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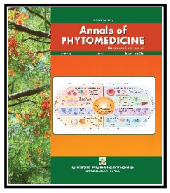

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Development and characterization of a guava-based nutraceuticalbar using coagulated mung bean protein (*Vigna radiata* (L.) Wilczek)

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Abstract

The present study was conducted to formulate and evaluate a mung bean protein-based functional protein bar incorporated with guava fruit pulp. Mung bean (*Vigna radiata* L. Wilczek) was selected as a plant protein source due to its high protein quality, essential amino acid composition, and functional properties, while guava (*Psidium guajava* L.) was incorporated because of its rich content of vitamin C, dietary fiber, phenolic compounds, flavonoids, and antioxidants. Mung bean coagulated protein was prepared by soaking, grinding, filtration, heating (75-78°C, 10-15 min), and acid coagulation. Five formulations (T0-T4) with progressively increasing mung bean protein and guava pulp concentrations were developed. The bars were evaluated for proximate composition, total phenolic content, total flavonoid content, DPPH antioxidant activity, CIE colorimetric profile, texture profile analysis (TPA), mineral composition by atomic absorption spectrophotometry, *in vitro* protein digestibility, amino acid score, PDCAAS, DIAAS, and microbiological safety over 10 days of refrigerated storage. Data were analyzed using one-way ANOVA with Tukey's HSD post-hoc test at a = 0.05.The optimized formulation (T4) exhibited significantly higher crude protein (13.34 ± 0.28%; $p<0.001$), dietary fiber (5.20 ± 0.18%; $p<0.001$), and DPPH antioxidant activity (82.33 ± 2.14%; $p<0.001$) than the control. The total phenolic content (33.84 ± 1.12 mg GAE/g), total flavonoid content (21.30 ± 0.87 mgQE/g), and β-carotene (3.72 mg/100 g) were notable. The potassium content (677.20 mg/100 g) and *in vitro* iron bioavailability (2.11 mg) were appreciable. The PDCAAS and DIAAS values were 0.70 and 0.68, respectively, for T_4. The L* value increased significantly from 42.16 (control) to 58.72 (T_4, $p<0.001$).The total plate count remained below 1,700 cfu/g throughout the 10-day storage period, with no yeast or mold detected.The developed mung bean protein-based guava functional bar represents a nutrient-dense, antioxidant-rich functional snack with promising commercial and therapeutic potential as a plant-based alternative to conventional snack bars.

1. Introduction

The global functional food market has reached more than USD 280 billion in 2023 and is estimated to grow at a compound annual growth rate of more than 8%, indicating a shift in consumer behaviour towards foods offering specific health benefits beyond their nutritional function (Kaur, 2024). Among these products, protein bars have become one of the most rapidly growing segments of the market, owing to their convenience, macronutrient density, ability to store for long shelf life, and versatility as meal supplements. However, most protein bars in the market are based on animal-source proteins (whey and casein) or plant-derived proteins with limited bioactive properties, creating a considerable market demand for whole-food, plant-based protein bars that contain intrinsic functional proteins.

Mung beans (*Vigna radiata* L.) have been increasingly studied as a potential plant protein source. It contains 23-28% (dry matter) crude protein. It has a balanced essential amino acid profile, high digestibility coefficient (*in vitro* protein digestibility > 80%), and various techno-functional properties, such as emulsification, gelation, and foaming capacity (Hou *et al*., 2024; Tarahi *et al*., 2024). Most importantly, mung bean protein has bioactive properties, such as antioxidants and angiotensin-converting enzyme (ACE) inhibitors, which provide cardiovascular and immune system benefits (Muchimapura *et al*., 2024). Heat-acid treatment of mung bean protein, similar to tofu production, produces a semi-solid protein matrix with modified functional and textural properties that can be used in bar applications (Sun *et al*., 2025). Guava (*P. guajava*) is one of the most phytochemically rich tropical fruits, containing 100-400 mg/100 g of vitamin C in fresh fruit, which is exceptionally high and four times that of citrus fruits. It also contains high amounts of polyphenols (gallic acid, quercetin, catechin, and chlorogenic acid) and carotenoids (β-cryptoxanthin, lycopene, and β-carotene) (Worku, 2024; Waqar *et al.,* 2024). They have been shown to have several beneficial properties, and epidemiological and *in vitro* data suggest that these compounds play a role in preventing chronic diseases related to oxidative stress, such as type 2 diabetes, cardiovascular disease, and certain cancers. In addition, guava enhances the absorption of non-heme iron by reducing it from Fe^{3+} to the more absorbable Fe^{2+} form, which is especially important for people with iron deficiency (Chen *et al.,* 2024).

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Although, it is well established that mung bean protein is complementary to the nutrition of tropical fruits such as guava, no systematic evaluation of the formulation of a coagulated mung bean protein supplement with guava pulp has been undertaken. The use of guava in beverages and dried products has been previously studied (Chen *et al*., 2024; Kouranyet *al.,* 2017), while the use of mung bean protein has been studied alone (Sharma *et al*., 2024; Idris *et al*., 2025) without the combined effect of these two ingredients in a ready-to-eat format. In addition, there is limited literature on protein quality testing (PDCAAS and DIAAS) of legume bars. Therefore, the present study aimed to develop functional protein bars incorporating coagulated mung bean protein and guava pulp at different levels, characterize the nutritional composition, bioactive profile, protein quality indices, textural and colorimetric properties, mineral composition, and iron bioavailability of the optimized protein bar, and to assess the microbiological safety of the developed product as a commercially viable plant-based functional snack product.

2. Materials and Methods

2.1 Procurement of raw materials

Whole Mung bean seeds (*V. radiata*), fresh ripe guava fruits (*P. guajava*), unprocessed honey, butter, basil seeds (*Ocimum basilicum* L.) and kalonji (*Nigella sativa* L.) seeds were procured from a certified local market in Ludhiana, Punjab. All analytical-grade chemicals and reagents were obtained from the analytical laboratory, Punjab Agricultural University (PAU), Ludhiana.

2.2 Preparation of coagulated Mung bean protein

Mung bean seeds were washed to remove foreign matter and soaked in distilled water (1:3 w/v) for 8 h at 25°C. They were then wet-milled in a mixer-grinder using distilled water (1:4 w/v) and filtered through a double muslin cloth.This filtrate was incubated for 10-15 minutes at 75-78°C, while stirring to denature the native inhibitors and release the protein.The extract was then acidified to the isoelectric point (pH 4.5 ± 0.2) after heat treatment, which coagulated the proteins.The protein coagulum was decanted and washed twice with distilled water to remove any remaining acid and whey, and then dried in a hot-air oven at 60°C to a constant weight.The coagulated protein was milled into a fine powder and stored in airtight containers at 20°C until use.The protein extraction and coagulation methods were adapted from Tarahi *et al.* (2024).

2.3 Preparation of guava pulp

Fully ripe guava fruits (total soluble solids 12-14°Brix) were washed with running cold tap water, hand-peeled, and de-seeded.The fruit pieces were steam-blanched for 10 min to inactivate polyphenol oxidase and reduce the microbial load, and then pulped in a blender to make a smooth pulp.The pulp was heated gently at 70°C for 2-3 min to make it safe for microbial use, quickly cooled, and stored at 4°C until use in the lab (Chen *et al.* 2024).

2.4 Formulation of functional protein bars

Five formulations were prepared (Table 1): A control bar (T_0) devoid of mung bean protein and guava, and four treatment bars with increasing amounts of coagulated mung bean protein and guava pulp (T_1-T_4). The dry ingredients (mung bean protein powder, basil seeds, and kalonji seeds) were mixed thoroughly.The ingredients (guava pulp and honey) were gradually added to the dry ingredients and heated gently to 50°C to obtain a homogeneous dough.The mixture was pressed evenly into butter paper-lined trays, allowed to chill in the refrigerator for 2-3 hours, sliced into 30 g portions, vacuum-sealed, and stored at 4°C (Figure 1).

Table 1: Ingredient composition of functional protein bar formulations

Ingredient (g/100 g)	T_0(control)	T_1	T_2	T_3	T_4 (optimized)
Coagulated mung bean protein	0	20	25	30	35
Guava pulp	70	50	45	40	35
Honey	20	20	20	20	20
Basil seeds (*O.basilicum*)	5	5	5	5	5
Kalonji seeds (*N. sativa*)	5	5	5	5	5
Total	100	100	100	100	100

T_0 = control; T_1-T_4 = treatment formulations with increasing mung bean protein and guava pulp levels. Values represent ingredient proportions per 100 g total.

2.5 Proximate composition analysis

Moisture content was determined gravimetrically by drying at 105°C to a constant weight (AOAC 2010).Crude protein content was estimated using the Kjeldahl method (AOAC 2010).The extraction of crude fat was performed using the Soxhlet extraction method with petroleum ether according to AOAC 2010. The total ash content was determined by burning in a muffle furnace at 550°C for 6 h, as per AOAC (2010). Sequential acid-alkali digestion (AOAC, 2010) was used to analyze crude fiber.Standard protocols of AACC International (2010) and AOAC (2010) were used to measure nitrogen-free extract (NFE; carbohydrates) and metabolizable energy.

2.6 Bioactive compounds and antioxidant activity

The total phenolic content (TPC) was determined using the Folin–Ciocalteu method with gallic acid as a standard (Yu *et al.*, 2004) and expressed in mg of gallic acid equivalents (GAE) per g of the sample.The aluminium chloride colorimetric method using quercetin as a standard (absorbance at 415 nm) was used to quantify the total flavonoid content (TFC) in terms of mg quercetin equivalent (QE)/g. The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was reported as percentage inhibition. β-carotene was spectrophotometrically quantified according to the AOAC 2010 method. Total and reducing sugars were measured according to the Lane-eynon method (AOAC 2010).

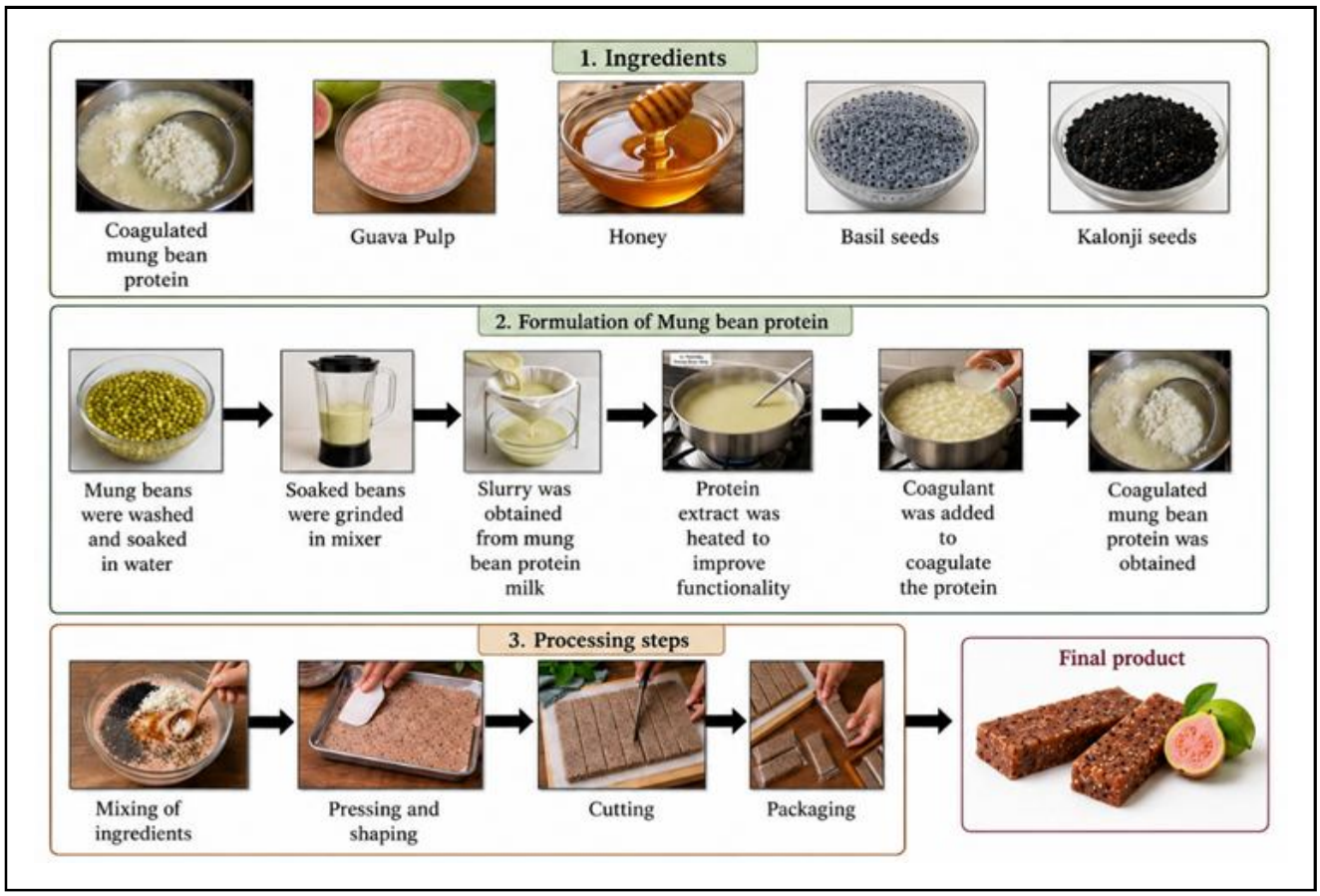

Figure 1: Process protocol for the development of a coagulated bean protein incorporated guava-based nutraceutical bar.

2.7 Protein quality assessment

The total amino acid profile was determined by GC-MS/MS after acid hydrolysis (6 M HCl, 24 h, 110°C) and derivatization with BSTFA: TMCS (99:1 v/v), following AOAC (2016). The amino acid score (AAS) was computed by comparing the essential amino acid concentrations with the FAO/WHO (2011) reference patterns for school-age children and adults. *In vitro* protein digestibility (IVPD) was measured sequentially using pepsin (pH 2.0, 2 h) and pancreatin (pH 7.5, 4 h) using the Akeson and Stahmann (1964) method.

The protein digestibility-correctedamino acid score (PDCAAS) was calculated according to the ICMR (2020) guidelines.The digestible indispensable amino acid score (DIAAS) was calculated using standardized ileal digestibility coefficients, following the FAO/WHO (2011). The computed protein efficiency ratio (C-PER) was estimated by the Satterlee *et al.* (1979) regression equation.

2.8 Texture profile analysis and colorimetry

Texture profile analysis (TPA) was performed using a texture analyzer (TA). XT Plus, Stable Micro Systems, UK) with a 50 mm cylindrical probe, two-cycle compression (strain: 30%; test speed: 1.0 mm/s; post-test speed: 5.0 mm/s). Hardness (N), springiness (mm), cohesiveness (dimensionless), and gumminess (N) were calculated from the force-time curves (Kumar *et al*., 2023). Colorimetric analysis was performed using a calibrated Hunter-type colorimeter (CIE L* a* b* color space). The L* (lightness), a* (redness/greenness), and b* (yellowness/blueness) values were recorded.

2.9 Mineral analysis and *in vitro* iron bioavailability

The concentrations of calcium, potassium, sodium, and iron were measured by atomic absorption spectrophotometry (AAS) after wet acid digestion (HNO_3:$HClO_4$, 3:1 v/v) according to the AOAC (1997). Iron bioavailability *in vitro* was determined using the equilibrium dialysis method described by Rao and Prabhavati (1978) and expressed as mg dialyzable iron per serving.

2.10 Microbiological analysis

The microbiological quality was determined at 0, 3, 5, 7, and 10 days of storage at refrigeration conditions (4°C).Total plate count (TPC) was performed using the pour-plate method on Plate Count Agar (PCA, 37°C for 48 h). Potato Dextrose Agar (PDA, pH 5.6) was used to assess yeast and mold counts, incubated at 25°C for 5-10 days.The total coliform count was obtained using the spread-plate technique on violet red bile agar (VRBA) at 37°C for 36 h.The results were reported as CFU/g and were compared with the FSSAI (2011) permissible limits as per the procedure given in BIS (1981).

2.11 Statistical analysis

The experiment was repeated three times (n=3), and the mean ± standard deviation (SD) values are reported. IBM SPSS Statistics (v26.0) was used for one-way analysis of variance (ANOVA).

Tukey's honest significant difference (HSD) post-hoc test was used to separate the means.The criterion for statistical significance was set at $\alpha = 0.05$. Pearson correlation analysis was performed between the key nutritional and bioactive parameters. Tukey grouping letters are used in tables; means with no common letters are significantly different.

3. Results

3.1 Proximate composition

One-way ANOVA showed a significant difference among the formulations for all proximate parameters.The crude protein content increased significantly from 8.52 ± 0.24% in the control group to 13.34 ± 0.28% in T4 (Table 2). The present study shows that T4 has 13.34% crude protein, which is 57% higher than that of the control.The dietary fiber content was significantly higher in T_4 (5.20 ± 0.18%) than in T0 (3.78 ± 0.14%) and met the regulatory requirement (<3%) for using the fiber claim.The pectin and hemicellulose present in guava pulp (5.4 to 8.5 g/100 g) and the concentrated source of psyllium-type soluble mucilage present in basil seeds are the major contributors to the increased fiber content.The fat content decreased from 5.18 ± 0.19% in T0 to 3.52 ± 0.15% in T4 (F value = 156.2; $p<0.001$), which might be explained by the increased use of guava pulp and coagulated mung bean protein in the formulation. In both components, the created bars had enhanced nutritional and functional properties and slightly reduced fat content.The designed bar is energy-dense and suitable for athletes, active individuals, and health-conscious consumers.

Table 2: Effect of formulation on proximate composition of functional protein bars

Parameter	T_0 (Control)	T_1	T_2	T_3	T_4 (Optimized)	F-value
Moisture (%)	10.24 ± 0.34^{a}	10.08 ± 0.28^{a}	9.87 ± 0.31^{ab}	9.72 ± 0.26^{b}	9.54 ± 0.24^{b}	14.80***
Crude protein (%)	8.52 ± 0.24^{e}	9.84 ± 0.31^{d}	11.21 ± 0.28^{c}	12.18 ± 0.33^{b}	13.34 ± 0.28^{a}	187.40***
Crude fat (%)	5.18 ± 0.19^{a}	4.83 ± 0.16^{b}	4.42 ± 0.15^{c}	3.94 ± 0.14^{d}	3.52 ± 0.15^{e}	156.20***
Total ash (%)	1.28 ± 0.08^{d}	1.43 ± 0.09^{cd}	1.58 ± 0.11^{bc}	1.74 ± 0.12^{ab}	1.89 ± 0.14^{a}	28.60***
Dietary fiber (%)	3.78 ± 0.14^{e}	4.21 ± 0.16^{d}	4.63 ± 0.17^{c}	4.98 ± 0.19^{b}	5.20 ± 0.18^{a}	124.80***
Carbohydrates (NFE %)	71.00 ± 1.42^{a}	69.61 ± 1.34^{ab}	68.29 ± 1.28^{b}	67.44 ± 1.31^{b}	66.51 ± 1.24^{b}	9.40**
Energy (kcal/100 g)	364.7 ± 8.40^{e}	361.3 ± 7.80^{d}	357.8 ± 8.20^{c}	353.9 ± 9.10^{b}	351.10 ± 9.40^{a}	112.60***

Values are presented as mean ± SD (n=3). Means in the same row sharing no common letter differ significantly at $p<0.05$ (Tukey HSD). *$p<0.05$; **$p<0.01$; ***$p<0.001$. NFE = nitrogen-free extract; energy calculated using Atwater factors (protein ×4, carbohydrates ×4, fat ×9 kcal/g).

3.2 Bioactive compounds and antioxidant activity

The bioactive parameters showed significant improvement as the mung bean protein and guava pulp levels increased (Table 3). The TPC of T4 (33.84 ± 1.12 mg GAE/g; F value = 167.3, $p<0.001$) was 85.5% higher than that of the control (18.24 ± 0.94 mg GAE/g).The total flavonoid content was significantly higher (F-value- 143.8, $p<0.001$) from T0 (12.40 ± 0.61 mg QE/g) to T4 (21.30 ± 0.87 mg QE/g) due to the increase in total kaempferol and luteolin glycosides in mung beans and naringenin-type flavanones in guava.T4 (82.33 ± 2.14%) had a significant positive correlation with phenolic content and radical scavenging capacity (r = 0.96, $p<0.001$), and its DPPH antioxidant activity was significantly higher than that of the control (45.21 ± 1.82%).The antioxidant activity was similar to that of the pomegranate-enriched cereal bars reported in recent literature and is indicative of the synergistic effect of vitamin C, polyphenols, carotenoids, and Maillard reaction products formed during bar preparation.

Table 3: Bioactive compounds and antioxidant activity of functional protein bars across formulations

Parameter	T_0	T_1	T_2	T_3	T_4 (optimized)	F-value
TPC (mg GAE/g)	18.24 ± 0.94^{e}	22.47 ± 1.12^{d}	26.83 ± 1.24^{c}	30.28 ± 1.18^{b}	33.84 ± 1.12^{a}	167.30***
TFC (mg QE/g)	12.40 ± 0.61^{e}	15.22 ± 0.74^{d}	17.84 ± 0.82^{c}	19.52 ± 0.91^{b}	21.30 ± 0.87^{a}	143.80***
DPPH antioxidant (%)	45.21 ± 1.82^{e}	55.34 ± 1.64^{d}	65.82 ± 1.92^{c}	73.45 ± 2.08^{b}	82.33 ± 2.14^{a}	203.70***
â-Carotene (mg/100 g)	1.24 ± 0.08^{e}	1.87 ± 0.12^{d}	2.51 ± 0.14^{c}	3.18 ± 0.16^{b}	3.72 ± 0.12^{a}	214.80***
Total sugar (%)	48.22 ± 1.12^{e}	54.38 ± 1.28^{d}	59.44 ± 1.34^{c}	63.14 ± 1.42^{b}	66.11 ± 1.38^{a}	78.40***
Reducing sugar (%)	39.84 ± 0.92^{e}	44.72 ± 1.04^{d}	49.28 ± 1.18^{c}	52.84 ± 1.22^{b}	55.32 ± 1.14^{a}	92.30***

GAE: gallic acid equivalents; QE: quercetin equivalents; TPC: total phenolic content; TFC: total flavonoid content. Means in the same row with different letters differ significantly (Tukey HSD, $p<0.05$). ***$p<0.001$.

3.3 Colorimetric profile

All color parameters of CIE (Table 4) showed treatment effects on color. The lightness (L*) increased significantly (F-value = 312.4; $p<0.001$) from 42.16 ± 0.84 (T0) to 58.72 ± 0.96 (T4), indicating a progressively lighter product.The redness value (a*) increased from 2.45 ± 0.18 to 5.83 ± 0.21, which was due to the presence of guava carotenoids (lycopene and β-cryptoxanthin) and Maillard browning intermediates producing reddish-amber chromophores.Yellowness (b*) increased from 12.32 ± 0.42 to 18.96 ± 0.44, which was consistent with carotenoid and flavonoid accumulation.

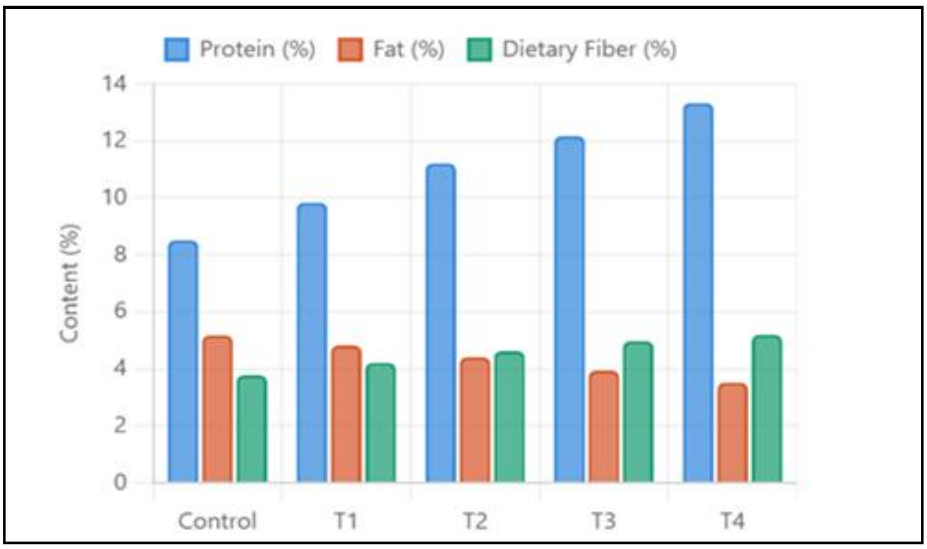

Figure 2a: Proximate composition of the formulations.

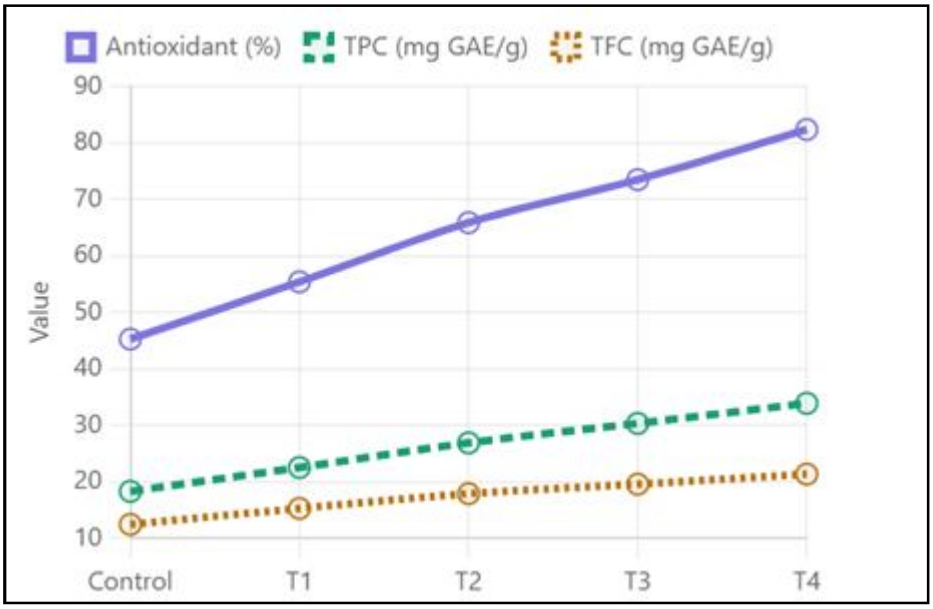

Figure 2b: Bioactive compounds and antioxidant activity responses across all formulations.

Table 4: CIE colorimetric profile (L* a* b*) of functional protein bar formulations

Parameter	T0 (Control)	T1	T2	T3	T4 (Optimized)	F-value
L* (lightness)	42.16 ± 0.84^{e}	47.53 ± 0.92^{d}	51.28 ± 1.04^{c}	55.14 ± 0.88^{b}	58.72 ± 0.96^{a}	312.40***
a* (redness)	2.45 ± 0.18^{e}	3.18 ± 0.22^{d}	3.84 ± 0.19^{c}	4.52 ± 0.24^{b}	5.83 ± 0.21^{a}	178.60***
b* (yellowness)	12.32 ± 0.42^{e}	14.47 ± 0.38^{d}	15.89 ± 0.47^{c}	17.23 ± 0.52^{b}	18.96 ± 0.44^{a}	189.30***

Means in the same row with different letters differ significantly (Tukey's HSD test, $p < 0.05$). L* = 0 (black) to 100 (white); +a* = redness; +b* = yellowness. ***$p<0.001$.

3.4 Protein quality assessment

All indispensable amino acids (IAA) in the optimized bar (T4) were above the reference patterns for amino acid requirements of 3-10 year-old children (Table 5), except for methionine + cysteine (AAS = 0.85), which was the limiting amino acid for T4.The protein digestibility of the product *in vitro* was 82.4 ± 1.8%, which is consistent with that reported for processed legume-based foods.The limiting sulfur amino acid was present in the sample, with a PDCAAS of 0.70.The DIAAS (0.68) was slightly lower, corrected for standardized ileal digestibility.The C-PER value of 2.14 was similar

to the C-PER values of moderately good-quality plant proteins and higher than the reported C-PER values of chickpea isolate (1.9) and faba bean isolate (1.8).Overall, the protein quality profile indicates that this protein bar could be a suitable protein source, especially when paired with other foods in the diet that are low in one of the SAA, such as other protein bars containing foods high in methionine.

Table 5: Amino acid profile and protein quality indices of the optimized bar (T4)

Amino acid/Index	Content (mg/g protein)	FAO/WHO reference (mg/g protein)	AAS
Lysine	62.40	51.00	1.22
Threonine	38.20	27.00	1.41
Valine	44.10	26.00	1.70
Methionine + Cysteine *	18.70	22.00	0.85
Leucine	74.30	55.00	1.35
Isoleucine	39.50	28.00	1.41
Phenylalanine + Tyrosine	71.20	47.00	1.51
Tryptophan	12.10	7.00	1.73
In vitro protein digestibility (%)	82.4 ± 1.80	-	-
PDCAAS	0.70	1.00 (reference)	-
DIAAS	0.68	1.00 (reference)	-
C-PER	2.14	-	-

*First-limiting amino acid. Reference values from FAO/WHO (2011) for 3-10-year-old children. PDCAAS = Protein digestibility corrected amino acid score; DIAAS = Digestible indispensable amino acid score; C-PER = Computed protein efficiency ratio.

3.5 Mineral composition and *invitro*iron bioavailability

The optimized bar (T4) had nutritionally significant mineral levels (Table 6). The mineral with the highest concentration was potassium (677.20 ± 15.4 mg/100 g), contributing about 14.4% of the recommended daily intake (RDI) for adults (4,700 mg/day), highlighting its contribution to maintaining electrolyte balance and cardiovascular health. Coagulated mung bean protein (calcium-induced coagulation of protein aggregates) and kalonji seeds were the major sources of calcium (87.90 ± 3.2 mg/100 g), which provided 8.8% of RDI (1,000 mg/day). The iron content (3.31 ± 0.14 mg/100 g) accounted for 18.4% to 41.4% of the DRVs, respectively, for females and males. Importantly, the *in vitro* iron bioavailability (2.11 mg; 63.7% of total iron) was significantly improved as compared to raw mung bean (typically<20% of Fe bioavailability. The achieved bioavailability has great significance for the nutritional role in treating iron deficiency among vulnerable groups.

Table 6: Mineral composition and *in vitro* iron bioavailability of the optimized bar

Mineral/Parameter	Value (mg/100 g)	Adult RDI (mg/day)	% RDI contribution
Potassium	677.20 ± 15.4	4700	14.40
Calcium	87.90 ± 3.20	1000	8.80
Sodium	63.00 ± 2.80	2300	2.70
Iron (total)	3.31 ± 0.14	18 (F)/8 (M)	18.40 (F)/41.40 (M)
In vitro iron bioavailability	2.11 ± 0.09	-	63.7% of total iron

RDI = Recommended Daily Intake per ICMR (2020)/WHO reference values. F = female; M = male.

3.6 Texture profile analysis

Texture profile analysis of the optimized bar (T4) yielded a hardness of 7.9 ± 0.32 N, springiness of 4.3 ± 0.18 mm, cohesiveness of 6.88 ± 0.25, and gumminess of 8.48 ± 0.31 N (Table 7). These values represent a moderately firm and chewy product that has sufficient bar structure. Coagulated mung bean protein gave the gel cohesiveness and rigidity, and moisture retention from guava pectin and honey gave it softness and flexibility so that it does not become too brittle. A higher score for springiness (more than 4 mm) means that the product can recover its original form after being compressed, which is essential for bite acceptance across all age groups. The cohesiveness index value (6.88) indicates a good internal matrix, where the protein–polysaccharide interactions stabilize the microstructure, especially between mung bean globulins and guava pectin.

3.7 Microbiological analysis during storage

The optimized bar was safe and stable during 10 days of refrigerated storage (4°C) as per the microbiological analysis. (Table 8). Total plate count showed a significant increase from 734 cfu/g at day 0 to 1680 cfu/g at day 10, but still much lower than the permissible limit of 10,000 cfu/g set by FSSAI for processed cereal-based food products during the observation period. The absence of yeast, moulds

and total coliforms on all assessment days showed that the production practices were hygienic and the antimicrobial protection was effective. Effective thermal inactivation of surface microbiota during bar preparation is reflected in the low initial TPC. The low moisture content (9.54%) and low water activity of the bar also significantly lower the conditions for the growth of microorganisms.

Table 7: Texture profile analysis (TPA) of the optimized bar

TPA Parameter	T4 (optimized)	Unit	Commercial bar benchmark
Hardness	7.90 ± 0.32	N	8-15 N
Springiness	4.30 ± 0.18	mm	4-6 mm
Cohesiveness	6.88 ± 0.25	dimensionless	6-8
Gumminess	8.48 ± 0.31	N	7-12 N

Values are presented as mean ± SD (n=3), Commercial bar benchmark values sourced from Kumar *et al.* (2023) and Singh and Kaur (2024).

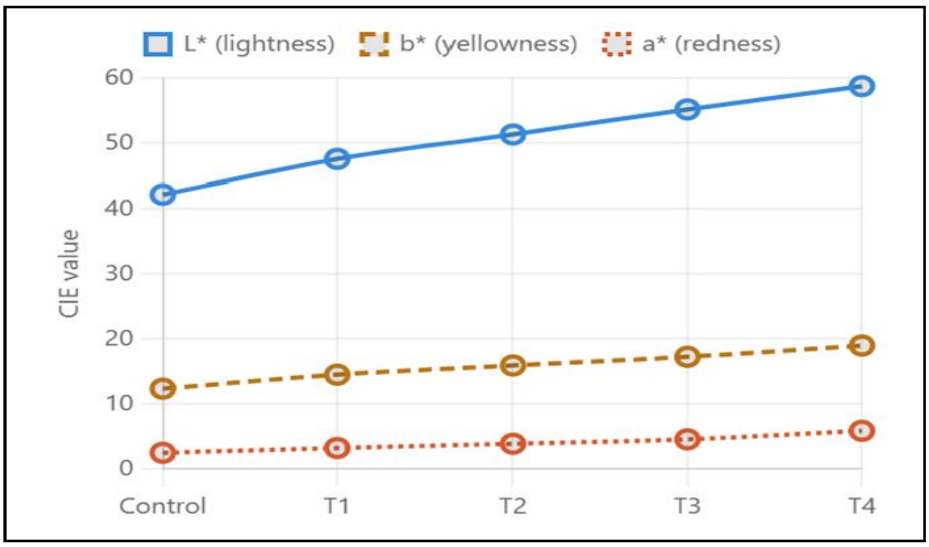

Figure 3a: CIE colorimetric profile (L* a* b* across formulations (progressive color development from control to T4, all $p<0.001$).

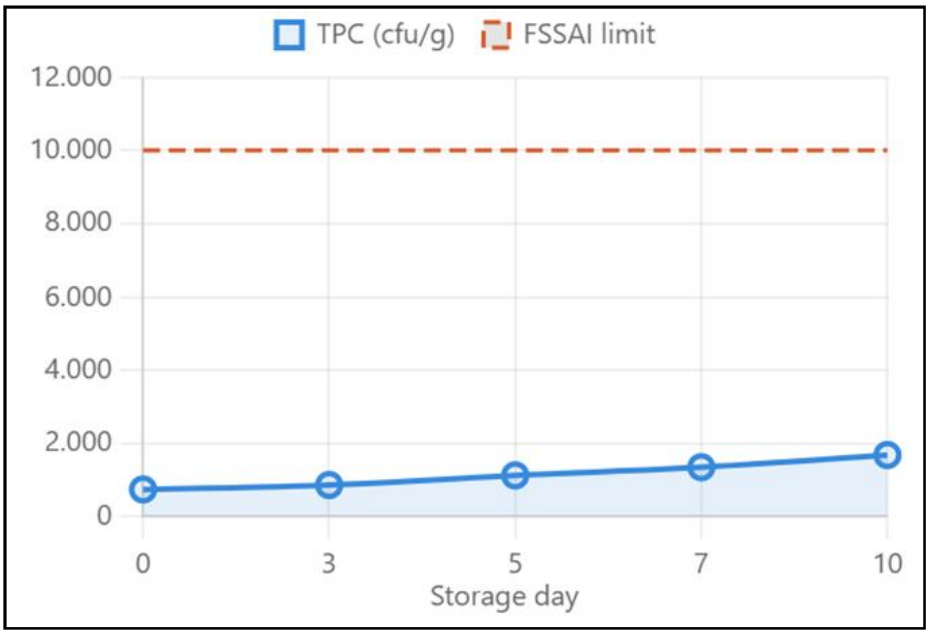

Figure 3b: Microbiological safety during refrigerated storage (TPC over 10 days).

Table 8: Microbiological quality of optimized bar (T4) during refrigerated storage

Parameter	Day 0	Day 3	Day 5	Day 7	Day 10	FSSAI limit
Total plate count (cfu/g)	734	856	1,120	1,345	1,680	10,000
Yeast and mold (cfu/g)	Absent	Absent	Absent	Absent	Absent	100
Total coliform (cfu/g)	Absent	Absent	Absent	Absent	Absent	10

FSSAI = Food Safety and Standards Authority of India (2011) permissible limits for processed cereal-based food products. Absent = below detection limit (<1 cfu/g).

4. Discussion

The present investigation revealed that the inclusion of coagulated mung bean protein and guava pulp greatly improved the nutritional, functional and antioxidant attributes of the nutraceutical bars. This progressive improvement in protein content from C to T4 may be explained by the high protein content of mung bean protein and the desirable amino acid profile of mung bean proteins, especially the globulins (vicilin and legumin), which are known to possess high digestibility and techno-functional properties. Hou *et al.* (2024) and Sharma *et al.* (2024) reported similar findings, noting mung bean protein as a promising sustainable plant protein that can be utilized for functional food products. The higher dietary fiber content in the optimized formulation is probably supplied by guava pectin and mucilage-rich basil seeds, which have been linked to enhancing gastrointestinal wellness and managing glycaemic levels (Singh *et al.,* 2024). Moreover, as the fat content decreased with the increase in the amount of guava pulp and coagulated protein incorporation, it may be possible to develop low-fat snack foods with a high nutrient value without sacrificing functionality.

A significant increase in total phenolic content, flavonoid concentration, β-carotene and DPPH radical scavenging activity was noted for the optimized formulation, which showed that the combination of mung bean and guava is synergistic in releasing bioactive compounds. Guava and mung bean are known as excellent sources of vitamin C; carotenoids, catechins, and quercetin derivatives for the former and vitexin and isovitexin for the latter, with proven health-promoting properties (Waqar *et al.*, 2024; Worku, 2024). This increased antioxidant activity observed in the current study could thus be attributed to the synergistic effect of polyphenols, flavonoids, carotenoids and Maillard reaction products produced during mild thermal treatment. The same enhancement of antioxidant properties has been reported with fruit bars or protein bars made with tropical fruits (Kourany *et al*., 2017). Furthermore, the positive correlation of phenolic content and antioxidant activity detected in the present work corroborates previous reports that phenolic compounds are one of the important contributors to free radical scavenging capacity in functional snack products (Yu *et al*., 2004). The optimized bar also provides a significant nutritional benefit with the greatly enhanced iron bioavailability. The ascorbic acid present in guavas might have increased the absorption of non-heme iron by converting ferric iron to the more absorbable ferrous form and by reducing the inhibitory effect of phytates and tannins, as observed by Joshi *et al.* (2024).

The protein quality, texture, color and microbiological stability of the developed bars also confirm their commercial and nutritional potential. The PDCAAS, DIAAS and C-PER values of the optimized formulation were satisfactory, showing good to moderate quality plant protein similar to other legume-based functional products (FAO/WHO, 2011). The sulfur-containing amino acids were still limiting, but it seemed that the product was suitable in diversified diets. The texture profile analysis showed that the hardness, cohesiveness and springiness values of the mung bean protein matrices were found within an acceptable range as compared to commercial snack bars, which could be attributed to stable interactions between mung bean protein matrices and guava pectin (Singh and Kaur, 2024). Furthermore, the gradual increase of L*, a* and b* values as the amount of guava incorporation increased, enhanced the visual attractiveness by the increased carotenoid pigmentation and suppression of the Maillard browning reactions. The microbiological evaluation showed that there was no growth of yeast, mold or coliform during the refrigerated storage period and that the bars always remained within the limits set by the FSSAI. Antimicrobial activity of the phytochemicals in guava, constituents of honey, and bioactive compounds of kalonji, along with low moisture content, may have contributed to the microbial stability of the product (Barakat, 2024). The present study has proved that the mung bean protein-guava nutraceutical bars have the potential to become a safe, functional, plant-based, antioxidant-rich snack for health-conscious athletes, vegans and nutritionally vulnerable populations.

5. Conclusion

The objective of this study was to develop and extensively characterize a novel mung bean protein-guava nutraceutical bar as a plant-based functional snack food. The optimized formulation (T4) showed significant improvement ($p<0.001$) in all the measured nutritional and bioactive properties as compared to the control. The key outcomes included crude protein content that was higher than the EFSA protein source threshold, DPPH antioxidant activity was 82.33%, TPC was 33.84 mg GAE/g, the mineral content was appreciable with a higher iron bioavailability (63.7% of total iron) and protein quality was satisfactory (PDCAAS = 0.70; DIAAS = 0.68) and maintained throughout 10-day refrigerated storage, and the product was microbiologically safe. The results suggest that the formulated nutraceutical-based snack bar is a promising and sustainable snack bar that can replace animal protein-based snack bars, especially for health-conscious consumers, vegans, athletes and those with iron deficiency anemia (IDA) and oxidative stress-induced chronic diseases. Scale-up study, techno-economic feasibility analysis, long-term storage stability (accelerated shelf-life testing), determination of glycemic index, and in vivo bioavailability studies are some of the points that should be explored for future work. Further research on other synergistic ingredients (such as moringa leaf powder, chia seeds) and other coagulants can further improve the nutritional and functional value.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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