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ARTICLE

# Changes in Lactic Acid Bacteria Counts in Fermented Milk and Fermented Milk Beverages During Refrigerated Storage Under Commercial Conditions

Min-Jung Jung[1], Kun-Ho Seo[1], Jung-Whan Chon[2], Kwang-Young Song[3*]

[1]College of Veterinary Medicine and Center for One Health, Konkuk University, Seoul, Korea
[2]Department of Food Science and Biotechnology, Kangwon National University, Chunchon, Korea
[3]Department of Companion Animal Health and Department of Pet Industry, Daegu Haany University, Gyeongsan, Korea

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*Corresponding author :
Kwang-Young Song
Department of Companion Animal Health and Department of Pet Industry, Daegu Haany University, Gyeongsan, Korea
Tel : +82-53-819-1605
Fax : +82-53-819-1273
E-mail : drkysong@gmail.com



**ORCID**
Min-Jung Jung
https://orcid.org/0009-0005-0334-2529
Kun-Ho Seo
https://orcid.org/0000-0001-5720-0538
Jung-Whan Chon
https://orcid.org/0000-0003-0758-6115
Kwang-Young Song
https://orcid.org/0000-0002-5619-8381

## Abstract

In this study, changes in lactic acid bacteria (LAB) counts were evaluated in commercially produced fermented milk and fermented milk beverage products from the day of manufacture to the end of their shelf life under refrigerated storage conditions. LAB viability was monitored at multiple time points to assess stability in industrial production and distribution environments. All products were stored at 4℃ ± 1℃, reflecting standard commercial refrigeration conditions. The fermented milk and fermented milk beverage products exhibited high initial LAB counts after manufacture. Although gradual reductions in viable counts were observed during refrigerated storage, LAB levels remained consistently above the internal quality criteria throughout the designated shelf life. In products containing *Bifidobacterium* spp., counts obtained using selective enumeration media were generally lower and showed a greater decline during refrigerated storage than total LAB counts. These findings are consistent with those of previous studies, highlighting the importance of initial inoculation density and product-specific formulation in maintaining LAB viability during storage. Notably, this study provides field-based empirical data obtained from commercially manufactured products under real storage conditions, thereby extending the findings of laboratory-scale studies. Overall, these results emphasize the importance of adequate initial LAB inoculation, continuous monitoring of LAB viability, and application of product-specific quality management strategies. This study provides practical and scientific evidence to support the establishment of robust quality-control criteria and optimization of manufacturing processes for fermented dairy products.

## Keywords

fermented milk, fermented milk beverage, lactic acid bacteria, storage stability, shelf life

## Introduction

Fermented milk and fermented milk beverages (drink-type products) are among the most widely consumed dairy products worldwide, in which lactic acid bacteria (LAB) play a central role in fermentation, product safety, sensory properties, and potential health-related functions [1,2]. The presence of viable LAB at sufficient levels is therefore considered a fundamental quality attribute of fermented dairy products, particularly those marketed with probiotic or functional claims [3].

The survival and stability of LAB during storage are critical determinants of product quality and functionality and are influenced by a range of factors, including starter culture composition, inoculation level, fermentation conditions, product formulation, oxygen exposure, and storage temperature [4,5]. In particular, refrigerated storage, although essential for microbial safety, may impose multiple stresses on LAB—such as organic acid accumulation, cold shock, nutrient limitation, and oxidative stress—leading to gradual reductions in viable counts during shelf life [6].

Regulatory frameworks in many countries, including Korea, specify minimum LAB counts for fermented dairy products to ensure baseline microbiological quality. The Korean Food Code defines threshold LAB levels for fermented milk products at the time of manufacture or sale; however, these regulatory limits are often insufficient to guarantee consistent quality throughout the entire shelf life [7]. Consequently, dairy manufacturers commonly implement additional internal quality criteria that exceed regulatory minimums in order to maintain product consistency, technological robustness, and consumer trust until the end of shelf life [8].

From an industrial perspective, maintaining stable LAB counts throughout storage is closely associated with product reliability, brand credibility, and consumer perception of freshness and functionality [9]. Declines in LAB counts below expected levels may compromise not only regulatory compliance but also the functional value of fermented dairy products, particularly those containing probiotic strains such as *Bifidobacterium* spp., which are known to exhibit relatively low tolerance to environmental stresses [10].

Despite the recognized importance of LAB stability, many previous studies have been conducted under laboratory-scale or model-system conditions, which may not fully reflect the complexity of commercial manufacturing and distribution environments. Recent reviews have highlighted the need for field-based studies that monitor LAB populations in commercially produced fermented dairy products under actual storage conditions, from manufacture to the end of shelf life [11,12].

Therefore, generating empirical data on LAB dynamics in real commercial products is essential for identifying critical control points in manufacturing, evaluating the adequacy of inoculation strategies, and establishing evidence-based quality management practices. In this context, the objective of this study was to evaluate changes in LAB counts from the day of manufacture to the end of shelf life in commercially produced fermented milk and fermented milk beverage products stored under refrigerated conditions, and to compare LAB stability among different product types produced under industrial conditions.

# Materials and Methods

## 1. Test products

This study evaluated three commercially produced fermented dairy products (FM-A, FM-B, and FM-C), representing different categories of fermented dairy products commonly distributed in the Korean dairy market (Table 1). For each product, three independent batches produced under commercial manufacturing conditions were obtained and analysed separately. Microbiological analyses were performed in triplicate for each batch at each sampling point. Each batch was considered an independent biological replicate.

The selected products differed in formulation, fermentation type, and target LAB levels, allowing comparative evaluation of LAB stability across product categories. Such

**Table 1.** Characteristics of fermented dairy products evaluated in this study

| Product type | Product code* | Starter culture | Target LAB count (CFU/mL) | Shelf life** |
|---|---|---|---|---|
| Probiotic yogurt drink | FM-A | *Bifidobacterium* spp. | ≥ 1.0 × $10^6$ | 14 days |
| Fermented milk | FM-B | ABT-7 mixed starter culture | ≥ 1.0 × $10^7$ | 20 days |
| | | *Bifidobacterium* spp. | ≥ 1.0 × $10^5$ | |
| Concentrated fermented milk | FM-C | Mixed yogurt starter | ≥ 1.0 × $10^8$ | 20 days |

*Product names and manufacturers were anonymized for research purposes.
**Shelf-life periods were determined according to manufacturer specifications indicated on the product labels.

an approach has been widely used in previous studies investigating microbial stability in commercial fermented dairy products [5,13].

Sampling schedules were established according to the designated shelf life of each product. FM-A was analysed on Days 0, 7, and 14, whereas FM-B and FM-C were analysed on Days 0, 10, and 20.

## 2. Storage conditions and sampling

All products were stored under refrigerated conditions at 4℃ ± 1℃, which reflects standard commercial storage and distribution conditions for fermented dairy products in Korea. The designated shelf life of each product was determined according to manufacturer specifications indicated on the product labels. Samples were analysed at three key time points: (i) on the day of manufacture (Day 0), (ii) during refrigerated storage, and (iii) at the end of the designated shelf life.

Sampling time points were selected to capture both early and late-stage changes in LAB viability during storage. Refrigerated storage is known to impose multiple stress factors on LAB, including low-temperature stress, organic acid accumulation, and nutrient limitation, which may lead to gradual reductions in viable cell counts over time [6]. All analyses were performed within the stated shelf-life period indicated on product packaging.

## 3. Enumeration of lactic acid bacteria

LAB counts were determined using the standard plate count method in accordance with commonly accepted microbiological procedures for fermented dairy products. Depending on product characteristics and target LAB groups, either bromocresol purple (BCP) agar or tryptose–sulfite–cycloserine agar supplemented with 4-methylumbelliferyl phosphate (TOS-MUP) agar was used as the selective medium.

Samples (10 mL) were homogenised with 90 mL of sterile 0.1% peptone water and serially diluted in the same diluent. Appropriate dilutions were plated in duplicate onto selective media. Plates containing 30–300 colonies were selected for enumeration, and results were expressed as colony-forming units per millilitre (CFU/mL).

BCP agar was used for the enumeration of total LAB in fermented milk and fermented milk beverages, as it supports the growth of a broad range of lactic acid bacteria while suppressing background microflora. BCP agar plates were incubated aerobically at 37℃ for 48 h.

TOS-MUP agar, supplemented with mupirocin, was used for the selective enumeration of *Bifidobacterium* spp. in probiotic milk products, as recommended in previous studies and international guidelines [14,15]. TOS-MUP agar plates were incubated anaerobically at 37℃ for 72 h using an anaerobic jar system.

Changes in LAB counts during storage were evaluated by comparing viable counts obtained at each sampling point, following approaches commonly applied in shelf-life studies of fermented dairy products [16]. Internal quality criteria were established based on manufacturer standards and Korean Food Code requirements for fermented dairy products.

### 4. Statistical analysis

All experiments were performed in triplicate, and results are expressed as mean ± SD. LAB counts were converted to log CFU/mL prior to analysis. Changes in LAB counts during storage were evaluated using one-way analysis of variance (ANOVA) to assess differences among sampling time points. When significant differences were observed, Tukey's post-hoc test was applied for multiple comparisons. Statistical significance was considered at $p < 0.05$.

All statistical analyses were performed using GraphPad Prism version 10 (GraphPad Software, San Diego, CA, USA). This statistical approach has been commonly employed in previous studies evaluating LAB stability in fermented dairy products during refrigerated storage [6].

## Results and Discussion

### 1. Changes in lactic acid bacteria counts in yogurt drink (FM-A) during storage

The viability of bifidobacteria enumerated on TOS-MUP agar in the yogurt drink gradually decreased during refrigerated storage over a 14-day period. A significant reduction was observed between Day 0 and later storage periods ($p < 0.05$). The initial viable count was $1.2 \times 10^7$ CFU/mL (Day 0), which decreased to $7.8 \times 10^6$ CFU/mL (Day 7) and further to $5.3 \times 10^6$ CFU/mL (Day 14), as shown in Table 2. Overall, this reduction corresponds to an approximate decrease of 0.3–0.4 log CFU/mL.

Despite this decrease, viable cell counts remained within the specified quality criteria throughout the entire storage period, indicating that the yogurt drink maintained microbiological stability under refrigerated conditions. This suggests that the probiotic yogurt drink maintained acceptable microbiological stability for at least 14 days under

**Table 2.** Changes in lactic acid bacteria counts of a yogurt drink (FM-A) throughout the shelf life (unit: CFU/mL)

| Sample | Medium | Day 0 | Day 7 | Day 14 | Evaluation |
|---|---|---|---|---|---|
| FM-A | TOS-MUP agar | $(1.20 \pm 0.08) \times 10^{7a}$ | $(7.80 \pm 0.05) \times 10^{6b}$ | $(5.30 \pm 0.11) \times 10^{6b}$ | Met internal quality criterion ($\geq 1.0 \times 10^6$ CFU/mL) |

Values are presented as mean ± SD (n = 3).
Differences among sampling points were analysed by one-way ANOVA followed by Tukey's test ($p < 0.05$).
Values with different superscript letters within the same row indicate significant differences ($p < 0.05$).
CFU/mL, colony-forming units per millilitre; TOS-MUP, tryptose–sulfite–cycloserine agar supplemented with 4-methylumbelliferyl phosphate; one-way ANOVA, one-way analysis of variance.

refrigerated storage conditions.

These findings are consistent with previous studies reporting gradual reductions in LAB viability during refrigerated storage due to storage-related stress conditions [6,16]. Such viability losses have been attributed to intracellular stress accumulation and limited energy availability during cold storage conditions.

In particular, earlier studies have demonstrated that LAB populations in fermented dairy systems typically show minor but significant reductions during refrigerated storage, without critically affecting overall product quality. The magnitude of reduction observed in this study is comparable to those reported in similar systems, supporting the microbiological stability of the product under practical storage conditions.

From an industrial perspective, maintaining stable product quality is critical, as fluctuations in viable cell counts may influence product quality and microbial stability. The relatively small reduction observed in this study suggests that the manufacturing and storage conditions applied were appropriate for preserving microbiological stability during refrigerated storage, thereby supporting its applicability in controlled fermentation processes.

## 2. Changes in lactic acid bacteria counts in fermented milk during storage

Fermented milk products exhibited high initial LAB counts immediately after manufacture, indicating effective fermentation and sufficient inoculation levels (Table 3). During refrigerated storage, LAB counts showed a decreasing trend in both media, and the reductions were statistically significant over time ($p < 0.05$). However, the magnitude of reduction differed depending on the selectivity and recovery characteristics of the enumeration medium.

On BCP agar, viable cell counts decreased from $4.5 \times 10^8$ CFU/mL at Day 0 to $0.8 \times 10^8$ CFU/mL at Day 20, corresponding to an approximate reduction of 0.7–0.8 log CFU/mL. In contrast, counts determined using TOS-MUP agar showed a more gradual decline, from $7.0 \times 10^6$ CFU/mL to $5.2 \times 10^6$ CFU/mL over the same storage period, representing a reduction of approximately 0.1–0.2 log CFU/mL. These results indicate that a greater apparent reduction was observed on BCP agar compared to TOS-MUP agar, which could partially reflect differences in selectivity and recovery characteristics between the enumeration media rather than intrinsic differences in LAB stability.

**Table 3.** Changes in lactic acid bacteria counts of fermented milk (FM-B) throughout the shelf life (unit: CFU/mL)

| Sample | Medium | Day 0 | Day 10 | Day 20 | Evaluation |
|---|---|---|---|---|---|
| FM-B | BCP agar | $(4.50 \pm 0.12) \times 10^{8a}$ | $(2.50 \pm 0.07) \times 10^{8b}$ | $(0.80 \pm 0.04) \times 10^{8c}$ | Met internal quality criterion ($\geq 1.0 \times 10^7$ CFU/mL) |
| | TOS-MUP agar | $(7.00 \pm 0.10) \times 10^{6a}$ | $(6.30 \pm 0.06) \times 10^{6ab}$ | $(5.20 \pm 0.13) \times 10^{6b}$ | Met internal quality criterion ($\geq 1.0 \times 10^6$ CFU/mL) |

Values are presented as mean ± SD (n = 3).
Differences among sampling points were analysed by one-way ANOVA followed by Tukey's test ($p < 0.05$).
Values with different superscript letters within the same row indicate significant differences ($p < 0.05$).
CFU/mL, colony-forming units per millilitre; BCP, bromocresol purple; TOS-MUP, tryptose–sulfite–cycloserine agar supplemented with 4-methylumbelliferyl phosphate; one-way ANOVA, one-way analysis of variance.

Despite these decreases, viable cell counts obtained from both media consistently remained within the specified quality criteria throughout the storage period, suggesting that LAB populations in fermented milk products maintained acceptable levels of viability under refrigerated conditions for at least 20 days.

The observed reduction in LAB counts during storage is consistent with previous studies, which have attributed such declines to factors including organic acid accumulation, reduced metabolic activity at low temperatures, and nutrient limitation [6,15]. These stress conditions may gradually impair cell viability during storage, although they do not necessarily compromise overall product quality within typical shelf-life periods.

Notably, the extent of decline differed markedly between the two media. BCP agar, commonly used for general LAB enumeration, may recover a broader spectrum of LAB populations, including cells that are more susceptible to storage-related stress. In contrast, TOS-MUP agar selectively enumerates bifidobacteria, which may show different recovery characteristics under refrigerated storage conditions or be less affected by compositional changes in the fermented milk matrix. This discrepancy highlights the importance of considering methodological differences when interpreting microbiological data, as enumeration results may vary depending on medium selectivity, recovery efficiency, and target populations.

From a quality control perspective, the consistent maintenance of LAB counts above specified criteria underscores the importance of achieving sufficiently high initial inoculation levels during production. This ensures that, despite gradual declines during storage, viable LAB populations remain within acceptable limits throughout product shelf life.

Furthermore, the observed differences between selective media emphasize the need for careful interpretation of microbiological data. Variability arising from methodological factors suggests that additional confirmatory analyses or standardized enumeration approaches may be required to improve comparability and reproducibility across studies.

### 3. Lactic acid bacteria stability in fermented milk and concentrated milk beverages

Fermented milk beverages exhibited a decreasing trend in LAB counts during refrigerated storage, consistent with the patterns observed in fermented milk products, and the reductions were statistically significant over time ($p < 0.05$). However, viable cell counts consistently remained above the specified quality criteria throughout the storage period.

In concentrated fermented milk products (FM-C), high initial LAB counts were observed at Day 0, followed by a gradual decline during storage (Table 4). On BCP agar, viable counts decreased from $1.1 \times 10^9$ CFU/mL at Day 0 to $6.0 \times 10^8$ CFU/mL at Day 20, corresponding to an approximate reduction of 0.2–0.3 log CFU/mL. In contrast, counts determined using TOS-MUP agar decreased from $6.0 \times 10^6$ CFU/mL to $1.2 \times 10^6$ CFU/mL over the same period, representing a reduction of approximately 0.6–0.7 log CFU/mL. These results indicate a greater apparent reduction in counts obtained using TOS-MUP agar compared to BCP agar, which could partially reflect differences

**Table 4.** Changes in lactic acid bacteria counts of concentrated fermented milk (FM-C) throughout the shelf life (unit: CFU/mL)

| Sample | Medium | Day 0 | Day 10 | Day 20 | Evaluation |
|---|---|---|---|---|---|
| FM-C | BCP agar | $(1.10 \pm 0.11) \times 10^{9a}$ | $(7.30 \pm 0.07) \times 10^{8b}$ | $(6.00 \pm 0.10) \times 10^{8b}$ | Met internal quality criterion ($\geq 1.0 \times 10^8$ CFU/mL) |
| | TOS-MUP agar | $(6.00 \pm 0.09) \times 10^{6a}$ | $(3.20 \pm 0.12) \times 10^{6b}$ | $(1.20 \pm 0.03) \times 10^{6c}$ | Met internal quality criterion ($\geq 1.0 \times 10^6$ CFU/mL) |

Values are presented as mean ± SD (n = 3).
Differences among sampling points were analysed by one-way ANOVA followed by Tukey's test ($p < 0.05$).
Values with different superscript letters within the same row indicate significant differences ($p < 0.05$).
CFU/mL, colony-forming units per millilitre; BCP, bromocresol purple; TOS-MUP, tryptose–sulfite–cycloserine agar supplemented with 4-methylumbelliferyl phosphate; one-way ANOVA, one-way analysis of variance.

in selectivity and recovery characteristics between the enumeration media.

Despite these decreases, viable LAB counts obtained from both media remained within the specified quality criteria throughout the storage period, indicating that microbiological stability was maintained under refrigerated conditions. Notably, bifidobacteria counts (enumerated on TOS-MUP agar) were lower than total LAB counts (BCP agar) at the initial production stage and exhibited a larger decline during storage. This observation may suggest a greater sensitivity of bifidobacteria to storage-related stress conditions, although methodological factors, including medium selectivity and recovery efficiency, cannot be excluded.

Previous studies have reported that bifidobacteria are particularly sensitive to environmental stresses such as oxygen exposure, low pH, and cold storage conditions, which can negatively affect their survival in fermented dairy systems [10]. Such sensitivity may contribute to the greater decline observed during storage in this study. In contrast, total LAB populations, which include a broader range of species with varying stress tolerance, may exhibit relatively greater stability under similar conditions.

The observed differences between enumeration media highlight the importance of considering methodological factors when interpreting microbiological data. BCP agar reflects total LAB populations, whereas TOS-MUP agar selectively targets bifidobacteria, which may differ in survival characteristics depending on product composition and storage conditions. Therefore, variations in enumeration results may arise from differences in medium selectivity, recovery efficiency, and target microbial groups.

From a quality control perspective, these findings emphasize the importance of maintaining sufficiently high initial LAB counts during production to ensure that viable populations remain above acceptable limits throughout the product shelf life. Additionally, products containing bifidobacteria may require more stringent control of processing and storage conditions, including oxygen management, optimized inoculation levels, and appropriate formulation strategies, to maintain probiotic viability [17,18].

## Conclusion

This study evaluated changes in LAB counts in commercially produced fermented milk and fermented milk beverage products from the day of manufacture to the end of shelf life under refrigerated storage conditions. By monitoring LAB viability under actual industrial production and storage environments, this study provides practical insight into microbiological quality management in commercial fermented dairy products.

The results demonstrated that products with sufficiently high initial LAB counts generally maintained viable LAB levels above internal quality criteria throughout the designated shelf life, despite gradual reductions during refrigerated storage. These findings suggest that adequate initial inoculation levels and appropriate manufacturing control are important factors for maintaining LAB stability during storage. In products containing *Bifidobacterium* spp., counts obtained using selective enumeration media showed relatively greater reductions during storage compared with total LAB counts, indicating that probiotic-containing products may require more careful control of processing and storage conditions to maintain microbial viability.

The discrepancy observed between LAB counts obtained using BCP agar and TOS-MUP agar may be partially attributable to differences in the selectivity and recovery characteristics of the enumeration media. Therefore, microbiological results obtained using different selective media should be interpreted cautiously when evaluating LAB stability in fermented dairy products. One limitation of this study is that strain-specific molecular identification and viability assays were not performed. Further studies using molecular approaches are warranted to improve the understanding of LAB dynamics and probiotic survival under commercial storage conditions.

Overall, these findings highlight the importance of adequate initial LAB inoculation, product-specific quality management, and continuous monitoring of LAB viability to ensure microbiological stability throughout the shelf life of fermented dairy products. The results of this study may serve as useful scientific evidence for dairy manufacturers seeking to optimise fermentation processes, improve product consistency, and establish robust quality control strategies for commercial fermented dairy products.

## Conflict of Interest

The authors declare no potential conflict of interest.

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