Agriculture and Food

AIMS Agriculture and Food, 10(2): 293–313.
DOI: 10.3934/agrfood.2025015
Received: 28 November 2024
Revised: 25 March 2025
Accepted: 15 April 2025
Published: 13 May 2025

https://www.aimspress.com/journal/agriculture

Research article

Synergistic approach: Optimizing probiotic viability in ice cream through prebiotic based encapsulation and betalain fortification

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Abstract: This study explores the potential of ciceritol-based encapsulation alongside betalain fortification to improve the survival of *Bifidobacterium bifidum* (SP-9) in ice cream formulations. The encapsulated beads, which contain *B. bifidum*, were evaluated under simulated gastric and intestinal conditions, thus demonstrating enhanced survival rates compared to free cells. Incorporating ciceritol in combination with sodium alginate (M_2) significantly improved the survival in simulated intestinal conditions. Additionally, ice cream formulations enriched with encapsulated probiotics (M_2 micro capsules) and various concentrations of betalain extract (49, 29, 9%) were subjected to physicochemical and sensory evaluations. The results were statistically significant ($p < 0.05$). The ice cream variant IC-9, which contains 3301.02 mg/100g db betalains, exhibited favorable quality attributes with an increased hardness (0.38 kg) and viscosity (502.13 mPa.s), while the firmness (766.91 g) and consistency (2749.2 g.sec) remained comparable to the control. No significant change in pH (6.70–6.76)

was observed, although the acidity levels increased from 0.21% (control) to 0.31% (IC9). IC-9 received superior sensory scores for the overall acceptability (7.88) and whitening index (45.01%). Conclusively, the current research found that the combination of ciceritol and sodium alginate served as the best encapsulating material, and subsequently focused on the potential use of betalains as natural colorants in the development of functional ice cream in enhancing both health benefits and sensory appeal in frozen desserts.

Keywords: prebiotic; natural coloring; ice cream; encapsulation; probiotics; Betalains

1. Introduction

Milk based products constitute an important part of our life. They provide nutritional components such as fat, carbohydrates, protein, vitamins, and minerals for the body growth [1]. Ice cream, a frozen dairy dessert, is composed of 4–5% milk protein, 60–72% water, 7–15% fat, 28–40% total solids, 5–7% lactose, flavors, colors, 0.5% stabilizers and emulsifiers, and other sugars 12–16% [2]. Color plays an important role in the acceptance of ice cream. Various plant based derived bioactive components are used in ice cream formulations for coloring in addition to functional therapeutic purposes [3,4]. In the modern era, natural food colors are preferred over synthetic color additives by food producers due to their adverse human health impacts [5]. Beetroot extract (E162) is an important source of red coloring pigment (betalains). Beetroot betalains, which include betanin and isobetanin, are considered superior to enhance the color of low-acidic food products due to their color sustainability over a wide range of pH values (3–7) [6]. Betalains possess many positive human health benefits, including the improvement of immune systems, anti-inflammatory, antitumor, and hepatoprotective properties [7,8].

Due to its near-neutral pH and high solids content, ice cream provides an enabling environment for probiotic delivery to make it a functional product [9,10]. Probiotics are utilized for their numerous health benefits, including metabolic and immune system activation. These health-boosting effects depend upon their survival rate in the large intestine. Microencapsulation is employed to enhance the survival and targeted delivery of probiotics, especially *Lactobacilli* and *Bifidobacteria* [11]. Additionally, it prevents the bacteria from enacting fermentation of the product, helps to separate bacterial cells from the effects of harsh environments, and enhances their viability during processing and targeted delivery in the digestive tract, thus potentially preventing loss of cell [12,13]. Ciceritol, a non-digestible oligosaccharide, possesses prebiotic properties that can improve the viability of probiotics by serving as a substrate for fermentation by these bacteria [11]. Sodium alginate is also chosen for its excellent encapsulation property due to its non-toxicity and economical availability compared to other materials. A flavored alginate matrix to encapsulate *Lactobacillus fermentum* was formulated, which resulted in an increased viability compared to free cells [14]. However, scientists suggested pores in alginate beads and proposed a combination of materials to overcome this issue. Combining ciceritol and sodium alginate may help improve the beads' structure and enhance the probiotics' viability [15].

Globally, probiotics are generally incorporated into fermented dairy products to enhance the nutritional and therapeutic features of the functional products [16]. As ice cream is a non-fermented dairy product, fewer studies have explored encapsulated probiotic incorporation into ice cream [17]. Additionally, there's a lack of investigation into prebiotic-based encapsulation systems and the role of

betalains in enhancing ice cream's functional properties. Addressing these considerations is crucial to understanding the potential health benefits and consumer acceptability of probiotic-enriched ice cream formulations. Considering all the health impacts associated with ciceritol, beet betalains, and probiotics, the current study investigates the use of ciceritol and alginate based encapsulated beads to improve the survival of *Bifidobacterium bifidum* and utilizes encapsulated probiotics, along with red beet juice extract as a natural coloring, to develop gut-friendly functional ice cream. Subsequently, the ice cream is evaluated for its physico-chemical and sensory features.

2. Materials and methods

2.1. Procurement of material

All the materials, including the beet root, whip cream, skim milk, and powdered sugar, were procured from the local market of Multan. The probiotic cultures (*Bifidobacterium bifidum,* SP-9, DVS culture) were purchased from SACCO (Italy). All analytical grade chemicals and reagents were purchased from Sigma (USA).

2.2. Beet juice extraction

The procured sugar beets were washed to clean out mud and other debris material. After that, the sugar beets were peeled with a sharp knife and sliced into cubes for juice extraction through an electric juice extractor machine. The extracted juice was filtered through a muslin cloth and further centrifuged (5000 × *g*, 10min) to remove any suspended residues.

2.3. Chemical analysis of juice

2.3.1. Quantification of Betalains

A spectrophotometric method was used to estimate the content of betaxanthins (yellow pigment) and betacyanins (violet pigment) [18,19]. The extracted juice sample (1mL) was mixed with a phosphate buffer up to a 500 mL volume to make it 500-fold diluted. Then, the absorbance was measured at 476 nm and 538 nm through a UV-visible spectrophotometer to compute the vulgaxanthin-I and betanin concentrations, respectively. The 2nd absorbance of the samples was recorded at 600 nm and used to correct the absorbance values. The correction factor is also considered as samples and may contain small amounts of impurities. The equations used for the calculation of betalains are as follows:

$$C_B = \frac{[1.095\times(A538-A600)]\times fd\times 1000}{1120} \times \frac{1}{TSC}, \quad (1)$$

$$C_{V\text{-}I} = \frac{(A476-0.258\times A538-0.742\times A600)\times fd\times 1000}{750} \times \frac{1}{TSC}, \quad (2)$$

$$C_T = C_B + C_{V\text{-}I}, \quad (3)$$

where C_B represents the concentration of betanin, $C_{V\text{-}I}$ represents the concentration of vulgaxanthin,

C_T represents the total concentration of betalains, f_d is a dilution factor, and TSC is the total solid content.

2.3.2. Quantification of total phenolic contents (TPC)

The total phenolic content (TPC) in the sugar beet juice was estimated according to a standard procedure [20]. The sample aliquots (0.5 mL) were mixed with an Folin-Ciocalteu (FC) reagent (2.5 mL) and Na_2CO_3 (7.5%, 2 mL). A stay time of 30 min was given at 25 °C in the dark for the complete reaction. The absorbance was measured at 760 nm by a spectrophotometer (Model-SPRCORD 200 plus, Analytik-Jena, Germany). The gallic acid (10–100 ppm) concentrations were measured to plot the standard regression curve. The results were expressed as mg GAE/100 g, and the concentration was calculated from the standard curve of gallic acid.

2.3.3. Antioxidant activity (DPPH)

The antioxidant activity of aqueous extracts of the sugar beets was analysed using 2,2-Diphenyl-1-picrylhydrazy (DPPH) as the free radical scavenging activity [21]. The DPPH stock solution (0.024%) was diluted in methanol to prepare the working solution until it provided a 0.98 ± 2 absorbance value at 517 nm. The beet juice (100 µL) and DPPH (3 mL) were added separately to each test tube. Approximately 30 min of stay time was given to each sample in the darkness. Then, the absorbance was recorded in triplicate at 517 nm.

2.4. Encapsulation of probiotics

The final concentrations of probiotics (*B. bifidum*, SP-9, DVS culture, Sacco Italy) were maintained (10^{10} CFU/ mL) through the optical density measured spectrophotometrically at 600nm; then, they were microencapsulated using sodium alginate and ciceritol in different concentrations using the extrusion method [22]. A solution of sodium alginate (2%) and ciceritol (2%) was prepared. *B. bifidum* was mixed (1%) in a sterile condition with the encapsulation material solutions according to the treatment plan (Table 1). The material was passed through a needle (27 G) and the produced capsules were immersed for 30 min in a $CaCl_2$ (0.05 M) solution for complete gelation. The beads were harvested through filtration, washed with PBS (50 mM), and stored in an air-tight pack at −80 °C until use [15,23].

Table 1. Treatment plan for the encapsulation of *B. bifidum*.

Treatment	Sugar beet Juice (%)	Water (%)	Sugar (%)	Encapsulated probiotic (%)	Whip Cream (%)	Skim milk powder (%)
IC	0	44	10	1	25	21
IC49	49	0	5	1	25	21
IC29	29	15	10	1	25	21
IC9	09	35	10	1	25	21

2.4.1. Structural analysis of ciceritol-alginate matrix

The images were taken using a digital camera and scanning electron microscopy (SEM) (SU1510, Tokyo, Japan). The SEM micrographs were taken at 15 kV under a vacuum of 9.75×10^{-5} Torr at 5–40μm, with low and high resolutions of ≥ ×45 and ≥ ×1000, respectively.

2.4.2. Survival of *B. bifidum* in simulated gastrointestinal conditions

The stability of *B. bifidum* was evaluated following the protocol [15]. Free and encapsulated *B. bifidum* were subjected to low pH simulated gastric fluids (SGF) (pH 2.0 and 2.5). Ciceritol and sodium alginate beads (0.50 g) and free cells (0.50 mL) were added in SGF (4.5 mL) and incubated for 30, 60, and 90 min at 37 °C. The beads were taken out at specific intervals, dissolved in a sodium citrate solution (50mM, 4.5 mL), and plated on MRS media for further enumeration. Similarly, free and encapsulated *B. bifidum* were exposed to a porcine bile salt solution (2%) and incubated for 1 and 2 hrs. Furthermore, the recovered beads were released and plated on specific media for enumeration [24].

2.4.3. Release profile and storage of *B. bifidum*

The release study of encapsulated *B. bifidum* was carried out following a previous study [11]. The beads (0.50 g) were added to simulated intestinal fluid (SIF) (pH 6.8, 50 mM KH_2PO_4). The release profile was determined after 0, 30, 60, and 90 minutes. A storage study of encapsulated *B. bifidum* was carried out following a previous study [15]. The stability was determined for 1, 14, and 28 days of storage at 4 °C. The enumeration of free and encapsulated *B. bifidum* followed the same procedure as mentioned in Section 2.6.

2.5. Manufacturing of ice cream

Functional ice cream was manufactured according to the standard method with some modifications [25,26]. Sugar beet juice, sugar, skim milk powder, and water were added in whipped cream and mixed for 20–30 minutes. This premix was churned for 40 minutes in the ice cream maker. Ice cream was poured out in ice cream cups (100 g) and the encapsulated beads of probiotics (1%, M_2) were incorporated in all treatments. Four treatments—IC, IC49, IC29, and IC9—of ice cream were developed with the same concentration of 25% whip cream, 21% skim milk powder, and encapsulated *B. bifidum* (1%) as follows: IC: control ice cream; IC49: 49% sugar beet juice, 5% sugar, and 0% water; IC29: 29% sugar beet juice, 10% sugar, and 15% water; and IC9: 09% sugar beet juice, 20% sugar, and 25% water (Table 2).

Table 2. Treatment plan for the development of ice cream.

Treatment	Ciceritol (%)	Sodium alginate (%)	Probiotics (%)
M_o	0	0	1%
M_1	0	100	1%
M_2	50	50	1%
M_3	100	0	1%

2.6. Physico-chemical analysis of ice cream

2.6.1. Color

The coloring index was measured with a portable colorimeter displaying L*, a*, and b*. L* specified black to white (0 to 100), a* designated red to green (+/− red/green), and b* indicated yellow to blue (+/− yellow/blue). Additionally, the whiteness index (WI) and Hue^{o} were accordingly detected by their specific formulas [27,28].

2.6.2. Brix

The Brix parameter of the ice cream was estimated through a hand-held refractometer (Model RHB0-90, Cole-Parmer, USA) at an ambient temperature of 25 ± 2 ℃. A few drops of molten ice cream were positioned on the stage of the sloping prism of the refractometer. The particular values were examined after placing the flip cover [29].

2.6.3. Overrun

The volume of the maximum air incorporated into the ice cream was measured by a particular method [30].

2.6.4. Specific gravity

The specific gravity of the sugar beet ice cream was determined at 20 ℃ and measured in g/cm^3 [31].

2.6.5. Melting rate

The melting rate of functional ice cream was determined at ambient temperature (25 ± 2 ℃) by placing 70 g of ice cream on a sieve suspended over a cylinder (100 mL). The time when the first drop of ice cream fell into the cylinder was noted, and the volume was measured up to 45 min with a 5 min interval time. The melting rate from the linear portion of each melting curve was calculated [32].

2.6.6. Viscosity

The viscosity of the ice cream was measured in mPa·s units using a viscometer (Model NJP-BS). The spindle no. 2 was used to take the torque measurements of the ice cream mix (300 mL) at 4–5 ℃ and a speed of 60 rpm [32,33].

2.6.7. Textural profile

The texture of the ice cream was measured by a texture analyzer (Model TA-X2 plus, UK). A cylinder probe (P-75) was used to compress the sample (300 g) at a speed of 5mm/sec [20].

2.6.8. pH and acidity

The pH of the sugar beet ice cream was measured using a pH meter (HANNA, USA) fitted with a glass electrode. Before conducting the analysis, the pH meter was calibrated for optimum functionality with various buffer solutions (pH 4.0 and 7.0) [34]. The acidity of the functional ice cream was determined as the lactic acid (Equivalent weight of lactic acid = 90 g) percentage in the tested samples using phenolphthalein (1%) as an indicator [2].

2.7. Sensory evaluation

A sensory evaluation of the functional ice cream was performed using a Hedonic scale (9: Like extremely, 1: Dislike extremely) and endorsed by the ethical committee of the university. A panel of trained experts of ten judges (female and male) from the Faculty of Food Science & Nutrition, BZ-University evaluated the ice cream. Each sample treatment was secretly coded and evaluated for various sensory perceptions on a 9-point Hedonic scale in a well-lit and sound proof room [35,36].

2.8. Statistical analysis

An analysis of variance (ANOVA-one way) under a completely randomized design (CRD) design was applied to evaluate the effect of the juice extract on the ice cream using a statistical software (Statistix 8.1). The least significant differences (LSD) were defined at a 5% significance level among the mean values ($p \leq 0.05$), and considered highly significant at ($p \leq 0.01$) and non-significant at ($p \geq 0.05$). [37].

3. Results and discussion

3.1. Chemical analysis of juice extract

3.1.1. Betalains

Betalains, nitrogenous compounds found in red beets, cactus pearss, and amaranthus fruit, serve as colorants in food products. The mean values for betanin, vulgaxanthin-I, and the total betalain concentration in the juice extract were measured as follows: betanin (Cb), 233.92; vulgaxanthin-I (Cv-I), 295.35; and the total concentration of betalains (CT), 366.78 mg/100g db, as listed in Table 3. The ice cream samples containing different concentrations of beet juice (IC49-IC9) were analyzed, with IC49 exhibiting the highest betalain content due to its 49% juice concentration. The stability of the spray-dried beetroot powder extract was tested, and revealed betalain concentrations in the range of 223.88–218.38 mg/100 g db for betanins, 143.74–149.49 mg/100 g db for vulgaxanthin-I, and 367.60–367.87 mg/100 g db for total betalains, which are consistent with previous findings [18]. Moreover, the study demonstrated the stability of the spray-dried beetroot powder extract, thus indicating consistent betalain concentrations across different samples. These findings contribute to the understanding of betalain retention and its potential applications in food product development.

Table 3. Mean average values for Betalains, DPPH and TPC parameter of sugar beet juice.

Parameter	Mean value
C_b (mg/100 g db)	233.92 ± 3.21
$C_{v\text{-}I}$ (mg/100 g db)	295.35 ± 87.79
C_T (mg/100 g db)	366.78 ± 36.43
DPPH (%)	49.13 ± 7.39
TPC (GAE mg/100 g)	25.1 ± 0.2

C_b: betanin concentration, $C_{v\text{-}I}$: vulgaxanthin-I concentration, C_T: Total concentration of betalains, TPC: total phenolic content, DPPH: 2,2-diphenyl-1-picrylhydrazyl,GAE: galic acid eqavalent, db: dry basis; The values are mean ± SD (n = 3).

3.1.2. Antioxidant activity (DPPH)

DPPH is utilized to assess the antioxidant capacity of a tested sample by measuring its ability to scavenge free radicals. In this study, the sugar beet ice cream with encapsulated probiotics exhibited a mean DPPH value of 49.13%, as listed in Table 3. Variations in the ice cream samples, thereby incorporating different concentrations of beet juice, showed that higher beet juice concentrations, as seen in sample IC49 (49% juice), corresponded to increased radical scavenging activities, whereas lower concentrations, such as in sample IC9 (9% juice), displayed lower DPPH radical scavenging activities. These results align with previous findings [38], where the antioxidant and antimicrobial activities in beetroot pomace extracts were observed and a DPPH activity of 50% was reported for a water-based pomace extract. However, our findings differ from those in [39], which observed a DPPH activity of 76.71% in ready-to-drink beetroot juice prior to the thermal treatment. This discrepancy may be attributed to variations in the beetroot variety, farming practices, and storage conditions. Further research is warranted to explore the factors which influence the DPPH activity in beet-derived products and their potential implications for human health.

3.1.3. Total phenolic contents

The TPC, which represents the antioxidant potential in fruits and vegetables, is comprised of compounds vital to shield the human body against oxidative damage and diseases such as cancer. The TPC in beetroot juice, which was quantified in GAE g/100 g, was determined to be 25.1 GAE mg/100g, as listed in Table 3. This value is consistent with findings in literature where the TPC values ranged from 25.12 to 24.06 mg GAE/100 g and 22.5 GAE mg/100 g [40,41]. These results underscore the rich phenolic content of beetroot juice, thus highlighting its potential as a valuable source of antioxidants. Further investigations are necessary to explore the health benefits associated with consuming beetroot-derived phenolics and their role in mitigating oxidative stress-related diseases. Additionally, studies which focus on the influence of the processing methods and storage conditions on the phenolic content of beetroot juice would provide valuable insights into maximizing its antioxidant properties.

3.2. Structural analysis of beads

The micrographs in Figure 1 provide insight into the structural characteristics of beads fabricated using different concentrations of sodium alginate and ciceritol, thus indicating successful encapsulation across all treatments [11,15]. Notably, beads with higher sodium alginate concentrations

exhibited a spherical shape, while those that incorporated ciceritol appeared elongated. A SEM analysis revealed the presence of pores within the alginate beads; however, composite beads that contained ciceritol displayed an enhanced structural integrity. Despite ciceritol beads showing less effective encapsulation, their integration into composite beads led to an improved structure, likely due to pore filling within the alginate matrix. This observation suggests that the presence of pores may stem from the bonding of bi- or trivalent calcium cations with sodium alginate [15]. Furthermore, the shrivelled appearance of the ciceritol beads can be attributed to their water-soluble nature, while dents observed in the composite beads may result from the freeze-drying process [11]. These findings underscore the potential of composite beads, thereby combining alginate and ciceritol, to enhance the structural integrity of encapsulated probiotics. The study highlights the importance of optimizing bead composition to achieve the desired encapsulation properties, thus offering insights for the development of effective probiotic delivery systems. Future research could delve deeper into understanding the mechanisms that underlie the structural improvements observed in composite beads and explore additional materials to further enhance the encapsulation efficiency and stability.

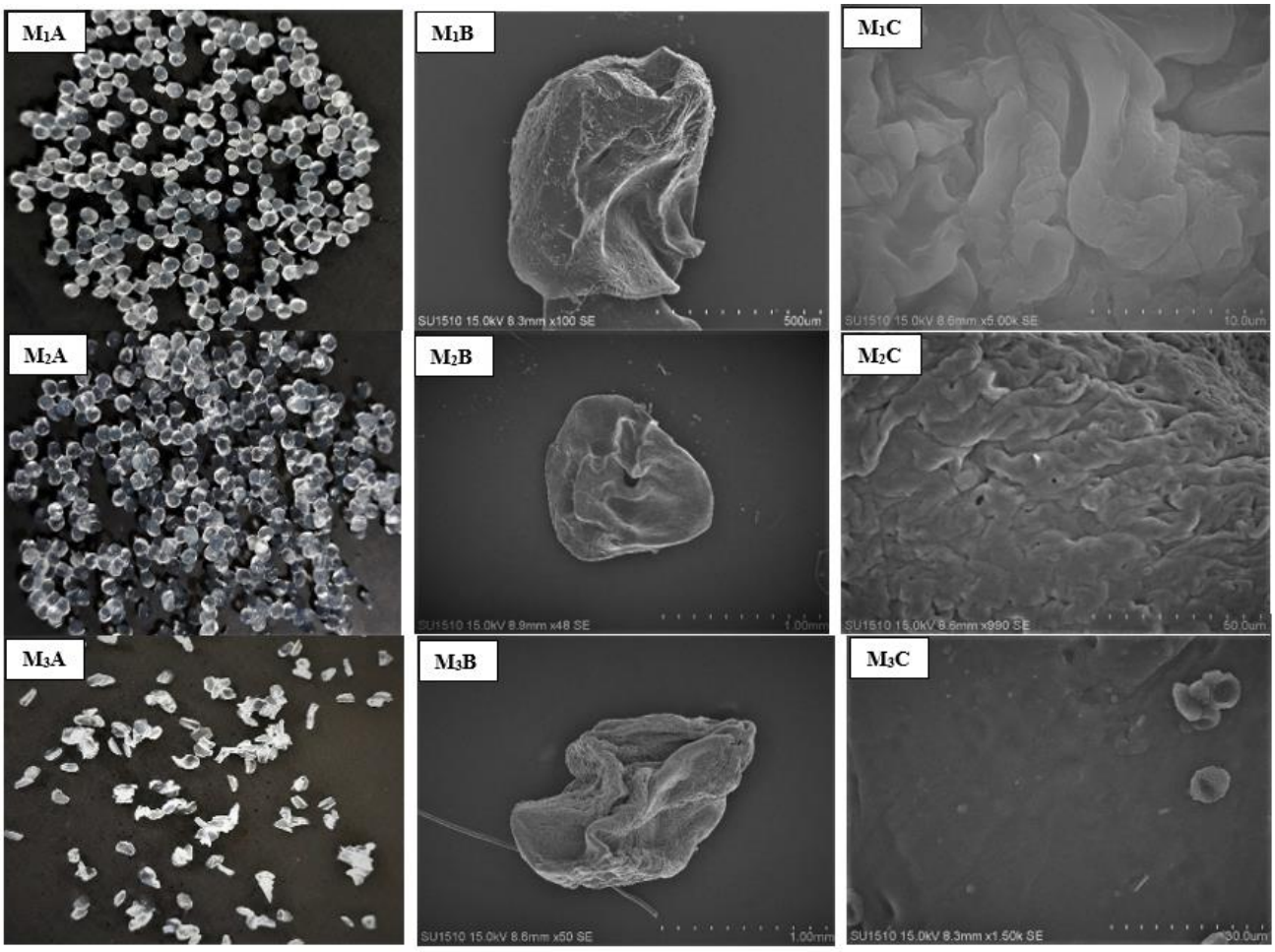

Figure 1. Structural microgram difference between sodium alginate and ciceritol beads with digital camera (A), SEM at high and low magnification ≥ × 1000X (B,C); M_1, sodium alginate 100%; M_2, sodium alginate 50% & ciceritol 50%; M_3, ciceritol 100%.

3.3. Survival of B. bifidum in Simulated gastric fluid (SGF)

The survival of free and encapsulated *B. bifidum* was evaluated in SGF with varying pH levels, thus revealing significant differences in the viability across treatments [11,15]. While the viability of *B. bifidum* ranged from 4.12 ± 0.12 to 9.77 ± 0.08 CFU/mL, the unencapsulated cells struggled to maintain the viability under acidic conditions, thereby showing a decrease from 9.76 ± 0.07 to 4.12 ± 0.12 CFU/mL at pH 2.0 and further to 5.1 ± 0.7 at pH 2.5 (Figure 2). This decline is attributed to the exposure of free cells to acidic environments, thus leading to an outer wall rupture. Conversely, encapsulated *B. bifidum* cells exhibited a sustained viability even at low pH levels, with M_2 displaying the maximum stability, followed by M_3. This enhanced survival suggests an effective protection conferred by sodium alginate and ciceritol. However, the alginate barrier may be prone to erosion, which results in the formation of tiny pores in the bead structure. Adding ciceritol facilitated a structural enhancement by forming a hydrogel barrier against these pores, therevt functioning as a prebiotic to bolster the probiotic viability. However, ciceritol as an encapsulation material proved less effective than alginate, likely due to its water-soluble nature, thus resulting in a decreased viability and shrivelled beads, as reported by [11]. Alginate does not swell in acidic media. However, the release was observed due to the erosion of the alginate hydrogel. Therefore, alginate was mixed with ciceritol to improve the cell viability and cell proliferation [24]. Addressing this, it was proposed to incorporate prebiotic encapsulation materials alongside sodium alginate to improve both the structure and probiotic viability. These findings underscore the potential of composite encapsulation systems to optimize the probiotic delivery and enhance their functionality in functional foods [42].

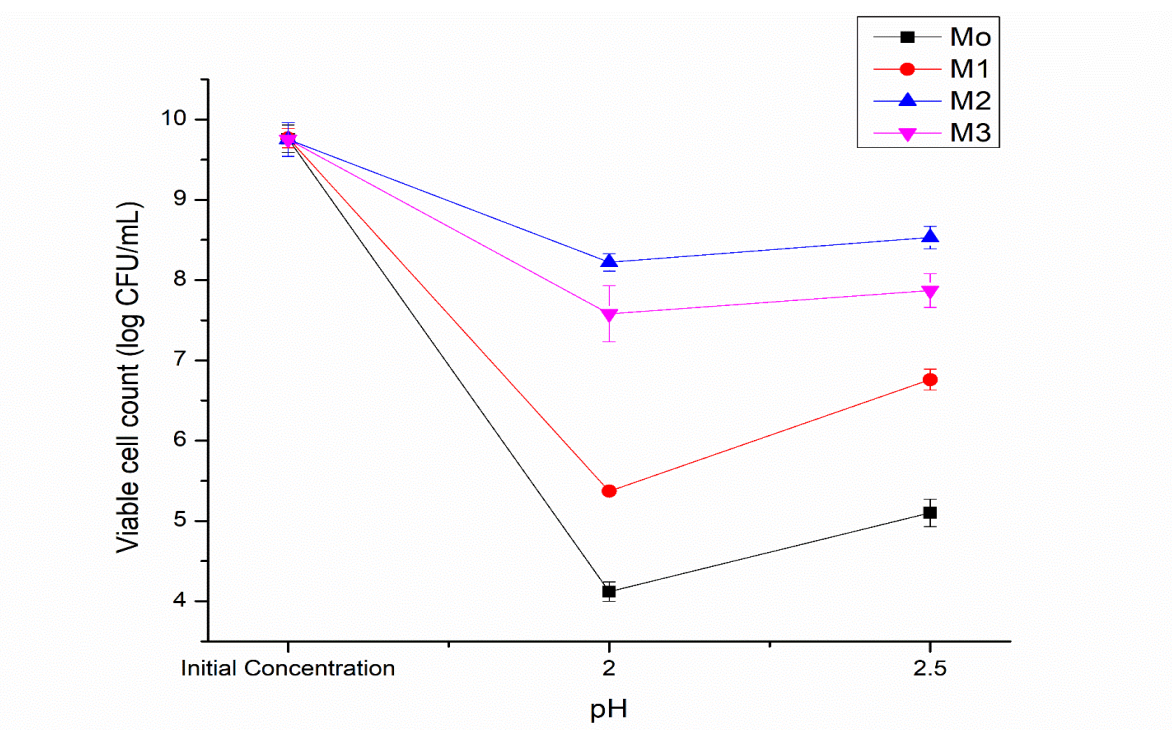

Figure 2. Survival of free and encapsulated *B. bifidum* in SGF (M_o, free probiotic cell; M_1, alginate encapsulated beads; M_2, alginate & ciceritol encapsulated beads; M_3, ciceritol encapsulated beads).

3.4. Survival of B. bifidum in bile salt

The survival of free and encapsulated *B. bifidum* was assessed in a bile salt solution, and revealed significant variations across treatments. The free cells exhibited a decreased survival in the bile salt solution even after 2 hours of incubation, with the viability dropping from 9.78 ± 0.1 to 4.38 ± 0.13 log CFU/mL (Figure 3). In contrast, the encapsulated cells displayed an enhanced survival compared to their free counterparts, with the maximum survival observed for M_2 (8.17 ± 0.15 log CFU/mL), followed by M_3. This improved survival of the encapsulated cells suggests a more effective encapsulation of the probiotic cells. The increased in vitro survival in bile salt may contribute to an improved stability of *B. bifidum* in the intestine. The combination of sodium alginate and ciceritol as the encapsulation material proved effective to enhance the probiotic survival, which is consistent with the findings of [43], who utilized a combination of sodium alginate and whey protein for similar purposes. However, it is worth noting that sodium alginate as the sole encapsulation material may not be as effective as when used in combination with other materials. These results underscore the potential of composite encapsulation systems, such as the combination of sodium alginate and ciceritol, to optimize the probiotic delivery and improve their survival, which may have implications to enhance the functional properties of probiotic-containing products.

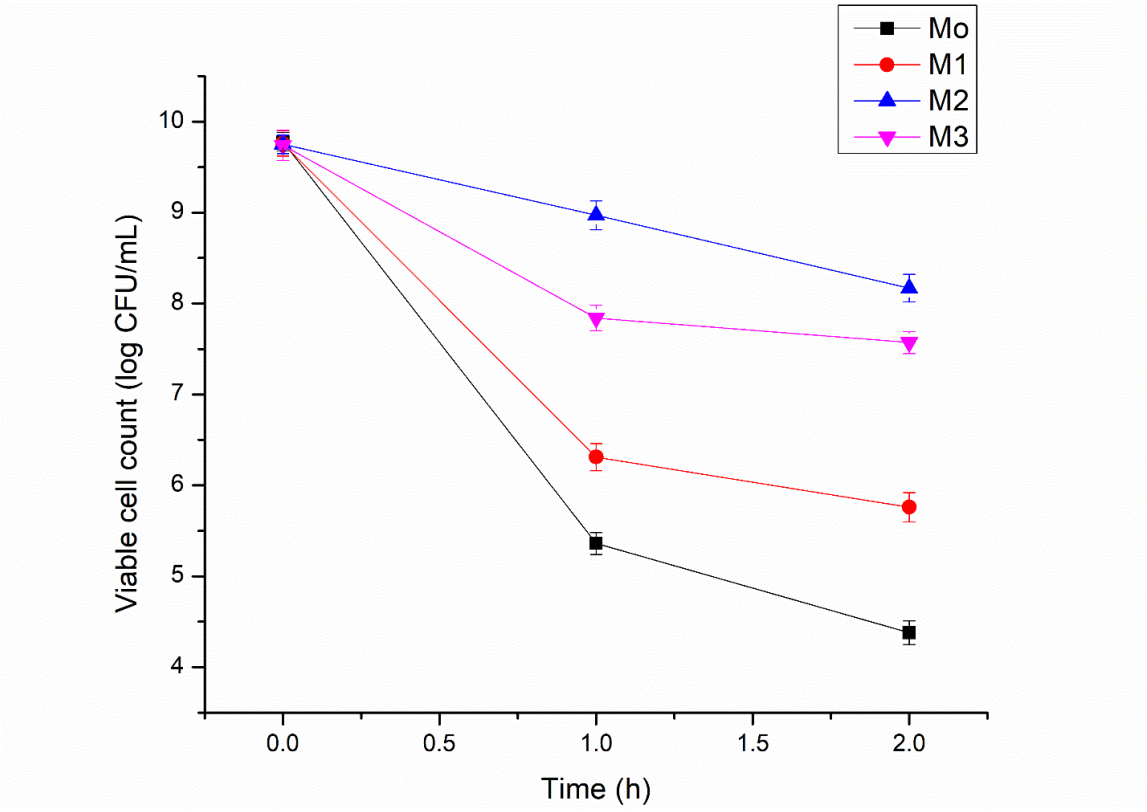

Figure 3. Bile salt tolerance of free and encapsulated *B. bifidum* (M_o, free probiotic cell; M_1, alginate encapsulated beads; M_2, alginate & ciceritol encapsulated beads; M_3, ciceritol encapsulated beads).

3.5. Release profile of encapsulated B. bifidum

The release profile of encapsulated *B. bifidum* was evaluated using SIF, and revealed significant variations among the treatments. The maximum release rate was observed for M_3 (100 ± 1.1%) within 90 minutes of exposure to alkaline conditions (Figure 4), while the minimum release rate was noted for 40 ± 3.0% after 30 minutes of exposure. Alginate beads have been reported to exhibit slow release rates; however, the increased release rate of *B. bifidum* in this study may be attributed to the water-soluble nature of ciceritol. The complete release of probiotics in the large intestine is crucial to provide health benefits. The release of *B. bifidum* was facilitated by an ion exchange phenomenon in alkaline conditions, whereby the carboxylic group of sodium alginate is exchanged with the SIF. In contrast, the ciceritol beads exhibited the highest release rate due to their water-soluble nature. These findings are consistent with those of [44], who encapsulated the probiotic cells in sodium alginate and carrageenan beads. Their study reported slower release mechanisms for the alginate beads compared to the carrageenan beads, which further higlighted the impact of the encapsulation materials on the release kinetics of probiotics.

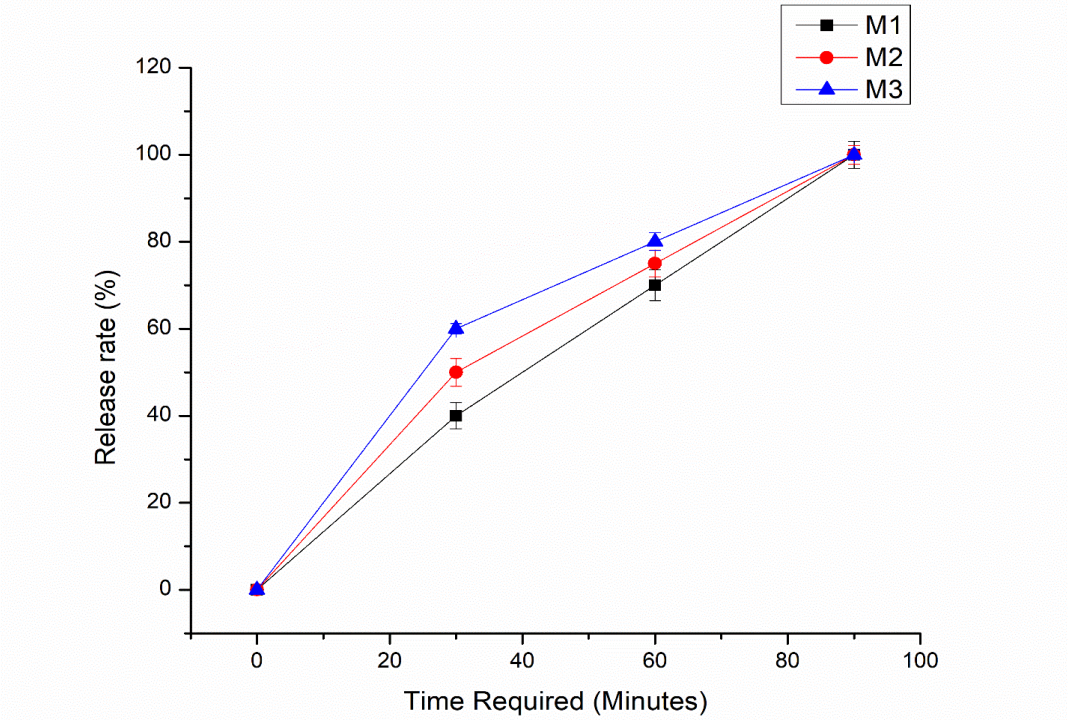

Figure 4. Release profile of encapsulated *B. bifidum* in SIF (M_o, free probiotic cell; M_1, alginate encapsulated beads; M_2, alginate & ciceritol encapsulated beads; M_3, ciceritol encapsulated beads).

3.6. Storage stability of B. bifidum

The storage stability of both free and encapsulated *B. bifidum* significantly varied across different treatments and storage days. The viability ranged from 5.46 ± 0.09 to 9.76 ± 0.1 log CFU/mL among the treatments and storage periods (Figure 5). A higher stability was observed for M_2 (8.71 ± 0.09 log

CFU/mL), while the minimum stability was noted for the M_o free cells (5.46 ± 0.09 log CFU/mL) after 28 days of storage. The free probiotic cells exhibited a decreased viability over the storage period, likely due to the low temperature (refrigeration, freezing) storage conditions. As compared to refrigeration, freezing may cause lethal injury by damaging their cell membrane or walls of probiotics cells, which ultimately caused a reduction in the vital metabolic activities of cells [45,46]. In contrast, the encapsulated cells across all treatments maintained the required viability, which demonstrated that microencapsulation offers a superior protection compared to free cells. However, the viability decreased with an increased storage time, possibly due to the storage conditions and the production of secondary metabolites. Interestingly, the ciceritol-encapsulated cells exhibited a higher viability compared to the alginate-encapsulated cells, thus suggesting that ciceritol as a prebiotic may enhance the viability during prolonged storage. These findings are consistent with those of [47], who investigated the survival of free and encapsulated probiotics under refrigerated conditions. They found that the encapsulated cells maintained their viability even after one month of storage, while the viability of the free cells significantly decreased over time.

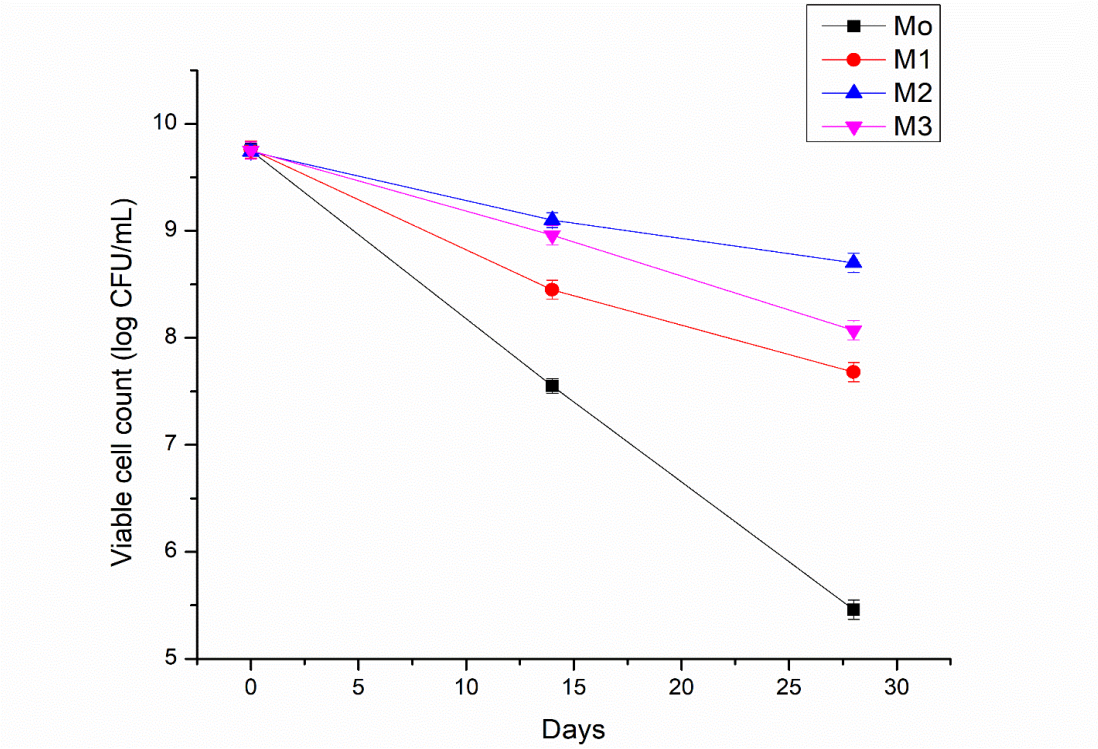

Figure 5. Storage stability (4 °C) of free and encapsulated *B. bifidum* (M_o, free probiotic cell; M_1, alginate encapsulated beads; M_2, alginate & ciceritol encapsulated beads; M_3, ciceritol encapsulated beads).

3.7. Physical parameters of ice cream

3.7.1. Color

Color is a primary determinant that influences our food choices. The results showed highly significant differences ($p \leq 0.01$) in the L*, a*, b*, Hue (°), and WI. The mean values for the color

parameters of the functional ice cream (IC9, IC29, and IC49) were higher (L*: 53.64, a*: 29.99, b*: −0.16) and exhibited varied behaviors when compared to the control values (Table 4). IC9 exhibited higher value (45.01) for the WI but were lower than the control. Furthermore, lower values were recorded for parameters L* (48.26), a* (21.45) in IC49, for b* (−5.08) in IC9, and for WI (41.68) in IC29. These findings align with the study [28], who investigated the impact of roselle extracts on the ice cream quality characteristics. They observed a decrease in the L* values from 80.30 to 65.07, with the lowest value recorded for IC20 (20% roselle extract). A similar decreasing trend was noted for the b* and WI values (7.13–0.43 and 79.00–65.03, respectively). Additionally, the hue angle (Hue °) exhibited the highest mean value for the control (87.60) and the lowest (36.63) for IC20. Similarly, research was conducted on the ice cream quality, and reported L* values that ranged from 90.500 to 379.750 and hue angle values from 0.581 to 5.008. These studies underscore the significant impact of additives, such as roselle extracts, on the ice cream color parameters, thereby influencing the consumer perception and acceptance [48].

Table 4. Color parameter of sugar beet ice cream with encapsulated probiotic.

Treatments	Color				
	L*	a*	b*	Hue (^{o})	WI
IC	79.85 ± 0.02^a	2.49 ± 0.02^c	9.26 ± 0.06^a	85.84 ± 0.07^a	77.69 ± 0.03^a
IC49	48.26 ± 3.46^d	21.45 ± 0.56^b	-0.16 ± 0.68^b	0.04 ± 0.05^d	43.98 ± 3.41^{cd}
IC29	50.24 ± 0.21^c	29.99 ± 0.22^a	-5.07 ± 0.02^c	1.64 ± 0.01^c	41.68 ± 0.29^d
IC9	53.64 ± 0.133^b	29.13 ± 0.04^a	-5.08 ± 0.02^c	1.82 ± 0.01^b	45.01 ± 0.09^{bc}

The value of L* indicates the light: dark black (0) & white (100); a* = green (-) & redness (+); b* = blue (-) & yellow (+), WI = whitening index 100; Hue angle indicates the chromaticity of color (0^o for red, for yellow 90^o ,180 ^{o} for green and 270 ^{o} for yellow), The values are mean ± SD.

3.7.2. Brix, Overrun, specific gravity and melting rate

The results for the brix, overrun, specific gravity and melting rate of developed ice creams were displayed in Table 5. Brix, which reflects the sugar concentration in the ice cream solution, is a critical parameter that influences its sweetness, texture, and overall quality. In the presented study, significant variations were observed in the Brix values among different treatments, with the highest value recorded in the IC9 treatment (40.0) and the lowest in IC49 (28.0). This trend can be attributed to the increased concentration of sugar in the formulations, which aligned with findings from previous research [49,50], who investigated the impact of sweeteners on the ice cream properties and reported similar trends in the Brix levels. Overrun, the amount of air incorporated during ice cream processing, is another crucial parameter that affects its texture and mouthfeel. In the study, significant differences were observed in the overrun values among treatments, with IC49 exhibiting the highest value (54.183%) and IC9 exhibiting the lowest (22.580%), though the control exhibited an overrun value of17.59%. This variation can be attributed to the presence of sugar at a higher value in the beet juice (IC49), which enhanced the value of total solids and, consequently, the overrun by incorporating more air cells into the structure. These findings are consistent to previous research [51], who discussed the impact of the cherry paste concentration on the ice cream overrun and reported similar increases in the overrun with higher paste concentrations. The specific gravity, which reflects the ice cream density, also showed significant variations among the treatments, with IC49 having the highest value (0.87 g/cm^3) and IC9

having the lowest (0.82 g/cm^3). This discrepancy can be attributed to the differing concentrations of the sugar beet juice: higher concentrations can lead to higher specific gravity values. These findings align with previous research [31], who studied ice cream supplemented with concentrated cactus pear pulp and observed similar trends in the specific gravity values, which had varying concentrations of pulp. The melting rate, which affects the ice cream's sensory attributes and consumer experience, also demonstrated significant differences among the treatments, with IC9 exhibiting the highest mean value (2.13 mL/min) and IC49 exhibiting the lowest (1.40 mL/min), while the control had an overrun value of 1.90 ml/min. The higher melting rate in IC9 can be attributed to its lower overrun, as ice cream with higher overrun tends to melt more slowly due to the increased number of air cells in the structure. This observation is consistent to previous research [52], who studied the rheological properties of stevia ice cream and reported similar trends in the melting rates with varying overrun levels. In summary, the parameters of Brix, overrun, specific gravity, and melting rate are essential indicators of the ice cream quality and consumer acceptability. The findings of the study highlight the significant impact of the ingredient composition and formulation on these parameters, thus emphasizing the importance of optimizing ice cream formulations to achieve the desired sensory attributes and overall quality. Further research in this area could focus on exploring additional factors which influence the ice cream characteristics and develop innovative formulations to meet the consumer preferences and market demands.

Table 5. Physico-chemical parameter of sugar beet ice cream with encapsulated probiotic.

Treatment	Brix (°)	Overrun (%)	Specific gravity (g/cm^3)	Melting rate (ml/min)	pH	Acidity (%)
IC	20.00 ± 0.01^{d}	17.59 ± 1.86^{d}	0.73 ± 0.005^{d}	1.90 ± 0.1^{b}	6.70 ± 0.10^{a}	0.21 ± 0.05^{cd}
IC49	28.00 ± 0.01^{c}	54.18 ± 2.11^{a}	0.87 ± 0.005^{a}	1.40 ± 0.2^{d}	6.70 ± 0.10^{a}	0.24 ± 0.06^{bc}
IC29	32.00 ± 0.02^{b}	39.57 ± 1.62^{b}	0.85 ± 0.01^{b}	1.75 ± 0.02^{c}	6.76 ± 0.050^{a}	0.27 ± 0.013^{ab}
IC9	40.00 ± 0.01^{a}	22.58 ± 2.33^{c}	0.82 ± 0.005^{c}	2.13 ± 0.01^{a}	6.73 ± 0.050^{a}	0.31 ± 0.09^{a}

The values are mean ± SD (n = 3), Mean with different letters are significantly different form each other. IC: Control ice cream; IC49: 49% sugar beet juice; 5 % sugar, 0% water, IC29: 29% sugar beet juice; 10% sugar, 15% water, IC9: 9% sugar beet juice; 20% sugar, 25% water.

3.7.3. Viscosity and texture analysis

The viscosity and textural properties for the functional ice creams were presented in Table 6. Viscosity, a fundamental physical property of ice cream, is crucial to determine its texture and mouthfeel. In the study, the viscosity values exhibited highly significant differences among the treatments, with IC9 recording the highest mean value (502.13mPas) and the control (IC) recording the lowest (126.33 mPas). This discrepancy can be attributed to the lower concentration of sugar beet juice (9%) in IC9, coupled with a higher concentration of sucrose (20%), which acted as a bulking agent and contributed to an increased viscosity. In another research study by [53], who investigated fat replacers in vanilla ice cream production, reported similar effects on the viscosity with low-calorie sweeteners such as aspartame, maltodextrin, inulin, oligofructose, and acesulfame. The cohesiveness, consistency, and firmness did not show significant differences among the treatments ($p \geq 0.05$), while the hardness demonstrated significant differences ($p \leq 0.05$). The current findings are supported by [54],

who examined the rheological properties of ice cream incorporated with inulin. They observed that as the value of total solids was enhanced, there was an increase in the hardness, which indicated a direct relationship between the hardness and the total solid content.

Table 6. Viscosity and texture parameter of sugar beet ice cream with encapsulated probiotic.

Treatment	Viscosity (mPa.s.)	Cohesiveness (g)	Consistency g(sec)	Firmness (g)	Hardness (kg)
IC	126.33 ± 0.57^{d}	-156.69 ± 27.57^{ab}	2609.9 ± 0.77^{b}	761.92 ± 14.36^{a}	0.13 ± 0.007^{c}
IC49	227.80 ± 4.85^{c}	-179.19 ± 0.93^{b}	2749.2 ± 0.78^{a}	425.99 ± 1.56^{c}	0.32 ± 0.0007^{a}
IC29	272.57 ± 24.02^{b}	-131.80 ± 0.15^{ab}	2286.1 ± 2.29^{c}	472.54 ± 0.74^{b}	0.23 ± 0.007^{ab}
1C9	502.13 ± 35.46^{a}	-122.28 ± 1.20^{a}	2749.2 ± 1.20^{a}	766.91 ± 1.17^{a}	0.38 ± 0.010^{a}

The values are mean ± SD, IC: Control ice cream; IC49: 49% sugar beet juice; IC29: 29% sugar beet juice; IC9: 9% sugar beet juice.

3.7.4. Chemical parameters (pH and acidity)

The pH parameter, which measures the concentration of hydrogen ions in a solution, yielded non-significant results ($p \geq 0.05$) (Table 5). Conversely, the titratable acidity, which gauges the total acid concentration in a food, showed significant differences among the treatments ($p \leq 0.05$). The highest mean acidity value was recorded in IC9 (0.31%), whereas the lowest was observed in IC49 (0.24%). The control samples exhibited an acidity value of 0.21%. The higher acidity in IC9 can be attributed to the lower concentration of the sugar beet juice, which typically has a pH ranging from 6.3 to 6.5. In contrast, the lower acidity in IC49 can be attributed to the higher volume of juice used (49 ml), which resulted in a lower acidity due to dilution. These findings align with those of [55], who investigated the effects of *Lactobacillus rhamnosus* GG addition on ice cream and reported similar variations in the pH. In another study, fluctuations in the pH were observed in range from 1.5 to 6.5 on the ice cream formulations [53].

3.8. Sensory evaluation

The ice cream prepared with various concentrations of beet juice including probiotic *B. bifidum* yielded varying sensory scores (Table 7). Significance was noted in the sensory parameters such as taste, flavor, color, odor, smoothness, mouthcoating, texture, firmness, aftertaste, and the overall acceptability ($p \leq 0.01$). Notably, as the level of beet juice extract gradually reduced from IC49 to IC9, the score for the overall acceptability was increased. The highest score of the overall acceptability was recorded for IC9, with 9% concentrations of beet juice extract, alongside the control sample IC, which had no added flavor or color. The beet juice contained a high amount of moisture and free saccharides (fructose and glucose) that may result in an impact of the physico-chemical and the enhancement of the sensory features of the ice cream [56,57]. The addition of fructo-oligosaccharide provided suitable sensory properties to symbiotic ice cream, thereby adding a better mouthfeel, sustainable flavor, and slight sweetness [9].

Table 7. Sensory attributes of sugar beet ice cream with encapsulated probiotic.

Treatments	Color	Taste	Flavor	Odor	Mouth coating	Smoothness	Firmness	Texture	After taste	Overall acceptability
IC	7.55 ± 1.11^{ab}	7.70 ± 1.05^{ab}	7.50 ± 1.5^{ab}	7.70 ± 0.94^{a}	7.10 ± 0.39^{ab}	7.30 ± 0.82^{ab}	7.60 ± 0.96^{a}	7.4 ± 1.17^{ab}	7.75 ± 0.92^{a}	7.90 ± 1.07^{a}
IC49	5.55 ± 1.86^{d}	5.45 ± 1.95^{c}	5.20 ± 1.5^{d}	5.60 ± 1.71^{c}	5.40 ± 1.26^{d}	5.70 ± 1.49^{cd}	5.15 ± 1.15^{c}	5.3 ± 1.33^{d}	4.70 ± 1.33^{c}	5.15 ± 1.29^{c}
IC29	6.05 ± 1.01^{cd}	5.90 ± 1.19^{c}	6.05 ± 0.95^{cd}	5.90 ± 1.44^{bc}	6.20 ± 0.78^{bcd}	5.90 ± 1.10^{cd}	5.95 ± 0.89^{bc}	6.3 ± 1.06^{cd}	6.20 ± 1.13^{b}	6.10 ± 0.73^{bc}
IC9	7.95 ± 0.75^{a}	7.90 ± 0.73^{a}	7.90 ± 0.77^{a}	7.55 ± 0.98^{a}	7.80 ± 0.82^{a}	7.95 ± 0.83^{a}	7.55 ± 0.83^{a}	7.7 ± 1.05^{a}	7.45 ± 1.06^{a}	7.88 ± 0.73^{a}

The values are mean ±SD (n = 10), Mean with different letters are significantly different form each other. IC: Control ice cream; IC49: 49% sugar beet juice; 5 % sugar, 0% water, IC29: 29% sugar beet juice; 10% sugar, 15% water, IC9: 9% sugar beet juice; 20% sugar, 25% water.

4. Conclusion

In conclusion, the incorporation of beetroot extract in ice cream offers a natural alternative to synthetic additives, thereby enriching the product with antioxidants and phenolic compounds. This addition not only enhanced the nutritional value of ice cream but also presented diversity to the commercially available options. Ciceritol and alginate served as effective encapsulation matrices, thus enhancing the survival of probiotics against simulated gastro-intestinal conditions. Furthermore, the utilization of encapsulated *B. bifidum* prevented fermentation in the ice cream and provided additional health benefits without compromising the product quality. The physico-chemical and sensory analysis revealed that the ice cream formulations with lower concentrations of beetroot extract (IC9) were preferred. Overall, this research highlighted the potential of beetroot extract and encapsulated probiotics to enhance the nutritional profile and health-promoting properties of ice cream, thus paving the way for the development of functional food products with improved health benefits and consumer appeal. Furthermore, additional studies may be carried out to check the fermentation perspectives to boost the functional perspectives.

Use of AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

Acknowledgments

The authors also extend their appreciation to the Researchers Supporting Project number (RSP2025R502), King Saud University, Riyadh, Saudi Arabia, for funding this study.

Conflict of interest

The authors declare no conflicts of interest.

Author contributions

Majid Hussain: Conceptualization, Data curation, Writing-review and editing; Hifsa Izhar: Formal analysis, Writing, Formating; Taha Rababah, Vaida Bartkutė-Norkūniene: Funding acquisition, Data curation, Writing-review and editing; Muhammad Azam: Formal analysis, Conceptualization, Writing-review, and editing; Numan AL-Rayyan, Bandar N. Hamadneh: Conceptualization, Funding acquisition, Data curation, Writing-review, and editing; Ali Almajwal, Rania M. Jammal: Writing-review and editing, Data curation, Funding acquisition, and Conceptualization. All authors have approved the final version of the manuscript.

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