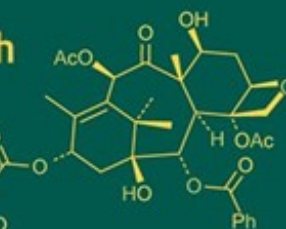
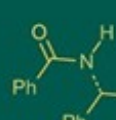


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Refrigerated storage stability, probiotic survivability and techno-economic feasibility of dragon-fruit-enriched synbiotic buttermilk

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ABSTRACT

This study evaluated the refrigerated-storage behaviour, probiotic survivability, packaging response and production economics of an optimized synbiotic buttermilk containing 10% red dragon fruit (*Hylocereus polyrhizus*) pulp. Cow milk was fermented with 2% mixed lactic starter and 2% *Bifidobacterium bifidum*, blended with fruit pulp, packed in pre-sterilized bottles and stored at $4 \pm 1^\circ\text{C}$ for 8 days. Microbial quality and viable populations were assessed at 2-day intervals. The general lactic acid bacterial count declined from 4.45×10^8 CFU/mL on day 0 to 3.85×10^8 CFU/mL on day 8. In the selective survivability assessment, LAB and *Bifidobacterium* counts decreased only slightly, from 7.75 to 7.50×10^7 CFU/mL and from 8.92 to 8.62×10^7 CFU/mL, respectively. Coliforms remained undetected throughout storage, whereas yeast and mould were undetected through day 6 and appeared at 1.12×10^4 CFU/mL on day 8. Post-acidification occurred in both glass and PET bottles: pH changed from 4.40 to 4.00 in glass and from 4.50 to 3.90 in PET, while titratable acidity increased from 0.51% to 0.70% and 0.69%, respectively. The estimated production cost was ₹71.10/L or ₹14.22 per 200-mL serving. These findings indicate that the dragon-fruit-enriched beverage retained probiotic populations above commonly cited desirable levels over the evaluated period and was economical to produce. However, yeast and mould detection on day 8 suggests that, under the tested conditions, a conservative refrigerated shelf life should not exceed 6 days unless further validation establishes an acceptable microbiological limit.

Keywords: *Bifidobacterium bifidum*, Dragon fruit, Functional dairy beverage, Post-acidification, Probiotic viability, Refrigerated shelf life, Synbiotic buttermilk

1. Introduction

Fermented dairy beverages are widely used as carriers for beneficial microorganisms because the milk matrix supplies fermentable nutrients and can protect bacterial cells during manufacture, storage and passage through the gastrointestinal tract. A probiotic is a live microorganism that, when administered in an adequate amount, confers a health benefit on the host (Hill *et al.*, 2014) [1]. A synbiotic combines live microorganisms with substrate(s) selectively utilized by host microorganisms to provide a health benefit (Swanson *et al.*, 2020) [2]. Accordingly, the functional value of a synbiotic beverage depends not merely on adding a culture but on retaining an adequate viable population until consumption.

Bifidobacterium species are valued in functional foods but are technologically demanding because their survival is affected by strain, oxygen exposure, acidity, storage temperature and the physicochemical characteristics of the food matrix. Refrigerated fermented products commonly undergo post-acidification, with declining pH and increasing titratable acidity, and these changes may progressively reduce probiotic survival (Mani-López *et al.*, 2014; Shori, 2022) [3, 4]. Reviews of dairy probiotic foods emphasize that formulation and matrix composition are decisive determinants of *bifidobacterial* stability (Sibanda *et al.*, 2024) [5].

Red dragon fruit is a promising adjunct for cultured dairy beverages. Its carbohydrates and soluble fibre may support microbial metabolism, while its natural pigments and phytochemicals can improve product appeal and functional positioning. However, fruit addition also changes the acidity, nutrient environment and spoilage risk of a fermented beverage. A storage study is therefore essential before claims concerning probiotic delivery, shelf life or commercial feasibility can be made.

Packaging may further influence stability through differences in oxygen, moisture and light barriers. Glass is chemically inert and offers a strong gas barrier, whereas PET is lighter and less breakable but can differ in permeability. Comparing product acidity and pH in the two packages during refrigeration provides a practical indication of whether either container accelerates post-acidification.

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The present work focused on the two downstream objectives of the product-development study: (i) evaluating the viability of *Bifidobacterium* during refrigeration and (ii) assessing shelf life and techno-economic feasibility. The optimized beverage containing 10% dragon-fruit pulp was monitored for viable LAB and *Bifidobacterium*, hygiene indicators, packaging-related pH and acidity changes, and estimated production cost.

2. Materials and Methods

2.1 Product formulation and preparation

The optimized formulation (T₂) comprised 90% buttermilk and 10% red dragon-fruit pulp and contained 2% mixed lactic starter culture and 2% *Bifidobacterium bifidum* culture. Cow milk was heated at 90 °C for 5 min, cooled to 40–42 °C, inoculated with both cultures and incubated at 37 ± 1 °C for 6–8 h until a firm coagulum and a titratable acidity of approximately 0.7–0.9% lactic acid were obtained. The curd was churned, blended with pasteurized dragon-fruit pulp and permitted salt/spices, homogenized, filled into pre-sterilized bottles and held at 4 ± 1 °C.

2.2 Culture activation and maintenance

The freeze-dried *B. bifidum* and mixed lactic starter were activated through three successive transfers using 1% inoculum at 37 °C for 24 h. TPY broth supplemented with L-cysteine hydrochloride was used for *B. bifidum*; sterile skim milk or MRS broth was used for the mixed starter. *Bifidobacterium* cultures were incubated anaerobically at 37 °C for 24–48 h. Purity was checked by Gram staining and catalase reaction. Working cultures containing approximately 1 × 10⁸ CFU/mL were stored at 4 ± 1 °C and subcultured at fortnightly intervals.

2.3 Refrigerated-storage design

The optimized beverage was examined on days 0, 2, 4, 6 and 8 of storage at 4 ± 1 °C. These actual sampling points were used for microbial quality, selective probiotic survivability and packaging comparisons. Glass and PET bottles were evaluated separately for pH and titratable acidity. All microbiological determinations were performed in triplicate.

2.4 Microbiological quality

For general aerobic enumeration, a 10-mL beverage sample was homogenized with 90 mL sterile 0.1% peptone water and serially diluted. One-millilitre aliquots were pour-plated with 15–20 mL molten plate-count agar at 45 ± 1 °C and incubated at 37 ± 1 °C for 48 ± 2 h. Yeasts and moulds were enumerated by spreading 0.1 mL of appropriate dilutions on potato dextrose agar acidified to pH 3.5 ± 0.1, followed by incubation at 25 ± 1 °C for 5 days. Coliforms were assessed by the violet-red bile agar pour-plate procedure with an overlay and incubation at 37 ± 1 °C for 24 ± 2 h. Results were expressed as CFU/mL; absence of colonies at the tested dilution was recorded as not detected (ND). The procedures were adapted from ISO 4833-1:2013 [6], ISO 21527-1:2008 [7] and ISO 4832:2006 [8], respectively.

2.5 LAB and *Bifidobacterium* survivability

For selective LAB enumeration, serial dilutions were pour-plated on de Man-Rogosa-Sharpe agar and incubated anaerobically at 37 ± 1 °C for 48–72 h. *B. bifidum* was enumerated on MRS agar supplemented with 0.05% L-cysteine hydrochloride and incubated anaerobically at 37 ± 1 °C for 72 h. Plates containing 30–300 colonies were counted, and viable populations were calculated from colony number, dilution factor and plated volume and expressed as CFU/mL. MRS medium was based on de Man *et al.* (1960) [9].

2.6 Packaging response

The pH of beverage samples stored in glass and PET bottles was measured on each sampling day using a calibrated digital pH meter. Titratable acidity was determined by neutralization with standard alkali and expressed as percentage lactic acid. Standard error (SE) and

critical difference (CD) at the 5% level were available for each storage-day comparison.

2.7 Techno-economic assessment

Ingredient, culture, fruit, packaging and labelling costs were calculated using the prevailing purchase prices recorded during the experiment. Processing cost was taken as 20% of raw-material cost. Total batch cost, cost per litre and cost per 200-mL serving were then calculated. The estimate excludes profit margin, distribution, marketing, depreciation and taxes and therefore represents production cost rather than a final retail price.

3. Results and Discussion

3.1 Microbial quality during refrigerated storage

Table 1. Microbial quality of optimized synbiotic buttermilk during storage at 4 ± 1 °C

Storage (day)	General LAB count (×10 ⁸ CFU/mL)	Yeast and mould (×10 ⁴ CFU/mL)	Coliforms
0	4.45	ND	ND
2	4.40	ND	ND
4	4.24	ND	ND
6	4.05	ND	ND
8	3.85	1.12	ND

ND, not detected at the tested dilution. Values were reported from the thesis storage dataset.

The general LAB count decreased progressively from 4.45 × 10⁸ CFU/mL at day 0 to 3.85 × 10⁸ CFU/mL at day 8, an absolute decline of 0.60 × 10⁸ CFU/mL (13.5%). Despite this reduction, the final count remained high. Refrigerated storage slows bacterial metabolism but does not completely prevent injury caused by acidity, nutrient depletion or accumulated fermentation products. A gradual loss of viable cells is consequently expected in cultured dairy products.

Coliforms were not detected at any sampling point, supporting satisfactory hygienic preparation and handling under the conditions used. Yeast and mould were absent through day 6 but reached 1.12 × 10⁴ CFU/mL on day 8. This late appearance is the clearest shelf-life constraint in the dataset. Although the probiotic population remained abundant, a functional count alone cannot establish acceptable shelf life when spoilage organisms emerge. Therefore, the evidence supports a conservative refrigerated shelf life of up to 6 days for the present process unless day-8 acceptability is demonstrated against an applicable microbiological specification and confirmed in replicate production batches.

3.2 Survival of LAB and *Bifidobacterium*

Table 2. Selective viable counts in optimized synbiotic buttermilk during refrigerated storage

Storage (day)	LAB (×10 ⁷ CFU/mL)	<i>Bifidobacterium</i> spp. (×10 ⁷ CFU/mL)
0	7.75	8.92
2	7.69	8.85
4	7.62	8.78
6	7.56	8.70
8	7.50	8.62

Counts are expressed as CFU/mL, consistent with the numerical form of the source table; they are not log-transformed values.

Selective LAB counts declined from 7.75 × 10⁷ to 7.50 × 10⁷ CFU/mL, corresponding to a 3.23% decrease. *Bifidobacterium* counts changed from 8.92 × 10⁷ to 8.62 × 10⁷ CFU/mL, a 3.36% decrease. The smooth, limited decline indicates that the beverage matrix sup

ported both populations during the 8-day observation period. Bifidobacterial counts remained above 10^7 CFU/mL, within the range frequently cited as desirable for probiotic dairy carriers (Shori, 2022) [4]. This comparison describes numerical retention; statistical significance cannot be inferred because dispersion estimates for the viability table were not reported.

The retention may reflect the nutrient-rich milk matrix and the contribution of fermentable carbohydrates or soluble fibre from dragon fruit. Conversely, the downward trend is consistent with the sensitivity of bifidobacteria to increasing acidity and oxygen. Shori (2022) [4] emphasized that food matrix, strain, active ingredients and storage conditions jointly govern bifidobacterial survival, while Sibanda *et al.* (2024) [5] identified shelf-life viability as a continuing challenge in

dairy foods. The present decline was smaller than the substantial reductions that can occur in some commercial fermented products, although direct comparisons require identical strains, media and storage durations.

The general LAB dataset in Table 1 and the selective survivability dataset in Table 2 were generated for different analytical purposes and should not be treated as interchangeable. Their different numerical levels likely reflect the enumeration media and operational definitions used. Future studies should specify the starter organisms recovered by each medium and present counts as $\log_{10}$ CFU/mL with standard deviations to improve comparability.

3.3 Effect of packaging on post-acidification

Table 3. Changes in pH and titratable acidity of optimized synbiotic buttermilk in glass and PET bottles

Day	Glass pH	PET pH	SE	CD (5%)	Glass acidity (%)	PET acidity (%)	SE	CD (5%)
0	4.4	4.5	0.031	0.094	0.51	0.51	0.005	0.017
2	4.3	4.4	0.031	0.094	0.56	0.55	0.003	0.008
4	4.2	4.3	0.027	0.083	0.60	0.60	0.025	0.077
6	4.1	4.1	0.027	0.083	0.65	0.64	0.028	0.085
8	4.0	3.9	0.027	0.083	0.70	0.69	0.015	0.046

SE, standard error; CD, critical difference at 5%.

Both packages showed the expected pattern of post-acidification. In glass, pH decreased by 0.40 unit (4.40 to 4.00), while acidity rose by 0.19 percentage point (0.51% to 0.70%). In PET, pH decreased by 0.60 unit (4.50 to 3.90), while acidity rose by 0.18 percentage point (0.51% to 0.69%). These inverse trends are consistent with continued formation of organic acids by viable starter and probiotic organisms during refrigeration. Mani-López *et al.* (2014) [3] similarly reported lower pH and higher titratable acidity during storage of yogurts and fermented milks.

At days 0, 2, 4 and 8, the absolute glass-PET pH difference was 0.10 unit and exceeded the reported CD for the corresponding day (0.083–0.094); there was no difference on day 6. For acidity, the packages were identical on days 0 and 4, and their 0.01-percentage-point differences on days 2, 6 and 8 exceeded the reported CD only on day 2 (0.008). Thus, statistically detectable point differences occurred, but neither package showed consistently superior control of both variables. The two materials produced broadly comparable acidification profiles over the short storage period.

3.4 Techno-economic feasibility

Table 4. Estimated production cost for a 10-L batch of optimized synbiotic buttermilk

Cost item	Market price	Quantity	Cost (₹)
Cow milk	₹45/L	4.5 L	202.50
Starter culture	₹500 (basis used)	2 g	10.00
<i>B. bifidum</i> culture	₹3,000/100 g	1 g	30.00
Red dragon fruit	₹100/L	1 L	100.00
Packaging and labelling	₹5/bottle	50 bottles	250.00
Total raw-material cost	-	-	592.50
Processing cost	20% of raw-material cost	-	118.50
Production cost, 10 L	-	-	711.00
Production cost, 1 L	-	-	71.10
Production cost, 200 mL	-	-	14.22

Costs reflect prices recorded for the experiment and should be updated before commercial planning.

The estimated cost of a 10-L batch was ₹711.00, equivalent to ₹71.10/L and ₹14.22 per 200-mL serving. Packaging and labelling accounted for ₹250.00, or 35.2% of total production cost, and were the largest single component. Cow milk contributed ₹202.50 (28.5%), dragon fruit ₹100.00 (14.1%), processing ₹118.50 (16.7%), *B. bifidum* culture ₹30.00 (4.2%) and starter culture ₹10.00 (1.4%). The dominance of packaging suggests that bulk procurement, reusable glass systems or optimized bottle sourcing could materially influence the final cost.

The estimate indicates technical promise for small-scale manufacture, but it is not a complete commercial feasibility study. Labour, energy measured directly, cold-chain distribution, quality assurance, equipment depreciation, product losses, retailer margins and taxes were not separately itemized. A commercialization decision would require sen-

sitivity analysis using current local prices, validated scale-up yields and an intended selling price.

4. Conclusion

Dragon-fruit-enriched synbiotic buttermilk retained high viable populations during 8 days at 4 ± 1 °C. Selective LAB and *Bifidobacterium* counts decreased by only 3.23% and 3.36%, respectively, and *Bifidobacterium* remained above 8.6×10^7 CFU/mL. Glass and PET bottles both supported comparable short-term storage, although continued post-acidification occurred. Coliforms were absent throughout; however, yeast and mould appeared on day 8. The safest conclusion from the available evidence is therefore a provisional refrigerated shelf life of 6 days rather than 8 days. The calculated production cost of ₹14.22 per 200-mL serving suggests affordability at the experimental scale, with packaging as the largest cost component. Further work should validate shelf life across independent batches, include sensory

data at every storage point, report replicate variability and determine an applicable microbiological acceptance limit before commercial release.

5. Study Limitations

The storage experiment covered only 8 days and did not report replicate dispersion for viable counts. Sensory acceptance during storage and package-specific probiotic counts were not available. The cost analysis used prevailing experimental prices and omitted several commercial overheads. In addition, the original study used the term 'synbiotic' operationally for the combined culture-and-fruit formulation; definitive classification under the current ISAPP definition would require evidence that the fruit-derived substrate is selectively utilized and that the combined product confers a health benefit (Swanson *et al.*, 2020) [2]. These limitations restrict generalization but do not alter the observed direction of change.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability

The data supporting the findings of this study are contained within the article and the underlying thesis records.

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