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Strain- and Matrix-Dependent Survival of *Lactobacillus acidophilus* LA-5 and *Lacticaseibacillus paracasei* 431 in Commercial Ice Cream Bases

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ABSTRACT

The survival of *Lactobacillus acidophilus* LA-5 and *Lacticaseibacillus paracasei* 431 was evaluated in commercially available ice cream base mixtures differing in dairy and plant-based fat content. The objective was to determine strain-dependent stability during frozen storage and to model viability loss using the Weibull inactivation equation. Following inoculation, samples were frozen and stored at −20 °C for 90 days. Viable counts were monitored periodically, and survival kinetics were described by non-log-linear regression. A significant reduction in cell numbers was observed after a single freeze–thaw cycle in both strains. During subsequent storage, gradual inactivation occurred, with distinct strain- and matrix-dependent differences. *L. acidophilus* LA-5 showed higher stability in formulations with dairy-based fat compared with the plant-based fat, whereas *L. paracasei* 431 exhibited shorter predicted times to equivalent log-reductions in dairy systems. Survival curves were adequately described by the Weibull model, indicating heterogeneous resistance within bacterial populations. The results demonstrate marked strain- and formulation-specific differences in probiotic stability during frozen storage and provide quantitative parameters for predicting viability loss in ice cream matrices.

Keywords: ice cream, probiotics, Lactobacillus acidophilus, Lacticaseibacillus paracasei, frozen storage

INTRODUCTION

The human gastrointestinal tract harbors a complex microbial ecosystem that plays a crucial role in host physiology. Beyond its traditional functions in nutrient metabolism, immune modulation, and pathogen exclusion, the gut microbiota has been associated with systemic processes, including neuroendocrine signaling, psychological well-being, respiratory health, and chronic non-communicable diseases (Andreu-Sánchez et al., 2025; Colica & Vecchio, 2026; Maqsood et al., 2025). Consequently, modulation of the gut microbiome has become an important strategy for supporting host health.

Among microbiome-modulating approaches, probiotics remain the most extensively investigated. According to the FAO/WHO definition, later refined by ISAPP, probiotics are live microorganisms that confer a health benefit on the host when administered in adequate amounts (FAO/WHO, 2001; Hill et al., 2014). Traditionally, probiotic microorganisms have mainly belonged to lactic acid bacteria and related genera, although the spectrum of investigated species and strains has expanded with advances in microbiome research. Regardless of the specific organism, sufficient viable counts must be maintained throughout product shelf life to ensure efficacy, commonly in the range of 10^8–10^9 CFU per daily dose (FAO/WHO, 2001; Hill et al., 2014).

Fermented dairy products have historically been the predominant vehicles for probiotic delivery. However, the diversification of functional foods has led to the exploration of non-traditional carriers. Ice cream has emerged as a promising alternative due to its high consumer acceptance, particularly among children, and its storage at subzero temperatures, which may prolong microbial stability compared with refrigerated products such as yogurt (Soodbar et al., 2024). Nevertheless, probiotic incorporation into ice cream presents technological challenges. The freeze–thaw process represents the primary stress factor affecting probiotic survival in frozen systems. Ice crystal formation, osmotic imbalance, oxygen incorporation during overrun, and temperature fluctuations during storage can

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significantly reduce viable cell counts. Survival is influenced by freezing rate, storage temperature, initial inoculation level, and matrix composition. Protein content, fat level, sugar concentration, and buffering capacity have been identified as key factors affecting probiotic stability in ice cream (Lisboa et al., 2025). Although frozen storage generally slows microbial inactivation compared with liquid or semi-solid systems, a gradual decline in viability is still expected. Typical probiotic ice cream shelf life has been reported to be at least six months, with expected reductions of approximately 1–2 log units over 3–6 months at −18 °C (Ziarno et al., 2024).

Compared with dairy matrices, plant-based formulations may present additional challenges due to differences in protein structure, lipid composition, and buffering capacity. Technological strategies such as protein fortification, favourable storage temperature, oxygen content, redox potential, water activity and pH have been proposed to improve probiotic stability in these systems (Tripathi & Giri, 2014).

Most studies on probiotic ice cream have focused on strains of *Lactobacillus* and related genera because of their documented safety and functional properties. Among these, *Lacticaseibacillus casei* and *Lactobacillus acidophilus* have been reported to exhibit comparatively high survival in frozen dairy systems (Lisboa et al., 2025). However, strain-specific differences and matrix-dependent effects remain insufficiently characterized, particularly in commercially optimized industrial ice cream bases. Therefore, the aim of the present study was to evaluate the survival of *L. acidophilus* LA-5 and *L. paracasei* 431 in commercial dairy and plant-based ice cream mixes differing in fat content. Viability decline during frozen storage was described using the Weibull inactivation model in order to characterize non-log-linear survival kinetics and to estimate shelf life of the probiotic formulations.

MATERIALS AND METHODS

Ice cream base

Four different standard ice cream base mixtures were prepared by Ljubljanske mlekarne d.d. (Ljubljana, Slovenia). The base mixtures were unfermented and pasteurized, and they contained varying concentrations of milk fat (low, medium, high) or plant-based fat (Table 1). No flavourings were incorporated, and oxygen was not added during processing. The formulation with added plant-based fat contained glucose-fructose syrup, milk proteins, water, and the stabilizer Pulsgard Extrulce 274. In contrast, the milk-fat formulations were prepared using milk, cream, milk powder, and the stabilizer LACTOPOL Pulsgard Extrulce 252.

Table 1: Composition of ice cream formulations (w/w, %)

Dairy-based fat						
Formulation	Milk fat	Milk (3.5%)	Cream (49% fat)	Milk powder	Stabilizer	Sucrose
6% fat	6.0	69.6	10.2	4.7	0.5	9.0
10% fat	10.0	59.0	20.8	4.7	0.5	15.0
12% fat	12.0	52.8	27.0	4.7	0.5	15.0
Plant-based fat						
Formulation	Plant-based fat	Water	Glucose–fructose syrup	Milk proteins	Stabilizer	Sucrose
8% fat	8.0	64.9	7.0	9.0	0.6	10.5

Survival of probiotic bacteria in ice cream

Commercially available freeze-dried cultures of *L. acidophilus* LA-5 and *L. paracasei* 431 were obtained from Chr. Hansen (Denmark). According to the manufacturer's instructions, freeze-dried strain was separately and gradually incorporated into 100 mL of ice cream base mixture to achieve a final concentration of 1 × 10^9 CFU/mL. Due to the high viscosity of the ice cream bases, thorough mixing was performed to ensure homogeneity of the inoculated mixtures. The mixtures were subsequently aliquoted (5 mL per sample) and frozen at −20 °C in a standard laboratory freezer. Thereafter, samples were thawed at two-week intervals over a storage period of three months. The viability of probiotic bacteria was determined using De Man-Rogosa-Sharpe (MRS) agar (Merck, USA) plate count method, as described below. All experiments were conducted in triplicates.

Freeze–thaw effect and statistical analysis

To evaluate the effect of a single freeze–thaw cycle, probiotic samples with an initial concentration of 10^9 CFU/mL, suspended in a dairy matrix containing 10% fat, were frozen and subsequently thawed after 120 min. The experiment was performed in five replicates. Viable counts were determined after thawing as described in Chapter 2.2. Viability reduction following the freeze–thaw cycle was expressed as the percentage decrease relative to the initial counts. Due to the small sample size, nonparametric statistical tests were applied. Differences between the initial and post-thaw viable counts within each bacterial species were analyzed using the Mann–Whitney U test. To compare the magnitude of reduction between the two species, the difference between initial and final viable counts (Δ = final − initial) was calculated for each replicate. The resulting Δ values were then compared between the two bacterial species using the Mann–Whitney U test. Statistical significance was established at $p < 0.05$.

Mathematical modeling of probiotic viability

The decline in probiotic viability during frozen storage was described using the Weibull model, which enables characterization of non-log-linear survival kinetics frequently observed in food matrices (Van Boekel, 2002). The model was expressed as:

$$\log (N_t/N_0) = -b \times t^{\eta}$$

where N_0 represents the initial viable count, N_t the viable count at time t, b the scale parameter, and η the shape parameter. Experimental data were fitted using GraphPad Prism (version 9; GraphPad Software, San Diego, CA, USA). The model parameters (b and η) were estimated by least-squares regression analysis. Shelf life was defined as the time required for the probiotic population to decline from the initial level (N_0 = 10^9 CFU/mL) to the minimum recommended level of 10^6 CFU/mL (i.e., a 3-log reduction). The corresponding time was calculated analytically from the fitted Weibull parameters.

RESULTS

Probiotic survival curves in ice cream

Data on bacterial viability were collected at different time points and fitted to the Weibull inactivation model for both species, with the parameters b and η estimated from the experimental data (Table 2). The survival curves of both strains during frozen storage showed clear strain- and matrix-dependent differences in inactivation behaviour. *L. acidophilus* LA-5 exhibited predominantly non-log-linear survival kinetics ($\eta < 1$), characterized by an initial rapid decline followed by a more gradual decrease (tailing effect) (Figure 1A). Among the tested matrices, the most pronounced initial reduction was observed in the dairy matrix containing 10% fat, as reflected by the highest inactivation rate parameter (b), resulting in the steepest early decline. In the plant-based matrix containing 8% fat, the curve shape was also characterized by $\eta < 1$, indicating initial sensitivity of a subpopulation. However, despite a less pronounced initial drop compared to the 10% fat dairy matrix, this condition showed the fastest overall decline over time, reflected in the progressively steeper slope during storage. Overall, although *L. acidophilus* LA-5 experienced a strong initial reduction in certain matrices, its decline over the same storage interval was generally slower compared to *L. paracasei* 431, indicating greater persistence of the resistant subpopulation during prolonged frozen storage.

In contrast, *L. paracasei* 431 exhibited survival kinetics closer to log-linear behaviour ($\eta \approx 1$) in most ice cream matrices, indicating a relatively constant rate of inactivation over time (Figure 1A). The survival curves across different matrices were more similar to each other compared to *L. acidophilus* LA-5, demonstrating less pronounced matrix-dependent variability. This suggests a more uniform population response to frozen storage conditions.

Table 2: Estimated parameters (b and η) obtained from the best-fit Weibull inactivation model for *L. acidophilus* LA-5 and *L. paracasei* 431 in different ice cream formulations. Parameters were calculated by non-log-linear regression of mean viability data during frozen storage

Estimated parameters	Dairy 6% fat	Dairy 10% fat	Dairy 12% fat	Plant-based 8% fat
L. acidophilus LA-5				
b	0.03206	0.2502	0.1420	0.08769
η	0.5755	0.1980	0.2347	0.5478
L. paracasei 431				
b	0.02725	0.02895	0.004575	0.07124
η	0.9351	0.9164	1.272	0.6957

Effect of freeze–thaw cycle on probiotic survival

Following a single freeze–thaw cycle, viability decreased by 49.9% in *L. acidophilus* LA-5 and by 35.4% in *L. paracasei* 431. The reductions were statistically significant within each species ($p < 0.05$), and the magnitude of reduction differed significantly between strains ($p < 0.05$). These results indicate that *L. acidophilus* LA-5 was more susceptible to acute freezing stress, whereas *L. paracasei* 431 exhibited greater resistance to the freeze–thaw cycle and more uniform long-term inactivation kinetics across different matrices. This trend is further supported by the data presented in Figure 1.

Shelf life of probiotic ice cream

The predicted shelf life of probiotic ice cream formulations differed markedly between the two strains (Table 3). *L. acidophilus* LA-5 exhibited high stability in dairy-based matrices, with predicted survival exceeding 12 months and, in several cases, extending beyond 24 months before reaching critical log reductions. These values are within or above the typical commercial shelf life of ice cream with 4-12 months of shelf life depending on the storage temperature (Goff et al., 2025), indicating that dairy formulations did not represent a limiting factor for the long-term viability of this strain. In contrast, matrix with added plant-based fat proved to be more critical for *L. acidophilus* LA-5. In this formulation, a 2-log reduction was predicted to occur within approximately one year, and a 3-log reduction within two years, suggesting reduced long-term stability compared with dairy-based systems.

For *L. paracasei* 431, substantially shorter predicted survival times were observed. In dairy-based matrices, a 3-log reduction was estimated to occur after approximately five months, indicating that strain viability may become limiting within the typical commercial shelf life of frozen products. Interestingly, in the plant-based matrix, *L. paracasei* 431 exhibited relatively improved stability compared with *L. acidophilus* LA-5, with slower progression toward higher log reductions. Overall, strain-dependent differences were evident, with *L. acidophilus* LA-5 demonstrating superior long-term stability in dairy matrices, whereas *L. paracasei* 431 showed faster inactivation kinetics under the same conditions.

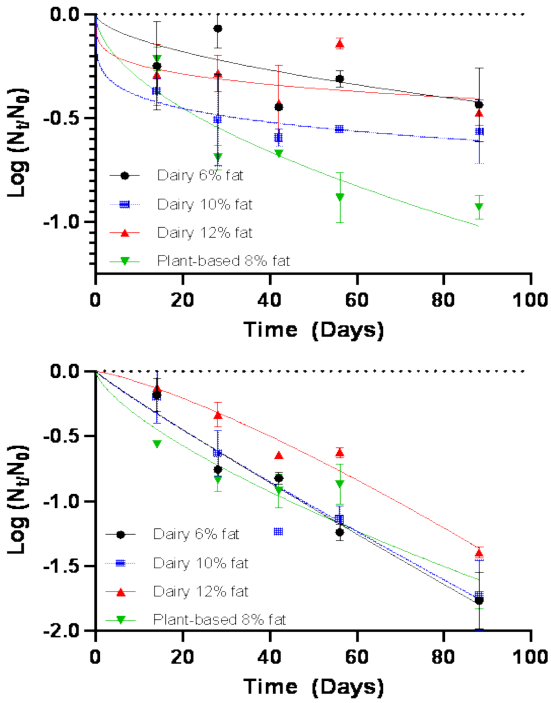

Figure 1: Decline of probiotic viability in dairy and plant-based fat ice cream mixtures during frozen storage. Data are presented as mean values ± standard deviation. Microbial counts at time zero were determined prior to freezing. Mean values were fitted using the Weibull inactivation model $\log (N_t/N_0) = -b \times t^{\eta}$, and the model parameters b and η were estimated by non-log-linear regression. A: *L. acidophilus* LA-5; B: *L. paracasei* 431

Table 3: Calculated shelf life of ice cream based on Weibull probiotic deactivation model

Reduction ($\log (N_t/N_0)$)	Dairy 6% fat	Dairy 10% fat	Dairy 12% fat	Plant-based 8% fat
L. acidophilus LA-5				
-1	394 d	687 d	>1000 d	85 d
-2	>1000 d	>1000 d	>1000 d	301 d
-3	>1000 d	>1000 d	>1000 d	632 d
L. paracasei 431				
-1	47 d	48 d	69 d	45 d
-2	99 d	102 d	119 d	121 d
-3	153 d	158 d	164 d	216 d

d – days

DISCUSSION

The present study demonstrates that probiotic survival in ice cream is primarily strain dependent and strongly influenced by matrix composition. The most pronounced viability loss occurred during the freezing step, confirming that acute freezing stress represents the main technological constraint in probiotic ice cream production. Similar observations have been reported previously, where freezing and overrun were identified as critical factors affecting lactobacilli survival in frozen dairy matrices (Magariños et al., 2007; Nascimento et al., 2023).

During subsequent frozen storage, gradual inactivation was observed and it was adequately described by the Weibull model. The observed non-log-linear survival kinetics – particularly for *L. acidophilus* LA-5 – may reflect heterogeneous resistance within the bacterial population, as has been discussed in recent analyses of microbial survival curves where concave and convex patterns are interpreted as evidence of subpopulations differing in stress tolerance and inactivation dynamics (Membré & Leguérinel, 2024). The use of a non-log-linear reduction model allowed a more realistic description of survival behaviour than traditional log-linear assumptions.

Clear differences between strains were evident. The higher long-term stability of *L. acidophilus* LA-5 in dairy matrices suggests that milk-derived components, including fat globules and milk proteins, may exert protective effects during frozen storage through structural stabilization and mitigation of ice crystal damage (Akalın et al., 2024). In contrast, *L. paracasei* 431 exhibited greater susceptibility to progressive inactivation in dairy systems, indicating that resistance to acute freezing stress does not necessarily predict long-term storage stability. Such strain-dependent variability in response to low-temperature and osmotic stress has been consistently reported in recent studies on probiotic survival in frozen and refrigerated food systems (Hurtado-Romero et al., 2025). Comparative investigations further indicate that *L. paracasei* 431 strains may demonstrate improved stability relative to *L. acidophilus* LA-5 under certain conditions, for example during refrigerated storage in fermented dairy beverages (Sadaghdar et al., 2012), highlighting that strain performance is highly context dependent. Therefore, the observation that matrix with added plant-based fat provided comparable support for *L. paracasei* 431 survival but substantially lower stability for *L. acidophilus* LA-5 was not unexpected.

Although the Weibull model enabled estimation of long-term stability, predictions beyond the 90-day experimental period rely on extrapolation and should therefore be interpreted cautiously. Particularly when pronounced tailing behaviour is present, extended predicted survival may reflect model characteristics rather than experimentally validated storage data.

Overall, the results confirm that incorporation of probiotics into commercially optimized ice cream bases is technologically feasible without the addition of other functional ingredients increasing the survival of bacteria; however, appropriate strain selection remains essential for ensuring functional stability throughout frozen storage.

CONCLUSIONS

This study confirms that the development of probiotic ice cream based on industrial ice cream mixes is technically achievable without reformulation of the matrix. The stability of incorporated probiotic cultures, however, depends strongly on strain characteristics and on the compositional properties of the ice cream base. Distinct differences in long-term performance between *L. acidophilus* LA-5 and *L. paracasei* 431 highlight the importance of strain selection when frozen storage is required. Formulations with dairy-based fat provided more favourable conditions for maintaining probiotic viability in certain cases, whereas matrices with plant-based fat showed strain-specific effects. The application of non-log-linear modelling provided a useful framework for comparing survival behaviour and for estimating potential product performance during extended storage. These findings support the use of predictive modelling as a complementary tool in the formulation and optimization of functional frozen desserts. Overall, successful probiotic ice cream production requires an integrated approach that considers strain physiology, matrix composition, and storage conditions in order to ensure functional stability throughout the intended shelf life.

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Od seva in matriksa odvisno preživetje *Lactobacillus acidophilus* LA-5 in *Lacticaseibacillus paracasei* 431 v komercialnih osnovah za sladoled

IZVLEČEK

V tej raziskavi smo ovrednotili preživetje sevov *Lactobacillus acidophilus* LA-5 in *Lacticaseibacillus paracasei* 431 v komercialno dostopnih mešanicah za osnovo sladoleda, ki se razlikujejo po vsebnosti mlečnih in rastlinskih maščob. Določili smo sevno specifično stabilnost med skladiščenjem po zamrzovanju ter modelirali izgubo vitalnosti z uporabo Weibullove enačbe inaktivacije. Študijo smo izvedli tako, da smo po inokulaciji bakterij v sladoledno osnovo vzorce zamrznili in jih 90 dni skladiščili pri −20 °C. Število živih mikroorganizmov smo periodično spremljali, kinetiko preživetja pa opisali z nelogaritmično regresijo. Pri obeh sevih smo po enem ciklu zamrzovanja in odtaljevanja zaznali znatno zmanjšanje števila celic. Med nadaljnjim skladiščenjem smo opazili postopno inaktivacijo bakterij, pri čemer so se jasno pokazale razlike glede na sev in matriks. Sev *L. acidophilus* LA-5 je izkazal večjo stabilnost v formulacijah z maščobo mlečnega izvora v primerjavi z rastlinsko maščobo, medtem ko je *L. paracasei* 431 v mlečnih sistemih dosegal krajše predvidene čase do enakovrednih logaritemskih zmanjšanj. Weibullov model je ustrezno opisal krivulje preživetja, kar kaže na heterogeno odpornost znotraj bakterijskih populacij. Rezultati potrjujejo izrazite razlike v stabilnosti probiotikov med zamrznjenim skladiščenjem, ki so specifične za sev in formulacijo, ter omogočajo napovedovanje izgube vitalnosti v sladolednih osnovah s pomočjo kvantitativnih parametrov.

Ključne besede: sladoled, probiotiki, *Lactobacillus acidophilus*, *Lacticaseibacillus paracasei*, zamrznjeno skladiščenje