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Compositional protein and lipid quality indices in Salak (*Salacca zalacca*) snack bars fortified with *Moringa oleifera*

ITA FATKHUR ROMADHONI[1,♥], DWI IRIYANI[2], RITA ISMAWATI[3], KRISTIAN TRIATMAJA RAHARJA[4]

[1]Study Program of Applied Bachelor of Culinary, Universitas Negeri Surabaya. Jl. Ketintang No. 1, Surabaya 60231, East Java, Indonesia. Tel./fax.: +62-812-3910-1600, ♥email: itaromadhoni@unesa.ac.id

[2]Study Program of Bachelor of Biology, Faculty of Sains and Technology, Universitas Terbuka. Jl. Dr. Ir. H. Soekarno No. 567, Surabaya 60293, East Java, Indonesia

[3]Undergraduate of Nutrition, Universitas Negeri Surabaya. Jl. Rektorat Unesa, Surabaya 60213, East Java, Indonesia

[4]Bachelor of Applied Health Promotion, Politeknik Negeri Madura. Jl. Raya Camplong Km. 4, Madura 69281, East Java, Indonesia

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Abstract. *Romadhoni IF, Iriyani D, Ismawati R, Raharja KT. 2026. Compositional protein and lipid quality indices in Salak (*Salacca zalacca*) snack bars fortified with* Moringa oleifera*. Asian J Agric 10 (1): g100165. https://doi.org/10.13057/asianjagric/g100165.* This study evaluated whether graded incorporation of *Moringa oleifera* Leaf Powder (MOLP) induces coordinated remodeling of protein and lipid composition in salak (*Salacca zalacca*) flour-based snack bars. A completely randomized design was used with five fortification levels (0-8% w/w MOLP substitution in salak flour), each replicated in three independent batches (n = 3). Indispensable amino acid composition of the snack bars was quantified using High-Performance Liquid Chromatography (HPLC) after acid hydrolysis and pre-column derivatization. Amino acid adequacy was evaluated relative to the FAO/WHO/UNU adult reference pattern through Amino Acid Scoring (AAS). Fatty acid composition was determined by gas chromatography with flame ionization detection (GC-FID), and lipid distribution was interpreted using composite indices including the Atherogenic Index (AI), Thrombogenic Index (TI), and the n-6/n-3 ratio. MOLP fortification produced a clear dose-dependent increase in the density of indispensable amino acids. Lysine increased from 36.13 to 58.71 mg g^{-1} protein, correcting the intrinsic lysine limitation of the fruit-derived matrix, whereas sulfur amino acids remained limiting across treatments. Fatty acid redistribution resulted in moderate increases in polyunsaturated fatty acids (up to 36.05% of total fatty acids). Composite lipid indices remained within balanced compositional ranges, with AI decreasing to 0.95, TI reaching 0.62, and the n-6/n-3 ratio maintained at 1.32 despite a constant palm oil source. These findings demonstrate that moderate MOLP inclusion (6-8%) can simultaneously modify indispensable amino acid adequacy and lipid compositional indices in a fruit-based snack matrix. Because digestibility and bioavailability were not assessed, the results represent compositional indicators rather than physiological metrics of protein quality.

Keywords: Amino acid score, fatty acid profile, *Moringa oleifera*, protein quality, *Salacca zalacca*

Abbreviations: AAS: Amino Acid Scores, AI: Atherogenic Index, AOAC: Association of Official Agricultural Chemists, DIAAS: Digestible Indispensable Amino Acid Score, EAA: Essential Amino Acids, FAO: Food and Agriculture Organization, MOLP: *Moringa oleifera* Leaf Powder, MUFA: Monounsaturated Fatty Acids, PUFA: Polyunsaturated Fatty Acids, SFA: Saturated Fatty Acids, TI: Thrombogenic Index, w/w: Weight per Weight

INTRODUCTION

Consumer demand for plant-based foods has encouraged the development of nutritionally improved products derived from locally available agricultural commodities (Çakıtlı and Nurko 2025). Beyond increasing total macronutrient content, contemporary reformulation strategies emphasize nutrient quality, particularly essential amino acid adequacy and fatty acid balance, as indicators of compositional functionality in plant-based food systems (Peñalver et al. 2022). Within tropical agricultural systems, diversification and value addition of underutilized crops represent critical strategies for enhancing farmer income stability, reducing post-harvest losses, and strengthening regional food resilience.

Salak fruit (*Salacca zalacca* (Gaertn.) Voss) is widely cultivated in Indonesia and several Southeast Asian countries; however, its utilization remains largely confined to fresh fruit markets (Kamalia et al. 2021; Mardiani et al. 2021; Prihatini et al. 2023). Seasonal production and limited shelf-life render salak vulnerable to post-harvest deterioration during peak harvest periods. Converting salak into flour improves storability and enables the production of value-added products. However, fruit flours often have low protein density and unbalanced amino acids, especially lysine and sulfur amino acids, limiting their biological quality (Ifmalinda et al. 2019). Snacks with vegetable oils or syrups often contain saturated and n-6 fatty acids from palm or refined oils, affecting lipid profiles. Therefore, salak-based nutrition optimization needs protein densification, not just more calories.

Moringa oleifera Lam. is recognized as a nutrient-dense plant resource with high protein content and a relatively balanced essential amino acid composition (Qadir et al. 2022). Dried moringa leaves contain lysine concentrations of approximately 55-65 mg g^{-1} protein and total protein approaching 30% on a dry weight basis (Coello et al. 2022).

In addition to protein richness, moringa leaf lipids contain appreciable levels of α-linolenic and linoleic acids, contributing to favorable Polyunsaturated Fatty Acid (PUFA) fractions (Ziani et al. 2019). Moringa attributes suggest it can enhance fruit-based matrices as a complementary strategy. However, leaf fortification faces constraints like green color, vegetal flavor, slight bitterness, and anti-nutritional factors such as phytates and polyphenols. Excessive levels may harm texture or taste. Therefore, finding the optimal fortification level is essential to balance nutrition and product quality.

Previous studies on the incorporation of moringa have primarily focused on proximate composition, antioxidant capacity, and increases in crude protein (Moyo et al. 2011; Peñalver et al. 2022). However, crude protein content alone does not reflect the adequacy of indispensable amino acids. Evaluation relative to FAO reference patterns provides a more biologically meaningful assessment of protein quality (WHO/FAO/UNU 2007). In parallel, lipid quality is frequently described using composite indices such as the Atherogenic Index and Thrombogenic Index (Ulbricht and Southgate 1991). These indices are mathematical descriptors derived from fatty acid distribution and integrate weighted contributions of specific saturated and unsaturated fatty acids. Importantly, AI and TI represent compositional predictors rather than clinical endpoints, and their interpretation should remain within the context of relative fatty acid modeling.

Despite the analytical relevance of FAO-based amino acid scoring and composite lipid indices, their combined application within fruit-derived snack matrices remains limited. Most fortification studies focus on cereal systems, with fruit flours rarely evaluated for their amino acid adequacy. The potential for protein remodeling in fruit-based matrices remains underexplored. Combining salak flour with MOLP offers nutritional densification and agricultural value. This approach enables shelf-stable use of a perishable fruit and increases amino acid density per biomass, supporting sustainable intensification. Based on the compositional complementarity between fruit-derived flours and leaf-derived protein sources, this study hypothesized that graded incorporation of *M. oleifera* Leaf Powder (MOLP) into salak (*S. zalacca*) flour snack bars would improve indispensable amino acid adequacy while simultaneously influencing fatty acid distribution within the lipid fraction. Specifically, MOLP fortification was expected to correct lysine limitations inherent in fruit-based matrices and to induce proportional redistribution of fatty acids reflected in composite lipid quality indices. Therefore, this study aimed to evaluate how increasing levels of MOLP (0–8% w/w) affect indispensable amino acid composition relative to FAO reference patterns and alter lipid compositional descriptors, including the Atherogenic Index (AI), Thrombogenic Index (TI), and the n-6/n-3 ratio.

Therefore, this study aimed to evaluate protein remodeling and lipid redistribution in salak-based snack bars fortified with graded levels of MOLP (0-8% w/w). Indispensable amino acid composition was assessed relative to the WHO/FAO/UNU (2007) adult reference pattern, and fatty acid distribution was analyzed using AI and TI as compositional descriptors. Particular emphasis was placed on identifying a fortification range that maximizes indispensable amino acid adequacy while maintaining balanced fatty acid patterning.

MATERIALS AND METHODS

Experimental design

A Completely Randomized Design (CRD) was employed with five levels of *M. oleifera* Leaf Powder (MOLP) fortification: 0% (control), 2%, 4%, 6%, and 8% (w/w substitution of salak flour). Each formulation was produced in three independent production batches (biological replicates, n = 3). The order of treatment production was randomized across production days to minimize potential systematic processing bias.

For each batch, chemical analyses were conducted in triplicate (technical replicates). Technical replicate values were averaged within batch prior to statistical analysis to avoid pseudoreplication. Therefore, the experimental unit for statistical inference was the independent production batch (n = 3). The overall analytical workflow used to evaluate protein and lipid quality is illustrated in Figure 1.

Raw materials

Ripe salak (*S. zalacca*) fruits were obtained from a local producer in Ponggok, Blitar, East Java, Indonesia. Fresh *M. oleifera* leaves with green petioles were sourced from a food-grade-certified supplier registered with the local agricultural authority (Diskoperindag Sumenep, Indonesia). All analytical reagents used in chemical analyses were of analytical grade.

Preparation of salak flour

Salak fruits were peeled, deseeded, and sliced (3–5 mm thickness). Slices were dried in a forced-air oven at 55°C for 24 h until the moisture content was <12%. Dried material was milled using a laboratory grinder and moved through a 60-mesh sieve to obtain uniform flour. Flour was stored in airtight polyethylene containers at room temperature (25 ± 2°C) until use.

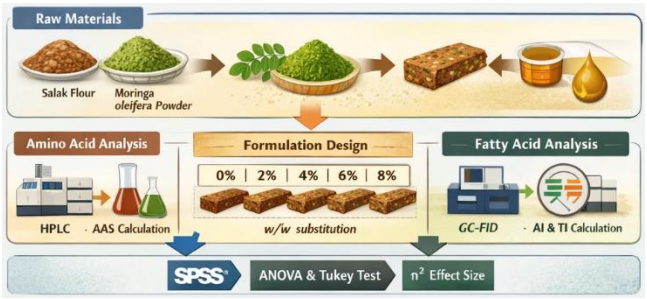

Figure 1. Experimental workflow for protein and lipid quality evaluation

Preparation of *M. oleifera* leaf powder (MOLP)

Fresh moringa leaves were washed, drained, and dried at ≤50°C for 18 h to minimize thermal degradation of amino acids and unsaturated fatty acids. Dried leaves were milled and sieved (60 mesh). MOLP was stored in sealed containers protected from light and moisture until formulation.

Snack bar formulation and processing

Snack bars were prepared using salak flour as the primary matrix with partial substitution by MOLP at five levels (0-8% w/w). The base formulation was adapted from Gunyaphan et al. (2020) with modifications. Ingredients consisted of salak flour, MOLP, glucose syrup, honey, palm-based vegetable oil, and egg white. All dry ingredients were thoroughly mixed prior to the addition of liquid ingredients. The mixture was homogenized at ambient temperature until a uniform dough was obtained. The dough was molded into rectangular bars (approximately 10 × 3 × 1 cm) and dried in a forced-air oven at 60°C for 6 h while waiting for the final moisture content was below 10% (AOAC 925.10) (AOAC International 2019). After drying and cooling, snack bars were sealed in polyethylene pouches and stored at room temperature (25 ± 2°C) prior to chemical analyses. The formulation of salak-moringa snack bars used to evaluate protein and lipid quality is shown in Table 1.

Amino acid analysis

For amino acid analysis, snack bar samples were finely ground into powder prior to hydrolysis. Amino acid composition of the snack bar samples was determined using High-Performance Liquid Chromatography (HPLC) following acid hydrolysis and pre-column derivatization with ortho-phthalaldehyde (OPA). Approximately 50 mg of the powdered snack bar sample was hydrolyzed with 2 mL of 6 N HCl in vacuum-sealed tubes under a nitrogen atmosphere to prevent oxidative degradation. Hydrolysis was performed at 110°C for 24 h (Fidyasari et al. 2024). After hydrolysis, the solution was allowed to cool to room temperature and then evaporated to dryness using a rotary evaporator. The hydrolysate was then reconstituted in 0.01 N HCl to a final volume of 10 mL. Prior to chromatographic analysis, amino acids were derivatized using OPA reagent. The OPA derivatization reagent consisted of 50 mg ortho-phthalaldehyde dissolved in 4 mL methanol, 0.025 mL β-mercaptoethanol, 0.050 mL Brij-30, and 0.5 M borate buffer (pH 10.4).

HPLC analysis was performed using a Thermo Scientific Hypersil ODS-2 column coupled with a fluorescence detector. Two mobile phases were used: Buffer A: sodium acetate buffer containing methanol, Na-EDTA, and tetrahydrofuran (THF) adjusted to pH 6.5. Buffer B: methanol (95%). Prior to injection, 25 µL of OPA reagent was added to the hydrolysate and incubated for 1 min. Subsequently, 5 µL of the derivatized sample was injected into the HPLC system. Amino acid concentrations were expressed as milligrams of amino acid per gram of protein (mg g^{-1} protein). Tryptophan was not determined because it is degraded during acid hydrolysis.

Amino acid scoring

Essential Amino Acid (EAA) scores were calculated relative to the WHO/FAO/UNU (2007) adult amino acid requirement pattern (≥18 years). The amino acid score (AAS) was determined using the following equation: AAS = (mg indispensable amino acid per g protein in sample) / (mg indispensable amino acid per g protein in FAO adult reference pattern)

The WHO/FAO/UNU (2007) adult reference pattern (mg g^{-1} protein) was used as the standard of comparison for each indispensable amino acid.

Sulfur amino acids were evaluated as the combined sum of methionine and cysteine (Met + Cys), in accordance with FAO recommendations. The sulfur amino acid score was therefore calculated as: Sulfur AAS = [(Met + Cys) sample (mg/g protein)] / [(Met + Cys) FAO reference (mg/g protein)].

$$\text{AAS} = \frac{\text{mg indispensable amino acid per g protein in sample}}{\text{mg indispensable amino acid per g protein in FAO adult reference pattern}}$$

$$\text{AAS}_{(Met+Cys)} = \frac{\text{(Met + Cys) content in sample (mg g}^{-1}\text{ protein)}}{\text{(Met + Cys) content in FAO adult reference pattern (mg g}^{-1}\text{ protein)}}$$

Aromatic amino acids were similarly evaluated as the combined sum of phenylalanine and tyrosine (Phe + Tyr) following FAO grouping principles. The limiting amino acid for each formulation was identified as the indispensable amino acid (or grouped amino acid) with the lowest amino acid score. Because true ileal digestibility was not determined in this study, the reported AAS values represent compositional indicators of indispensable amino acid adequacy rather than Digestible Indispensable Amino Acid Scores (DIAAS).

Fatty acid analysis

The fatty acid composition of the snack bar samples was determined after in situ preparation of Fatty Acid Methyl Esters (FAMEs) using the method of Park and Goins (1994). Approximately 50 mg of powdered snack bar sample was placed into a test tube and mixed with 100 µL dichloromethane and 1 mL of 0.5 N NaOH in methanol. The tube was flushed with nitrogen gas and heated at 90°C for 10 min to promote lipid saponification.

After cooling, 1 mL of 14% boron trifluoride (BF_3) in methanol was added, and the mixture was reheated at 90°C for 10 min to convert fatty acids to methyl esters. After the reaction, the mixture was cooled, then 1 mL of distilled water and 500 µL of hexane were added. The solution was vortexed and centrifuged to facilitate phase separation. The upper hexane layer containing FAMEs was collected for gas chromatographic analysis.

Table 1. The formulation of salak-moringa snack bars used to evaluate protein and lipid quality

Ingredient	0% MOLP	2% MOLP	4% MOLP	6% MOLP	8% MOLP
Salak flour	60	58	56	54	52
MOLP	0	2	4	6	8
Glucose syrup	20	20	20	20	20
Honey	10	10	10	10	10
Palm-based vegetable oil	5	5	5	5	5
Egg white	5	5	5	5	5

Note: Formulations are expressed as percentages of total mixture weight (w/w). Egg white was added as part of the binder system to facilitate mixing and molding of the snack bar matrix and was partially removed during oven drying

Gas chromatography analysis

Fatty Acid Methyl Esters (FAMEs) were analyzed using an Agilent 7890B gas chromatograph equipped with a flame ionization detector (GC-FID) and an HP-88 capillary column (100 m × 0.25 mm × 0.2 µm). The oven temperature program was set to start at 100 °C and increase to 240°C at a rate of 4°C min^{-1}, followed by a 15 min holding period. The detector and injector temperatures were maintained at 260°C. A split injection ratio of 10:1 was used with helium as the carrier gas and nitrogen as the make-up gas.

Fatty acids were detected by comparing retention times with those of a Supelco 37-component FAME standard mixture (Sigma-Aldrich). The fatty acid profile shows the relative concentration of each fatty acid. All analyses were conducted in triplicate per batch. Lipid health indices were calculated according to Ulbricht and Southgate (1991): AI = [C12:0 + (4 × C14:0) + C16:0] / (ΣMUFA + Σn-6 + Σn-3). TI = (C14:0 + C16:0 + C18:0) / [0.5 × ΣMUFA + 0.5 × Σn-6 + 3 × Σn-3 + (Σn-3 / Σn-6)]

$$\text{AI} = \frac{C12{:}0 + (4 \times C14{:}0) + C16{:}0}{\Sigma MUFA + \Sigma n\text{-}6 + \Sigma n\text{-}3}$$

Where, C12:0: Lauric acid, C14:0: Myristic acid, C16:0: Palmitic acid, ΣMUFA: Total monounsaturated fatty acids, Σn-6: Total omega-6 fatty acids, Σn-3: Total omega-3 fatty acids

Only identified fatty acids detected by GC-FID were included in the calculation.

$$\text{TI} = \frac{C14{:}0 + C16{:}0 + C18{:}0}{(0.5 \times \Sigma MUFA) + (0.5 \times \Sigma n\text{-}6) + (3 \times \Sigma n\text{-}3) + \left(\frac{\Sigma n\text{-}3}{\Sigma n\text{-}6}\right)}$$

Where, C14:0: Myristic acid; C16:0: Palmitic acid, C18:0: Stearic acid, ΣMUFA: Sum of monounsaturated fatty acids, Σn-6: Sum of omega-6 fatty acids, Σn-3: Sum of omega-3 fatty acids

All fatty acids were expressed as a percentage of the total identified fatty acids prior to index calculation. For statistical analysis and composite index calculation, fatty acid percentages were derived from full-precision normalized peak areas prior to rounding for tabulation, ensuring internal computational consistency across treatments.

The snack bar formulation contained a constant amount of vegetable oil (palm-based oil) across all treatments. No additional lipid sources were varied among fortification levels. Therefore, observed differences in fatty acid distribution and composite indices reflect proportional compositional changes associated with the inclusion of MOLP rather than differential oil supplementation. Fatty acid identification was based on retention time comparison with a Supelco 37-component FAME standard mixture. Minor peaks detected at very low abundance were interpreted cautiously due to the potential for co-elution in complex plant matrices.

Statistical analysis

The experimental unit was defined as the batch of snack bars produced independently. Each treatment level (0%, 2%, 4%, 6%, and 8% MOLP) was prepared in three independent production batches (n = 3), each manufactured on a separate day under identical conditions. For each batch, all chemical analyses (amino acid and fatty acid composition) were performed in triplicate (technical replicates). Technical replicate values were averaged within each batch prior to statistical analysis to avoid pseudoreplication. Thus, statistical comparisons were performed using batch means (n = 3 per treatment).

The statistical analysis procedure was as follows: initially, technical triplicate measurements were averaged within each batch. Next, batch means were evaluated for normality (Shapiro–Wilk test) and for equal variances (Levene's test). A one-way ANOVA was performed with batch means as the experimental unit. When significant differences were found ($\alpha = 0.05$), Tukey's post hoc test was used. Effect sizes were determined with eta-squared (η^2) to measure the strength of treatment effects on indispensable amino acid composition and composite lipid quality indices. Alongside p-values, 95% confidence intervals (CI) for treatment means were also computed to express uncertainty. Effect size classifications followed standard thresholds (0.01 = small; 0.06 = medium; ≥0.14 = large). All analyses were conducted with SPSS version 26.

RESULTS AND DISCUSSION

Statistical analyses were performed using independent batch means (n = 3 per treatment). Technical replicates within each batch were averaged before analysis to avoid pseudoreplication.

Indispensable amino acid composition

Graded MOLP fortification significantly affected the concentration of all indispensable amino acids ($p < 0.001$). Tukey HSD analysis confirmed that each fortification level differed significantly within individual amino acids ($p < 0.05$), demonstrating a consistent dose-dependent response (Table 2).

Lysine increased from 36.13 to 58.71 mg g^{-1} protein across treatments, representing the most nutritionally relevant

shift. Sulfur amino acids (Met + Cys) increased progressively but remained below adequacy (AAS < 1.0). Other indispensable amino acids exceeded adult reference requirements across all formulations.

Effect sizes were uniformly large (η^2 = 0.978–0.999), indicating that fortification level accounted for the vast majority of observed variance. These results confirm treatment-driven compositional remodeling rather than analytical variability.

Amino acid score relative to FAO/WHO/UNU (2007) adult pattern

Amino acid scores calculated against the WHO/FAO/UNU (2007) adult reference patterns are presented in Table 3. In the control formulation (0% MOLP), lysine (0.80) and sulfur amino acids (Met + Cys; 0.25) were below adequacy (score < 1.0), identifying sulfur amino acids as the primary limiting amino acid group.

With MOLP fortification, lysine scores increased to 1.09-1.30 at 2-8% inclusion, exceeding the adequacy threshold and indicating correction of lysine limitation. Sulfur amino acid scores increased progressively from 0.25 in the control to 0.48 at 6% inclusion, then decreased slightly to 0.46 at 8%; however, values remained below unity across all treatments, confirming persistent sulfur amino acid limitation.

Other indispensable amino acids consistently exceeded adult reference requirements across all formulations. Histidine ranged from 1.21 to 2.37, isoleucine from 1.16 to 1.59, leucine from 1.01 to 1.45, threonine from 1.24 to 1.95, and valine from 0.95 in the control to 1.53 at 8% inclusion. Aromatic amino acids (phenylalanine + tyrosine) showed high adequacy throughout treatments (2.36–2.55). Overall, MOLP fortification resulted in selective correction of lysine deficiency and progressive improvement of sulfur amino acid scores, while maintaining adequacy for all other indispensable amino acids relative to adult reference requirements.

Fatty acid composition

The proportional fatty acid distribution of the snack bars is presented in Table 4. Fatty acid composition reflected proportional redistribution across saturated, monounsaturated, and polyunsaturated fractions. Palmitate (C16:0) remained the dominant saturated fatty acid, increasing from 26.17% to 36.97% across treatments. Linoleate (C18:2n-6) and α-linolenate (C18:3n-3) were the primary PUFA fractions and increased substantially following fortification.

Composite indices demonstrated significant treatment effects ($p < 0.001$). The n-6/n-3 ratio increased from 0.93 to 1.32 in a dose-dependent manner. AI decreased to 0.95 at 8% inclusion, while TI reached a minimum of 0.55 at 4% inclusion and remained lower than control values across fortified treatments. Effect sizes were large to very large (η^2 = 0.923-0.996), indicating strong treatment influence on lipid redistribution. These findings indicate structured fatty acid reweighting rather than simple enrichment.

Table 3. Amino acid scores relative to FAO/WHO/UNU (2007) adult reference pattern

Amino Acid (or Group)	Reference (mg g^{-1} protein)	0% MOLP	2% MOLP	4% MOLP	6% MOLP	8% MOLP
Histidine	15	1.21	1.78	1.99	2.21	2.37
Isoleucine	30	1.16	1.37	1.40	1.54	1.59
Leucine	59	1.01	1.25	1.26	1.36	1.45
Lysine	45	0.80	1.09	1.11	1.26	1.30
Sulfur AA (Met + Cys)	22	0.25	0.40	0.44	0.48	0.46
Aromatic AA (Phe + Tyr)	38	2.36	2.40	2.55	2.50	2.51
Threonine	23	1.24	1.65	1.67	1.88	1.95
Valine	39	0.95	1.27	1.32	1.43	1.53

Note: Scores are calculated using mean indispensable amino acid values from Table 2. Tryptophan was not determined and therefore not scored. Amino Acid Score (AAS) = (mg indispensable amino acid per g protein in sample) / (mg indispensable amino acid per g protein in FAO adult reference pattern)

Table 2. Indispensable amino acid composition (mg g^{-1} protein) of salak snack bars fortified with *Moringa oleifera*

Amino acid	0% MOLP	2% MOLP	4% MOLP	6% MOLP	8% MOLP	η^2
Histidine	18.08±0.02^{e}	26.72±0.01^{d}	29.88±0.03^{c}	33.19±0.36^{b}	35.62±0.03^{a}	0.993
Isoleucine	34.72±0.00^{d}	41.24±0.07^{c}	41.93±0.04^{c}	46.20±0.01^{b}	47.82±0.03^{a}	0.986
Leucine	59.42±0.31^{d}	73.55±0.12^{c}	74.19±0.18^{c}	80.29±0.02^{b}	85.72±0.01^{a}	0.999
Lysine	36.13±0.04^{e}	48.84±0.02^{d}	49.99±0.04^{c}	56.86±0.33^{b}	58.71±0.11^{a}	0.994
Methionine	5.16±0.05^{e}	8.39±0.02^{d}	9.10±0.02^{c}	9.78±0.06^{a}	9.37±0.01^{b}	0.984
Cysteine	0.40±0.04^{d}	0.43±0.01^{c}	0.56±0.05^{b}	0.69±0.12^{a}	0.76±0.03^{a}	0.997
Phenylalanine	87.53±1.10^{a}	87.52±1.08^{a}	91.17±1.11^{b}	88.94±0.50^{a}	88.98±0.89^{a}	0.998
Tyrosine	2.20±0.03^{c}	3.78±0.04^{d}	5.89±0.05^{c}	6.15±0.06^{b}	6.56±0.04^{a}	0.992
Threonine	28.45±0.09^{e}	37.86±0.10^{d}	38.48±0.12^{c}	43.15±0.02^{b}	44.93±0.01^{a}	0.999
Valine	37.19±0.02^{e}	49.34±0.03^{d}	51.50±0.04^{c}	55.67±0.01^{b}	59.66±0.02^{a}	0.978

Note: Values are mean ± SD (n = 3). Different superscript letters within rows indicate significant differences among treatments (Tukey, $p < 0.05$). η^2 represents the effect size from a one-way ANOVA. Tryptophan was not determined due to degradation during acid hydrolysis and was excluded from statistical analysis. Statistical comparisons were performed using full-precision analytical values prior to rounding; therefore, treatments with similar rounded means may still differ significantly

Composite lipid quality indices

Composite lipid descriptors derived from quantified fatty acids are presented in Table 5. One-way ANOVA revealed significant treatment effects for all indices ($p < 0.001$), with very large effect sizes for n-6/n-3 ratio ($\eta^2 = 0.996$) and Atherogenic Index (AI; $\eta^2 = 0.977$), and a large effect for Thrombogenic Index (TI; $\eta^2 = 0.923$).

The n-6/n-3 ratio increased progressively from 0.93 in the control to 1.32 at 8% MOLP inclusion, with each treatment level differing significantly from one another (distinct superscript letters, $p < 0.05$). This indicates a dose-dependent proportional shift toward greater relative n-6 representation.

The Atherogenic Index (AI) showed a non-linear pattern. The highest value was observed at 2% inclusion (1.09^{d}), while the lowest value occurred at 8% inclusion (0.95^{a}). Intermediate treatments displayed statistically distinct groupings, with 6% inclusion (0.98^{b}) significantly lower than control (1.07^{c}) and 2% treatment.

The Thrombogenic Index (TI) reached its minimum at 4% inclusion (0.55^{a}), which differed significantly from the control (0.64^{c}). The 6% treatment (0.61^{bc}) showed partial overlap with adjacent levels, consistent with Tukey subgrouping, indicating moderated redistribution rather than strictly linear change. Collectively, these results demonstrate that MOLP fortification significantly influenced composite lipid descriptors, with treatment level accounting for more than 90% of the total variance across all indices.

Table 5. Composite lipid quality indices of salak–moringa snack bars

Parameter	0%	2%	4%	6%	8%	η^2
n-6/n-3 ratio	0.93^{a}	1.14^{b}	1.17^{c}	1.27^{d}	1.32^{e}	0.996
Atherogenic Index (AI)	1.07^{c}	1.09^{d}	1.07^{c}	0.98^{b}	0.95^{a}	0.977
Thrombogenic Index (TI)	0.64^{c}	0.59^{b}	0.55^{a}	0.61^{bc}	0.62^{c}	0.923

Note: The n-6/n-3 ratio was calculated from quantified C18:2n-6, C20:3n-6, C20:4n-6, C22:2n-6 and C18:3n-3, C20:5n-3, C22:6n-3 fractions. TI and AI were calculated according to a previous study (Ulbricht and Southgate 1991). η^2 values indicate extremely large treatment effects for n-6/n-3 and AI (>0.97) and a large effect for TI (>0.90), demonstrating that fortification level explained the majority of observed variance in composite lipid descriptors. Composite indices were calculated directly from normalized individual fatty acid values, prior to rounding, to ensure internal computational consistency

Table 4. Individual fatty acid composition of salak–moringa snack bars (% of identified fatty acids)

Saturated Fatty Acids (SFA)	0% MOLP	2% MOLP	4% MOLP	6% MOLP	8% MOLP
C10:0 (Caprate)	0.11±0.05	0.26±0.03	0.31±0.03	0.28±0.03	0.33±0.10
C12:0 (Laurate)	0.42±0.04	0.91±0.03	0.95±0.07	0.78±0.02	0.82±0.02
C14:0 (Myristate)	2.14±0.39	3.93±0.46	3.96±0.21	3.20±0.02	3.25±0.03
C16:0 (Palmitate)	26.17±0.64	35.62±0.78	36.18±5.11	36.89±0.57	36.97±0.59
C18:0 (Stearate)	1.98±0.03	2.65±0.01	2.91±0.01	3.54±0.01	3.76±0.02
C20:0 (Arachidate)	1.05±0.03	1.70±0.01	0.92±0.01	1.76±0.01	1.82±0.02
C22:0 (Behenate)	0.36±0.03	0.62±0.01	0.71±0.01	0.79±0.01	0.82±0.02
C24:0 (Lignocerate)	0.94±0.02	1.81±0.06	1.82±0.07	1.76±0.10	1.80±0.08
Total SFA (ΣSFA)	33.17	47.50	47.76	49.00	49.57
Monounsaturated Fatty Acids (MUFA)					
C16:1n-7 (Palmitoleate)	2.16±0.03	3.97±0.01	4.05±0.01	3.57±0.02	3.66±0.01
C18:1n-9 (Oleate)	2.33±0.02	3.23±0.01	3.79±0.01	3.88±0.02	3.92±0.01
trans-C18:1n-9 (Elaidate)	4.75±0.73	6.82±0.87	6.90±0.43	6.13±0.13	6.89±0.14
C20:1n-9 (Eicosenoate)	0.51±0.03	0.89±0.01	1.12±0.00	5.96±0.68	6.05±0.72
Total MUFA (ΣMUFA)	9.75	14.91	15.86	19.54	20.52
Polyunsaturated Fatty Acids (PUFA)					
C18:2n-6 (Linoleate)	9.23±0.10	17.91±0.01	19.23±0.08	16.96±0.99	17.11±0.77
C18:3n-3 (α-Linolenate)	11.18±0.72	16.36±0.61	16.92±0.60	15.99±0.34	16.15±0.22
C18:3n-6 (γ-Linolenate)	0.31±0.23	0.38±0.01	0.82±0.01	0.78±0.01	0.86±0.01
C20:3n-6 (Dihomo-γ-linolenate)	0.76±0.01	0.85±0.02	1.54±0.01	1.49±0.01	1.53±0.01
C20:4n-6 (Arachidonate)	0.67±0.04	0.82±0.07	1.11±0.28	1.82±0.83	1.94±0.60
C20:5n-3 (Eicosapentaenoate)	0.40±0.08	0.69±0.09	1.73±0.01	0.93±0.00	0.43±0.01
C22:2n-6 (Docosadienoate)	0.09±0.02	0.20±0.01	0.20±0.03	0.88±0.00	0.92±0.10
C22:6n-3 (Docosahexaenoate)	0.34±0.03	0.64±0.02	0.93±0.03	0.29±0.08	0.31±0.06
Total PUFA (ΣPUFA)	22.98	37.85	42.48	39.14	39.25

Note: Values are expressed as mean ± SD of three independent production batches (n = 3). Fatty acids are described as percentages of total identified fatty acids detected by GC-FID in each chromatogram. Peak areas were normalized to 100% prior to statistical analysis and composite index calculation. Reported values are rounded to two decimal places for presentation; therefore, minor deviations from 100% may occur when summing individual fatty acids due to rounding. Fatty acids present at <0.10% were included in normalization and index calculations but are not individually displayed

Comparative nutritional positioning

A comparative overview of selected nutritional indicators is presented in Table 6. The table compares the present formulation with representative studies published between 2020 and 2026.

As shown in Table 6, the 8% MOLP snack bars formulation had a lysine content of 58.71 mg/g protein, significantly higher than the 14.9-16.9 mg/g reported for Strychnos madagascariensis fruit flour (Chemane et al. 2022). This demonstrates that fortifying with MOLP greatly increased the density of essential amino acids, especially lysine, which is often limited in plant-based ingredients. In terms of lipid quality, the formulation contained 36.05% total PUFA, placing it within the mid-range of cereal-insect composite flours (30.01-64.24%) and exceeding the ~8-10% found in fruit-based flours. The Atherogenic Index (AI = 0.95) and Thrombogenic Index (TI = 0.62) reflect a favorable lipid profile, with TI values lower than those of cricket powders from industrial by-product diets (0.83-1.10) (Domiszewski et al. 2026). Although these studies provide useful reference points, direct comparison should be interpreted cautiously because the food matrices differ substantially (fruit flours, insect powders, and cereal extrudates). These matrices differ in ingredient composition, processing conditions, and lipid sources, which can influence nutrient distribution and lipid quality indices. The n-6/n-3 ratio of 1.32 is particularly notable, as it is well below the typical Western ratio (>10) and significantly lower than insect powder ratios (6.80-16.44), indicating a more balanced polyunsaturated fatty acid profile that is within ranges considered compositionally balanced (Willett et al. 2019).

From a protein quality perspective, the lysine density of the 8% MOLP formulation substantially exceeds that reported in fruit- or cereal-based functional ingredients, indicating that MOLP inclusion effectively mitigates common lysine limitations observed in plant-derived snack matrices. These findings position the formulation within the range of protein-quality-enhanced plant-based snack systems. In terms of lipid functionality, although the total PUFA level is moderate rather than maximal, the qualitative distribution of fatty acids (as reflected by TI and n-6/n-3) is more favorable than several insect-derived flours reported in recent literature. This distinction is important: the impact on lipid health is driven not solely by PUFA quantity but by fatty acid patterning and relative compositional balance.

Furthermore, the combination of a low thrombogenic index (0.62) and a balanced n-6/n-3 ratio (1.32) suggests that the present formulation achieves a compositional lipid index profile within the range of several emerging compositional positioning ingredients. Collectively, these findings indicate that MOLP fortification confers a dual functional enhancement, improving both indispensable amino acid adequacy and lipid health indices, and thereby strengthening the product's positioning within the functional snack and plant-based system.

MOLP inclusion induced structural remodeling of indispensable amino acid density rather than merely increasing crude protein content. The substantial lysine increase addresses the primary limitation of fruit-derived matrices, which are typically lysine-deficient. This demonstrates effective botanical complementation within a non-cereal system, extending traditional plant protein blending paradigms beyond cereal-legume frameworks. MOLP inclusion did not simply elevate total nitrogen but reconfigured indispensable amino acid composition, yielding a lysine density (58.71 mg/g protein) substantially above values reported for fruit-based flours (Chemane et al. 2022). This distinction is critical. Fruit-derived matrices are inherently lysine-limited and rarely serve as protein-quality carriers (Ifmalinda et al. 2019). By integrating leaf-derived protein into a fruit system, this study operationalizes plant protein complementation in a matrix where such correction is not traditionally explored (WHO/FAO/UNU 2007). In doing so, it expands classical cereal-legume paradigms into a leaf–fruit compositional integration model, thereby redefining the functional potential of fruit-based snack systems.

Table 6. Comparative nutritional indicators between the present study (8% MOLP) and selected literature (2020-2026)

Study	Product type	Lysine (mg/g protein)	Total PUFA (% total FA)	AI	TI	n-6/n-3 ratio
Present study (8% MOLP)	Salak snack bar fortified with *Moringa oleifera* Lam.	58.71	36.05	0.95	0.62	1.32
Chemane et al. (2022)	*Strychnos madagascariensis* Poir. fruit flour	14.9-16.9	~8-10	0.28	0.60	-
(Domiszewski et al. 2026)	Cereal and insect flours (millet, rice, cricket, mealworm)	-	30.01-64.24	0.10-0.48	0.22-1.14	-
Igual et al. (2025)	Corn extrudates enriched with hemp (12.5%)	-	59.6	0.207	0.377	-
Andrade et al. (2025)	*Acheta domesticus* (Linnaeus, 1758) powder (industrial by-product diets)	-	29.6-33.1*	0.51-0.53	0.83-1.10	6.80-16.44

Note: Present study values (8% MOLP) correspond to the final recalculated lipid indices reported in Table 5 of the manuscript (AI = 0.95; TI = 0.62; n-6/n-3 = 1.32; PUFA = 36.05%). PUFA range in Domiszewski et al. (2026) reflects different cereal-insect matrices (30.01-64.24%). PUFA values in Andrade et al. (2025) correspond to reported PUFA percentages for FI and FII powders (29.6-33.1%). "-" indicates a parameter not reported in the original study. Values are presented exactly as reported in the respective publications; no recalculation was performed for external studies

Although sulfur amino acids remained limiting, the selective correction of lysine substantially improved overall indispensable amino acid balance. Because digestibility was not assessed, amino acid scores should be interpreted as indicators of compositional adequacy rather than physiological utilization metrics. Nonetheless, the magnitude of lysine densification confirms MOLP as a protein quality modulator within horticultural matrices. As emphasized by FAO (2013) and Rutherfurd et al. (2015), compositional scores (AAS) do not substitute for Digestible Indispensable Amino Acid Scores (DIAAS). Nevertheless, within a compositional modeling framework, the present data demonstrate that moderate MOLP inclusion can structurally elevate indispensable amino acid adequacy without reformulating the base matrix or relying on isolated protein concentrates. This positions MOLP not as a protein additive, but as a protein quality modulator within horticultural systems.

Trace levels of long-chain n-3 fatty acids, such as EPA and DHA, have occasionally been reported in plant-derived matrices, including *M. oleifera* leaf powder, although their concentrations are generally very low and may arise from minor biosynthetic pathways or from chromatographic co-elution with structurally related polyunsaturated fatty acids (Fidyasari et al. 2024). Importantly, the relative contribution of these minor peaks to the total fatty acid pool was negligible and did not materially influence the calculated lipid quality indices. Composite descriptors such as the Atherogenic Index (AI), Thrombogenic Index (TI), and the n-6/n-3 ratio are primarily driven by the dominant fatty acid fractions, particularly C16:0, C18:1, C18:2n-6, and C18:3n-3. Therefore, the observed compositional trends in lipid indices remain robust and reflect a proportional redistribution of major fatty acid classes associated with MOLP incorporation rather than the presence of trace amounts of long-chain PUFA.

AI remained below unity, and TI declined relative to control, indicating favorable restructuring of pro- and anti-thrombogenic fatty acids. Total PUFA (36.05%) remains moderate compared to highly enriched extrudates (Igual et al. 2025), but must be interpreted through composite indices (Ulbricht and Southgate 1991). The AI staying below 1 (0.95) and the TI decreasing to 0.62 indicate a favorable rebalancing of pro- and anti-thrombogenic fatty acids. Notably, the TI was lower than that observed in insect-derived powders made from industrial by-product diets (Andrade et al. 2025), highlighting improved proportional balance rather than quantitative excess. As Chen and Liu (2020) clarify, AI and TI are standardized compositional comparators rather than clinical predictors. This formulation shows lipid restructuring without implying direct cardiometabolic health claims. The n-6/n-3 ratio (1.32) further supports controlled redistribution; although not approaching unity, it remains substantially lower than ranges reported for insect systems (6.80-16.44) (Andrade et al. 2025) and well below typical western dietary levels linked to inflammation.

Importantly, this balance was maintained despite constant palm oil input, underscoring MOLP's role in stabilizing omega patterns in refined oil-based snack systems. These changes occurred without altering the primary lipid source, demonstrating that botanical integration can modulate fatty acid patterning within fixed oil systems. Within this framework, MOLP inclusion optimized lipid patterns rather than enriched lipids. The broader novelty of this work lies in its systems integration. Few studies concurrently apply FAO amino acid scoring and composite lipid indices within plant-based snack matrices (Chemane et al. 2022; Igual et al. 2025; Domiszewski et al. 2026). Most moringa formulations emphasize antioxidant activity or crude protein elevation (Dhawi et al. 2020; Farooq and Koul 2020). Here, both the adequacy of indispensable amino acids and the redistribution of the lipid index were evaluated simultaneously, enabling multidimensional nutritional positioning.

From an agricultural systems perspective, this approach increases nutrient output per unit of harvested biomass without expanding farmland. Processing salak flour reduces its post-harvest spoilage, while adding moringa leaves increases the essential amino acid content in the same production system. This modification supports sustainable intensification by prioritizing nutritional yield over land expansion (Willett et al. 2019; Ndhlala and Tshabalala 2023). The key innovation of this study lies not only in ingredient fortification but also in engineering nutrient density within current horticultural value chains.

Overall, the data demonstrate that moderate MOLP inclusion induces coordinated remodeling of proteins and lipids within a fruit-derived snack matrix. Instead of simply increasing individual nutrients, the formulation targets the provision of essential amino acids and the balanced redistribution of fatty acids. This dual approach to modification redefines fortification as a matter of compositional adjustment rather than just increasing nutrient quantities. By incorporating leaf biomass into a fruit-based system, the study demonstrates how nutritional density can be improved using existing agricultural infrastructure, without needing major reformulation or external protein sources.

These findings provide proof of concept for compositional remodeling in fruit-derived snack matrices. This provides a scalable pathway to create nutrient-rich, shelf-stable horticultural products consistent with sustainable intensification principles. The next section summarizes these results in the context of developing functional foods and adding value to agriculture. While the current findings show an increase in indispensable amino acid density, additional research on digestibility and bioavailability is necessary to verify how these nutrients are physiologically utilized. Although compositional improvements in nutritional quality were observed, the practical development of MOLP-fortified snack products will ultimately depend on economic feasibility, raw material availability, and production scalability in existing food processing frameworks. Despite the observed improvements in nutritional composition, the inclusion of MOLP may modify sensory attributes such as color, flavor, and overall palatability. Accordingly, future studies should incorporate sensory evaluation to assess the acceptability of MOLP-fortified snack products among consumers.

In conclusion, moderate incorporation of *M. oleifera* leaf powder (6-8%) induced coordinated protein and lipid remodeling within a fruit-derived snack matrix. Increasing MOLP levels from 0 to 8% (w/w) significantly enhanced indispensable amino acid density, particularly lysine, which increased from 36.13 to 58.71 mg g^{-1} protein, thereby correcting the intrinsic lysine limitation of the fruit-based matrix (AAS increased from 0.80 to 1.30). Despite this improvement, sulfur amino acids (Met + Cys) remained the limiting group, with scores ranging from 0.25 to 0.48 across treatments. Lipid redistribution was also observed, with total PUFA reaching 36.05% of identified fatty acids, while composite lipid descriptors remained within favorable compositional ranges. The Atherogenic Index (AI) decreased to 0.95, the Thrombogenic Index (TI) reached 0.62, and the n-6/n-3 ratio increased modestly to 1.32, indicating balanced fatty acid patterning despite the constant palm-oil input. The results provide a scalable framework for nutrient densification and post-harvest value addition in tropical crop systems.

However, validation through digestibility, bioavailability, oxidative stability, and sensory evaluation remains necessary to confirm translational applicability. Future studies should incorporate digestibility-based protein quality assessments (DIAAS), GC–MS confirmation of fatty acid profiles, oxidative stability analysis, and sensory evaluation to verify the nutritional functionality and consumer acceptability of MOLP-fortified salak snack products. Such investigations will strengthen the translational potential of leaf–fruit compositional integration as a strategy for nutritional densification and post-harvest value addition in tropical horticultural systems.

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