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Nutritional, Physicochemical, and Microbiological Quality of Soy Yoghurt Fortified with Fresh *Moringa oleifera* Leaf Aqueous Extract During Refrigerated Storage

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Abstract. Growing interest in plant-based fermented foods has encouraged the development and evaluation of non-dairy yoghurt formulations. This study evaluated the effects of formulation and refrigerated storage time on the nutritional, physicochemical, and microbiological characteristics of soy yoghurt containing fresh *Moringa oleifera* leaf aqueous extract. Four formulations containing different combinations of moringa, garlic, and lime extracts were prepared in three independent production batches. Samples were stored at 4 ± 1 °C and analyzed on Days 7 and 14 for proximate composition, calculated energy value, pH, titratable acidity, aerobic plate count (APC), and lactic acid bacteria (LAB). Two-way analysis of variance showed significant formulation, storage time, and/or interaction effects on most measured variables, while titratable acidity was primarily affected by storage time. Formula B had the highest crude protein, fat, and calculated energy values at both sampling times, whereas Formula C had the highest carbohydrate content. Formula D showed the lowest APC on Day 7 and the highest LAB population at both sampling times, reaching 6.79 $\log_{10}$ CFU/mL on Day 14. LAB populations remained above 6 $\log_{10}$ CFU/mL in all formulations at both sampling times. These findings indicate that formulation composition influenced the measured characteristics of soy yoghurt between Days 7 and 14, although the independent contribution of fresh *Moringa oleifera* leaf aqueous extract could not be separated from those of garlic and lime.

Keywords: aerobic plate count; calculated energy value; lactic acid bacteria; plant-based fermentation

1. Introduction

The global market for plant-based foods has grown rapidly over the last decade, fueled by shifting dietary habits, rising awareness of environmental sustainability, and demand for alternatives for people with lactose intolerance or milk allergies (Longworth et al., 2024; Xie et al., 2023). In this expanding sector, fermented plant-based products are especially notable because fermentation not only prolongs shelf life but also enhances flavor, texture, and overall acceptability. Among plant substrates, soybeans remain a popular choice due to their excellent nutritional profile, which includes high-quality protein, unsaturated fats, and essential micronutrients that support the production of fermented foods (Fan et al., 2024;; Wang et al., 2025). Consequently, soy yoghurt has become an important non-dairy alternative to conventional dairy yoghurt.

Despite these advantages, producing soy yoghurt with consistent quality remains challenging. Unlike dairy milk, soy milk does not contain casein micelles, and its gel structure is mainly formed through the acid-induced aggregation of denatured soy proteins. These structural differences influence water retention, buffering capacity, acid development, and microbial viability during storage (Yang et al., 2024). As a result, formulation methods effective for dairy yogurt are not always suitable for soy-based products. Therefore, understanding ingredient interactions within the soy protein matrix is crucial for developing products with stable nutritional value, desirable physical and chemical properties, and good

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microbiological quality. Plant-based matrices also differ in protein structure, fermentable carbohydrate content, and emulsion behavior. These differences may result in weak gel formation, undesirable flavor, unstable acidity, and inconsistent survival of lactic acid bacteria, thereby reducing product quality and consumer acceptance (Montemurro et al., 2021;). These formulation challenges have encouraged the use of specialized starter cultures and stabilizers to improve texture and fermentation consistency in plant-based yoghurt (Brugnoli et al., 2023; Yasar et al., 2022).

Soy milk provides a promising counterpoint to this perception. Soybeans are nutrient-dense and widely cultivated, and soy milk is naturally lactose-free, while providing essential macronutrients and micronutrients that support fermentation and product functionality (Fan et al., 2024). Nevertheless, despite soy's established role in food systems, systematic datasets describing the nutritional, physicochemical, and microbiological behavior of fermented soy yogurt—particularly under different functional fortification strategies and during refrigerated storage—remain comparatively limited relative to dairy-based yogurt or other plant substrates (Ma et al, 2023). This gap is important because storage stability is a key determinant of product feasibility, quality consistency, and consumer satisfaction, particularly for fermented foods that undergo ongoing biochemical changes after fermentation.

Previous studies have often evaluated only selected attributes, such as sensory acceptance, antioxidant activity, or individual physicochemical characteristics, making it difficult to understand how formulation simultaneously affects nutritional composition, physicochemical behavior, and microbiological quality during storage (Bhusal et al., 2022; Mehta et al., 2023; Romadhoni et al., 2026). Studies using fresh *Moringa oleifera* leaf aqueous extract in soy yoghurt also remain scarce compared with those using dried leaf powder (Peñalver et al., 2022). Comparative evidence on soy yoghurt formulated with fresh moringa leaf aqueous extract and evaluated simultaneously for nutritional, physicochemical, and microbiological characteristics during refrigerated storage remains limited (Adepoju & Selezneva, 2020; Adewumi et al., 2022). Detailed research on moringa leaves found that the vitamin E content is 82.858 mg/100 g, demonstrating that moringa leaves have the potential to enhance the benefits of yogurt products (Rambu et al, 2025). Rather than focusing on a single quality attribute, the present study therefore adopted an integrated approach to evaluate several quality indicators within a single experimental design.

Therefore, this study evaluated the effects of formulation and refrigerated storage time on the nutritional composition, physicochemical characteristics, and microbiological quality of soy yoghurt containing fresh *Moringa oleifera* leaf aqueous extract. Measurements were conducted on Days 7 and 14 of refrigerated storage. This approach provides comparative evidence on formulation-related quality changes in soy yoghurt prepared with different combinations of moringa, garlic, and lime extracts.

2. Materials and Methods

This experimental study evaluated the effects of formulation and refrigerated storage time on the nutritional, physicochemical, and microbiological characteristics of soy yoghurt fortified with fresh *Moringa oleifera* leaf aqueous extract. A completely randomized factorial design was used, consisting of four formulations and two refrigerated storage times, namely Days 7 and 14. Three independent production batches were prepared for each formulation (n = 3). Product preparation was conducted in Udanawu, Blitar, East Java, Indonesia, while laboratory analyses were performed at the Food Science Laboratory, Faculty of

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Dried soybeans were obtained from a local supplier in Blitar, East Java. Fresh *Moringa oleifera* leaves, garlic (*Allium sativum*), and key lime (*Citrus aurantiifolia*) were purchased from a local market on the day of processing. A commercially activated starter culture (Assorted Starter, Agavie®, AGAVILab, Indonesia), declared by the manufacturer to contain *Lactiplantibacillus plantarum*, was added to soy milk at 10% (v/v).

Fresh moringa leaves were washed to remove adhering impurities. Fifty grams of leaves were homogenized with 50 mL of distilled water for 90 s using a blender. The homogenate was filtered through muslin cloth to obtain approximately 55 mL of aqueous extract. The extract was prepared immediately before yoghurt production, and 10 mL was added to each moringa-containing formulation. The phytochemical composition of the extract was not characterized in the present study.

Fresh garlic cloves were peeled, washed, crushed, and homogenized with distilled water. The homogenate was filtered to remove coarse particles, and the resulting aqueous filtrate was used immediately as garlic extract. Aqueous extraction is appropriate for recovering water-soluble garlic constituents; published procedures commonly involve crushing or homogenizing fresh garlic in distilled water followed by filtration (Hayat et al, 2018).

Fresh key lime fruits were washed, cut aseptically, and manually squeezed. The juice was filtered to remove seeds, pulp, and coarse residues before being added to the formulations. A published procedure for *Citrus aurantiifolia* prepared the juice by cleaning the fruits, cutting them with a sterile knife, aseptically squeezing them into a sterile vessel, and clarifying the juice to remove residues (Vong et al, 2018).

Soybeans were soaked in water for approximately 12 h, washed, and blended with distilled water at a 1:1 ratio. The resulting slurry was filtered through cheesecloth to obtain soy milk. The soy milk was pasteurized at 85 °C for 15 min and then cooled to approximately 40 °C before the addition of the plant ingredients and starter culture.

Four formulations were prepared. Formula A contained 0.5% garlic extract and 0.5% lime extract. Formula B contained 10 mL of fresh moringa leaf aqueous extract and 1.0% lime extract. Formula C contained 10 mL of fresh moringa leaf aqueous extract and 1.0% garlic extract. Formula D contained 10 mL of fresh moringa leaf aqueous extract, 0.5% garlic extract, and 0.5% lime extract. The percentages of garlic and lime extracts were calculated relative to the total formulation volume. Each formulation was transferred into sterile 200 mL containers before fermentation.

Fermentation was conducted at 40 °C for 21 h. The temperature was selected because temperatures around 37–40 °C are commonly used to support the growth and acidification activity of lactic acid bacteria in soy-based fermented products. Previous studies have reported successful soy yoghurt fermentation using lactic acid bacteria at approximately 37 °C for up to 24 h, indicating that prolonged fermentation may be required to achieve gel formation in soy matrices (Jo et all., 2025). Because pH was not recorded as a fermentation endpoint in the present study, fermentation was terminated after 21 h when a continuous coagulated matrix had formed without visible phase separation. This operational endpoint was used instead of a predetermined pH value. After fermentation, the products were stored at 4 ± 1 °C and analyzed on Days 7 and 14.

Proximate composition, including carbohydrate, crude protein, crude fat, crude fiber, moisture content, and gross energy, was determined using standard methods of the AOAC (AOAC, 2019). The calculated energy value was estimated from carbohydrate, crude protein, and crude fat contents using the general Atwater factors of 4 kcal/g for carbohydrate, 4 kcal/g for

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protein, and 9 kcal/g for fat. These factors metabolizable energy rather than gross energy measured by bomb calorimetry (Ziarno, M., et al, 2023). The pH of soy yoghurt was measured at room temperature using a calibrated digital pH meter (Mettler Toledo®). Titratable acidity was determined by titration with standardized 0.1 N NaOH and expressed as percentage lactic acid. yoghurt. Titratable acidity (TTA) was determined by titration with a standardized sodium hydroxide solution and expressed as the percentage of lactic acid, following established analytical procedures (De Santis et al., 2019).

Aerobic plate count (APC) and total lactic acid bacteria (LAB) were determined using the standard plate count technique. Serial decimal dilutions were prepared using sterile physiological saline before plating on the appropriate culture media. APC and LAB populations were expressed as $\log_{10}$ colony-forming units per milliliter ($\log_{10}$ CFU/mL**)** following incubation under the recommended conditions (De Santis et al., 2019). The microbiological analyses quantified only total LAB populations and APC. No molecular identification or strain-specific characterization of bacterial isolates was performed.

Data are presented as mean ± standard error (SE) of three independent production batches. Two-way analysis of variance was used to evaluate the main effects of formulation and refrigerated storage time and their interaction. When significant differences among formulations were detected, mean comparisons were conducted using Duncan's Multiple Range Test at $p < 0.05$. Statistical analyses were performed using IBM SPSS Statistics version 27.0.

3. Results and Discussion

The proximate composition and calculated energy value of soy yoghurt were affected differently by formulation and refrigerated storage time (Table 1). Two-way analysis of variance showed significant effects of formulation and storage time on carbohydrate, crude protein, fat, moisture, and calculated energy value. Significant interactions between formulation and storage time were observed for carbohydrate, crude fiber, crude protein, fat, and moisture, indicating that storage-related changes depended on formulation composition. Crude fiber was significantly affected by storage time and the formulation-storage time interaction, whereas the overall formulation effect was not significant. The interaction between formulation and storage time was not significant for the calculated energy value.

Carbohydrate content was significantly affected by formulation, storage time, and their interaction. Formula C had the highest carbohydrate content on both sampling days, whereas Formula B had the lowest. Carbohydrate content increased slightly from Day 7 to Day 14 in all formulations, although the magnitude of the increase differed among formulations. The significant interaction indicates that storage-related changes depended on formulation composition.

The observed differences may reflect variation in ingredient composition and in the amount of carbohydrate remaining after fermentation. The utilization of carbohydrates in fermented soymilk depends on the LAB strain, substrate composition, and processing conditions. Fermentation with different LAB strains has been shown to produce distinct changes in the nutrient composition of soybean yoghurt alternatives (Gan et al., 2023). Starter culture selection may also influence carbohydrate-related changes during refrigerated storage of fermented soy beverages (Ziarno et al., 2023). However, individual sugars were not quantified in the present study; therefore, the carbohydrate fractions responsible for the observed differences could not be identified.

Crude protein was significantly affected by formulation, storage time, and their interaction. Formula B had the highest crude protein content on Days 7 and 14, whereas Formula D had the lowest. Protein content decreased between Days 7 and 14 in

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all formulations, but the magnitude of the decrease differed among formulations.

The substrate composition and metabolic characteristics of the starter culture can influence protein-related changes in fermented soy products. LAB strains used in soy yoghurt fermentation differ in growth, acidification, and protease activity, which may contribute to differences in the resulting product characteristics (Jo et al., 2025). Nevertheless, only crude protein was measured in the present study. Peptide composition, amino acids, and proteolytic activity were not determined. Therefore, the observed decrease should not be interpreted as direct evidence of proteolysis or peptide formation.

Table 1. Proximate composition and calculated energy value of soy yoghurt formulations during refrigerated storage

Parameter	Day	Formula A	Formula B	Formula C	Formula D	*p*-formulation	*p*-storage time	*p*-interaction
Carbohydrate (%)	7	6.76±0.03^{c}	6.54±0.01^{d}	7.49±0.02^{a}	6.84±0.01^{b}	<0.001	<0.001	0.010
	14	7.03±0.04^{b}	6.71±0.01^{d}	7.71±0.02^{a}	6.95±0.02^{c}			
Crude fibre (%)	7	0.82±0.03^{a}	0.83±0.03^{a}	0.85±0.02^{a}	0.83±0.04^{a}	0.086	0.009	0.021
	14	0.85±0.01^{a}	0.83±0.01^{a}	0.70±0.02^{b}	0.74±0.04^{b}			
Crude protein (%)	7	5.84±0.01^{c}	6.51±0.02^{a}	5.92±0.03^{b}	5.49±0.01^{d}	<0.001	<0.001	0.002
	14	5.52±0.03^{c}	6.22±0.01^{a}	5.76±0.01^{b}	5.31±0.02^{d}			
Fat (%)	7	2.29±0.01^{c}	2.74±0.01^{a}	1.55±0.01^{d}	2.33±0.01^{b}	<0.001	<0.001	0.032
	14	2.11±0.01^{b}	2.61±0.02^{a}	1.42±0.02^{c}	2.12±0.01^{b}			
Moisture (%)	7	86.61±0.02^{ab}	86.58±0.02^{b}	86.57±0.01^{b}	86.65±0.02^{a}	<0.001	<0.001	<0.001
	14	86.18±0.02^{b}	85.0 ±0.02^{d}	85.13±0.02^{c}	86.83±0.01^{a}			
Calculated energy value (kcal/100 mL)	7	70.98±0.27^{b}	76.89±0.18^{a}	67.62±0.33^{c}	70.26±0.07^{b}	<0.001	<0.001	0.071
	14	69.16±0.28^{b}	75.15±0.16^{a}	66.70±0.18^{d}	68.12±0.17^{c}			

Note: Values are presented as mean ± standard error of three independent production batches (n = 3). Within the same parameter and storage day, means followed by different superscript letters differ significantly according to Duncan's Multiple Range Test at $p < 0.05$. The p-values represent the main effects of formulation and refrigerated storage time, and the formulation and storage time interaction obtained using two-way analysis of variance

Fat content was significantly affected by formulation, storage time, and their interaction. Formula B had the highest fat content at both sampling times, whereas Formula C had the lowest. Fat content decreased slightly from Day 7 to Day 14 in all formulations. The significant interaction indicates that the magnitude of the decrease depended on formulation composition.

Refrigerated storage may affect selected lipid-related characteristics of fermented soy beverages, while the direction and magnitude of the changes depend on the formulation and starter culture. Ziarno et al. (2023) reported that fatty acid profiles and other compositional characteristics of yoghurt-type fermented soy beverages could vary during refrigerated storage. However, free fatty acids, peroxide value, and lipid oxidation products were not measured in the present study. Consequently, the decrease in fat content cannot be attributed specifically to lipid hydrolysis or oxidation.

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Crude fibre was significantly affected by storage time and the formulation, and the storage time formulation interaction, whereas the overall formulation effect was not significant. On Day 7, crude fibre contents were statistically comparable among formulations. By Day 14, Formulas A and B had higher crude fibre values than Formulas C and D. This result indicates that storage-related changes in crude fibre depended on formulation composition.

Plant-based yoghurt matrices contain different types and amounts of structural carbohydrates, which may influence fermentation behavior and product characteristics. Their composition also varies according to the plant substrate and processing conditions (Montemurro et al., 2021). However, the present study measured crude fibre only and did not distinguish among soluble, insoluble, and fermentable fibre fractions. The specific fiber components associated with the observed changes therefore could not be identified.

Moisture content was significantly affected by formulation, storage time, and their interaction. On Day 7, moisture values differed only slightly among formulations. By Day 14, Formula D had the highest moisture content, whereas Formula B had the lowest. The significant interaction indicates that moisture changed differently among formulations during refrigerated storage.

Water distribution in plant-based yoghurt depends on protein structure, ingredient composition, gel formation, and processing conditions. Plant-based matrices differ from dairy matrices in their protein structure and gel-forming behavior, and these differences can influence water retention and texture (Montemurro et al., 2021). Starter culture and storage duration may also influence water-holding characteristics in fermented soy beverages (Ziarno et al., 2023), while strain selection can affect gel formation and water retention in soy yoghurt (Jo et al., 2025). Nevertheless, water-holding capacity, syneresis, and texture were not measured in the present study. Moisture content alone therefore cannot confirm the physical stability of the soy yoghurt matrix.

The calculated energy value was significantly affected by formulation and storage time, whereas the formulation and storage time interaction was not significant. Formula B had the highest calculated energy value at both sampling times, consistent with its comparatively high protein and fat contents. Formula C had the lowest calculated energy value. Calculated energy decreased from Day 7 to Day 14 in all formulations.

The calculated energy value was mathematically derived from carbohydrate, crude protein, and fat contents using general Atwater factors. It should therefore be interpreted as an estimated metabolizable energy value rather than as an independent analytical measurement or gross energy determined by bomb calorimetry.

Overall, formulation and refrigerated storage affected the proximate characteristics of soy yoghurt in different ways. Significant interactions for several variables indicate that storage-related changes depended on the combination of ingredients. However, the formulations differed simultaneously in moringa, garlic, and lime contents. Consequently, the independent contribution of fresh *Moringa oleifera* leaf aqueous extract could not be separated from the effects of the complete formulation.

The physicochemical characteristics of soy yoghurt were affected differently by formulation and refrigerated storage time (Table 2). The pH was significantly affected by formulation, storage time, and the formulation and storage time interaction. In contrast, titratable acidity was significantly affected by storage time, whereas the main effect of formulation and the formulation and storage time interaction were not significant.

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The pH was significantly affected by formulation, storage time, and their interaction. On Day 7, Formula C had the highest pH, followed by Formula B, whereas Formulas A and D had lower values. By Day 14, pH values had converged to a narrow range of 5.84–5.86, and all formulations were statistically comparable.

Table 2. Physicochemical characteristics of soy yoghurt formulations during refrigerated storage

Parameter	**Day**	**Formula A**	**Formula B**	**Formula C**	**Formula D**	***p*-formulation**	***p*-storage time**	***p*-interaction**
pH	7	5.63±0.01^{c}	5.98±0.03^{b}	6.19±0.01^{a}	5.73±0.02^{c}	<0.001	0.020	<0.001
	14	5.86±0.02^{a}	5.85±0.01^{a}	5.85±0.02^{a}	5.84±0.01^{a}			
TTA (% lactic acid)	7	0.44±0.01^{a}	0.49±0.02^{a}	0.45±0.02^{a}	0.42±0.02^{a}	0.157	<0.001	0.061
	14	0.33±0.01^{a}	0.31±0.01^{ab}	0.30±0.01^{b}	0.32±0.01^{ab}			

Note: Values are presented as mean ± standard error of three independent production batches (n = 3). Within the same parameter and storage day, means followed by different superscript letters differ significantly according to Duncan's Multiple Range Test at $p < 0.05$. The p-values represent the main effects of formulation and refrigerated storage time and the formulation × storage time interaction obtained using two-way analysis of variance.

The significant interaction indicates that pH did not change uniformly among formulations. The pH of Formulas B and C decreased from Day 7 to Day 14, whereas Formulas A and D showed slight increases. Therefore, the effect of storage cannot be described as a uniform increase or decrease across all treatments.

Plant-based yoghurt matrices differ in buffering capacity, fermentable carbohydrate composition, and protein structure, all of which may influence acidification behavior (Montemurro et al., 2021). LAB strain selection can also affect growth, acidification, gel formation, and other fermentation characteristics in soy yoghurt (Jo et al., 2025). In fermented soy beverages, starter culture and refrigerated storage duration have also been shown to influence pH and other physicochemical characteristics (Ziarno et al., 2023).

The pH values measured on Day 7 should not be interpreted as fermentation endpoint values because pH was not recorded at the end of fermentation. They describe the physicochemical condition of the products after seven days of refrigerated storage. The convergence of pH values by Day 14 indicates that formulation-related differences became less pronounced during storage. However, this finding does not demonstrate improved gel stability or sensory quality because water-holding capacity, syneresis, texture, and consumer acceptance were not evaluated.

Titratable acidity was significantly affected by refrigerated storage time, whereas the main effect of formulation and the formulation and storage time interaction were not significant. TTA decreased from Day 7 to Day 14 in all formulations. Values ranged from 0.42% to 0.49% lactic acid on Day 7 and from 0.30% to 0.33% on Day 14.

The non-significant formulation effect indicates that overall TTA did not differ consistently among formulations when both storage times were considered together. Similarly, the non-significant interaction indicates that the storage-related reduction followed a broadly comparable pattern across formulations. The results should therefore not be interpreted as evidence that one formulation promoted or inhibited acid development more strongly than the others.

The pH and TTA followed different patterns because the two measurements describe related but distinct characteristics of the fermented matrix. The pH reflects hydrogen ion activity, whereas TTA reflects

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the amount of base required to neutralize titratable acids and is influenced by buffering components. Plant-based yoghurt matrices differ from dairy yoghurt in protein composition, buffering capacity, and acid-binding behavior (Montemurro et al., 2021). Ziarno et al. (2023) similarly evaluated pH together with compositional and water-retention characteristics in fermented soy beverages because these measurements provide complementary rather than interchangeable information.

Individual organic acids were not quantified in the present study. Consequently, the decline in TTA cannot be attributed to acid degradation, microbial utilization of organic acids, changes in acid dissociation, or another specific biochemical mechanism. The findings should be interpreted only as observed changes in the measured acid-related characteristics between Days 7 and 14.

Overall, formulation had a stronger influence on pH than on TTA. No abrupt changes in the measured pH and TTA were observed during the evaluated storage interval. Nevertheless, these measurements alone are insufficient to establish overall physicochemical stability, sensory acceptability, or commercial shelf life.

The microbiological characteristics of soy yoghurt were significantly influenced by formulation and refrigerated storage time (Table 3). APC was significantly affected by formulation, storage time, and the formulation and storage time interaction. LAB populations were significantly affected by formulation and storage time, whereas the interaction was not significant.

Table 3. Microbiological characteristics of soy yoghurt formulations during refrigerated

Parameter	Day	Formula A	Formula B	Formula C	Formula *D*	*p-formulation*	*p-storage time*	*p-interaction*
Aerobic plate count ($\log_{10}$ CFU/mL)	7	6.84±0.01^{a}	5.09±0.01^{b}	5.11±0.01^{b}	4.44±0.02^{c}	<0.001	<0.001	<0.001
	14	6.91±0.02^{a}	5.16±0.02^{b}	5.12±0.01^{b}	5.16±0.01^{b}			
Lactic acid bacteria ($\log_{10}$ CFU/mL)	7	6.56±0.01^{c}	6.70±0.04^{b}	6.84±0.10^{a}	6.90±0.03^{a}	<0.001	0.003	0.995
	14	6.43±0.02^{c}	6.57±0.05^{b}	6.72±0.07^{a}	6.79±0.02^{a}			

Note: Values are presented as mean ± standard error of three independent production batches (n = 3). Within the same parameter and storage day, means followed by different superscript letters differ significantly according to Duncan's Multiple Range Test at $p < 0.05$. The p-values represent the main effects of formulation and refrigerated storage time and the formulation × storage time interaction obtained using two-way analysis of variance.

APC was significantly affected by formulation, refrigerated storage time, and their interaction. On Day 7, Formula D had the lowest APC, whereas Formula A had the highest. Formulas B and C had intermediate and statistically comparable values. By Day 14, Formula A remained the highest, while Formulas B, C, and D were statistically comparable.

The significant formulation and storage time interaction indicates that APC did not change uniformly among formulations. Formula D showed the largest increase from Day 7 to Day 14, whereas Formulas B and C changed only slightly. Formula D should therefore not be described as having the lowest APC throughout the entire storage interval. A more accurate interpretation is that

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Formula D had the lowest APC on Day 7 and an APC comparable to those of Formulas B and C on Day 14.

The lower APC values observed in Formulas B, C, and D were associated with the complete formulations. These formulations contained fresh moringa leaf aqueous extract together with different amounts of garlic and/or lime extract, whereas Formula A contained garlic and lime but no moringa. Because phytochemical concentrations and the identities of microorganisms contributing to APC were not determined, the differences cannot be attributed specifically to moringa or to a particular antimicrobial compound.

APC represents aerobic microorganisms capable of growing under the selected culture and incubation conditions. It does not distinguish beneficial microorganisms from spoilage organisms or pathogens. APC alone therefore cannot establish product safety, microbial community composition, or maximum shelf life.

LAB populations were significantly affected by formulation and refrigerated storage time, whereas the formulation and storage time interaction was not significant. Formulas C and D had the highest LAB populations on both sampling days, Formula B had intermediate populations, and Formula A had the lowest. Because Formulas C and D shared the same superscript group, the data do not demonstrate a significant difference between these formulations within either sampling day.

LAB populations decreased slightly from Day 7 to Day 14 in all formulations. The non-significant interaction indicates that the direction and magnitude of this decline were broadly comparable among formulations. Formulation therefore influenced the overall LAB population but did not substantially alter the pattern of change during refrigerated storage.

Starter culture and matrix composition can influence viable microbial populations and storage behavior in fermented soy beverages. Ziarno et al. (2023) reported that starter culture selection affected viable microorganism populations during refrigerated storage. Jo et al. (2025) similarly demonstrated strain-dependent differences in LAB growth and fermentation performance in soymilk. These findings support the interpretation that differences in LAB populations may be associated with interactions between the starter culture and the complete formulation rather than with a single ingredient.

All formulations maintained culturable LAB populations above 6 $\log_{10}$ CFU/mL on Days 7 and 14. This result indicates that viable LAB persisted during the evaluated storage interval. However, total LAB enumeration does not establish probiotic functionality. Evaluation of probiotic potential requires strain-level identification and functional testing, including tolerance to low pH, bile salts, pepsin, and pancreatin (Megur et al., 2023). These properties were not evaluated in the present study.

The microbiological analyses were limited to total culturable APC and LAB populations. Molecular identification, strain-specific characterization, pathogen testing, yeast and mould enumeration, and microbial community analysis were not performed. The results therefore describe changes in total culturable populations rather than changes in microbial ecology or product safety.

Overall, formulation and refrigerated storage influenced APC and LAB populations differently. Formula D had the lowest APC on Day 7, whereas Formulas C and D had the highest LAB populations at both sampling times. These findings provide baseline information on culturable aerobic microorganisms and LAB between Days 7 and 14 but do not determine product safety, probiotic functionality, maximum shelf life, or suitability for commercial refrigerated distribution.

4. Limitations and Future Directions

This study was limited by the use of only two storage times, the absence of a plain soy yoghurt control and single-ingredient

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treatments, and the lack of phytochemical, sensory, textural, and strain-specific microbiological analyses. Because the formulations differed simultaneously in moringa, garlic, and lime composition, the independent contribution of each ingredient could not be determined. Future studies should use a factorial design with appropriate controls, include measurements immediately after fermentation and at additional storage times, characterize the bioactive composition of the moringa extract, and evaluate sensory quality, texture, syneresis, organic acids, spoilage organisms, pathogens, and microbial community composition. Pilot-scale production and extended shelf-life testing are also needed before the formulation can be considered for commercial application.

5. Conclusion

Formulation and refrigerated storage time significantly affected the nutritional, physicochemical, and microbiological characteristics of soy yoghurt evaluated on Days 7 and 14. Formula B retained the highest crude protein, fat, and calculated energy values; Formula C had the highest carbohydrate content; and Formula D showed the most favorable microbiological profile, with the lowest aerobic plate count on Day 7 and the highest lactic acid bacteria population at both sampling times. The significant formulation to storage time interactions observed for several parameters indicate that storage-related changes depended on ingredient composition. Overall, soy yoghurt containing fresh Moringa oleifera leaf aqueous extract maintained acceptable measured quality attributes during the evaluated storage interval. Future studies should include appropriate controls, additional storage times, characterization of moringa bioactive compounds, sensory and textural evaluation, pathogen and spoilage testing, and pilot-scale validation.

Declaration of Generative AI and AI-Assisted Technologies in the Writing Process

During the preparation of this work, the authors used ChatGPT to enhance clarity. After using ChatGPT, the authors reviewed and edited the content as needed and assumed full responsibility for the publication.

Authorship Contribution Statement

Ita Fatkhur Romadhoni: Conceptualization, Methodology, Software, Validation; Lilis Sulandari: Formal analysis, Investigation, Data curation, Writing – original draft, Visualization; Niken Purwidiani: Conceptualization, Methodology, Writing – review & editing; Dwi Iriyani: Methodology, Validation, Formal analysis, Investigation, Writing – review & editing; Rita Ismawati: Validation, Writing – review & editing; Kristian Triatmaja Raharja: Validation, Formal analysis, Investigation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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