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Impact of microencapsulation and milk matrix on *Lactobacillus acidophilus* survival in yoghurt-based ice creams

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Abstract: This study examined the effects of milk origin (cow versus buffalo), probiotic delivery form (free cells versus alginate-encapsulated *Lactobacillus acidophilus* ATCC 4356), and inulin supplementation on microbial viability, physicochemical properties, and melting behaviour of yoghurt ice cream over a 60 day storage period. Ten formulations were evaluated on days 1, 15, 30, and 60 for total and probiotic counts, yeast and mould levels, pH, titratable acidity, drip time, viscosity (measured at 30 and 50 rpm), and overrun. Buffalo milk samples containing encapsulated probiotics and inulin showed the highest *Lactobacillus acidophilus* counts (reaching 5.97 log CFU·g^{-1}), the greatest resistance to melting (first drip: 97.14 min), the highest viscosity (20.166 Pa.s at 30 rpm), and the highest overrun (34.77%), whereas cow milk controls exhibited the lowest microbial survival and melting performance. Encapsulation markedly improved probiotic stability; in most encapsulated samples, yeast and mould counts remained below 2.00 log CFU·g^{-1} after 30 days, and this was accompanied by higher pH values (maximum 4.99 ± 0.04) and more controlled titratable acidity levels (0.57–0.87%). These findings indicate that alginate microencapsulation, particularly within buffalo milk matrices, effectively enhances probiotic viability and the functional quality of frozen dairy products.

Keywords: *Bubalus bubalis*; synbiotic; prebiotic; texture profile; sensory properties; shelf-life

This study examines the effects of milk origin (cow versus buffalo), probiotic delivery form (free cells versus alginate-encapsulated *Lactobacillus acidophilus* ATCC 4356), and inulin supplementation on microbial viability, physicochemical characteristics, and melting behaviour of yoghurt-based ice creams. Buffalo milk has gained increasing recognition in functional food research owing to its superior macro- and micronutrient profile, notably higher levels of fat, protein, calcium, and bioactive molecules such as immunoglobulins, lactoferrin, and lysozyme, compared with conventional cow milk (Abesinghe et al. 2020). These compositional advantages, combined with its naturally dense viscosity and white opacity, which are attributable to retinol rather than carotenoids, offer notable technological functionality, especially in fermented and frozen dairy matrices such as yoghurt-based ice cream. Its structural richness provides a unique opportunity for improving the delivery and stability of health-promoting bioactives, particularly probiotics.

Probiotic integration into dairy systems, especially using *L. acidophilus*, has been widely documented with respect to its role in modulating the intestinal microbiota, enhancing mucosal immunity, and providing general gastrointestinal benefits. However, *L. acidophilus* remains highly vulnerable to physicochemical

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stressors encountered during processing, especially during freeze–thaw cycles and long-term frozen storage. Factors such as pH shifts, oxygen exposure, low temperatures, and matrix interactions are known to significantly reduce viable cell counts, thereby limiting the functional claims of probiotic products (Shah 2000; Krasaekoopt et al. 2003).

To address this challenge, microencapsulation techniques, particularly those utilising sodium alginate, chitosan, or resistant starch, have been investigated as protective delivery systems that enhance microbial viability by creating semi-permeable barriers against external stressors (Picot and Lacroix 2004). In parallel, the incorporation of prebiotics such as inulin has demonstrated synergistic potential by selectively stimulating probiotic proliferation while simultaneously modulating the sensory and textural properties of dairy matrices (Fooks et al. 1999; Van Loo 2007). Synbiotic applications, combining encapsulated probiotics with functional fibres such as inulin, thus constitute a promising frontier in frozen dairy innovation.

Despite the growing body of work on probiotic survival in ice cream, only a limited number of studies have systematically examined how milk origin (cow versus buffalo), probiotic delivery form (free versus encapsulated), and prebiotic co-supplementation (inulin) interactively affect both the viability of *L. acidophilus* and the physicochemical attributes of yoghurt-based ice creams. In particular, the role of buffalo milk as a stabilising matrix for encapsulated probiotics during frozen storage has not been thoroughly investigated. Moreover, comparative datasets addressing how these formulation parameters influence melting resistance, overrun, pH dynamics, and textural behaviour over shelf life remain scarce, when assessed using metrics such as pH, drip time, overrun, and microbial viability.

Therefore, this study sets out to comprehensively assess the impact of milk type (cow versus buffalo), probiotic form (free versus encapsulated), and inulin presence on microbiological viability, melt resistance, overrun, and structural stability in yoghurt-based ice creams stored over 30 days. By simultaneously evaluating microbial counts (including yeast–mould dynamics), drip time, pH, and overrun properties across formulations, this research addresses an important gap in the synbiotic frozen-dairy literature. The findings are intended to inform formulation strategies for more resilient, probiotic-enriched frozen dairy products with enhanced shelf life, textural integrity, and functional authenticity.

MATERIAL AND METHODS

Material

Yoghurt ice cream production was conducted at the dairy processing unit of Atatürk University, Faculty of Agriculture, under the supervision of the Food Engineering Department. MRS Broth (Merck, Germany) was utilised for the activation of bacterial strains and for the enumeration of microbiological counts. Buffered Peptone Water (Merck) was used to facilitate serial dilutions and microbiological analyses. Sodium alginate (Sigma-Aldrich, USA) served as the encapsulation matrix, and $Na_2HPO_4 \cdot 7H_2O$ (Merck) was used in the preparation of buffer solutions. Raw buffalo milk (*Bubalus bubalis*) and raw cow milk were obtained from the Atatürk University Agricultural Faculty dairy research farm (Erzurum, Turkey). Both milks were refrigerated at 4 °C and processed within 24 h of collection.

Preparation procedures

Activation and encapsulation of probiotic culture. Lyophilised *Lactobacillus acidophilus* ATCC 4356 (Peyma Hansen, Denmark) was inoculated into 9 mL of MRS Broth and incubated anaerobically at 37 °C for 18 h. The culture was then transferred to 100 mL of MRS Broth (Biokar BK070HA) for secondary incubation under identical conditions. After centrifugation (6 000 rpm, 4 °C, 10 min), the bacterial pellet was washed twice with sterile peptone water and resuspended in a 0.1% sterile peptone solution. Absorbance was measured at 600 nm to estimate viable counts (approximately 10^{10} CFU·g^{-1}).

Encapsulation was carried out using a modified Krasaekoopt method. A 20 mL probiotic suspension (approximately 10^7 CFU·mL^{-1}) was homogenised with 80 mL of 2.5% sterile sodium alginate for 10 min. The mixture was extruded dropwise into a 0.2 M $CaCl_2$ solution using a sterile 21G syringe to form spherical gel beads via ionic cross-linking. The beads were collected by filtration (Whatman No. 4) and stored at 4 °C.

Yoghurt and ice cream production. Cow and buffalo milks were standardised to 12% non-fat solids using skimmed milk powder, and the fat content of cow milk was adjusted to that of buffalo milk (5.5%) by the addition of cow cream. Pasteurisation was carried out at 90 °C for 10 min, after which the mix was cooled to 43 °C and supplemented with 0.5% inulin (Peyma Hansen), 2% yoghurt starter (Chr. Hansen DVS, Denmark), and probiotic cells. Fermentation at 43 °C continued until a pH of 4.60, after which encapsulated probiotics were added at 37 °C. Samples were stored

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at 4 °C for 24 h. The yoghurt base was then mixed with 0.5% stabiliser (salep, 99% dry matter), 0.25% emulsifier (Tito, Turkey), and 18% sucrose (99% purity), heated at 70 °C for 10 min and cooled to 42 °C; encapsulated probiotics (approximately 1×10^9 CFU·mL^{-1}) were then added. Mixes prepared from cow and buffalo milk were divided into five equal parts. Probiotic bacteria and inulin were not added to the first part of the mix samples [cow control (CC) and buffalo control (BC)], and these were considered controls.

The following abbreviations were used throughout the study: cow milk (C), buffalo milk (B), probiotic culture (*Lactobacillus acidophilus* ATCC 4356) (P), encapsulated probiotic culture (EP), and inulin (I).

Unencapsulated probiotic bacteria were added to the second part (C+P and B+P), and encapsulated bacteria were added to the third part (C+EP and B+EP). In the fourth part, 1% inulin was added as a prebiotic along with unencapsulated probiotic bacteria (C+P+I and B+P+I), and in the fifth part, 0.5% inulin was added along with encapsulated probiotic bacteria (C+EP+I and B+EP+I). Thus, ten different yoghurt ice cream samples were prepared. The mixture was held at 42 °C for 30 min, cooled to 5 °C over 6 h, churned, packaged, and hardened. Ten formulations were assessed on days 1, 15, 30, and 60 in a 10 by 4 factorial, completely randomised design. All results are presented as the mean of two blocks.

Analytical procedures

Sampling and storage protocol. On each designated storage day, samples were aseptically collected from deep-frozen containers. Ten distinct formulations were prepared, and the entire experiment was conducted in two independent blocks.

Microbiological analysis. Total aerobic mesophilic bacteria (TAMB) were enumerated in accordance with ISO 4833:2013 guidelines. TAMB were enumerated on Plate Count Agar (PCA; Neogen NCM0010A, USA) via the spread-plate method. Serial dilutions were prepared in 0.85% (*w/v*) NaCl. *Lactobacillus delbrueckii* subsp. *bulgaricus* counts were determined on MRS agar (de Man et al. 1960) and *Streptococcus thermophilus* counts were determined on M-17 agar (Hagen and Narvhus 1999). *Lactobacillus acidophilus* was enumerated on MRS-sorbitol agar (Neogen). Yeast and mould counts were assessed on Potato Dextrose Agar (PDA; Lab 098).

Physicochemical measurements. pH was measured using a calibrated digital pH meter. Titratable acidity was assessed by titration with standardised sodium hydroxide (NaOH). Viscosity was determined using a Fungilab viscometer at 20 and 50 rpm. Melting characteristics were evaluated using mesh systems under ambient conditions, and both initial drip time and total melting time were recorded.

Statistical analysis

All quantitative data were subjected to two-way analysis of variance (ANOVA) and Duncan's post hoc test ($P < 0.01$), and the analyses were performed using SPSS 22.0 (SPSS Inc., USA).

RESULTS AND DISCUSSION

This section presents the microbiological findings of yoghurt-based ice cream samples formulated with different milk sources, probiotic types, and encapsulation strategies. Each microbial parameter was statistically evaluated over a 60-day storage period, and the results were interpreted with respect to treatment effects and storage dynamics. The outcomes are discussed with reference to the relevant literature to contextualise probiotic viability and the microbiological safety of the formulations.

Microbiological analysis results

Microbiological analysis focused on five key indicators: total aerobic mesophilic bacteria, *L. delbrueckii* subsp. *bulgaricus*, *Streptococcus thermophilus*, *L. acidophilus*, and total yeast and mould counts. For each parameter, the impact of treatment group, storage period and their interaction was assessed. The following subsections present the results in detail, supported by Duncan's multiple comparison test and previous scientific findings.

Total aerobic mesophilic bacteria (TAMB) count of ice cream samples. The TAMB counts of the ice cream samples ranged from 6.00 to 9.16 log CFU·g^{-1} during the 60-day storage period. Analysis of variance revealed that treatment type, storage duration, and their interaction had a statistically significant effect on TAMB counts ($P < 0.01$). The detailed results of Duncan's multiple comparison test are presented in Table 1, while the interaction effect is also presented in Table 1.

Buffalo milk ice cream with probiotics (B+P) showed the highest total aerobic mesophilic bacteria (TAMB) count (8.34 ± 0.69 log CFU·g^{-1}), whereas the cow milk control (CC) exhibited the lowest value (6.73 ± 1.40 log CFU·g^{-1}). TAMB levels increased progressively over the 60-day storage period, which likely reflects the combined contribution of probiotic

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Table 1. Total aerobic mesophilic bacteria (TAMB) counts (log CFU·g^{-1}) in ice cream samples during 60-day storage (mean ± SD, n = 2 blocks)

Treatments	TAMB (log CFU·g^{-1})	Storage period (days)	TAMB (log CFU·g^{-1})
CC	6.73 ± 1.40^{e}	1	6.75 ± 1.11^{c}
C+P	7.05 ± 1.72^{d}	15	7.61 ± 1.02^{b}
C+P+I	7.76 ± 1.01^{b}	30	7.43 ± 0.95^{b}
C+EP	7.81 ± 0.84^{b}	60	7.89 ± 0.76^{a}
C+EP+I	7.54 ± 0.61^{bc}	Sign.	**
BC	7.58 ± 0.28^{bc}	–	–
B+P	8.34 ± 0.69^{a}	–	–
B+P+I	7.39 ± 0.65^{c}	–	–
B+EP	7.64 ± 0.65^{bc}	–	–
B+EP+I	6.33 ± 0.26^{f}	–	–
Sign.	**	–	–
T × SP	**	–	–

$^{a–f}$different letters within the same column (treatments or storage period) indicate significant difference (Duncan's test, $P < 0.01$); statistical significance of main effects and interactions was determined by two-way ANOVA ($P < 0.01$); C – cow milk; B – buffalo milk; CC – cow control; BC – buffalo control; P – probiotic; I – inulin; EP – encapsulated bacteria; T × SP – treatment × storage period interaction; Sign. – statistical significance

Table 2. Duncan's multiple comparison test results of *Lactobacillus delbrueckii* subsp. *bulgaricus* counts of ice cream samples (log CFU·g^{-1})

Treatments	*Lactobacillus bulgaricus* (log CFU·g^{-1})	Storage period (days)	*Lactobacillus bulgaricus* (log CFU·g^{-1})
CC	6.59 ± 1.36^{d}	1	6.35 ± 1.15^{c}
C+P	6.39 ± 0.94^{e}	15	7.19 ± 0.48^{b}
C+P+I	7.33 ± 0.39^{b}	30	7.15 ± 0.84^{b}
C+EP	7.40 ± 0.55^{b}	60	7.78 ± 0.72^{a}
C+EP+I	7.02 ± 0.94^{c}	–	–
BC	7.37 ± 0.63^{b}	–	–
B+P	7.08 ± 0.83^{c}	–	–
B+P+I	7.27 ± 0.98^{b}	–	–
B+EP	8.37 ± 0.65^{a}	–	–
B+EP+I	6.35 ± 0.53^{e}	–	–
Sign.	**	T × SP	**

$^{a–e}$different letters indicate significant difference between treatments (Duncan's multiple comparison test, $P < 0.01$); **statistical significance of main effects and interactions was determined by two-way ANOVA ($P < 0.01$); treatments abbreviations as explained in Table 1

cultures and starter bacteria. Earlier work by Hekmat and McMahon (1992) reported initial *L. acidophilus* counts of approximately 10^8 CFU·mL^{-1} following freezing, with a decline of about 1 log during storage, while Modler et al. (1990) observed no significant loss ($P > 0.05$) in *L. bulgaricus* and *S. thermophilus* over an 11-week period. These findings are consistent with previous reports on the microbiological stability of probiotic ice cream products.

Lactobacillus delbrueckii subsp. bulgaricus count of ice cream samples. According to analysis of variance, *L. delbrueckii* subsp. *bulgaricus* counts were significantly affected by treatment type, storage period, and their interaction ($P < 0.01$). The detailed results of Duncan's multiple comparison test are provided in Table 2.

Buffalo milk ice cream containing encapsulated probiotics (B+EP) showed the highest *Lactobacillus bulgaricus* count (8.37 ± 0.65 log CFU·g^{-1}), whereas buffalo milk with encapsulation plus inulin (B+EP+I) exhibited the lowest value (6.35 ± 0.53 log CFU·g^{-1}). In cow milk samples, the non-encapsulated probiotic formulation (C+P) recorded the lowest count (6.39 ± 0.94 log CFU·g^{-1}). Storage duration significantly increased bacterial counts from day 1 to day 60 ($P < 0.01$), which may be attributed to post-acidification and residual metabolic activity of the cultures. Compared with the findings of Lopez et al. (1998), who reported values ranging from 4.30 to 6.80 log CFU·g^{-1} in commercial yoghurt ice creams, the higher counts observed in the present study suggest that specific formulation and processing conditions are conducive to enhanced probiotic survival.

Streptococcus thermophilus subsp. thermophilus counts of ice cream samples. The results of two-way analysis of variance (ANOVA) revealed significant effects of treatment type, storage period, and their interaction on *S. thermophilus* counts in ice cream samples ($P < 0.01$). The detailed mean values and results of Duncan's multiple comparison test are presented in Table 3.

Buffalo milk with free probiotics (B+P) exhibited the highest *S. thermophilus* count (7.62 ± 0.42 log CFU·g^{-1}), whereas the buffalo formulation containing encapsulated probiotics with inulin (B+EP+I) showed the lowest value (5.85 ± 1.02 log CFU·g^{-1}). In cow milk, the non-encapsulated probiotic sample (C+P) recorded the lowest count (6.08 ± 0.94 log CFU·g^{-1}). *S. thermophilus* counts increased significantly over the 60-day storage period ($P < 0.01$), which may reflect sustained metabolic activity and protective effects of the dairy matrix. Variations are likely attributable to production tech-

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Table 3. Duncan's multiple comparison test results of *Streptococcus thermophilus* subsp. *thermophilus* averages of ice cream samples (log CFU·g^{-1})

Treatments	*Streptococcus thermophilus* (log CFU·g^{-1})	Storage period (days)	*Streptococcus thermophilus* (log CFU·g^{-1})
CC	6.77 ± 0.36^{d}	1	6.14 ± 0.84^{b}
C+P	6.08 ± 0.94^{e}	15	7.04 ± 0.78^{a}
C+P+I	6.75 ± 0.81^{d}	30	7.11 ± 0.63^{a}
C+EP	7.11 ± 0.65^{c}	60	7.11 ± 0.59^{a}
C+EP+I	6.91 ± 0.61^{d}	–	–
BC	7.27 ± 0.41^{bc}	–	–
B+P	7.62 ± 0.42^{a}	–	–
B+P+I	7.31 ± 0.43^{b}	–	–
B+EP	6.84 ± 0.71^{d}	–	–
B+EP+I	5.85 ± 1.02^{f}	–	–
Sign.	**	T × SP	**

$^{a–f}$different letters indicate significant difference (Duncan's multiple comparison test, $P < 0.01$); **statistical significance of main effects and interactions was determined by two-way ANOVA ($P < 0.01$); treatments abbreviations as explained in Table 1

Table 4. Duncan's multiple comparison test results of Lactobacillus acidophilus counts of ice cream samples (log CFU·g^{-1})

Treatments	*Lactobacillus acidophilus* (log CFU·g^{-1})	Storage period (days)	*Lactobacillus acidophilus* (log CFU·g^{-1})
CC	5.47 ± 0.42^{d}	1	5.07 ± 0.34^{d}
C+P	5.86 ± 0.17^{ab}	15	5.94 ± 0.35^{a}
C+P+I	5.63 ± 0.54^{c}	30	5.68 ± 0.68^{c}
C+EP	5.86 ± 0.55^{ab}	60	5.85 ± 0.66^{b}
C+EP+I	5.53 ± 0.76^{c}	Sign.	**
BC	5.23 ± 0.48^{e}	–	–
B+P	5.60 ± 0.41^{c}	–	–
B+P+I	5.73 ± 0.76^{bc}	–	–
B+EP	5.91 ± 0.33^{a}	–	–
B+EP+I	5.97 ± 1.12^{a}	–	–
Sign.	**	–	–
T × SP	**	–	–

$^{a–e}$different letters within one column indicate significant difference (Duncan's multiple comparison test, $P < 0.01$); **statistical significance of main effects and interactions was determined by two-way ANOVA ($P < 0.01$); Sign. – statistical significance; treatments abbreviations as explained in Table 1

niques and starter-strain interactions, notably between *L. bulgaricus* and *S. thermophilus*. Similar studies have reported lower counts in cow milk-based frozen dairy products (Hagen and Narvhus 1999; Shah 2000), suggesting that the optimised formulations and probiotic supplementation employed in the present study supported enhanced *S. thermophilus* survival.

***Lactobacillus acidophilus* counts of ice cream samples.** Ensuring the viability of probiotic microorganisms such as *L. acidophilus* is crucial for maintaining the functional health benefits of fermented dairy products. It is widely accepted that a viable count exceeding 10^6–10^7 CFU·g^{-1} is necessary to achieve the desired probiotic effect (Shortt 1999; Shah 2000). However, the manufacturing and storage conditions of frozen dairy desserts, particularly freezing, pose a major threat to the survival of such strains (Champagne et al. 2005). Analysis of variance demonstrated that treatment (T), storage period (SP), and their interaction (T × SP) significantly influenced the viability of *L. acidophilus* ($P < 0.01$). The detailed multiple comparison outcomes are presented in Table 4.

Buffalo milk ice cream containing alginate-encapsulated *L. acidophilus* with inulin showed the highest viable counts (5.91–5.97 log CFU·g^{-1}), compared with buffalo milk samples without probiotics (5.23 log CFU·g^{-1}), indicating that encapsulation, particularly within buffalo milk matrices, enhances probiotic survival under frozen storage conditions. Counts increased during the first 15 days, which may be attributable to post-acidification or adaptation to the dairy matrix, and subsequently declined but remained above the therapeutic threshold. These results are consistent with the findings of Hagen and Narvhus (1999), Homayouni et al. (2008), Ranadheera et al. (2012), Karthikeyan et al. (2014), and Kailasapathy and Sultana (2003), who reported superior viability of encapsulated *Lactobacillus acidophilus* and *Bifidobacterium lactis* strains compared with free probiotics, and they further support the observations of Silva et al. (2018) regarding the protective effect of encapsulation over 60 days in frozen dairy systems.

Yeast and mould counts of ice cream samples. Analysis of variance revealed that the differences in yeast and mould counts were statistically significant across treatment groups (T), storage periods (SP), and their interaction (T × SP) ($P < 0.01$). Mean values and multiple comparison results are presented in Table 5.

Buffalo milk containing free probiotics (B+P) exhibited the lowest yeast and mould counts (2.17 ± 0.32 log CFU·g^{-1}), whereas cow milk with free probiotics (C+P; 3.02 ± 1.09 log CFU·g^{-1}) and buffalo milk with encapsulation plus inulin (B+EP+I;

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Table 5. Duncan's multiple comparison test results of yeast and mould average counts (log CFU·g^{-1}) in ice cream samples

Treatments	Yeast and mould count (log CFU·g^{-1})	Storage period (days)	Yeast and mould count (log CFU·g^{-1})
CC	2.55 ± 0.67^{c}	1	3.03 ± 0.67^{b}
C+P	3.02 ± 1.09^{a}	15	3.06 ± 0.66^{a}
C+P+I	2.35 ± 0.37^{d}	30	2.00 ± 0.00^{c}
C+EP	2.26 ± 0.28^{e}	60	2.00 ± 0.00^{c}
C+EP+I	2.24 ± 0.25^{e}	–	–
BC	2.41 ± 0.44^{d}	–	–
B+P	2.17 ± 0.32^{f}	–	–
B+P+I	2.35 ± 0.38^{d}	–	–
B+EP	2.90 ± 0.96^{b}	–	–
B+EP+I	3.01 ± 1.07^{a}	–	–
Sign	**	–	–
T × SP	**	–	–

$^{a–e}$different letters within one column indicate significant difference (Duncan's multiple comparison test, $P < 0.01$); **statistical significance of main effects and interactions was determined by two-way ANOVA ($P < 0.01$); treatments abbreviations as explained in Table 1

Table 6. Duncan's multiple comparison test results of pH values of ice cream samples

Treatment	pH (± SD)	Storage period (days)	pH (± SD)
CC	4.60 ± 0.29^{f}	1	4.89 ± 0.37^{a}
C+P	4.65 ± 0.32^{ef}	15	4.79 ± 0.12^{a}
C+P+I	4.61 ± 0.29^{f}	30	4.70 ± 0.00^{b}
C+EP	4.65 ± 0.19^{ef}	60	4.50 ± 0.12^{c}
C+EP+I	4.92 ± 0.13^{b}	–	–
BC	4.83 ± 0.31^{c}	–	–
B+P	4.95 ± 0.08^{ab}	–	–
B+P+I	4.77 ± 0.28^{d}	–	–
B+EP	4.67 ± 0.15^{e}	–	–
B+EP+I	4.99 ± 0.04^{a}	–	–
Sign.	**	–	–
T × SP	**	–	

$^{a–f}$different letters within one column indicate significant difference (Duncan's multiple comparison test, $P < 0.01$); **statistical significance of main effects and interactions was determined by two-way ANOVA ($P < 0.01$); treatments abbreviations as explained in Table 1

3.01 ± 1.07 log CFU·g^{-1}) showed the highest values. Yeast and mould counts peaked on day 15, followed by a decline by day 30, and remained at or below 2.00 log CFU·g^{-1} through Day 60, indicating effective suppression of fungal growth during frozen storage. These trends are consistent with the findings of Kailasapathy and Sultana (2006) and Ranadheera et al. (2012), who reported that encapsulated probiotics contribute to improved microbial stability and the inhibition of fungal growth during long-term cold storage.

Analysis results of ice cream samples

Analysis of variance indicated that treatment groups (T), storage periods (SP), and their interaction (T × SP) had a statistically significant effect on the pH values of yoghurt ice cream samples ($P < 0.01$). The results of Duncan's multiple comparison test are presented in Table 6. According to the findings, the highest pH value was observed in the buffalo milk sample containing encapsulated probiotic bacteria and inulin (4.99 ± 0.04), whereas the lowest pH value was recorded in the cow milk sample containing encapsulated probiotic bacteria (4.60 ± 0.29). Across storage periods, a significant decline in pH was observed over time. The highest mean pH was detected on Day 1 (4.89 ± 0.37), which gradually decreased to 4.50 ± 0.12 by Day 60. This reduction may be attributed to ongoing metabolic activity of probiotic cultures, leading to lactic acid production and a consequent decrease in pH during storage.

Milk type and alginate encapsulation were found to have a pronounced influence on pH stability. Cow milk samples exhibited consistently lower pH values, indicating a greater extent of acidification, whereas buffalo milk demonstrated a higher buffering capacity against acidity. Hekmat and McMahon (1992) suggested that a pH value of approximately 5.50 represents an optimal range for probiotic ice cream acceptability. Similarly, Pinto et al. (2012) reported pH values ranging from 4.18 to 4.94 in yoghurt ice creams containing encapsulated probiotics, which aligns with the findings of the present study. Furthermore, Martinou-Voulasiki and Zerfiridis (1990), together with Homayouni et al. (2008), documented that the incorporation of prebiotic components such as inulin, maltodextrin, and stabilising agents can regulate pH within the range of 4.40–5.30. Taken together, these findings indicate that probiotic encapsulation and the use of prebiotic ingredients play a critical role in modulating acidification, thereby improving both sensory attributes and probiotic stability in frozen dairy products.

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Table 7. Duncan's multiple comparison test results of the titration acidity values of ice cream samples

Treatment	Titration acidity (%)	Storage period (days)	Titration acidity (%)
CC	0.57 ± 0.13^{d}	1	0.55 ± 0.08^{c}
C+P	0.69 ± 0.16^{bcd}	15	0.56 ± 0.10^{c}
C+P+I	0.66 ± 0.15^{bcd}	30	0.82 ± 0.31^{b}
C+EP	0.71 ± 0.19^{bc}	60	0.96 ± 0.14^{a}
C+EP+I	0.82 ± 0.35^{ab}	–	–
BC	0.78 ± 0.25^{abc}	–	–
B+P	0.87 ± 0.42^{a}	–	–
B+P+I	0.79 ± 0.31^{ab}	–	–
B+EP	0.61 ± 0.11^{cd}	–	–
B+EP+I	0.75 ± 0.19^{abc}	–	–
Sign.	**	–	–
T × SP	**	–	–

$^{a–d}$different letters within one column indicate significant difference (Duncan's multiple comparison test, $P < 0.01$); **statistical significance of main effects and interactions was determined by two-way ANOVA ($P < 0.01$); treatments abbreviations as explained in Table 1

Table 8. Duncan's multiple comparison test results of the first dripping time of yoghurt ice cream samples

Treatment	First drip time (min)	Storage period (days)	First drip time (min)
CC	83.72 ± 15.74^{b}	1	77.12 ± 14.72^{c}
C+P	87.65 ± 14.41^{b}	15	72.96 ± 19.27^{c}
C+P+I	71.17 ± 19.92^{c}	30	86.45 ± 15.26^{b}
C+EP	51.68 ± 8.33^{d}	60	92.74 ± 20.08^{a}
C+EP+I	89.77 ± 8.62^{ab}	–	–
BC	82.47 ± 24.76^{b}	–	–
B+P	89.26 ± 17.01^{b}	–	–
B+P+I	85.18 ± 13.38^{b}	–	–
B+EP	97.14 ± 11.31^{a}	–	–
B+EP+I	85.14 ± 12.94^{b}	–	–
Sign.	**	–	–
T × SP	**	–	–

$^{a–d}$different letters within one column indicate significant difference (Duncan's multiple comparison test, $P < 0.01$); **statistical significance of main effects and interactions was determined by two-way ANOVA ($P < 0.01$); treatments abbreviations as explained in Table 1

Titration acidity analysis results of ice cream samples

The analysis of variance indicated that treatment groups (T), storage periods (SP), and their interaction (T × SP) had a statistically significant effect on the titratable acidity of yoghurt ice cream samples ($P < 0.01$). The detailed results of Duncan's multiple comparison test are presented in Table 7. Among the formulations, the highest titratable acidity was recorded in the buffalo milk yoghurt ice cream containing probiotic bacteria (0.87 ± 0.42%), whereas the lowest value was observed in the cow milk control sample (0.57 ± 0.13%). With increasing storage time, titratable acidity increased significantly, rising from 0.55 ± 0.08% on Day 1 to 0.96 ± 0.14% on Day 60. This gradual increase reflects the continued metabolic activity of lactic acid bacteria and the progressive accumulation of lactic acid during frozen storage.

The results demonstrate that both milk type and the incorporation of probiotic and prebiotic ingredients significantly affect the rate of acidification. The higher titratable acidity observed in buffalo milk-based formulations may be attributed to its greater buffering capacity, distinct protein composition, and specific fermentation kinetics. These findings are consistent with those of Ravula and Shah (1998), who reported titratable acidity values ranging from 0.77% to 0.79% in fermented dairy desserts, thereby supporting the findings of the present study. Similarly, Pinto et al. (2012) documented acidity values between 0.70% and 0.74% in yoghurt ice creams containing encapsulated *Bifidobacterium* BB-12, which are in close agreement with the results reported here.

First dripping time analysis results of ice cream samples

The first dripping time of yoghurt ice cream is regarded as a critical indicator of structural integrity and melting resistance during consumption (Bolliger et al. 2000). This parameter is largely determined by emulsion stability and the interactions between water molecules and the product matrix (El-Nagar et al. 2002). As shown in Table 8, the analysis of variance demonstrated statistically significant effects of treatment (T), storage period (SP), and their interaction (T × SP) on first dripping time ($P < 0.01$).

Buffalo milk yoghurt ice cream containing encapsulated probiotics (B+EP) exhibited the longest first dripping time (97.14 min), whereas the cow milk formulation with encapsulated probiotics (C+EP) showed the shortest value (51.68 min). Drip resistance increased progressively throughout storage, suggesting a gradual stabilisation of the ice cream matrix over time. Pinto et al. (2012) reported first dripping times ranging from 921 to 1366 s in yoghurt ice creams containing microencapsulated *Bifidobacterium* BB-12, while Stenman et al. (2016) observed delayed melting behaviour

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Table 9. Duncan's multiple comparison test results of the complete melting time of yoghurt ice cream samples

Treatment	Complete melting time (min)	Storage period (days)	Complete melting time (min)
C	119.10 ± 21.68^{bc}	1	96.62 ± 9.47^{c}
C+P	118.94 ± 28.75^{bc}	15	95.58 ± 27.46^{c}
C+P+I	94.36 ± 13.82^{d}	30	138.21 ± 18.13^{a}
C+EP	97.68 ± 36.52^{d}	60	124.61 ± 16.33^{b}
C+EP+I	114.11 ± 18.53^{c}	–	–
BC	99.51 ± 29.98^{d}	–	–
B+P	122.34 ± 19.29^{b}	–	–
B+P+I	117.97 ± 14.51^{bc}	–	–
B+EP	120.34 ± 27.46^{b}	–	–
B+EP+I	133.17 ± 26.97^{a}	–	–
Sign.	**	T × SP	**

$^{a–d}$different letters within one column indicate significant difference (Duncan's multiple comparison test, $P < 0.01$); **statistical significance of main effects and interactions was determined by two-way ANOVA ($P < 0.01$); treatments abbreviations as explained in Table 1

in products fortified with *Lactobacillus rhamnosus* GG, highlighting the contribution of encapsulation to matrix integrity. The comparatively lower drip resistance observed in cow milk formulations may be attributed to less stable emulsion formation, as El-Nagar et al. (2002) emphasised the importance of a stable and homogeneous fat–water emulsion for improved melting resistance.

Complete melting time analysis results of yoghurt ice cream samples

Complete melting time is a key functional quality parameter that is directly affected by the structural composition, emulsion stability, and formulation characteristics of frozen dairy products. Analysis of variance (Table 9) demonstrated that treatment (T), storage period (SP), and their interaction (T × SP) had statistically significant effects on complete melting time ($P < 0.01$).

Buffalo yoghurt ice cream containing encapsulated probiotics and inulin (B+EP+I) exhibited the longest melting time (133.17 min), whereas cow milk ice cream with free probiotics and inulin (C+P+I) showed the shortest melting time (94.36 min). These findings indicate that encapsulation, particularly in buffalo milk-based formulations, enhances structural resistance to melting. El-Nagar et al. (2002) reported that inulin supplementation positively influences melt cohesion. In agreement with the present findings, Kailasapathy and Sultana (2003) and Stenman et al. (2016) observed delayed melting behaviour in microencapsulated probiotic and inulin-enriched dairy products, supporting encapsulation as an effective strategy for improving ice cream stability.

Viscosity analysis results of yoghurt ice cream samples

Viscosity, a key rheological parameter, indicates ice cream mix flow behaviour, air entrapment, texture, and sensory quality (Bahramparvar and Tehrani 2011; Goff and Hartel 2013).

Measurements at 30 and 50 rpm were used to evaluate shear-thinning and formulation effects. ANOVA revealed that treatment (T), storage period (SP), and their interaction (T × SP) significantly affected 30 rpm viscosity ($P < 0.01$), whereas only the treatment effect was significant at 50 rpm ($P < 0.01$). The results of Duncan's multiple comparison test are presented in Table 10.

Buffalo yoghurt ice cream containing encapsulated probiotics and inulin (B+EP+I) exhibited the longest complete melting time (133.17 min), whereas cow milk yoghurt ice cream with free probiotics and inulin (C+P+I) showed the shortest melting time (94.36 min). These results indicate that encapsulation, particularly in buffalo milk matrices, enhances structural resistance to melting. El-Nagar et al. (2002) reported that inulin addition positively influences melt cohesion. The present findings are in agreement with those of Kailasapathy and Sultana (2003) and Stenman et al. (2016), who observed delayed melting behaviour in microencapsulated probiotic and inulin-enriched dairy products, supporting encapsulation as an effective strategy for improving ice cream stability.

Analysis results of overrun values of ice cream samples

Overrun (defined as the increase in volume resulting from air incorporation) plays a critical role in determining ice cream texture and palatability (Arbuckle 1986; Muse and Hartel 2004). In this study, the effects of milk type (cow vs. buffalo), probiotic form (free vs. alginate-encapsulated), and inulin addition on overrun in yoghurt ice creams were evaluated. Analysis of variance indicated that treatment (T), storage period (SP), and their interaction (T × SP) had statistically significant effects on overrun values ($P < 0.01$). The highest overrun (34.77 ± 1.47%) was observed in buffalo milk ice cream containing encapsulated probiotics, whereas the lowest value (31.47 ± 1.96%)

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Table 10. Duncan's multiple comparison test results for viscosity values (Pa.s) of yoghurt ice cream samples

Treatment	Viscosity (30 rpm)	Viscosity (50 rpm)	Storage period (days)	Viscosity (30 rpm)	Viscosity (50 rpm)
CC	16.704 50 ± 3.160 27^{a}	11.158 5 ± 0.757 09^{ab}	1	17.579 45 ± 1.064 62^{b}	10.083 14 ± 0.657 03^{c}
C+P	16.754 25 ± 2.137 31^{a}	11.160 38 ± 0.833 69^{ab}	15	19.240 95 ± 0.655 74^{a}	11.336 69 ± 0.637 59^{a}
C+P+I	16.579 00 ± 1.961 28^{a}	10 878. 6 ± 0.093 455^{ab}	30	14.738 50 ± 0.823 65^{c}	11.625 25 ± 0.420 57^{a}
C+EP	14.922 61 ± 3.566 42^{c}	10.191 6 ± 30.876 10^{c}	60	13.677 79 ± 1.341 68^{d}	10.787 49 ± 0.595 63^{b}
C+EP+I	16.617 37 ± 2.254 69^{a}	11.346 50 ± 0.561 44^{a}	–	–	–
BC	16.242 87 ± 1.963 20^{ab}	11.437 37 ± 0.559 44^{a}	–	–	–
B+P	16.823 87 ± 2.177 78^{a}	10.717 16 ± 0.884 00^{bc}	–	–	–
B+P+I	15.678 50 ± 2.645 39^{b}	11.182 00 ± 0.670 41^{ab}	–	–	–
B+EP	15.799 37 ± 2.018 93^{b}	10.867 73 ± 0.914 37^{ab}	–	–	–
B+EP+I	16.969 37 ± 2.596 82^{a}	10.641 63 ± 0.782 33^{bc}	–	–	–
Sign.	**	T × SP	**	–	–

$^{a–d}$different letters within one column indicate significant difference (Duncan's multiple comparison test, $P < 0.01$); **statistical significance of main effects and interactions was determined by two-way ANOVA ($P < 0.01$); treatments abbreviations as explained in Table 1

occurred in cow milk ice cream with encapsulation and inulin. These findings suggest that encapsulation, particularly when combined with inulin, may limit air incorporation efficiency. Overrun values were highest on Day 1 (34.46 ± 1.27%) and gradually decreased to 33.31 ± 1.55% by Day 60, indicating a progressive loss of incorporated air during storage. Duncan's multiple comparison test confirmed significant differences among formulations and storage periods (Table 11).

Table 11. Duncan's multiple comparison test results of overrun (%) in ice cream samples

Treatment	Overrun (%)	Storage period (days)	Overrun (%)
CC	32.81 ± 0.93^{de}	1	34.46 ± 1.27^{a}
C+P	32.17 ± 1.17^{ef}	15	32.83 ± 1.34^{b}
C+P+I	31.47 ± 1.96^{f}	30	32.93 ± 1.40^{b}
C+EP	33.94 ± 1.75^{abc}	60	33.31 ± 1.55^{b}
C+EP+I	33.84 ± 0.62^{abc}	–	–
BC	33.34 ± 1.54^{cd}	–	–
B+P	32.86 ± 0.74^{de}	–	–
B+P+I	33.51 ± 0.84^{bcd}	–	–
B+EP	34.77 ± 1.47^{a}	–	–
B+EP+I	34.39 ± 0.76^{ab}	–	–
Sign.	**	–	–
T × SP	**	–	–

$^{a–f}$different letters within one column indicate significant difference (Duncan's multiple comparison test, $P < 0.01$); **statistical significance of main effects and interactions was determined by two-way ANOVA ($P < 0.01$); treatments abbreviations as explained in Table 1

Overrun values obtained in the present study (31.47–34.77%) were higher than those reported by Homayouni et al. (2008) and Mohammadi and Mortazavian (2011) for symbiotic and low-fat frozen dairy desserts containing prebiotics or alternative sweeteners. In contrast, Eisinaite et al. (2016) reported reduced aeration in formulations enriched with inulin and oat β-glucan. Broader overrun ranges observed in ice cream formulations incorporating fruit purées, transglutaminase, or sugar substitutes (Esmerino et al. 2013) further highlight the influence of fibre type and carbohydrate structure on air incorporation. Similarly, Kailasapathy and Sultana (2003) reported significant variations in overrun associated with probiotic culture addition. Taken together, these findings demonstrate that milk base, probiotic form, and inulin enrichment substantially affect aeration behaviour, providing practical guidance for optimising texture and stability in functional frozen dairy products.

CONCLUSION

This study evaluated ten yoghurt-based ice-cream formulations prepared from cow and buffalo milk and containing either free or encapsulated *Lactobacillus acidophilus*, with or without inulin, over storage periods of 1, 15, 30, and 60 days, using a 10 × 4 factorial, completely randomised design with duplicate blocks. Microbiological and physicochemical data collected

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throughout the 60-day storage period were analysed by two-way ANOVA followed by Duncan's multiple range test ($P < 0.01$). All values reported represent the mean of two experimental blocks.

The principal finding concerned probiotic viability. The highest *L. acidophilus* count (mean 5.97 log CFU·g^{-1}) was observed in the buffalo milk formulation combining encapsulation and inulin (B+EP+I). Encapsulation conferred a clear advantage. While the buffalo milk control (BC) averaged 5.23 log CFU·g^{-1}, the encapsulated treatments B+EP and B+EP+I reached 5.91 and 5.97 log CFU·g^{-1}, respectively. Probiotic counts peaked on Day 15 and remained close to levels generally regarded as therapeutically relevant by the end of storage. These findings indicate that the combined application of microencapsulation and prebiotic supplementation is effective in preserving functional probiotic populations during frozen storage.

The enhanced survival of encapsulated probiotics can be attributed to the formation of a semi-permeable alginate gel network, which mitigates freezing stress, restricts oxygen diffusion, and buffers pH fluctuations. This protective effect was further supported by the compositional characteristics of buffalo milk, as well as by the water-binding capacity and prebiotic functionality of inulin. Quantitatively, encapsulated treatments consistently exhibited higher viable counts than their non-encapsulated counterparts, typically by several tenths to nearly one logarithmic unit, in agreement with previous reports on alginate-based encapsulation systems.

Physicochemical and structural properties were consistent with the microbiological outcomes and highlighted the technological advantages of combining buffalo milk with encapsulation and inulin. Melting resistance, assessed through first drip and total melting times, was markedly improved in encapsulated buffalo formulations. The longest first drip time was recorded for B+EP (97.14 min), compared with C+EP (51.68 min), while the longest total melting time occurred in B+EP+I (133.17 min). Overrun values were also higher in encapsulated buffalo treatments, with the maximum overrun (34.77%) observed in B+EP, indicating enhanced air incorporation and a creamier texture. Viscosity measurements supported these observations, as buffalo milk formulations, particularly those containing encapsulated probiotics and inulin, displayed the highest viscosity values (e.g. 20.166 Pa·s), reflecting improved body and structural stability.

Microbiological safety was maintained across all formulations throughout storage. Yeast and mould counts declined to ≤ 2.00 log CFU·g^{-1} by Days 30 and 60, especially in encapsulated samples, indicating effective microbial control under frozen conditions. pH trends further favoured the encapsulated–inulin buffalo formulation, which exhibited the highest pH value (4.99), reflecting more controlled acidification, whereas cow milk formulations generally showed lower pH values. As expected, titratable acidity increased progressively during storage, reaching an average of approximately 0.96% by Day 60.

Overall, the findings demonstrate that the use of buffalo milk in combination with alginate-based microencapsulation of *L. acidophilus* and inulin co-supplementation substantially enhances probiotic viability while improving melting resistance, viscosity, and overrun in yoghurt-based ice creams. This formulation successfully integrates microbiological functionality with desirable physicochemical properties and represents a promising approach for the development of synbiotic frozen dairy products. Future research may focus on consumer sensory evaluation, extended storage studies under commercial conditions beyond 60 days, microstructural analyses (e.g. SEM and ice-crystal characterisation), and techno-economic assessments to support industrial-scale application.

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