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Research article

Development of Probiotic Soy Yogurt Containing Kale Powder: Evaluation of Functional and Plant-Based Properties

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Abstract: Probiotic soy yogurt containing kale powder represents a promising plant-based functional product suitable for lactose-intolerant individuals and health-conscious consumers. This study aimed to develop and characterize soy-based probiotic yogurts containing kale powder at concentrations of 0%, 1%, 3%, and 5% (w/w). All formulations were standardized to an initial total soluble solids (TSS) content of 15 °Brix and fermented at 43 ± 1 °C for 8 hours using a commercial yogurt starter and probiotic culture. The yogurt containing 1% kale powder exhibited the most desirable physicochemical and functional properties, including favorable fermentation characteristics (pH 4.63, titratable acidity 0.75 ± 0.03%), the highest viable lactic acid bacteria (LAB) count (8.37 ± 0.05 log CFU/g), and optimal curd structure. Sensory evaluation using a 9-point hedonic scale with thirty untrained panelists confirmed its highest overall acceptability (7.93 ± 0.74). Chemical composition analysis revealed slight variations among formulations, with the 1% kale-containing yogurt showing relatively higher levels of protein (3.49 g/100 g), dietary fiber (0.95 g/100 g), carbohydrate (10.6 g/100 g), energy (65.6 Cal/100 g), and vitamin K_1 (4.94 μg/100 g), reflecting the natural contribution of kale powder rather than fortification. During 21 days of storage at 4 ± 1 °C, the 1% kale-containing yogurt maintained probiotic viability above 7.0 log CFU/g, consistent with internationally recognized efficacy criteria for probiotic products. No yeast and mold growth was detected during storage. These findings highlight, for the first time, the feasibility of formulating probiotic soy yogurt containing kale powder with desirable product quality and functional potential, aligning with current trends in plant-based fermented foods.

Keywords: Soy yogurt; probiotics; kale powder; plant-based dairy

1. Introduction

In recent years, global interest in plant-based functional foods has grown substantially, driven by heightened consumer awareness of health benefits, environmental sustainability, and ethical concerns associated with animal-derived products. This dietary shift is widely acknowledged, with recent academic literature highlighting the strong trend toward healthier and more sustainable food options enriched with bioactive compounds, thereby propelling significant market growth and innovation [1]. Among the various innovations in this sector, plant-based dairy alternatives, such as yogurts made from legumes, grains, and nuts, have become particularly prominent. These products cater to populations with lactose intolerance, milk allergies, or those adopting vegetarian or vegan lifestyles [2]. In the ASEAN region, where health-related non-communicable diseases and aging demographics are increasingly pressing issues, functional foods have been emphasized for their potential to maintain wellness and delay disease onset [3].

Soy milk is widely acknowledged as a suitable base for developing plant-based yogurts due to its high-quality protein, beneficial lipid profile, and ability to support microbial fermentation. It also provides a lactose-free, cholesterol-free, and sustainable alternative to dairy milk, thus appealing to individuals with lactose intolerance and those seeking heart-friendly diets [4]. Fermented soy-based products, including soy yogurt, have been associated with health-promoting effects such as improved gut microbiota balance, reduced blood cholesterol, and antioxidant activity [5-6]. Furthermore, soy yogurt is recognized as an effective carrier for probiotic strains, promoting their survival through the gastrointestinal tract [7]. Despite these benefits, plant-based yogurts made from soy often require formulation improvements to enhance their sensory and textural characteristics and overall consumer acceptability.

An emerging formulation strategy is the enrichment of soy yogurt with functional vegetables such as kale (*Brassica oleracea* L. var. *acephala* DC), a leafy green vegetable increasingly regarded as a superfood. Kale is rich in essential nutrients, including fiber, vitamins, particularly K_1 and β-carotene, minerals, and biologically active phytochemicals like glucosinolates and flavonoids. These compounds offer antioxidative and anti-inflammatory benefits and are associated with reduced risks of chronic diseases such as cardiovascular disorders and cancer [8]. In Thailand, kale cultivation and consumption have been rising steadily, especially among health-focused consumers, contributing to its growing incorporation into smoothies, salads, and functional food products alongside the expansion of organic agriculture [9].

Although kale is recognized for its nutritional value and increasing popularity, it has not been extensively studied for application in fermented plant-based food products. A review of contemporary scientific literature reveals a notable absence of research exploring the integration of kale into probiotic soy yogurt, indicating an opportunity for product innovation. Prior studies have examined the use of cereals, legumes, and fruit-based powders in dairy and soy yogurts [10-11], but no peer-reviewed publications have yet evaluated kale's inclusion in soy-based fermented systems. The high content of fiber and micronutrients in kale makes it a compelling additive to enhance nutritional quality. However, its fibrous nature and pigmentation could potentially alter yogurt texture and visual appeal if used in excessive amounts. Therefore, determining the optimal fortification level is essential to maintain product quality while maximizing health benefits.

Given the growing demand for clean-label and functional plant-based fermented products, particularly in Southeast Asia, where soy-based diets are culturally entrenched, this study aims to formulate and evaluate probiotic soy yogurt containing kale powder at concentrations of 0%, 1%, 3%, and 5% (w/w). The research evaluates fermentation performance, sensory quality, physicochemical and microbiological properties, and chemical composition. Results from this work are expected to inform future development of consumer-accepted, functionally improved, and value-added plant-based yogurts that align with health and sustainability trends.

2. Materials and Methods

2.1 Preparation of Kale Powder and Soy Milk

Fresh kale leaves (1 kg) were thoroughly washed under running water to remove surface impurities and then finely chopped. The chopped leaves were dehydrated in a convection oven at 60 ± 1 °C until constant weight was achieved. The dried leaves were subsequently ground using a high-speed blender and sieved through a 425 μm mesh to obtain a uniform fine powder. The final yield of kale powder was approximately 65 g per kilogram of fresh leaves, corresponding to a drying yield of about 6.5% (w/w). The resulting kale powder was stored in airtight zip-lock bags at 4 ± 1 °C until further use. For soy milk preparation, split soybeans (*Glycine max* (L.) Merr.) (1 kg) were washed and soaked in potable water at ambient room temperature for 6 hours. To reduce off-flavors and microbial load, the soaked beans were blanched in boiling water for 2 minutes. The blanched beans were then blended with potable water at a 1:3 (w/w) ratio to form a homogenized suspension. This suspension was filtered through a double-layered muslin cloth to extract soy milk. The extracted soy milk was pasteurized at 85± 1 °C for 15 minutes, followed by a second filtration using sterile muslin to remove any remaining solids. The prepared soy milk was cooled to room temperature and stored at 4 ± 1 °C until further use.

2.2 Fermentation of Probiotic Soy Yogurt Containing Kale Powder

For the preparation of kale containing probiotic soy yogurt, soy milk was used as the fermentation substrate, and its total soluble solids were adjusted to 15 °Brix by adding sucrose. All formulations were thoroughly mixed to ensure homogeneity, pasteurized at 85 ± 1 °C for 15 minutes, and then cooled to 45 ± 1 °C. A commercial plain soy yogurt available in Thailand was used as the inoculum source to provide lactic acid bacteria for soy milk fermentation. The yogurt starter, containing *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidophilus*, and *Bifidobacterium animalis* subsp. *lactis*, originally optimized for plant-based fermentations, was added at 10% (w/w). Kale powder was incorporated at concentrations of 1%, 3%, and 5% (w/w), while a control sample without kale (0%) was also prepared for comparison. The mixtures were transferred into 200 mL sterile glass containers and incubated at 43 ± 1 °C for 8 hours until the pH reached 4.6–4.8, which is considered suitable for set-style yogurt. The resulting yogurts were stored at 4 ± 1 °C until further analysis.

2.3 Sensory evaluation

A sensory evaluation was conducted to assess the acceptability of probiotic soy yogurt samples containing kale. Thirty untrained panelists participated in the evaluation, rating five sensory attributes: color, odor, flavor, texture, and overall acceptability. A 9-point hedonic scale, ranging from 1 (dislike extremely) to 9 (like extremely), was used to quantify participants' preferences. The collected data were statistically analyzed to identify consumer preferences and determine the most preferred formulation based on overall liking scores. This human-subject study was conducted in accordance with the Declaration of Helsinki and the institutional ethical guidelines of Mahamakut Buddhist University. Ethical approval was obtained under protocol number COA-0056-2567(2024).

2.4 Evaluation of Shelf-Life Stability of Probiotic Soy Yogurt Containing Kale Powder

The shelf-life stability of both the control and the most acceptable kale-containing probiotic soy yogurt formulations was assessed over a 21-day refrigerated storage period at 4 ± 1 °C. All samples were stored in tightly sealed transparent glass bottles wrapped with aluminum foil to protect them from light exposure and placed in a dark refrigerated environment at 4 ± 1 °C throughout the entire storage period. Samples were collected and analyzed on days 0, 7, 14, and 21 to determine changes in physicochemical properties, microbiological quality, and product stability during storage. This evaluation focused on monitoring potential alterations in physicochemical parameters and microbiological quality during storage. Particular attention was given to the enumeration of lactic acid bacteria and the detection of yeast and mold at regular intervals to ensure microbial safety and product stability throughout the storage duration [12].

2.5 Textural profile analysis

After fermentation, all yogurt samples were refrigerated at 4 ± 1 °C for 48 hours before texture analysis. Before testing, each sample (approximately 50 mL) was gently transferred into a cylindrical plastic cup (50 mm diameter × 30 mm height) to obtain a uniform surface. A BRAVO Food Texture Analyzer (Model TA Prime) was used to evaluate the texture characteristics following a modified procedure adapted from Cheng et al. [13]. The analysis simulated the chewing action by performing a two-cycle compression test using a cylindrical probe (TA4/1000) with a diameter of 36 mm. The test was conducted under the following settings: pre-test speed at 1 mm/s, test speed at 1 mm/s, post-test speed at 20 mm/s, and a compression distance of 25 mm. All measurements were carried out in triplicate, and the average values were reported. Texture profile analysis included measurements of hardness, cohesiveness, springiness, and gumminess to assess the quality and consistency of each yogurt formulation.

2.6 Physicochemical analysis

The pH of the yogurt samples was measured using a digital pH meter (Mettler Toledo, USA), and total soluble solids (TSS) were determined using a digital refractometer (ATAGO MASTER-50-H, Japan). Titratable acidity was expressed as a percentage of lactic acid according to the AOAC (2023) method [14].

Color attributes (L*, a*, b*) were evaluated using a colorimeter (Konica Minolta CR-400, Japan) calibrated with a standard white plate. Syneresis was determined by centrifuging 10 g of yogurt at 4,500 rpm for 10 min at 4 °C, and the separated liquid fraction was expressed as a percentage of the total sample weight. Water-holding capacity (WHC) was measured by quantifying the retained fraction after centrifugation [15–16]. All measurements were conducted in triplicate (n = 3), and results were expressed as mean ± SD. The proximate composition, including energy, protein, carbohydrate, total fat, dietary fiber, moisture, ash, and vitamin K_1, was analyzed by an accredited laboratory following AOAC (2023) official methods. Beta-carotene was analyzed instead of vitamin A because kale primarily contains carotenoids rather than preformed vitamin A (retinol). The compound was quantified using high-performance liquid chromatography (HPLC) to represent the measurable provitamin A fraction naturally present in plant-based ingredients, following validated procedures performed by a certified private laboratory.

2.7 Microbiological determinations

The microbial analysis of kale-containing probiotic soy yogurt was performed following the methodology adapted from Mehaya et al. [17]. Total bacterial count was determined using standard plate count techniques. Lactic acid bacteria were enumerated by spread-plating on de Man, Rogosa, and Sharpe (MRS) agar, followed by incubation at 37 ± 1 °C for 48 hours under anaerobic conditions. Yeasts and molds were assessed on potato dextrose agar (PDA) and incubated at 30 ± 2 °C for 5 to 7 days. All microbial counts were expressed as log colony-forming units per gram (log CFU/g).

2.8 Statistical Analysis

A completely randomized design (CRD) was employed to evaluate the physical, chemical, and microbiological characteristics of the yogurt samples, while sensory evaluation followed a randomized complete block design (RCBD) to account for panelist variability. All data are expressed as mean ± standard deviation (SD) based on three independent replicates. One-way analysis of variance (ANOVA) was performed to determine significant differences among treatments, and mean separation was conducted using Duncan's multiple range test (DMRT) at a 95% confidence level ($p \leq 0.05$). Statistical analyses were performed using IBM SPSS Statistics software, version 28 (IBM Corp., Armonk, NY, USA).

3. Results and Discussion

3.1 Fermentation Characteristics of Probiotic Soy Yogurt Containing Kale Powder

The effect of different kale powder concentrations on the fermentation performance and structural development of probiotic soy yogurt was studied. Four treatments were prepared: a control sample (0% kale) and three samples containing 1%, 3%, and 5% kale powder. Before fermentation, each formulation was adjusted to 15 °Brix and inoculated with a commercially available probiotic culture. After incubating at 43 ± 1 °C for 8 hours, noticeable variations in fermentation dynamics and curd formation were recorded. The quality of curd structure was observed to deteriorate progressively with increasing concentrations of kale. While acceptable gel textures were retained in the control, 1%, and 3% formulations, the 5% kale yogurt presented weak coagulation and a watery appearance. This decline in curd firmness at higher levels of kale fortification may be linked to interactions between bioactive compounds in kale and the soy protein matrix during acidification, potentially disrupting the aggregation and gelation of protein structures [18].

As presented in Figure 1, the pH values of all yogurt formulations consistently decreased during the 8-hour fermentation period, ultimately falling within the range of 4.63 to 4.80. Notably, the 1% kale-containing sample exhibited the most substantial acidification, achieving the lowest final pH (4.63 ± 0.03), which may reflect enhanced lactic acid bacterial activity stimulated by moderate levels of kale addition. The acidification process plays a vital role in curd formation, as casein begins to coagulate when the pH nears its isoelectric point (approximately 4.6), promoting gel network development [10, 17]. In parallel with the reduction in pH, titratable acidity steadily increased across all samples. During the 8-hour incubation period, lactic acid contents ranged between 0.62% to 0.75%. The highest acidity was found in the 1% kale formulation (0.75 ± 0.03%), while the 5% kale sample recorded the lowest value (0.62 ± 0.02%). All measured values exceeded the minimum lactic acid content of 0.6%, as required by Thailand's Ministry of Public Health under Notification No. 353 [19] for fermented milk products.

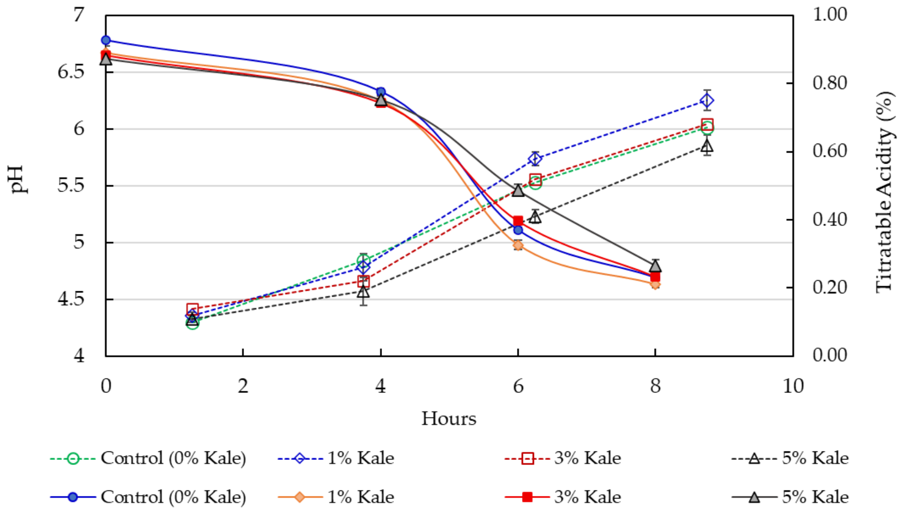

Figure 1. pH (—) and titratable acidity (---) profiles of probiotic soy yogurt containing kale powder (0%, 1%, 3%, and 5%) during 8 hours of fermentation at 43 ± 1 °C.

The TSS of the fermented soy yogurt samples ranged from 10.67 ± 0.58 to 12.33 ± 0.58 °Brix (Table 1). A statistically significant increase in TSS was observed as the kale powder concentration increased from 0% to 5%. This trend is likely due to the addition of kale powder, which contains naturally occurring sugars, soluble fibers, and other water-soluble phytochemicals. These components may have dissolved during heat treatment and contributed to the higher TSS values observed following pasteurization and after fermentation [10, 20]. Although sugar consumption by fermenting microbes typically reduces TSS during yogurt production, the enrichment with kale appears to have mitigated this reduction, particularly in the 5% formulation, which retained the highest TSS after the 8-hour fermentation. Syneresis, an indicator of gel network stability, was found to increase proportionally with higher kale inclusion. The yogurt containing 5% kale showed the greatest syneresis value (39.17 ± 1.04%) (Table 1), suggesting that elevated kale levels may weaken gel integrity. It is generally acknowledged that soy-based yogurts exhibit more pronounced syneresis compared to their dairy counterparts due to inherent differences in protein and lipid composition [21-22]. Conversely, WHC demonstrated a declining pattern, ranging from 71.33 ± 1.25% in the control to 60.17 ± 0.76% in the 5% kale sample. This decline suggests that increased kale content might interfere with the protein network formation, thereby leading to greater whey separation. These findings align with results from Arab et al. [23], which indicated that WHC in soy-based yogurts is influenced by fermentation dynamics, stabilizer use, and the protein content of the formulation. Kale addition also significantly altered the color properties of the yogurt. The visual appearance and color of the kale containing yogurt samples at four concentration levels are shown in Figure 2. As the kale concentration increased, the lightness value (L*) decreased, indicating a darker appearance. Simultaneously, the a* values shifted negatively, reflecting increased greenness, while b* values progressively decreased, indicating reduced yellowness. These changes are largely attributed to pigments such as chlorophylls and carotenoids inherent in kale, which not only affect product appearance but also offer health-related benefits [10, 24]. The addition of kale powder may enhance the functional quality of soy yogurt due to the presence of bioactive compounds such as vitamin K_1, β-carotene, lutein, and dietary fiber, which are known to support antioxidant activity and digestive health [10, 24, 25]. Although the 1% addition used in this study is relatively small, these components can still contribute to improving the nutritional and functional properties of the product rather than providing direct health claims.

In terms of microbiological quality, all yogurt formulations maintained LAB counts above 7 log CFU/g (approximately 10^7 CFU/g), which exceeds the minimum level generally recommended for probiotic foods to ensure functional efficacy [25]. The 1% kale formulation recorded the highest LAB population (8.37 ± 0.05 log

CFU/g), possibly due to prebiotic support from moderate kale enrichment. This finding aligns with Kim et al. [8], who demonstrated enhanced LAB survival in kale-based fermentations due to antioxidant-rich fibers, and with Rashwan et al. [10], who reported stabilized probiotic viability in yogurts containing plant-based bioactives. Additionally, no yeast and mold growth was detected in any sample, underscoring the microbiological safety of all formulations. Collectively, the soy yogurt containing 1% kale demonstrated the most desirable combination of quality traits, including strong gel structure, minimal syneresis, enhanced LAB viability, and improved color, making it the most favorable option among the variants tested.

Figure 2. Visual appearance and color of probiotic soy yogurt containing kale powder (0%, 1%, 3%, and 5%) at 8 hours of fermentation at 43 ± 1 °C.

Table 1. Physicochemical properties of probiotic soy yogurt containing different levels of kale powder after fermentation

Properties	Control (0% Kale)	1% Kale	3% Kale	5% Kale
Physical:				
TSS (˚Brix)	10.67 ± 0.58^{b}	10.83 ± 0.29^{b}	11.50 ± 0.50^{ab}	12.33 ± 0.58^{a}
Syneresis (%)	28.83 ± 2.02^{c}	31.16 ± 1.04^{c}	35.17 ± 0.76^{b}	39.17 ± 1.04^{a}
Water-Holding Capacity (WHC, %)	71.33 ± 1.25^{a}	68.17 ± 1.04^{b}	65.10 ± 1.00^{c}	60.17± 0.76^{d}
Color L*	78.83 ± 0.51^{a}	70.48 ± 0.71^{b}	59.22 ± 1.00^{c}	52.41 ± 0.74^{d}
a*	-1.71 ± 0.04^{a}	-3.64 ± 0.06^{b}	-4.57 ± 0.12^{c}	-4.72 ± 0.07^{d}
b*	17.32 ± 0.85^{a}	15.83 ± 0.87^{ab}	14.37 ± 0.65^{b}	12.33 ± 0.89^{c}
Microbiology:				
Total Bacteria (log CFU/g)	8.30 ± 0.02^{b}	8.42 ± 0.06^{a}	8.23 ± 0.05^{b}	7.11 ± 0.08^{c}
Lactic Acid Bacteria (log CFU/g)	8.28 ± 0.03^{ab}	8.37 ± 0.05^{a}	8.19 ± 0.08^{b}	7.05 ± 0.07^{c}
Yeast and Mold (log CFU/g)^{ns}	ND	ND	ND	ND

Note: Values are presented as mean ± SD (n = 3). Different superscript letters in the same row indicate significant differences ($p \leq 0.05$), ND indicates not detected, while ns indicates non-significant differences ($p > 0.05$), as determined by one-way ANOVA.

3.2 Textural Attributes of Probiotic Soy Yogurt Containing Kale Powder

Texture Profile Analysis (TPA) demonstrated that the inclusion of kale powder had a dose-dependent impact on the structural attributes of probiotic soy yogurt (Table 2). The control sample (0% kale) exhibited the highest hardness (2.39 ± 0.05 N), cohesiveness (0.51 ± 0.07), and gumminess (2.72 ± 0.08 N), with moderate springiness (0.18 ± 0.04 s). When 1% kale was added, hardness decreased slightly to 2.25 ± 0.02 N, and cohesiveness remained statistically unchanged (0.49 ± 0.05). Interestingly, springiness significantly increased to 0.27 ± 0.07 s ($p \leq 0.05$), while gumminess was maintained (2.66 ± 0.06 N), indicating that moderate kale supplementation preserved gel integrity and even improved the elastic recovery of the matrix. However, higher concentrations of kale (3% and 5%) severely compromised the yogurt's textural parameters. Hardness dropped sharply to 0.40 ± 0.03 N and 0.32 ± 0.02 N, respectively, and gumminess was reduced to 0.00 N in both cases. Cohesiveness and springiness were also dramatically reduced, with values approaching zero, signifying an almost complete breakdown of the gel structure. The total loss of springiness (0.00 ± 0.00 s) particularly reflects the absence of elasticity, a key factor in yogurt mouthfeel. This complete loss of springiness and gumminess clearly indicates a gel failure or non-gelling structure rather than merely a compromised texture. These formulations were therefore considered unsuccessful products in terms of textural integrity, as the excessive kale content disrupted the protein–polysaccharide network formation and reduced water-binding capacity, leading to weak or non-existent gel matrices. This phenomenon is consistent with the inherently weaker gelation ability of plant proteins compared with casein micelles in dairy systems [28]. These results align with previous findings that low levels of dietary fiber may enhance yogurt structure, whereas excessive fiber can disrupt the formation of a stable protein network [26–27]. The degradation observed at higher levels of kale is likely due to the interference of insoluble fibers with protein–protein interactions and competition for water within the matrix, impairing both casein and soy protein gelation. In plant-based yogurt systems, where the gel structure is inherently weaker than in dairy-based counterparts due to the absence of casein micelles, such interference becomes more pronounced and often leads to gel collapse [28].

Therefore, the findings suggest that a 1% kale addition level provides an optimal balance by enhancing the functional and quality attributes of the yogurt without compromising its texture. In contrast, higher concentrations (≥3%) negatively affect the physical structure of the yogurt, highlighting the importance of precise formulation when incorporating plant-based ingredients into functional food systems.

Table 2. Texture profile analysis (TPA) results of probiotic soy yogurt containing kale powder

Texture profile analysis (TPA)	Hardness (N)	Cohesiveness (-)	Springiness (s)	Gumminess (N)
Control (0% Kale)	2.39 ± 0.05^{a}	0.51 ± 0.07^{a}	0.18 ± 0.04^{b}	2.72 ± 0.08^{a}
1% Kale	2.25 ± 0.02^{b}	0.49 ± 0.05^{a}	0.27 ± 0.07^{a}	2.66 ± 0.06^{a}
3% Kale	0.40 ± 0.03^{c}	0.03 ± 0.02^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}
5% Kale	0.32 ± 0.02^{c}	0.01 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}

Note: Values are presented as mean ± SD. Different superscript letters within the same column indicate significant differences ($p \leq 0.05$), as determined by one-way ANOVA.

3.3 Sensory Acceptability of Probiotic Soy Yogurt Containing Kale Powder

The impact of kale powder supplementation on the sensory attributes of probiotic soy yogurt was assessed using a 9-point hedonic scale with 30 untrained panelists evaluating color, odor, flavor, texture, and overall liking (Table 3). The yogurt sample containing 1% kale powder received the highest overall acceptability score (7.93 ± 0.74), which was significantly higher than the control (7.43 ± 1.04; $p \leq 0.05$), indicating enhanced consumer preference at this fortification level. The 1% kale sample also achieved favorable scores across all other sensory attributes, including flavor (7.57 ± 0.94) and texture (7.33 ± 1.06), suggesting that low-dose kale incorporation may contribute to a well-balanced mouthfeel and a pleasant vegetal note without compromising product quality. These findings align with prior studies reporting that moderate inclusion of green vegetable powders can improve consumer perception in plant-based and dairy matrices by subtly enhancing flavor complexity and functional appeal [29]. The mild enrichment may also have influenced perceived creaminess and color uniformity due to kale's chlorophyll and fiber content, which has been associated with a smoother texture and more vibrant appearance in fermented products [30–31]. In contrast, higher fortification levels (3% and 5%) resulted in significantly lower scores across all sensory parameters, with the 5% kale yogurt receiving the lowest overall liking score (5.63 ± 0.72). Panelist feedback indicated that these samples exhibited unpleasant bitterness, off-flavors, and an overly dark green coloration, which negatively affected consumer acceptance. These undesirable attributes are likely due to elevated concentrations of chlorophylls, phenolic compounds, and insoluble fibers, which are known to contribute to sensory imbalances when used excessively in food formulations [32–33]. Overall, the results indicate that incorporating 1% kale powder provides the most favorable formulation, improving sensory characteristics and overall product acceptability. This finding supports its potential application in developing plant-based yogurt alternatives that are both palatable and suitable for consumers seeking dairy-free options.

Table 3. Sensory evaluation scores of kale-containing probiotic soy yogurt samples

Treatments	Color	Odor	Flavor	Texture	Overall liking score
Control (0% Kale)	7.27 ± 0.78[a]	7.23 ± 0.82[a]	7.50 ± 1.01[a]	7.27 ± 1.08[a]	7.43 ± 1.04[b]
1% Kale	7.43 ± 1.55[a]	7.26 ± 0.98[a]	7.57 ± 0.94[a]	7.33 ± 1.06[a]	7.93 ± 0.74[a]
3% Kale	6.60 ± 1.22[b]	6.07 ± 1.25[b]	6.50 ± 1.07[b]	6.73 ± 1.11[b]	6.87 ± 0.63[c]
5% Kale	5.93 ± 1.36[c]	4.40 ± 1.00[c]	5.10 ± 0.92[c]	5.47 ± 0.82[c]	5.63 ± 0.72[d]

Note: Values are presented as mean ± SD (n = 30). Different superscript letters within the same column indicate significant differences ($p \le 0.05$), as determined by one-way ANOVA.

3.4 Chemical Composition of Probiotic Soy Yogurt Containing Kale Powder

The chemical components of the two most consumer-preferred formulations of control (0% kale) and 1% kale-containing soy yogurt were compared to evaluate the functional impact of kale supplementation (Table 4). The yogurt containing kale powder exhibited modest yet meaningful improvements in several key nutritional metrics. Energy content increased by approximately 6.3% (65.6 vs. 61.7 Cal/100 g), coinciding with slight rises in protein (3.49 vs. 3.24 g/100 g), total fat (1.03 vs. 0.88 g/100 g), and total carbohydrate (10.6 vs. 10.2 g/100 g). These enhancements are consistent with the compositional attributes of kale, which is rich in macronutrients and phytochemicals. Dietary fiber content also improved in the 1% kale yogurt (0.95 vs. 0.81 g/100 g), which aligns with kale's established role as a dietary fiber source. Notably, vitamin K_1 (phylloquinone) levels increased significantly, from 0.68 to 4.94 μg/100 g (approximately sevenfold), highlighting kale's potential as a concentrated source of phylloquinone, a nutrient important for blood coagulation and bone health [34]. However, this amount is still below 10% of the Thai Recommended Daily Intake (RDI) for vitamin K (60 μg/day) as specified in the Thailand Ministry of Public Health Notification No. 445 (B.E. 2566) and reflects a functional improvement in formulation. Although beta-carotene (provitamin A) was not detected under the analytical conditions used, previous research has identified kale as a valuable source of carotenoids, including β-carotene and lutein [35]. The relatively low vitamin K_1 level compared with the theoretical content in kale powder may be due to its susceptibility to degradation by heat, light, and oxygen during drying, pasteurization, and fermentation processes. Oxidative reactions and dilution within the yogurt matrix could also contribute to further losses during storage. To improve vitamin K_1 retention in future studies, researchers may consider using higher kale concentrations, applying gentle drying methods such as freeze-drying, protecting the product from light and oxidation, incorporating microencapsulation techniques, or adding natural antioxidants before incorporation. While the addition of 1% kale powder improved the nutritional profile, particularly in dietary fiber and vitamin K_1, the quantity used in this study does not meet the minimum thresholds for nutrient fortification or enrichment claims according to the Thailand Ministry of Public Health Notification No. 445 (B.E. 2566). A slight increase in ash content (0.43 vs. 0.40 g/100 g) and a minor reduction in moisture (84.5% vs. 85.3%) were also observed, suggesting a more nutrient-dense matrix in the kale-added sample. These trends are in line with previous findings that reported compositional improvements in soy-based fermented products following the addition of green vegetable powders [36]. The results confirm that incorporating 1% kale powder into soy yogurt can enhance its chemical composition and functional properties, particularly in protein, dietary fiber, and vitamin K_1 content, without compromising sensory attributes. This formulation presents a promising plant-based functional product for consumers seeking improved product quality and functional potential in non-dairy alternatives.

Table 4. Chemical composition of probiotic soy yogurt with and without kale powder

Parameters	Control (0% Kale)	Soy Yogurt with 1% Kale
Energy (Cal/100g)	61.7	65.6
Protein (g/100g)	3.24	3.49
Total Carbohydrate (g/100g)	10.2	10.6
Total Fat (g/100g)	0.88	1.03
Moisture (g/100g)	85.3	84.5
Ash (g/100g)	0.40	0.43
Total Dietary Fiber (g/100g)	0.81	0.95
Vitamin K_1 (ug/100g)	0.68	4.94
Vitamin A (Beta-carotene) (ug/100g)	ND	ND

Note: ND = Not detected.

3.5 Storage Stability of Probiotic Soy Yogurt Containing Kale Powder

The shelf-life characteristics of probiotic soy yogurt containing 1% kale powder were evaluated over 21 days, focusing on key physicochemical and microbiological parameters. The storage stability of both control (0% kale) and 1% kale-containing probiotic soy yogurts was monitored on days 0, 7, 14, and 21 under refrigerated conditions (4 ± 1 °C) to assess changes in their physicochemical and microbiological properties. In both formulations, the pH gradually decreased throughout storage, indicating post-acidification caused by the residual metabolic activity of LAB. The control sample decreased from 4.68 ± 0.01 (Day 0) to 4.47 ± 0.05 (Day 21), whereas the 1% kale-containing yogurt showed a slightly greater reduction, from 4.64 ± 0.02 to 4.39 ± 0.02 (Table 5). Titratable acidity increased significantly ($p \leq 0.05$) in both formulations, from 0.67% to 0.93% for the control and from 0.72% to 1.05% for the kale yogurt, which is consistent with continued LAB fermentation during refrigerated storage [7, 37]. Syneresis (%) decreased steadily in both yogurts, from 28.85 ± 1.29% to 25.02 ± 2.24% in the control and 31.53 ± 0.62% to 25.30 ± 0.33% in the kale yogurt, suggesting improved serum retention and gel stability over time. The improvement observed in the kale formulation may be attributed to the WHC of kale fiber and its contribution to the stabilization of the protein–polysaccharide network [23, 38]. WHC and color parameters remained stable ($p > 0.05$) in both samples, with only a slight reduction in lightness (L) observed in the 1% kale yogurt, likely due to partial oxidation of chlorophyll pigments. Microbiologically, both formulations maintained high probiotic viability throughout the 21-day storage period. Total bacterial counts in the control yogurt decreased slightly from 8.25 log CFU/g (Day 0) to 7.21 log CFU/g (Day 21) (data not shown), while the 1% kale containing yogurt retained 8.32 log CFU/g to 7.41 log CFU/g. Likewise, LAB counts showed minimal reductions, decreasing from 8.21 to 7.14 log CFU/g in the control and from 8.31 to 7.37 log CFU/g in the 1% kale yogurt, indicating that kale addition contributed to better microbial protection, possibly due to its prebiotic fibers and antioxidant compounds [8, 38]. Microbiologically, both formulations maintained viable counts of lactic acid bacteria (LAB) exceeding 7 log CFU/g, which meets the requirement for probiotic foods as specified by the Thailand Ministry of Public Health Notification No. 346 (B.E. 2555). According to the accompanying Thai FDA guidelines, probiotic-containing foods are recommended to contain at least 10^6 CFU/g (6 log CFU/g) of viable microorganisms throughout the shelf life to ensure functional efficacy. No yeast and mold growth was detected in any samples, confirming the microbiological safety of the products during storage. Overall, both yogurt formulations exhibited stable physicochemical and microbiological properties during refrigerated storage. The addition of 1% kale slightly improved LAB survivability and gel consistency, indicating that kale fortification can serve as a functional strategy to enhance probiotic stability and product quality in soy-based yogurts during cold storage [7, 8, 38].

Table 5. Physicochemical properties of probiotic soy yogurt during refrigerated storage for 21 days

Properties /Days	pH	Titratable acidity(%)	Syneresis (%)	WHC (%)	Color		
					L*	a*	b*
Control (0% Kale):							
0	4.68 ± 0.01^{a}	0.67 ± 0.01^{d}	28.85 ± 1.29^{ns}	71.55 ± 1.29^{ns}	78.31 ± 0.60^{a}	-1.73 ± 0.02^{ns}	17.30 ± 0.15^{ns}
7	4.61 ± 0.02^{b}	0.73 ± 0.02^{c}	27.83 ± 1.60^{ns}	72.17 ± 1.60^{ns}	78.13 ± 0.57^{a}	-1.70 ± 0.01^{ns}	17.28 ± 0.24^{ns}
14	4..58 ± 0.02^{c}	0.84 ± 002^{b}	27.27 ± 1.96^{ns}	72.73 ± 1.96^{ns}	77.53 ± 0.10^{b}	-1.71 ± 0.03^{ns}	17.10 ± 0.27^{ns}
21	4.47 ± 0.05^{d}	0.93 ± 0.02^{a}	25.02 ± 2.24^{ns}	74.98 ± 2.24^{ns}	76.53 ± 0.38^{c}	-1.70 ± 0.03^{ns}	17.08 ± 0.11^{ns}
Soy Yogurt (1% Kale):							
0	4.64 ± 0.02^{a}	0.72 ± 0.02^{c}	31.53 ± 0.62^{a}	68.62 ± 0.45^{d}	70.73 ± 0.10^{a}	-3.64 ± 0.15^{c}	16.57 ± 0.26^{a}
7	4.60 ± 0.01^{b}	0.76 ± 0.01^{c}	28.08 ± 0.45^{b}	71.92 ± 0.45^{c}	69.63 ± 0.19^{b}	-3.52 ± 0.12^{bc}	16.33 ± 0.18^{a}
14	4.57 ± 0.01^{c}	0.89 ± 002^{b}	26.62 ± 0.20^{c}	73.38 ± 0.20^{b}	69.40 ± 0.39^{b}	-3.43 ± 0.14^{b}	16.12 ± 0.22^{a}
21	4.39 ± 0.02^{d}	1.05 ± 0.03^{a}	25.30 ± 0.33^{d}	74.70 ± 0.33^{a}	69.27 ± 0.51^{b}	-3.27 ± 0.13^{a}	15.52 ± 0.25^{b}

Note: Values are presented as mean ± SD (n = 3). Different superscript letters within the same column indicate significant differences ($p \leq 0.05$) among storage periods for each sample, while *ns* indicates non-significant differences ($p > 0.05$), as determined by one-way ANOVA.

4. Conclusions

This study demonstrated the successful development of probiotic soy yogurt containing kale powder, confirming its potential as a functional, plant-based fermented product. Among the tested formulations, the yogurt containing 1% kale powder exhibited the most desirable characteristics, including optimal fermentation performance, improved texture, high sensory acceptability, and favorable chemical composition, particularly in protein, dietary fiber, and vitamin K_1. This formulation also maintained probiotic viability above 7 log CFU/g throughout 21 days of refrigerated storage, with no detectable yeast and mold, indicating good microbiological safety and shelf-life stability. In contrast, higher kale concentrations (3% and 5%) negatively affected gel structure, taste, and overall product quality, likely due to interference of excessive fiber with the protein network. These findings highlight the feasibility of incorporating low levels of kale powder to enhance the functional quality of soy yogurt without compromising its sensory and structural integrity. Further research is recommended to explore the use of stabilizers or fiber modification techniques to improve product quality and consumer acceptance of plant-based yogurt products.

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