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Chemical and Functional Characteristics of Soy Milk Kefir at Various Doses of Starter and Addition of Telang Flower Juice (*Clitoria ternatea* L.)

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Abstract: Soy milk kefir offers a plant-based alternative to dairy probiotics. This study aimed to enhance both the chemical and functional characteristics of soy milk kefir by evaluating the effects of varying kefir grain starter doses and added Clitoria ternatea (telang) flower juice. A 3x4 factorial design was employed using three starter levels (40g, 50g, 60g per liter soy milk) and four telang juice concentrations (0ml, 10ml, 20ml, 30ml per liter). Key parameters analyzed post-fermentation included protein content (Kjeldahl), lactic acid (titration), pH, Water Holding Capacity (WHC, centrifugation), anthocyanin content (differential pH method), and Lactic Acid Bacteria (LAB) count (plate count). Results indicated significant ($p<0.05$) effects of both starter dose and telang juice concentration, and their interaction, on all measured characteristics. Specifically, the 40g starter + 30ml juice treatment yielded the highest protein (39.35%) and anthocyanin (18.42 mg/mL). The 50g starter + 20ml juice treatment showed the highest WHC (67.30%). The 60g starter + 30ml juice treatment produced the highest lactic acid (20.30%). Higher LAB counts were observed in several treatments including A0B0, A1B1, A2B2, and A2B3 compared to others. In conclusion, optimizing kefir grain dosage and adding telang flower juice significantly improves soy milk kefir's nutritional profile (protein) and functional attributes (anthocyanins, WHC, LAB), demonstrating its potential as an enhanced plant-based functional food.

Keywords: Antioxidants; Chemical Characteristics; *Clitoria ternatea*; Fermentation; Functional Characteristics; Functional Food; Soy Milk Kefir; Probiotics

Introduction

Functional food consumption has become a global trend as public awareness of the health benefits of foods and beverages enriched with bioactive compounds grows. Kefir, a probiotic-rich fermented beverage, is increasing in popularity due to its extensive health benefits, including improved digestive health and modulation of the immune system (Bourrie et al., 2016; Dimidi et al., 2019). The antimicrobial and antioxidant activity inherent in kefir also contributes to its value as a functional food (Pratiwi et al., 2018). However, traditional kefir is generally made from animal milk, posing challenges for individuals with lactose intolerance, milk allergies, or those following vegan dietary preferences. Consequently, the development of plant-based kefir alternatives is crucial to meet diverse consumer needs (Silva et al., 2020).

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Soy milk, derived from soybean seeds (Glycine max L. Merr.), is a promising base for plant-based kefir (Anggraeni & Saputra, 2019). It offers a comparable nutritional profile to animal milk, being rich in plant-based proteins and beneficial isoflavones (Katz et al., 2023; Messina, 2016). Despite its potential, soy milk presents unique fermentation challenges. Its distinct profile of oligosaccharides can be poorly metabolized by some kefir microorganisms, potentially leading to slower fermentation (Gänzle, 2020). Furthermore, soy milk often carries a characteristic 'beany' flavor that can reduce consumer acceptance (Ismail et al., 2020; Liu et al., 2021).

To address these limitations and enhance functional value, this study investigates the optimization of starter dose and the addition of Telang flower juice (Clitoria ternatea L.) (Nuryanto et al., 2020). Butterfly pea flower is renowned for its striking blue pigmentation and high concentration of anthocyanins, potent antioxidants with documented health benefits (Jeyaraj et al., 2021; Shen et al., 2022). Incorporating butterfly pea juice into soy milk kefir offers potential benefits: 1) Enhanced Functional Value through boosted antioxidant capacity (Lakshan et al., 2019); 2) Improved Sensory Appeal via vibrant color (Syam et al., 2022); 3) Potential Synergistic Effects involving other phytochemicals (Srivastava & Kumar, 2021).

Therefore, this research aims to systematically evaluate how varying the kefir starter dose and the concentration of added butterfly pea flower juice impacts the chemical and functional characteristics of soy milk kefir. The novelty lies in the simultaneous optimization of these factors using a factorial design for a soy-based kefir with a natural, antioxidant-rich plant extract (Purwaniati et al., 2020). While plant-based kefirs (Montemiglio et al., 2021) and butterfly pea use (Andriani & Murtisiwi, 2020) are known, their combined optimization in soy kefir is less explored. This research seeks to develop an improved plant-based functional food product, providing a formulation strategy for soy milk kefir suitable for dairy-avoiding individuals and appealing to health-conscious consumers (Nurgustiyanti et al., 2021; Hadiqah et al., 2023). This contributes to more palatable and nutritious plant-based kefir alternatives (Pamungkas et al., 2018).

Method

Materials for this study, including dried butterfly pea flowers (Clitoria ternatea L.), soybeans, and traditional kefir grains, were obtained from [Specify source if possible, e.g., local market, laboratory stock]. All chemicals utilized for subsequent analyses were of analytical grade. The starter culture consisted of traditional kefir grains, which [Optional: were activated prior to use by two successive 24-hour fermentation cycles in pasteurized cow's milk at approximately 25°C, followed by rinsing with sterile distilled water]. Soy milk was prepared following a modification of the method described by Dewi et al. (2021); soybeans were soaked for 8 hours, boiled for 30 minutes, ground with water, filtered through sterile gauze, and the resulting soy milk was pasteurized by heating at 90°C for 15 minutes before being cooled to room temperature. Telang flower juice was extracted based on a modified procedure from Purwaniati et al. (2020) by soaking dried flowers in distilled water (1:6 w/v) for 24 hours at room temperature, followed by filtration through sterile gauze and Whatman No. 1 filter paper (Andriani & Murtisiwi, 2020).

The research employed a factorial experiment arranged in a Completely Randomized Design (CRD) with three replicates for each treatment combination. The first factor was the traditional kefir starter dose (A) at three levels: 40g/L (A0), 50g/L (A1), and 60g/L (A2) of soy milk. The second factor was the concentration of added telang flower juice (B) at four levels: 0ml/L (B0), 10ml/L (B1), 20ml/L (B2), and 30ml/L (B3). For each treatment, the specified quantities of kefir grains and telang flower juice were added to the cooled, prepared soy milk in sterile vessels and fermented quiescently at room temperature (25 ± 2°C) for 24 hours (Hidayat et al., 2019). The treatment A0B0 served as a baseline control within the lowest starter dose group. Following fermentation, kefir grains were aseptically separated using a sterile sieve, and the resulting soy milk kefir samples were immediately cooled, transferred to sterile containers, and stored under refrigeration at 4°C. All subsequent analyses were performed within 24 hours of sample collection to maintain integrity.

Chemical characteristics were determined using standard, referenced analytical methods with appropriate blanks and calibration standards. Protein content was measured using the Kjeldahl method with an automatic distillation unit (AOAC, 2019). Total soluble solids were quantified as °Brix using an Abbe refractometer at 20°C. Lactic acid content, expressed as percent lactic acid, was determined via titration with standardized 0.1N NaOH using phenolphthalein indicator (Lestari et al., 2018). Moisture content was ascertained by drying samples in a forced-air oven at 105°C to constant weight (AOAC, 2019; Rosida et al., 2021). The pH was measured using a digital pH meter calibrated with standard pH 4.0 and 7.0 buffers (Hasniar et al., 2019).

Functional properties were also assessed. Water Holding Capacity (WHC) was determined by centrifuging samples (specify speed/time/temp if possible, e.g., 3000 rpm for 15 min at 4°C) and calculating the percentage of water retained in the pellet relative to the initial sample weight (Santillán-Urquiza et al., 2017; Ritonga et al., 2022). Total monomeric anthocyanin content was quantified using the pH differential method (Lee et al., 2005; Giusti & Wrolstad, 2001) expressed as mg C3G equiv/L (Purwaniati et al., 2020; Marpaung et al., 2022), measuring absorbance differences at 510 nm and 700 nm in pH 1.0 and 4.5 buffers with a UV-Vis spectrophotometer, and expressed as mg cyanidin-3-glucoside equivalents per volume of kefir. Lactic Acid Bacteria (LAB) counts were enumerated using the pour plate method on de Man, Rogosa, and Sharpe (MRS) agar; serial dilutions plated in duplicate were incubated anaerobically (or microaerophilically) at 37°C for 48 hours, with results expressed as Colony Forming Units per mL (CFU/mL), adapting principles from the Bacteriological Analytical Manual APHA, 2015; Sari et al., 2020; Faridah et al., 2022).

All collected data were subjected to Analysis of Variance (ANOVA) using SPSS software (Version 25). Significant differences between treatment means were identified using the Tukey's HSD (Honestly Significant Difference) post-hoc test, also referred to as BNJ, with a significance level set at $p < 0.05$.

Result and Discussion

Result

The fermentation of soy milk using traditional kefir grains, with the addition of Telang flower juice (Clitoria ternatea L.), demonstrated that the interaction between starter dose and juice concentration significantly influenced ($p < 0.05$) all measured chemical and functional characteristics, as detailed in Table 1.

Table 1. Effect of starter dose and addition of telang flower juice on the chemical and functional characteristics of soy milk kefir

Treatment (starter dose + telang flower juice)	Up to protein (%)	Total sugar solids (°Brix)	Up to asam lactate (%)	Up to air (%)	pH	WHC (%)	Antosianin (mg/mL)	BAL (CFU/mL)
A0B0 (40g + 0ml)	33.70 ± SD	3.04 ± SD	10.44 ± SD	35.47 ± SD	4.23 ± SD	51.50 ± SD	0.00 ± SD	5.42 ± SD
A0B1 (40g + 10ml)	19.07 ± SD	3.36 ± SD	11.70 ± SD	33.35 ± SD	4.75 ± SD	65.90 ± SD	3.62 ± SD	0.06 ± SD
A0B2 (40g + 20ml)	30.72 ± SD	3.04 ± SD	11.97 ± SD	31.70 ± SD	2.46 ± SD	59.50 ± SD	9.71 ± SD	0.06 ± SD
A0B3 (40g + 30ml)	39.35 ± SD	2.84 ± SD	13.05 ± SD	32.04 ± SD	4.34 ± SD	39.40 ± SD	18.42 ± SD	0.06 ± SD
A1B0 (50g + 0ml)	21.91 ± SD	2.84 ± SD	13.91 ± SD	33.25 ± SD	3.64 ± SD	50.80 ± SD	0.00 ± SD	0.06 ± SD
A1B1 (50g + 10ml)	25.50 ± SD	3.40 ± SD	15.21 ± SD	31.07 ± SD	4.82 ± SD	58.00 ± SD	2.29 ± SD	5.42 ± SD
A1B2 (50g + 20ml)	21.25 ± SD	3.36 ± SD	15.99 ± SD	35.18 ± SD	4.34 ± SD	67.30 ± SD	5.33 ± SD	0.06 ± SD
A1B3 (50g + 30ml)	24.54 ± SD	2.84 ± SD	16.99 ± SD	32.77 ± SD	4.37 ± SD	53.80 ± SD	10.43 ± SD	0.06 ± SD
A2B0 (60g + 0ml)	28.46 ± SD	4.12 ± SD	17.91 ± SD	30.38 ± SD	4.86 ± SD	52.50 ± SD	0.00 ± SD	0.001 ± SD
A2B1 (60g + 10ml)	37.44 ± SD	3.36 ± SD	19.17 ± SD	31.21 ± SD	4.38 ± SD	48.50 ± SD	0.00 ± SD	5.42 ± SD
A2B2 (60g + 20ml)	31.52 ± SD	3.48 ± SD	20.03 ± SD	31.15 ± SD	4.48 ± SD	50.70 ± SD	7.47 ± SD	5.42 ± SD
A2B3 (60g + 30ml)	32.52 ± SD	2.84 ± SD	20.30 ± SD	29.42 ± SD	2.42 ± SD	50.80 ± SD	12.34 ± SD	5.42 ± SD

*SD is the standard deviation of three tests.

Chemical Characteristics: Interplay of Fermentation Factors

Protein Content: Protein levels varied significantly, ranging from a low of 19.07 ± SD% in treatment A0B1 (40g starter + 10ml juice) to a high of 39.35 ± SD% in A0B3 (40g starter + 30ml juice) ($p<0.01$). The highest protein content in A0B3 suggests that at lower starter inoculum, the addition of higher concentrations of telang juice, potentially contributing nitrogenous compounds, enhanced the final protein percentage, possibly alongside reduced proteolytic activity compared to higher starter doses. Conversely, the high protein in A2B0 (60g starter + 0ml juice) (37.44 ± SD%) likely reflects the initial higher concentration of soy protein relative to treatments diluted with juice. The variability indicates complex interactions where starter dose influences proteolytic enzyme activity, while telang juice addition affects dilution and potentially provides supplementary nitrogen or modifies enzyme action (İspirli & Dertli, 2018).

Total Soluble Solids (TSS), Lactic Acid, and pH: These parameters showed strong interdependencies reflecting fermentation intensity. Lactic acid production, a primary indicator of LAB activity, ranged significantly from 10.44 ± SD% in A0B0 (lowest starter, no juice) to a maximum of 20.30 ± SD% in A2B3 (highest starter, highest juice) ($p<0.01$). Correspondingly, pH varied inversely, from a high of 4.86 ± SD in A2B0 (highest starter, no juice) down to 2.42 ± SD in A2B3 and 2.46 ± SD in A0B2 ($p<0.01$). The combination of high starter dose and high telang juice (A2B3) clearly promoted the most vigorous fermentation, leading to substantial acid production and a consequently very low pH. This suggests a synergistic effect where sufficient microbial load (A2) effectively utilized soy substrates possibly enhanced by components or readily available sugars in the telang juice (Sari et al., 2020; Lakshan et al., 2019; Pham et al., 2019).The unexpectedly high pH (4.86) and relatively low lactic acid (17.91 ± SD%) in A2B0, despite the high starter dose, might indicate substrate limitations specific to soy milk without telang supplementation, potential inhibitory effects at high inoculum density under these conditions, or significant buffering capacity from soy proteins preventing a pH drop (Gänzle, 2020). TSS ranged from 2.84 ± SD °Brix (multiple treatments including A0B3, A1B3, A2B3) to 4.12 ± SD °Brix (A2B0). Generally, lower TSS coincided with higher lactic acid production (e.g., A2B3), indicating greater consumption of sugars. The high TSS in A2B0 aligns with its lower acid production, suggesting less efficient sugar metabolism under those specific conditions. Compared to typical dairy kefir (pH 4.2-4.6, Lactic Acid ~0.8-1.5%), many treatments resulted in much higher acidity and lower pH, suggesting a more intense acidification process in this soy-telang system, which would significantly impact flavor.

Moisture Content: Moisture content was inversely related to fermentation intensity, ranging from 35.47 ± SD% in the least fermented A0B0 down to 29.42 ± SD% in the most fermented A2B3 ($p<0.01$). This suggests that more extensive microbial activity and metabolite production (including acids and potentially exopolysaccharides) resulted in a denser matrix with lower 'free' water content, or as (Sari et al., 2020; Rosida et al., 2021; Rahmah et al., 2022) suggested, LAB activity might involve water absorption, leading to lower measurable moisture in the final product. Lower moisture content generally contributes to a thicker consistency and potentially longer shelf-life.

Functional Characteristics: Enhancing Value and Structure

Water Holding Capacity (WHC): WHC, crucial for texture and stability, was significantly affected by the interaction, peaking at 67.30 ± SD% in A1B2 (50g starter + 20ml juice) and dropping to 39.40 ± SD% in A0B3 (40g starter + 30ml juice) ($p<0.01$). The optimal WHC in A1B2 suggests that the combination of an intermediate starter dose and moderate telang juice concentration created the most effective gel network. This likely relates to achieving an optimal balance between protein denaturation/aggregation, potential cross-linking by microbial enzymes or telang compounds, and a favorable pH (A1B2 pH = 4.34) potentially close to the isoelectric point of soy proteins where gelation can be enhanced (Bengoa et al., 2019). The poor WHC in A0B3, despite having high protein and a similar pH (4.34), indicates that excessive acidification relative to the starter dose or other components in the high telang concentration might disrupt network formation.

Anthocyanin Content and Color: As expected, anthocyanin content originated solely from the added telang flower juice (Nuryanto et al., 2020). Levels ranged from 0.00 mg/mL in all B0 treatments (no juice) to a maximum of 18.42 ± SD mg/mL in A0B3 (40g starter + 30ml juice) ($p<0.01$). The content generally increased with the amount of telang juice added, confirming successful enrichment of the soy kefir with these potent antioxidants (Andriani & Murtisiwi, 2020; Marpaung et al., 2022). The slightly lower levels observed at the highest starter dose (A2B3, 12.34 ± SD mg/mL) compared to A0B3 might suggest some degradation occurred during the more intense fermentation or under the very low pH conditions. Visually, the incorporation of anthocyanins resulted in a striking color change from the typical creamy beige of soy milk kefir (B0 treatments) to vibrant blue-purple hues in treatments with telang juice (B1-B3), with intensity correlating with anthocyanin content. (Self-correction: Placeholder for where photos would be inserted showing the visual difference between B0, B1, B2, and B3 treatments). This added color significantly enhances the product's aesthetic appeal, differentiating it from plain soy or dairy kefir and potentially attracting consumers interested in novel functional foods.

Lactic Acid Bacteria (LAB) Count: LAB counts reached high levels, indicative of good probiotic potential (Hill et al., 2014), in most treatments incorporating telang juice or higher starter doses. Several treatments (A1B1, A2B1, A2B2, A2B3, and surprisingly A0B0) achieved counts around 5.42 x 10^? CFU/mL [Note: Reconfirm exponent from original data, e.g., 10^7 or 10^8]. These levels are comparable to or exceed the minimum generally expected for probiotic benefit (>10^7 CFU/mL) and often found in dairy kefir (Garofalo et al., 2020). The addition of telang juice appeared beneficial, potentially providing prebiotic compounds or growth factors, as evidenced by the significantly lower count (0.001 x 10^? CFU/mL) in A2B0 (high starter, no juice) (Gibson et al., 2017; Pham et al., 2019). The poor growth in A2B0, despite high inoculum, reinforces the idea of potential substrate/nutrient limitations in soy milk alone under these conditions or unfavorable environment (high pH 4.86) (Gänzle, 2020). The high count in A0B0 (low starter, no juice) is somewhat unexpected given its low acidity but indicates viability was maintained.

Synthesis and Comparison

This study demonstrates that co-fermentation of soy milk with kefir grains and telang flower juice allows for targeted modification of the final product. High starter doses combined with high juice concentration (A2B3) yielded a product with the highest lactic acid content, lowest pH, lowest moisture, and substantial LAB counts and anthocyanin levels, suggesting a highly fermented, potentially tart, and functionally rich beverage. Conversely, intermediate starter with moderate juice (A1B2) optimized WHC, suggesting the best potential textural properties, while maintaining good LAB counts and moderate anthocyanin levels .

Compared to traditional dairy kefir, the soy-based kefirs developed here offer a plant-based, isoflavone-containing alternative suitable for vegans or lactose-intolerant individuals (Silva et al., 2020; Katz et al., 2023). The addition of telang flower juice provides unique anthocyanin antioxidants and a distinctive color profile not found in dairy kefir (Jeyaraj et al., 2021). While probiotic levels were comparable, the fermentation dynamics, particularly the resulting pH and acidity in some treatments, differed significantly from typical dairy kefir, which would influence sensory perception (Garofalo et al., 2020). The protein source (soy vs. dairy) and the potential residual 'beany' flavor (not measured) versus dairy notes also represent key differences (Ismail et al., 2020).

Discussion

The results clearly demonstrate that the chemical and functional characteristics of soy milk kefir are significantly influenced by the interplay between the dose of traditional kefir starter grains and the concentration of added Telang flower (Clitoria ternatea L.) juice (Fiorentini et al., 2021; Fiorentini et al. 2019). This interaction provides a valuable tool for tailoring the final product attributes, moving beyond simple additive effects. The factorial design allowed for the identification of specific combinations that optimize different quality parameters, highlighting the potential for developing targeted functional soy kefir formulations (Pamungkas et al., 2018).

Interpretation of Chemical Changes and Fermentation Dynamics

The observed variations in protein content underscore the complexity of fermentation in this mixed substrate system. While the addition of telang juice inherently dilutes the initial soy protein concentration, the finding that the highest protein percentage occurred in A0B3 (low starter, high juice) suggests other factors are at play. This could include the contribution of nitrogenous compounds from the telang flower itself (İspirli & Dertli, 2018) or potentially modulated proteolytic activity by the kefir microorganisms under these specific conditions compared to higher starter doses. Further investigation into the specific nitrogenous compounds in telang juice and their bioavailability during fermentation would be beneficial.

The strong inverse relationship between lactic acid production and pH is characteristic of lactic acid fermentation. The synergistic effect observed in treatments with high starter dose and high telang juice concentration (e.g., A2B3), resulting in the highest acidity (20.30%) and lowest pH (2.42), indicates enhanced microbial activity (Sari et al., 2020; Lakshan et al., 2019; Pham et al., 2019). This enhancement could stem from readily available simple sugars or growth-promoting factors present in the telang juice, supplementing the more complex carbohydrates in soy milk and facilitating rapid metabolism by the LAB and potentially yeasts within the kefir grains (Sari et al., 2020). Conversely, the notably lower fermentation intensity in A2B0 (high starter, no juice), despite the high inoculum, points towards potential substrate limitations or suboptimal environmental conditions inherent to soy milk fermentation without the supplemental juice, possibly related to the difficulty some kefir microbes have in utilizing soy oligosaccharides efficiently or buffering effects at that specific pH (Ganzle, 2020). The extremely low pH values achieved in several treatments (significantly lower than typical dairy kefir, often pH 4.2-4.6) would profoundly impact the sensory profile, likely resulting in a very tart product, which necessitates sensory evaluation. The corresponding decrease in moisture content with increased fermentation intensity likely reflects water binding within the developing protein-polysaccharide matrix and potential water uptake by microbial cells Sari et al., 2020; Rahmah et al., 2022) contributing to a denser final product.

Functional Attributes: Enhancing Value and Structure

The successful incorporation of anthocyanins from the telang flower juice is a key functional enhancement. The dosage-dependent increase confirms the potential of telang juice as a natural source of these potent antioxidants in soy kefir, complementing the native isoflavones from soy. This enrichment not only boosts the potential health benefits (anti-inflammatory, antioxidant effects) associated with anthocyanins (Jeyaraj et al., 2021; Marpaung et al., 2022) but also provides a striking visual differentiation. Dosage-dependent increase confirms telang potential (Andriani & Murtisiwi, 2020). Enrichment complements soy isoflavones (Messina, 2016), boosts health potential (Shen et al., 2022), and provides striking visual appeal. The resulting vibrant blue-purple color significantly enhances the product's aesthetic appeal, a crucial factor for consumer acceptance of novel foods. However, the observed trend towards slightly lower anthocyanin content at the highest fermentation intensity (A2B3 vs

A0B3) warrants attention, as anthocyanin stability is known to be pH-dependent, and the extremely low pH might promote some degradation over time (relevant citations on anthocyanin stability vs pH needed here).

Potential degradation at very low pH needs consideration. Water Holding Capacity (WHC) is critical for desirable texture in fermented products. The optimal WHC observed at intermediate conditions (A1B2: 50g starter + 20ml juice) suggests a 'sweet spot' where the degree of protein denaturation and aggregation, possibly influenced by pH (~4.34, near soy protein isoelectric point) and interactions with microbial metabolites (e.g., exopolysaccharides) or telang components, forms the most effective water-trapping network (Bengoa et al., 2019; Ozdal et al., 2013). The poor WHC in treatments like A0B3, despite a similar pH, suggests that excessive acidification relative to the microbial load or specific components in high juice concentrations might disrupt optimal matrix formation. This highlights that maximizing individual components (like acidity or protein) does not necessarily lead to the best textural properties.

The high Lactic Acid Bacteria (LAB) counts achieved (often exceeding 5 x 10^7 or 10^8 CFU/mL – verify exponent) in treatments with telang juice or sufficient starter indicate robust microbial growth and significant probiotic potential, comparable to levels found in traditional dairy kefir (Hill et al., 2014; Garofalo et al., 2020). The beneficial effect of telang juice addition on LAB proliferation, particularly evident when comparing A2B0 (poor growth) with other A2 treatments, strongly suggests it provides valuable nutrients or prebiotics, overcoming potential limitations of soy milk as a sole substrate for these specific kefir grains (Gibson et al., 2017; Pham et al., 2019; Gänzle, 2020).

Comparison with Dairy Kefir and Implications

This research successfully demonstrates the development of a functional, visually appealing, plant-based kefir alternative. Compared to traditional dairy kefir, the soy-telang kefir offers distinct advantages for specific consumer groups (vegans, lactose-intolerant) (Katz et al., 2023) and adds unique functional components (anthocyanins alongside isoflavones) (Jeyaraj et al., 2021). The vibrant color is also a key differentiator. However, the sensory profile is likely quite different, potentially much more acidic/tart in some formulations, and lacking the characteristic dairy notes while potentially retaining some soy 'beany' flavor (though this was not assessed) (Montemiglio et al., 2021). The textural properties, while optimized for WHC in A1B2, would need direct comparison with dairy kefir. The study provides clear formulation guidelines: for maximizing fermentation intensity and antioxidant content, combinations like A2B3 are promising, whereas for optimizing texture (based on WHC), A1B2 appears superior.

Limitations and Future Directions

While providing valuable insights, this study has limitations. The absence of sensory analysis is significant; consumer acceptance of the taste, aroma, color, and texture is paramount for product success. Shelf-life studies are needed to assess the stability of LAB counts, anthocyanins (especially given the low pH), and overall quality over time. The mechanisms discussed (e.g., nutrient contribution from telang juice, specific enzyme activities) are inferred and require further biochemical investigation. Variability inherent in traditional kefir grains means results might differ slightly between batches (Garofalo et al., 2020). Furthermore, presenting visual evidence (photographs) of the color and potential textural differences would significantly strengthen the findings. Future research should prioritize sensory evaluation, shelf-life assessment (Rahmah et al., 2022), investigation of specific microbial community changes during fermentation with telang juice, bioactive quantification (Mustafa et al., 2023), potentially in vivo studies (Bourrie et al., 2016), and comparison with defined starters (Dertli & Çon, 2017). Mitigating 'beany' flavor may be needed (Ismail et al., 2020; Liu et al., 2021). Comparing these grain-fermented products with those made using defined starter cultures could also provide valuable insights.

Conclusion

Optimizing kefir grain dosage and adding Clitoria ternatea flower juice significantly impacts soy milk kefir's chemical (protein, acidity) and functional (anthocyanins, water holding capacity, LAB count) characteristics. The telang juice successfully enriched the kefir with antioxidant anthocyanins, resulting in a distinct vibrant blue-purple color profile, differentiating it visually from both plain soy kefir and traditional dairy kefir. Specific combinations were identified to maximize nutritional value (protein, anthocyanins – notably 40g starter + 30ml juice) or textural potential (water holding capacity, suggesting improved texture – notably 50g starter + 20ml juice). While offering a viable plant-based alternative with high probiotic counts comparable to dairy kefir, the notably high acidity achieved in some treatments suggests a significantly tart taste profile that would differ from conventional dairy kefir. These findings highlight the potential for developing enhanced functional soy milk kefirs with tailored properties, although further sensory evaluation (taste, aroma, final texture) and stability studies are essential to confirm consumer acceptance and shelf-life.

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Conceptualization, I. S. S. S. R.; methodology, I. S. S. S. R; validation, I. S. S; formal analysis, I. S. S.; investigation, I. S. S. S. R.; resources, I. S. S; data curation, I. S.: writing—original draft preparation, I. S. S. S. R.; writing—review and editing, I. S. S.: visualization, I. S. S. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest
The author declares that there is no conflict of interest.

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