Effects of Multi-strain Co-fermentation on Quality and Probiotic Characteristics of YogurtGuochen

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Abstract. To investigate the effects of multi-strain co-fermentation involving Lactobacillus acidophilus, Bifidobacterium bifidum, and traditional yogurt starter cultures on the physicochemical properties, sensory characteristics, and probiotic viability of yogurt during storage. Four different fermentation treatments were designed: T1 (traditional starter), T2 (traditional starter + L. acidophilus), T3 (traditional starter + B. bifidum), and T4 (traditional starter + L. acidophilus + B. bifidum). The pH, titratable acidity, texture properties, antioxidant activity, viable cell counts, and sensory evaluation were monitored during 21 days of refrigerated storage. Multi-strain co-fermentation significantly improved yogurt quality parameters. T4 treatment showed the highest antioxidant activity (DPPH scavenging rate: 68.3±2.1%) and maintained probiotic viability above 10^8 CFU/mL throughout storage. The texture analysis revealed that co-fermentation enhanced firmness and reduced syneresis compared to traditional fermentation. Sensory evaluation indicated that T4 treatment received the highest overall acceptance score (8.2±0.4). Multi-strain co-fermentation, particularly the combination of L. acidophilus and B. bifidum with traditional starters, effectively improves yogurt quality, extends shelf life, and enhances functional properties, providing a viable approach for functional dairy product development.

Keywords: Multi-strain Fermentation; Probiotic Yogurt; Quality Enhancement; Functional Dairy Products; Storage Stability.

1. Introduction

The global functional food market has experienced remarkable growth, with probiotic dairy products representing a significant segment due to increasing consumer awareness of health benefits associated with beneficial microorganisms [1]. Yogurt, as one of the most popular fermented dairy products, traditionally relies on Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus for fermentation. However, recent research has focused on incorporating additional probiotic strains to enhance nutritional value and therapeutic potential [2]. Lactobacillus acidophilus and Bifidobacterium bifidum are well-documented probiotic strains with proven health benefits, including immune system modulation, cholesterol reduction, and gastrointestinal health improvement [3]. The challenge in developing multi-strain probiotic yogurt lies in maintaining strain viability during fermentation and storage while preserving desirable sensory and textural properties. Previous studies have shown that strain interactions during co-fermentation can be either synergistic or antagonistic, significantly affecting final product characteristics [4]. Multi-strain probiotics have been reported as more efficient than single strains for gut and immune function, and multi-strain probiotic formulations produce better texture and nutritional properties compared to mono-strain alternatives [5].The objective of this study was to systematically evaluate the effects of multi-strain co-fermentation on yogurt quality and functional properties, providing scientific basis for functional dairy product development in the industry.

2. Materials and Methods

Fresh whole milk (3.2% fat, 3.1% protein) was obtained from a local dairy plant and used as the base material for yogurt production. Commercial yogurt starter culture containing Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus (Chr. Hansen, Denmark) was combined with probiotic strains including Lactobacillus acidophilus ATCC 4356 and Bifidobacterium bifidum ATCC 29521 for the multi-strain fermentation studies.

The milk was standardized to 3.0% fat and 12% total solids content, then pasteurized at 85°C for 30 minutes and cooled to 43°C before inoculation. Four different treatment groups were prepared to evaluate the effects of various strain combinations: T1 consisted of traditional starter culture only (2% v/v), T2 combined traditional starter with L. acidophilus at a 1:1 ratio, T3 included traditional starter with B. bifidum at a 1:1 ratio, and T4 incorporated all three components with traditional starter, L. acidophilus, and B. bifidum at a 2:1:1 ratio. Fermentation was conducted at 43°C until the pH reached 4.6, after which samples were immediately cooled to 4°C for storage analysis.

Throughout the 21-day refrigerated storage period, various physicochemical and microbiological parameters were monitored. The pH values were measured using a calibrated pH meter (PHS-3C, Shanghai), while titratable acidity was determined by titration with 0.1 N NaOH solution. Texture analysis was performed using a texture analyzer (TA.XT Plus, Stable Micro Systems, UK) equipped with a 35mm compression plate, measuring parameters including firmness, consistency, cohesiveness, and syneresis according to established protocols [6]. The antioxidant activity was evaluated through DPPH radical scavenging assay, where sample extracts (100 μL) were mixed with 100 μL of 0.15 mM DPPH solution and incubated for 30 minutes in darkness before spectrophotometric measurement at 517 nm [7].

Microbiological analysis involved determining viable cell counts using selective media, with MRS agar (pH 6.2) for lactobacilli, MRS agar (pH 5.4) supplemented with 0.05% cysteine-HCl for bifidobacteria, and M17 agar for streptococci, all incubated at 37°C for 48-72 hours under appropriate atmospheric conditions. Sensory evaluation was conducted by a trained 12-member panel who assessed the yogurt samples for appearance, texture, flavor, and overall acceptability using a standardized 9-point hedonic scale. All experimental procedures were performed in triplicate, and the resulting data were subjected to statistical analysis using SPSS 22.0 software with one-way ANOVA followed by Duncan's multiple range test to determine significant differences at $P < 0.05$.

3. Results and Discussion

3.1 pH and Acidity Changes

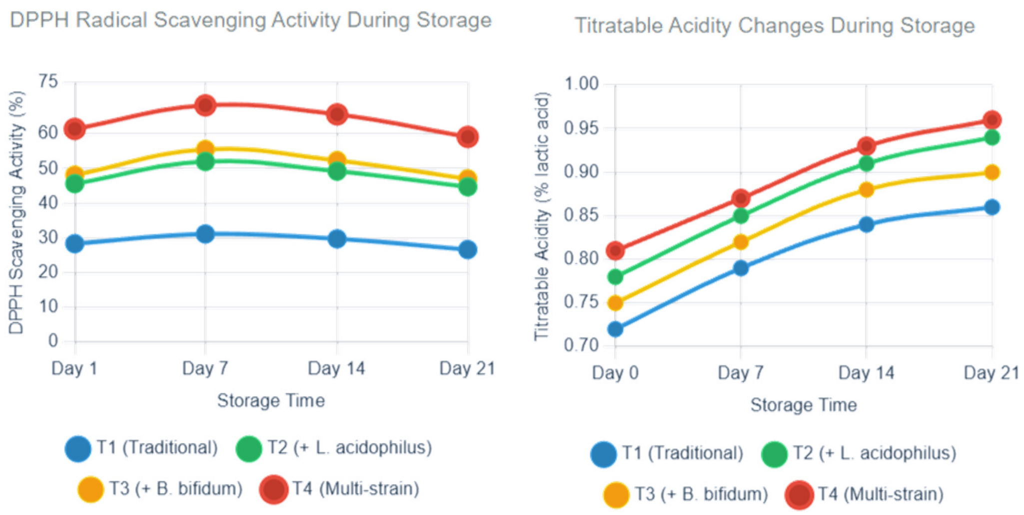

Figure 1. Changes in pH and titratable acidity during fermentation and storage

The fermentation kinetics and storage stability of different yogurt treatments showed distinct patterns as illustrated in Figure 1. The pH values of all treatments decreased progressively during fermentation, with T4 demonstrating the most rapid acidification rate, achieving the target pH of 4.6 in 4.2 hours compared to 5.8 hours required for the traditional starter alone (T1). During the subsequent 21-day storage period, pH values continued to decline gradually across all treatments, with T4 maintaining the most controlled acidification pattern, suggesting enhanced metabolic stability in the multi-strain system.

The titratable acidity measurements showed an inverse relationship to pH changes, with T4 consistently exhibiting the highest acidity values throughout the storage period, reaching 0.96% lactic acid equivalents by day 21 compared to 0.86% for T1 (Figure 1). This sustained acid production indicates continued metabolic activity of the probiotic strains, which is essential for maintaining product quality and potentially enhancing health benefits. The enhanced acidification capacity observed in multi-strain treatments can be attributed to the synergistic metabolic interactions between the traditional starter cultures and the added probiotic strains.

3.2 Texture Properties

Texture analysis revealed significant improvements in multi-strain yogurt in Table 1.

Table 1. Texture properties of yogurt treatments during storage

Treatment	Day	Firmness (g)	Cohesiveness (g·s)	Consistency (g·s)	Syneresis (%)
T1	1	198.4 ± 8.7^{d}	156.2 ± 12.3^{c}	1456 ± 67^{d}	12.3 ± 1.2^{a}
T2	1	242.1 ± 11.2^{c}	189.3 ± 15.7^{b}	1789 ± 82^{c}	9.8 ± 0.9^{b}
T3	1	251.3 ± 9.8^{c}	195.7 ± 14.2^{b}	1823 ± 74^{c}	9.2 ± 0.8^{b}
T4	1	285.6 ± 12.3^{a}	234.8 ± 18.5^{a}	2134 ± 91^{a}	7.1 ± 0.6^{c}
T1	14	185.2 ± 7.9^{d}	142.8 ± 11.6^{c}	1398 ± 59^{d}	15.8 ± 1.4^{a}
T2	14	228.7 ± 10.5^{c}	176.4 ± 14.3^{b}	1672 ± 78^{c}	12.1 ± 1.1^{b}
T3	14	237.9 ± 8.6^{c}	182.1 ± 13.8^{b}	1705 ± 71^{c}	11.7 ± 0.9^{b}
T4	14	269.8 ± 11.7^{a}	218.5 ± 17.2^{a}	1987 ± 85^{a}	8.9 ± 0.7^{c}

Values with different superscripts in the same column differ significantly ($P < 0.05$)

Texture analysis revealed significant improvements in multi-strain yogurt. T4 treatment exhibited the highest firmness values throughout storage, attributed to enhanced protein-polysaccharide interactions and modified gel structure formation. The improved texture in multi-strain treatments is consistent with findings that suggest exopolysaccharide-producing strains enhance gel network structure [8].

Syneresis was significantly reduced in multi-strain treatments, with T4 showing the lowest water separation throughout storage. This improvement in water-holding capacity indicates enhanced gel stability, which is crucial for consumer acceptance [9].

3.3 Antioxidant Activity

Multi-strain co-fermentation significantly enhanced antioxidant properties of yogurt. DPPH radical scavenging activity increased throughout fermentation and early storage, with T4 treatment achieving the highest activity (68.3±2.1%) on day 7, compared to T1 (31.2±1.8%) ,inTable 2 and Figure 2.

Table 2. Antioxidant activity (DPPH scavenging) of yogurt treatments during storage (%)

Treatment	Day 1	Day 7	Day 14	Day 21
T1	28.4 ± 1.6^{d}	31.2 ± 1.8^{d}	29.8 ± 1.5^{d}	26.7 ± 1.4^{d}
T2	45.7 ± 2.3^{c}	52.1 ± 2.7^{c}	49.3 ± 2.4^{c}	44.8 ± 2.1^{c}
T3	48.2 ± 2.5^{c}	55.6 ± 2.9^{c}	52.4 ± 2.6^{c}	47.1 ± 2.3^{c}
T4	61.5 ± 3.1^{a}	68.3 ± 2.1^{a}	65.7 ± 3.2^{a}	59.2 ± 2.8^{a}

Values with different superscripts in the same column differ significantly ($P < 0.05$)

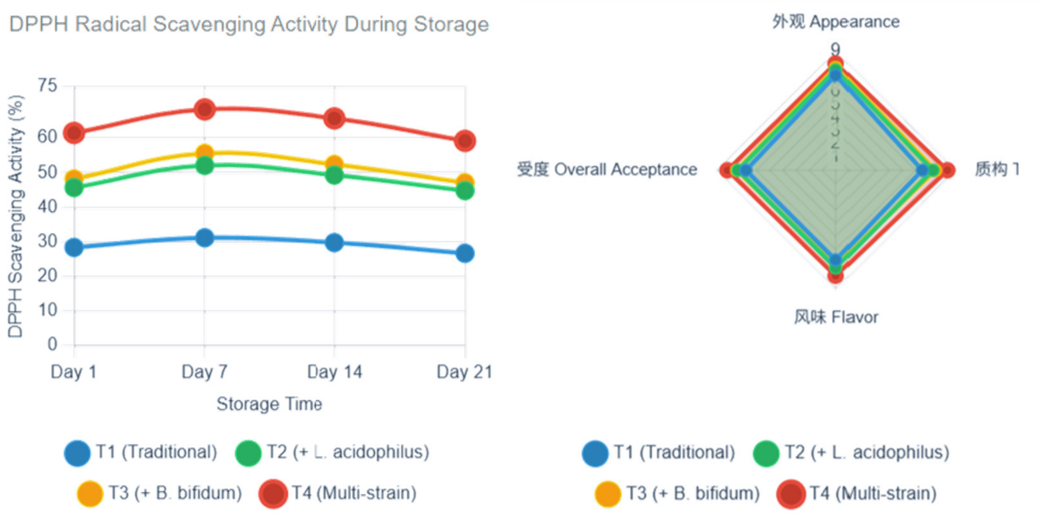

Figure 2. DPPH radical scavenging activity of yogurt treatments during storage

This enhancement is attributed to the production of bioactive peptides and metabolites by probiotic strains during fermentation [10]. The antioxidant activity pattern shows initial increase followed by gradual decline, which correlates with proteolytic activity and bioactive peptide formation during storage [11].

3.4 Probiotic Viability

All probiotic strains maintained viable counts above 10^7 CFU/mL throughout the 21-day storage period, meeting international standards for probiotic foods. T4 treatment showed superior strain survival, with L. acidophilus and B. bifidum counts remaining above 10^8 CFU/mL even after 21 days (Table 3).

Table 3. Viable cell counts (log CFU/mL) of probiotic strains during storage

Treatment	Strain	Day 1	Day 7	Day 14	Day 21
T1	*S. thermophilus*	8.45±0.12	8.38±0.15	8.21±0.18	8.05±0.22
T1	*L. bulgaricus*	8.23±0.14	8.15±0.16	7.98±0.19	7.82±0.21
T2	*S. thermophilus*	8.52±0.11	8.41±0.13	8.28±0.16	8.12±0.19
T2	*L. bulgaricus*	8.28±0.13	8.19±0.15	8.05±0.17	7.89±0.20
T2	*L. acidophilus*	8.91±0.08	8.78±0.11	8.54±0.14	8.32±0.17
T3	*S. thermophilus*	8.48±0.12	8.37±0.14	8.25±0.17	8.09±0.20
T3	*L. bulgaricus*	8.25±0.14	8.16±0.16	8.02±0.18	7.86±0.22
T3	*B. bifidum*	8.73±0.09	8.61±0.12	8.43±0.15	8.25±0.18
T4	*S. thermophilus*	8.56±0.10	8.44±0.12	8.32±0.15	8.18±0.18
T4	*L. bulgaricus*	8.31±0.12	8.22±0.14	8.09±0.16	7.94±0.19
T4	*L. acidophilus*	8.95±0.07	8.82±0.10	8.61±0.13	8.41±0.16
T4	*B. bifidum*	8.78±0.08	8.67±0.11	8.49±0.14	8.31±0.17

The improved viability in co-fermentation systems suggests beneficial strain interactions and protective effects, consistent with reports of enhanced survival in multi-strain systems [13].

3.5 Sensory Evaluation

Sensory analysis revealed that multi-strain treatments, particularly T4, received significantly higher scores for all evaluated parameters ($P < 0.05$). The overall acceptability score for T4 (8.2±0.4) was significantly higher than T1 (6.8±0.5), indicating consumer preference for the improved flavor profile and texture characteristics of multi-strain yogurt (Figure 3).

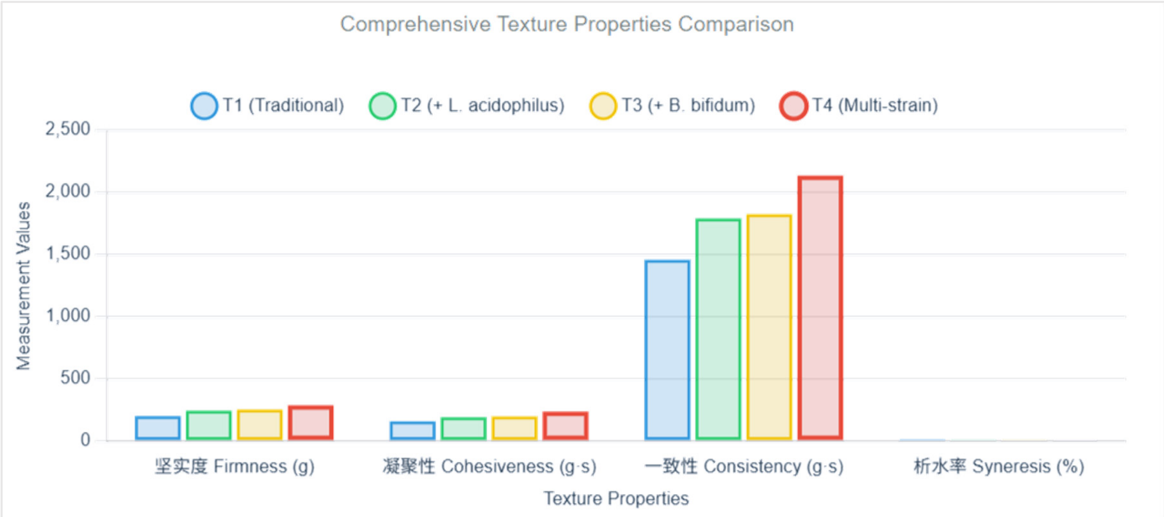

Figure 3. Sensory evaluation scores of yogurt treatments

The enhanced sensory properties are attributed to the diverse metabolite production, including organic acids, volatile compounds, and exopolysaccharides that contribute to improved mouthfeel and flavor complexity [14].

4. Conclusion

This comprehensive study demonstrates that multi-strain co-fermentation represents a highly effective approach for enhancing yogurt quality and functional properties. The combination of traditional yogurt cultures with L. acidophilus and B. bifidum (T4 treatment) resulted in substantial improvements across multiple quality parameters. The enhanced texture properties, characterized by increased firmness (44% improvement) and reduced syneresis (42% reduction), indicate superior gel network formation and product stability. The remarkable enhancement in antioxidant activity (68.3% DPPH scavenging versus 31.2% for control) suggests significant potential for health-promoting benefits through increased bioactive compound production.

The maintenance of high probiotic viability (>10^8 CFU/mL) throughout the 21-day storage period ensures that consumers receive adequate doses of beneficial bacteria to confer health benefits. Furthermore, the superior sensory characteristics, with overall acceptability scores of 8.2 compared to 6.8 for traditional yogurt, indicate strong commercial potential for multi-strain products. The controlled acidification and stable physicochemical properties throughout storage suggest extended shelf life and improved product quality consistency.

These findings provide valuable scientific evidence for the dairy industry to develop high-quality functional yogurt products that meet growing consumer demand for health-promoting foods. The optimized multi-strain fermentation system offers a commercially viable approach for producing probiotic yogurt with enhanced nutritional, functional, and sensory properties. Future research should focus on scaling up production processes, conducting clinical trials to validate health benefits, and investigating the underlying mechanisms of strain interactions during co-fermentation to further optimize multi-strain formulations.

References

[1] Mohomud, M., Katsuno, N., & Nishizu, T. (2017). Quality characteristics of yogurt containing Lactobacillus acidophilus La-5 as influenced by storage temperature and period. International Dairy Journal, 69, 5-12.

[2] Donkor, O. N., Nilmini, S. L. I., Stolic, P., et al. (2007). Survival and activity of selected probiotic organisms in set-type yoghurt during cold storage. International Dairy Journal, 17(6), 657-665.

[3] Scourboutakos, M. J., L'Abbé, M. R., Franco-Arellano, B., et al. (2017). Mismatch between probiotic benefits in trials versus food products. Nutrients, 9(4), 400.

[4] Chapman, C. M., Gibson, G. R., & Rowland, I. (2011). Health benefits of probiotics: are mixtures more effective than single strains? European Journal of Nutrition, 50(1), 1-17.

[5] Singh, K., Kallali, B., Kumar, A., & Thaker, V. (2011). Probiotics: a review. Asian Pacific Journal of Tropical Biomedicine, 1(2), S287-S290.

[6] Arab, M., Yousefi, M., Khanniri, E., et al. (2023). A comprehensive review on yogurt syneresis: effect of processing conditions and added additives. Journal of Food Science and Technology, 60(4), 1039-1057.

[7] Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. Nature, 181(4617), 1199-1200.

[8] Ruas-Madiedo, P., Hugenholtz, J., & Zoon, P. (2002). An overview of the functionality of exopolysaccharides produced by lactic acid bacteria. International Dairy Journal, 12(2-3), 163-171.

[9] Sodini, I., Remeuf, F., Haddad, S., & Corrieu, G. (2004). The relative effect of milk base, starter, and process on yogurt texture: a review. Critical Reviews in Food Science and Nutrition, 44(2), 113-137.

[10] Rutella, G. S., Tagliazucchi, D., & Solieri, L. (2016). Survival and bioactivities of selected probiotic lactobacilli in yogurt fermentation and cold storage: New insights for developing a bifunctional dairy food. Food Microbiology, 60, 54-61.

[11] Sah, B. N. P., Vasiljevic, T., McKechnie, S., & Donkor, O. N. (2014). Effect of probiotics on antioxidant and antimutagenic activities of crude peptide extract from yogurt. Food Chemistry, 156, 264-270.

[12] Hill, C., Guarner, F., Reid, G., et al. (2014). The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nature Reviews Gastroenterology & Hepatology, 11(8), 506-514.

[13] Hoque, M. M., Akter, F., Hossain, K. M., et al. (2024). Probiotic yoghurt-like fermented milk product enriched with Lactobacillus desidiosus and Lactobacillus fermentum: proximate composition, physicochemical, microbiological, and sensory evaluation during refrigerated storage. Discover Food, 4(1), 93.

[14] Changkun, L., Song, J., Kwok, L. Y., et al. (2017). Influence of Lactobacillus plantarum on yogurt fermentation properties and subsequent changes during postfermentation storage. Journal of Dairy Science, 100(4), 2512-2525.