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Shelf-life dynamics of key probiotic populations in dairy formulations: A temporal stability assessment

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ABSTRACT

This paper aims to conduct a preliminary temporal evaluation of the stability of *Lactobacillus* and *Bifidobacterium* spp. throughout the designated shelf life of probiotic dairy products. The enumeration of viable probiotic bacteria was conducted during the first week and at the end of the shelf life in seventeen commercial probiotic products. In the initial analysis, fourteen products contained live bacterial populations exceeding the recommended threshold of 6 log CFU/mL or CFU/g. Twelve products retained probiotic levels above this threshold at the end of the shelf life. *Lactobacillus* spp. counts were above 6 log CFU/mL or CFU/g in 13 products during the first week. Although a reduction was observed in 10 products throughout storage, 11 products maintained sufficient *Lactobacillus* viability by the end of the shelf life, while two products showed complete loss. Regarding *Bifidobacterium* spp., eight products met the 6 log CFU/mL level at the beginning and end of the shelf life. In contrast, the population declined to undetectable levels in three products. Notably, *Bifidobacterium* spp. indicated on the label were not detected in four products during the first week, and this number increased to seven products by the end of shelf life. These findings underscore the variability in probiotic survival across different products and raise concerns about the consistency between product labelling and actual microbial content over time. The viability and stability of *Lactobacillus* and *Bifidobacterium* populations in probiotic dairy products are crucial for their health benefits. Understanding the factors that influence their survival and optimising processing and storage conditions is essential for improving product effectiveness. Ongoing research into novel preservation techniques will be key to ensuring the reliability of these probiotics in dairy products.

KEYWORDS: Stability, Probiotics, Dairy Products, Microbial Count

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INTRODUCTION

Probiotics are implicated in diverse health benefits, encompassing enhanced nutrient bioavailability, improved intestinal homeostasis, and host immune modulation. Evidence suggests their potential to lower serum cholesterol concentrations, confer protection against oncogenesis and diminish allergy risk in susceptible individuals. Probiotics may also mitigate gastrointestinal disorders, prevent infantile diarrhoea, and alleviate urinary tract infections, osteoporosis, atopic diseases, and constipation. Notably, they have demonstrated efficacy in managing hypercholesterolemia and controlling inflammatory bowel diseases (Tripathi & Giri, 2014; Abatenh *et al.*, 2018; Das *et al.*, 2022; Latif *et al.*, 2023). Among the diverse probiotic microorganisms, species belonging to the genera *Lactobacillus* and *Bifidobacterium* represent the predominant taxa employed in probiotic formulations. (Vinderola *et al.*, 2011).

Dairy matrices are widely acknowledged as optimal vehicles for the delivery of probiotic bacteria, primarily attributable to their conducive physicochemical attributes and capacity to sustain probiotic viability and proliferation. These inherent characteristics render dairy formulations a superior medium for the incorporation of beneficial microorganisms, affording both a supportive environment for probiotics and the potential to augment their therapeutic efficacy (Vinderola *et al.*, 2011; Latif *et al.*, 2023). Consequently, dairy formulations represent the most prevalent accepted vehicles for probiotic microorganisms, with diverse presentations of probiotic-enriched dairy products constituting a substantial segment of the global functional food market. As primary delivery matrices for beneficial bacteria, yoghurt, buttermilk, kefir, cheese, and other fermented milk products constitute the most prevalent category of probiotic dairy products globally. The growing demand for functional foods has further solidified the role of these dairy products in global markets, making them indispensable in the promoting gut health and overall well-being (Kazemi *et al.*, 2024).

For probiotic foods to exert their intended effects, they must harbour a minimum threshold of viable microorganisms at the point of consumption. Consequently, maintaining viability represents a critical prerequisite for ensuring probiotic functionality. Industry benchmarks typically stipulate a minimum viable count of 10^6 CFU/g at the time of ingestion (Tripathi & Giri, 2014; Terpou *et al.*, 2019). A daily intake of 10^8 to 10^9 viable cells is generally deemed necessary to elicit the desired probiotic impact within the human host. Furthermore, it has been proposed that a daily consumption of approximately 100 grams of probiotic food is required to deliver 10^9 viable cells to the intestinal (Călinoiu *et al.*, 2016). However, a significant decline in viable probiotic cells frequently occurs during storage, potentially compromising the intended product efficacy. To mitigate this concern, an overage of probiotic microorganisms is often incorporated during manufacture to ensure an acceptable viable count at the point of consumption. Nevertheless, this practice can be economically restrictive and induce undesirable organoleptic alterations in the product matrix. Consequently, the sustained viability of probiotics throughout both the production and storage phases is paramount. At this juncture, selecting appropriate strains that exhibit adaptability to both the food matrix and the host intestinal milieu is paramount. Furthermore, documented variability in the survival competence of distinct strains within food matrices and the human gastrointestinal tract underscores the necessity for strain selection in developing probiotic foods (Dinkçi *et al.*, 2019).

When evaluating probiotic microorganisms in terms of their durability, *Lactobacillus* species are generally more resilient than *Bifidobacterium* species. *Lactobacilli*, naturally present in traditional fermented foods, exhibit greater resistance to low pH levels and demonstrate a stronger ability to adapt to milk and other food substrates. Additionally, oxygen tolerance is less of a concern for the survival of *Lactobacilli*, as they are more oxygen-tolerant than *Bifidobacteria*. As a result, many *Lactobacillus* species are considered more suitable for food applications from a technological perspective than *Bifidobacteria* (Terpou *et al.*, 2019).

A large number of factors have been identified as to the viability of probiotic microorganisms in food products during production, processing, and storage. These factors include food parameters (pH, titratable acidity, molecular oxygen, water activity, presence of salt, sugar and other compounds like hydrogen peroxide, bacteriocins, artificial flavouring and colouring agents), processing parameters (incubation temperature, heat treatment, cooling and storage conditions of the product, packaging materials, scale of production), and microbiological parameters (strain of probiotics, rate and proportion of inoculation) (Tripathi & Giri, 2014; Terpou *et al.*, 2019).

The objective of this study was to conduct a preliminary temporal evaluation of the stability of *Lactobacillus* and *Bifidobacterium* species throughout the designated shelf life of probiotic dairy products.

MATERIALS AND METHODS

Sampling

Seventeen commercially available probiotic dairy beverage products (identified as A through G) were collected from retail supermarkets in Ankara, Türkiye. To ensure temperature integrity during transit, samples were transported in an isothermal container and stored at 4 °C. The product selection encompassed a heterogeneous assortment of six probiotic dairy categories, representing seven distinct brands: plain kefir, chocolate kefir, fruit kefir, fruit smoothies, fruit shots, fruit yoghurt, cheese, and ayran. A comprehensive list of the analysed products is presented in Table 1. Microbiological analyses were performed at two discrete time intervals: during the initial week of the products' designated shelf life and at the end of the stated shelf life period.

Microbiological Quantification of *Lactobacillus* spp. and *Bifidobacterium* spp. Genera

Ten grams or ten millilitres of each sample were homogenised in 90 mL of Mitsuoka Buffer (composed of potassium dihydrogen phosphate, 4.5 g/L; disodium hydrogen phosphate, 6.0 g/L;

Table 1: The probiotic dairy products used in this study

Product	Product Description	Packaging Information	
A1	chocolate kefir	Probiotic microorganism	at least 10^6 CFU/g
A2	plain kefir	Probiotic microorganism	at least 10^6 CFU/g
A3	fruit yoghurt	Probiotic microorganism	at least 10^6 CFU/g
A4	fruit yoghurt	Probiotic microorganism	at least 10^6 CFU/g
A5	fruit yoghurt	Probiotic microorganism	at least 10^6 CFU/g
A6	fruit yoghurt	Probiotic microorganism	at least 10^6 CFU/g
B7	Cottage cheese	Probiotic microorganism	at least 10^6 CFU/g
C8	fruit kefir	Probiotic microorganism	at least 10^6 CFU/g
C9	fruit kefir	Probiotic microorganism	at least 10^6 CFU/g
D10	fruit shot	*Lactobacillus bulgaricus* *Streptococcus thermophilus* *Lactococcus lactis* *Bifidobacterium lactis*	at least 5×10^7 CFU/g
D11	fruit shot	*Lactobacillus bulgaricus* *Streptococcus thermophilus* *Lactococcus lactis* *Bifidobacterium lactis*	at least 2×10^6 CFU/g
D12	fruit shot	*Lactobacillus bulgaricus* *Streptococcus thermophilus* *Lactococcus lactis* *Bifidobacterium lactis*	at least 5×10^7 CFU/g
D13	fruit smoothie	*Lactobacillus bulgaricus* *Streptococcus thermophilus* *Lactococcus lactis* *Bifidobacterium lactis*	at least 1×10^7 CFU/g
E14	plain kefir	*Bifidobacterium lactis* *Lactobacillus acidophilus*	at least 10^6 CFU/g
F15	fruit kefir	*Bifidobacterium* spp. *Lactobacillus acidophilus*	at least 10^6 CFU/g
F16	plain kefir	*Bifidobacterium spp.* *Lactobacillus acidophilus*	at least 10^6 CFU/g
G17	ayran	*Bifidobacterium spp.* *Lactobacillus acidophilus*	at least 10^6 CFU/g

L-cysteine HCl, 0.5 g/L; Tween 80, 0.5 g/L) (Merck, Germany) and incubated at 37 °C for 30 minutes. Following incubation, serial dilutions of the homogenates were prepared up to 10^{-7} in Mitsuoka Buffer (Merck, Germany) (Champagne *et al.*, 2011; Vinderola *et al.*, 2019).

For the enumeration of *Lactobacillus* spp., 0.1 mL of each dilution was plated onto MRS agar supplemented with 0.05% cysteine (Merck, Germany) and incubated at 37 °C for 3 days. Following incubation, viable cell counts were determined by counting colonies within the range of 30 to 300 on the agar plates. Colony enumeration was performed using a Quebec colony counter. The results were expressed as colony-forming units per gram or millilitre (log CFU/g or CFU/mL) (Todorov *et al.*, 1999).

For the enumeration of *Bifidobacterium* spp., the pour plate method was employed using MRS-NNLP agar, which contained neomycin sulfate (100 mg/L), paromomycin (200 mg/L), nalidixic acid (15 mg/L), and LiCl (3 g/L). The inoculated plates were incubated under strictly anaerobic conditions at 37 °C for 3 days, using an anaerobic jar (BBL, Gaspack Anaerobic system, Franklin, New Jersey, USA). After incubation, viable cell counts were determined by enumerating colonies within the range of 30 to 300. Colony counting was performed with a Quebec colony counter, and the results were expressed as colony-forming units per gram or millilitre (log CFU/g or CFU/mL) (Todorov *et al.*, 1999).

To enhance the scientific rigour and reliability of the experimental findings, for each sample, a parallel set of experiments was conducted, and *Lactiplantibacillus plantarum* (formerly *Lactobacillus plantarum*) LMG2003, *Lactilactobacillus sakei* (formerly *Lactobacillus sakei*) NCDO2714, and *Bifidobacterium longum* ATCC15707 were employed as control strains. These strains were kindly provided by the culture collection of the Microbiology Laboratory, Department of Engineering, Faculty of Food Engineering, Ankara University.

Statistical Analysis

Statistical analysis was done by using SPSS (version 27) software. T test was applied to determine the difference between the groups. The statistical significance level was accepted as 5%.

RESULTS AND DISCUSSION

A total of seventeen probiotic dairy products were subjected to analysis, comprising four probiotic fruit yoghurts (brands A3, A4, A5, and A6), one probiotic ayran (brand G17), seven kefir (plain, chocolate, and fruit varieties; brands A1, A2, C8, C9, E14, F15, and F16), one probiotic fruit smoothie (brand D13), three probiotic fruit shots (brands D10, D11, and D12), and one probiotic cheese (brand B7), as detailed in Table 1. Based on the information provided on the product labels, eight of these formulations explicitly declared the inclusion of *Lactobacillus* spp. and *Bifidobacterium* spp. Conversely, the remaining nine products lacked specific information regarding the probiotic species.

To confer their intended health benefits, probiotic foods are required to contain a minimum of 10^6 CFU/g of viable microorganisms at the time of consumption (Tripathi & Giri, 2014). Maintaining a stable, viable cell count is crucial not solely between the production and expiration dates but also consistently throughout the entire storage duration (Kazemi *et al.*, 2024). In the current study, fourteen out of the seventeen analysed products (excluding samples D10, F15, and G17) exhibited viable probiotic counts exceeding 6 log CFU/mL,g during the first week of shelf life. At the end of the shelf life, twelve products (excluding samples B7, D10, D12, F15, and G17) maintained probiotic counts above the recommended minimum threshold of 6 log CFU/mL,g indicating general compliance with established probiotic viability standards (Table 2).

Table 2: Counts of *Lactobacillus* spp. and *Bifidobacterium* spp. in the probiotic dairy products

Product	Viable Count (log CFU/g or CFU/mL)			
	The first week of shelf life		The end of shelf life	
	Lactobacillus spp.	*Bifidobacterium spp.*	*Lactobacillus* spp.	*Bifidobacterium spp.*
A1	7.65±0.49	7.8±0.28	7.58±0.6	7.97±0.04
A2	7.19±0.27	7.27±0.37	7.15±0.21	7.35±0.21
A3	7.42±0.31	6.47±0.38	7.52±0.15	6.64±0.25
A4	7.4±0.28	7.26±0.19	6.98±0.04	7.12±0.16
A5	7.36±0.33	6.68±0.36	6.66±0.37	6.91±0.41
A6	6.85±0.32	5.58±0.33	7.02±0.12	5.89±0.3
B7	6.25±0.35	-	-	-
C8	7.25±0.35	7.65±0.09	7.4±0.08	7.57±0.1
C9	7.11±0.27	7.22±0.31	7.1±0.14	7.15±0.21
D10	5.07±0.09	4.53±0.46	-	-
D11	5.08±0.13	9.17±0.39	5.05±0.07	9.17±0.24
D12	6.64±0.51	-	5.86±0.08	-
D13	7.06±0.08	-	6.33±0.18	-
E14	6.61±0.3	5.59±0.27	6.38±0.15	-
F15	5.92±0.11	4.11±0.16	5.98±0.04	-
F16	7.36±0.16	-	7.26±0.14	-
G17	5.76±0.23	-	5.08±0.18	-

-: Not counted

In the first week of shelf life, *Lactobacillus* spp. counts exceeded 6 log CFU/mL,g in thirteen of the analysed products, while this number decreased to eleven by the end of the shelf life. In two products, *Lactobacillus* populations declined to undetectable levels at the end of storage. Except for product D13, no statistically significant differences were observed in *Lactobacillus* counts between the first week and the end of shelf life ($p>0.05$). However, product D13 exhibited a significantly lower count at the end of shelf life ($p=0.036$).

As for *Bifidobacterium* spp., only eight products maintained viable counts above 6 log CFU/mL at the beginning and the end of the shelf life. No statistically significant differences were found in *Bifidobacterium* counts between the two-time points in any of the products ($p>0.05$). Notably, in our study, *Bifidobacterium* spp., which were indicated on the label, were not detected in four products (D12, D13, F16, G17) during the first week of shelf-life and in seven products (D10, D12, D13, E14, F15, F16, G17) by the end of shelf life.

The findings of this investigation corroborate the observations of Farahmand *et al.* (2021), who documented that a significant proportion (22 out of 36) of commercially available probiotic fermented dairy products maintained *Lactobacillus* spp. counts exceeding 10^6 CFU/g at the end of their stated shelf life. Similarly, Haddad (2017) observed that viable counts of probiotic bacteria remained above 6.0 log CFU/g in all analysed samples except for 4 out of 10 products by the end of refrigerated storage. These results are consistent with our observations, where the majority of the tested products maintained *Lactobacillus* and *Bifidobacterium* counts at or above the recommended threshold of 6 log CFU/mL or CFU/g during storage. In our study, although a decline in viable cell numbers was observed in several products, particularly toward the end of shelf life, the viability remained sufficient in most samples to meet the established probiotic criteria.

In contrast, Jang *et al.* (2022) demonstrated that probiotic cell counts remained at or above 8 log CFU/mL throughout the storage period, indicating a higher level of viability preservation than observed in our study. Similarly, Yilmaz-Ersan *et al.* (2017) reported a gradual decrease in the viability of probiotic cultures during a 22-day storage period; however, viable cell counts consistently remained above the minimum effective dose of 6 log CFU/g. Such inter-study variability may be ascribed to disparities in product formulations, the specific probiotic strains employed, processing parameters, storage temperatures, and packaging methodologies, all of which significantly influence probiotic viability within dairy matrices.

The data obtained from this study indicated that although a decrease was observed in the counts of *Lactobacillus* and *Bifidobacterium* in sample A4 and *Lactobacillus* counts in A5 by the end of shelf life, all probiotic yoghurt products maintained viable probiotic populations above the critical threshold of 6 log CFU/g. Interestingly, an increase in both *Lactobacillus spp.* and *Bifidobacterium* spp. counts was detected in samples A3 and A6, and *Bifidobacterium spp.* in A5 during the same period. These findings are in agreement with those of Kailasapathy *et al.* (2008), who reported that all yoghurt samples maintained viable counts of *Lactobacillus acidophilus* and *Bifidobacterium animalis* ssp. within the recommended range of 10^6-10^7 CFU/g after 35 days of storage. Similarly, Menezes *et al.* (2022) found that the levels of *Lactobacillus acidophilus* in goat milk yoghurt remained above 6 log CFU/g throughout 35 days of storage. In contrast, Kazemi *et al.* (2024) reported that none of the probiotic yoghurt samples they analysed reached the minimum viable count for *Lactobacillus* spp.

Furthermore, Jayamanne and Adams (2006) observed that although 9 out of 10 yoghurt samples initially contained $>10^6$ CFU/g *Bifidobacterium*, a steady decline occurred during storage. Çakmakçi *et al.* (2012) also noted that while probiotic viability was stable during the first 7 days of storage, a significant decrease occurred thereafter. Alazzeh *et al.* (2020) reported that although *Lactobacillus delbrueckii* ssp. *bulgaricus* counts exceeded log 8 CFU/mL at the beginning of storage in all yoghurt products; the initial viability of *Bifidobacterium* spp. was considerably lower, ranging from 0.8 to 4.5 log CFU/mL. During a 4-week refrigerated period, the viability of *Lactobacillus* slightly decreased, while a drastic reduction in *Bifidobacterium* counts was observed after week three. Regarding cheese sample B7, while *Lactobacillus spp.* counts were measured at 6.25 log CFU/g during the first week of shelf life, and no viable *Lactobacillus* were detected at the end of shelf life. *Bifidobacterium* spp. were absent in both sampling periods. These findings partially contrast with those of Kasımoğlu *et al.* (2004), who reported that *L. acidophilus* populations in Turkish white cheese peaked at 10^{10} CFU/g on day 7 and, although declining during ripening, remained above 10^7 CFU/g in vacuum-packaged cheese and above 10^6 CFU/g in brine-ripened cheese throughout 90 days of storage. Similarly, Cichosz *et al.* (2014) noted only a slight reduction in *Lactobacillus rhamnosus* HN001 viability in Swiss-type and Dutch-type cheese during storage. Consistent with our findings, Kazemi *et al.* (2024) evaluated cheese samples for *Lactobacillus* viability and reported a highest average of 5.67 log CFU/mL. A significant decline in bacterial counts was observed over time, with no viable probiotic bacteria detectable in 3 out of 4 cheese samples by the end of the storage period.

In the first week of shelf life, the viable counts of *Lactobacillus spp.* in kefir samples A1, A2, C8, C9, E14, F15, and F16 were determined as 7.65, 7.19, 7.25, 7.11, 6.61, 5.92, and 7.36 log CFU/mL, respectively. Similarly, *Bifidobacterium* spp. counts in samples A1, A2, C8, C9, E14, and F15 were 7.80, 7.27, 7.65, 7.22, 5.59, and 4.11 log CFU/mL, respectively, while no viable *Bifidobacterium* cells were detected in sample F16. At the end of the shelf life, only the F15 sample exhibited *Lactobacillus* counts below the recommended threshold of 6 log CFU/mL. Additionally, *Bifidobacterium spp.* were undetectable in E14 and F15 samples by the end of the storage period. These findings contrast with those of Kazemi *et al.* (2024), who reported initial *Lactobacillus* counts of 5.84 and 6.75 log CFU/mL in kefir samples, followed by a rapid decline to undetectable levels during storage. Grønnevik *et al.* (2011) similarly observed a decrease in lactic acid bacteria over the first four weeks of storage in Norwegian kefir. In support of this trend, Irigoyen *et al.* (2005) noted that *Lactobacillus* populations in

kefir declined by approximately 1.5 log units between days 7 and 14 before stabilising until day 28.

In ayran samples, *Lactobacillus spp.* counts in product G17 decreased from 5.76 to 5.08 log CFU/mL throughout storage, and no *Bifidobacterium spp.* were recovered, despite label claims. A similar discrepancy was observed in the fruit smoothie sample D13, which exhibited a decrease in *Lactobacillus spp.* from 7.06 to 6.33 log CFU/mL during storage. At the same time, *Bifidobacterium* was not detected at any point in time. Among the fruit shot products, *Lactobacillus spp.* counts in D10, D11, and D12 were initially recorded as 5.07, 5.08, and 6.64 log CFU/mL, respectively. The corresponding *Bifidobacterium spp.* counts were 4.53 log CFU/mL in D10 and 9.17 log CFU/mL in D11, whereas no viable *Bifidobacterium* was observed in D12, despite its declaration on the label. By the end of the shelf life, *Lactobacillus spp.* were undetectable in D10, significantly reduced in D12, and maintained at 5.05 log CFU/mL in D11. Notably, D11 retained its *Bifidobacterium* population at 9.17 log CFU/mL. Contrary to these results, Kakisu *et al.* (2011) found that the viable counts of *Lactobacillus plantarum* (10^8 CFU/mL) in fermented milk remained stable throughout storage. Conversely, Moayednia *et al.* (2009) observed a general decline in probiotic viability during the storage of fermented milk drinks, aligning more closely with the current study.

The survival of probiotic microorganisms in food matrices is influenced by multiple factors, including strain characteristics, interactions with starter cultures, native microflora, enzyme activity, post-acidification, and contamination with spoilage or pathogenic microorganisms (Tripathi & Giri, 2014; Terpou *et al.*, 2019). Technological factors such as processing conditions, heat treatments, ripening protocols, and storage temperatures also play a critical role in maintaining probiotic viability (Bakr, 2015). Additionally, food additives such as salts, sugars, sweeteners, flavouring agents, and preservatives may negatively affect the survival of probiotics (Palanivelu *et al.*, 2022). Importantly, the response of probiotics to such factors is species- and strain-dependent. Therefore, selecting robust probiotic strains that can endure adverse conditions during processing and storage is essential, as strain-specific tolerance plays a pivotal role in ensuring probiotic-containing products' desired health benefits and microbial stability (Tripathi & Giri, 2014).

CONCLUSION

In conclusion, the viability and stability of *Lactobacillus* and *Bifidobacterium* populations in probiotic dairy products are critical determinants of these products' health benefits. Factors such as production processes, storage conditions, and the composition of the dairy matrix play significant roles in influencing the survival rates of these probiotic strains. While advances in encapsulation technologies, protective agents, and optimised production methods have shown promise in enhancing the stability of probiotics, further research is required to develop more efficient strategies for preserving these microorganisms throughout the product's shelf life.

The accurate monitoring of probiotic viability is essential for ensuring the effectiveness of these products and meeting consumer expectations regarding health benefits. As the demand for functional foods continues to grow, a deeper understanding of the factors affecting probiotic survival and the development of innovative solutions will be key to advancing the quality and reliability of probiotic dairy products. Future studies should focus on identifying new methods to enhance probiotic delivery, reduce the impact of environmental stressors, and ensure the consistency of health-promoting effects in dairy-based probiotics.

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AUTHORS' CONTRIBUTION

Seda SEYİRT: Methodology, Writing - Original Draft. Pınar ŞANLIBABA: Conceptualization, Methodology, Data Curation, Software, Writing - Original Draft, Writing - Review & Editing. Başar UYMAZ TEZEL: Conceptualisation, Methodology, Writing - Original Draft, Writing - Review & Editing, Supervision, Funding acquisition.

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