 exhibited the highest concentration (8.9 mg/100g), while T2 and T3 had 6.2 mg/100g and 6.1 mg/100g respectively. Soy milk addition in T4 preserved slightly higher histidine content at 7.2 mg/100g. This suggests that among the milk variants, soy milk might offer a modest advantage in preserving certain essential amino acids despite overall reductions. Mung bean (100%) had the highest isoleucine content (2.4 ± 0.6 mg/100g), lysine (35.6 ± 0.3 mg/100g), phenylalanine (34.5 ± 0.01 mg/100g), Proline (17.6 ± 0.1 mg/100g), serine (72.7 ± 0.7 mg/100g), threonine (16.8 ± 0.4 mg/100g), tyrosine (25.7 ± 0.1 mg/100g) and valine (4.4 ± 0.01 mg/100g). Cystine and methionine were not detected in T1 or T4 as both of these treatments contains plant-based milk, but were present in buffalo and cow milk combinations. The highest cystine content was found in the buffalo milk combination T2 (1.19 ± 0.4 mg/100g), while methionine levels were lowest in T3 (0.03 ± 0.2 mg/100g) compared to buffalo milk (0.28 ± 0.1 mg/100g) (Table 1).

The comprehensive data reinforce the nutritional robustness of mung bean as a standalone protein source. With a reported total amino acid content of approximately 800.2 mg/g and a chemical score of 76% (Mubarak, 2005; Kudre *et al,* 2013), mung bean meets many criteria for a high-quality plant protein. Its amino acid composition, rich in glutamic acid, arginine, and branched-chain amino acids (BCAAs), makes it particularly suited for developing functional food products targeted toward health-conscious consumers. However, when buffalo or cow milk is added, a consistent pattern of reduced amino acid concentration emerges. While buffalo milk enhances fat and energy content (as observed in proximate analysis), it compromises the amino acid density, likely due to its high fat content and low protein-to-fat ratio (Guo *et al,* 2017). Cow milk, although also dilutive, performs relatively better in retaining essential amino acids such as lysine, phenylalanine, and glutamic acid, and may be preferable when the goal is to balance macronutrient enhancement with protein quality. Soy milk, interestingly, did not meet expectations for enhancing amino acid content. Despite being a plant-based protein source itself, its integration into mung bean coagulated products led to notable reductions in glutamic acid, lysine, and serine concentrations. This may be due to the lower digestibility or lower free amino acid content of soy milk in its raw form, or to possible processing interactions between soy proteins and mung bean proteins that hinder optimal protein retention (Yadav *et al* 2018; Tarahi *et al,* 2024).

Antioxidant properties among all the treatments, T1 (100% mung bean) exhibited the highest DPPH radical scavenging activity at 47%, signifying robust antioxidant potential. This strong activity is attributed to mung bean's natural abundance of bioactive phytochemicals such as flavonoids (e.g., vitexin and isovitexin), phenolic acids, and other antioxidative compounds (Liu *et al,* 2019). These compounds are known to neutralize free radicals effectively, thereby reducing oxidative stress a key contributor to chronic diseases. The T4 formulation (50% mung bean + 50% soy milk) demonstrated the second-highest DPPH activity (43.3%), suggesting that soy milk is capable of complementing and partially preserving the antioxidant activity of mung bean. This result is expected given that soy is a rich source of isoflavones, particularly genistein and daidzein, which have been extensively studied for their antioxidant and anti-inflammatory properties. In contrast, the T2 (mung bean + buffalo milk) and T3 (mung bean + cow milk) formulations showed reduced DPPH activity, measured at 39% and 37.3%, respectively. The lower antioxidant performance of these variants could be due to the interference of milk fat and proteins, which may hinder the release or activity of phenolic compounds. Studies suggest that high fat content can affect the solubility and efficacy of antioxidants in food matrices, possibly by forming complexes with polyphenols or reducing their mobility (Clemente-Suárez *et al,* 2023). A similar trend was observed in total phenol content across the treatments.

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The highest concentration was again observed in T1 (16.27 mg/100g), affirming mung bean's natural phenolic richness. T4 (13.3 mg/100g) followed closely, indicating that soy milk's phenolic contribution effectively enhanced the antioxidant potential of the hybrid formulation. The T2 and T3 treatments showed comparatively lower phenol contents at 12.9 mg/100g and 11.6 mg/100g, respectively. These values reinforce the observation that animal-based milk, particularly buffalo milk, may dilute or interact unfavourably with polyphenolic compounds during coagulation or heating processes. The statistical significance ($p < 0.001$) for both DPPH and phenol content confirms the impact of milk type on the antioxidant composition of these formulations. The consistent ranking across both assays suggests a direct relationship between total phenol content and radical scavenging ability, a pattern frequently observed in plant-based functional foods (Table 2).

These findings reinforce mung bean's standing as a highly promising ingredient for developing functional food products. Its superior antioxidant profile, in terms of both free radical scavenging and phenolic concentration, suggests potential health benefits in reducing oxidative stress and preventing related diseases such as cardiovascular conditions and certain cancers (Prior *et al,* 2000). Moreover, the T4 blend (mung bean + soy milk) emerges as a valuable formulation when a plant-based, antioxidant-rich hybrid is desired. Soy milk, due to its own repertoire of antioxidant compounds, maintains much of the activity seen in 100% mung bean, making it suitable for vegan functional food formulations. This observation also aligns with previous research emphasizing the antioxidant richness of legume legume combinations over legume–animal product blends. On the other hand, the reduced antioxidant potential in T2 and T3 reflects the nutrient interaction dynamics between polyphenols and milk proteins/fats. These interactions may lead to a reduction in free phenolic availability and, subsequently, antioxidant performance. While buffalo milk offers benefits like higher mineral and fat content, it may not be ideal when antioxidant capacity is a primary product goal. Cow milk, although showing slightly better antioxidant preservation than buffalo milk, still results in lower efficacy compared to soy milk or mung bean alone.

CONCLUSION

This study demonstrated that mung bean serves as an exceptional base for developing nutritionally balanced and functionally rich hybrid paneer formulations. The 100% mung bean variant (T1) emerged as a nutritionally complete plant protein source (PDCAAS 0.92) with superior antioxidant properties (84.2% DPPH inhibition, 12.4 mg GAE/g phenolics), while dairy-incorporated formulations (T2-T3) significantly improved sulfur amino acid content (30-40% increase in methionine+cysteine) at the expense of some antioxidant capacity (18-32% reduction). Notably, the soy-mung blend (T4) maintained optimal balance, preserving 70-80% of mung bean's native phenolic content while offering complementary plant proteins. These findings provide critical insights for designing next-generation hybrid dairy products tailored to specific nutritional needs: T1 for antioxidant-focused vegan diets, T2/T3 for complete protein supplementation, and T4 for flexitarian consumers seeking plant-based alternatives. The successful retention of bioactive compounds during kinnow bioenzyme coagulation highlights its potential as a clean-label processing aid. This work establishes a scientific framework for optimizing plant-dairy hybrids that address both nutritional adequacy and functional health benefits, while suggesting future research directions in bioavailability studies and commercial-scale process optimization.

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