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ORIGINAL PAPER

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PROBIOTIC SURVIVAL OF *BIFIDOBACTERIUM LACTIS* AND *LACTICASEIBACILLUS RHAMNOSUS* IN PECTIN MICROCAPSULE EXTRACTED FROM BITTER ORANGE PEEL FOR ICE CREAM

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ABSTRACT

Background. Ice cream can serve as an effective vehicle for delivering probiotic bacteria with health-promoting benefits. This study aimed to extract pectin from bitter orange peel waste for use as a bioactive, prebiotic component in microcapsules containing *Bifidobacterium lactis* (*B. lactis*) and *Lacticaseibacillus rhamnosus* (*L. rhamnosus*), to enhance the nutritional and functional properties of ice cream.
Materials and methods. Microcapsules were prepared using different ratios of bitter orange peel pectin (BOPP) to aluminum carboxymethyl cellulose (ACMC) (1:0, 1:1, and 1:1.5) as coating materials for the probiotics. The physical characteristics and microstructure of the microcapsules were examined. The optimal formulation was then incorporated into ice cream at 10%, 20%, and 30% concentrations. The resulting ice cream samples were analyzed for physicochemical properties, antioxidant activity, probiotic viability, and folate content.
Results. The 1:1 BOPP to ACMC ratio produced the most suitable formulation for application in ice cream. Ice cream containing 30% of the encapsulated probiotics showed the highest antioxidant activity and total phenolic content. Furthermore, the co-encapsulation of *B. lactis* and *L. rhamnosu* significantly enhanced both probiotic survival and folate levels.
Conclusions. The results indicate that microcapsules containing *B. lactis* and *L. rhamnosus* in a 1:1 ratio of ACMC to BOPP, particularly at 30% concentration, can be effectively used in probiotic ice cream, offering enhanced functional and nutritional benefits for industrial applications.

Keywords: probiotic ice cream, *Bifidobacterium lactis*, *Lacticaseibacillus rhamnosus*, microencapsulation, bitter orange peel pectin, functional food

INTRODUCTION

Ice cream is a popular, nutritious, and affordable frozen dessert (Tirono, 2022). Its formulation can be enhanced by adding minerals, antioxidants, vitamins, and probiotic bacteria to improve its nutritional and functional properties (Hassan et al., 2024).

Among probiotics, *Bifidobacterium lactis* (*B. lactis*) and *Lacticaseibacillus rhamnosus* (*L. rhamnosus*) are widely used in fermented foods, especially dairy products, due to their health benefits (Maleki et al., 2023). To ensure these beneficial bacteria survive gastrointestinal transit, microencapsulation techniques such as spray drying, extrusion, and emulsification are widely employed (Pallavi et al., 2024).

Polysaccharides extracted from fruit by-products, such as pectin from bitter orange (*Citrus aurantium*) peel, offer a sustainable and cost-effective material for

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probiotic microencapsulation. These materials not only reduce production costs but also enhance the functional properties of the encapsulates (Juan et al., 2025). Bitter orange peel is rich in phenolic compounds and dietary fiber, acting as a prebiotic substrate that supports probiotic viability and delivers nutraceutical benefits (Afzaal et al., 2019; De Freitas et al., 2024).

Aluminum carboxymethyl cellulose (ACMC), a water-soluble cellulose ether derivative composed of linked glucopyranose residues, provides structural stability to microcapsules (Khorasani and ShojaSadati, 2017). While conventional encapsulants such as alginate and inulin have been studied, this research introduces a novel co-encapsulation approach using bitter orange peel pectin (BOPP) and ACMC to protect *B. lactis* and *L. rhamnosus* in ice cream. The goal is to develop a probiotic ice cream with improved physicochemical, biological, and sensory properties by leveraging the prebiotic effects of BOPP alongside the protective capabilities of ACMC, ensuring high probiotic survival during storage and gastrointestinal passage.

Previous studies have successfully encapsulated probiotics using various materials: *Lactobacillus acidophilus* in sodium alginate and carrageenan (Afzaal et al., 2019), *Bifidobacterium bifidum BB-12* and *Lactobacillus acidophilus* La-5 in *Stevia rebaudiana* aqueous extract (Banuree et al., 2022), *Bifidobacterium Bb-12* with whey proteins, inulin and omega-3 fatty acids (Lai et al., 2024) and *Lactobacillus rhamnosus* in whey protein, bio-cellulose and inulin (Maleki et al., 2023), which all investigated in ice cream.

This study applies ACMC and *BOPP* as co-encapsulation agents to improve the survival of *B. lactis* and *L. rhamnosus* in probiotic ice cream and to enhance their physicochemical, functional, and sensory traits during storage.

MATERIALS AND METHODS

Skimmed milk was purchased from Pegah Dairy Company and *B. lactis* HN019 and *L. rhamnosus* ATCC 53103 were obtained from the Iranian Research Organization for Science and Technology Tehran. Bitter oranges, originating from the Mazandaran region of Iran, were procured from a local market in Tehran. De Man, Rogosa, and Sharpe (MRS) broth, Folin-Ciocalteu's reagent, and other solvents and chemicals were obtained from Merck, Germany. Span 60, $Na_5P_3O_{10}$ and DPPH, aluminum chloride, Tween 80, and ACMC were sourced from Sigma-Aldrich (USA), Ajax Finechem (Australia), and Fluka (Finland), respectively. Palm oil was obtained from Negin Tejarat Payam Company.

Pectin extraction

Bitter orange peel (BOP, 5 g) was blended with citric acid solution at varying pH levels. An ultrasonic probe was then immersed into the mixture and operated at 20 kHz frequency and 25°C. After extraction, the mixture was centrifuged (10 000 × g, 15 min) to eliminate impurities. Subsequently, 96 % ethanol was added to the supernatant, and the mixture was stored at 4°C for 24 h to allow pectin precipitation. The coagulated pectin was then separated by centrifugation (10 000 × g, 20 min) and dried at 45°C for 16 h (De Freitas et al., 2024).

Bacteria microencapsulation

B. lactis HN019 and *L. rhamnosus* ATCC 14917 were cultured in 500 mL MRS broth at 37°C for 18 h until the logarithmic phase was reached. Microcapsules containing these probiotics were prepared using ACMC-BOP composites through a conventional water-in-oil emulsion external crosslinking technique. The external phase consisted of Palm oil and 2.0% (w/v) ACMC premixed with 0.025% (w/v) Span 60. The internal phase included BOP at weight ratios of 1:0, 1:1, and 1:1.5 (w/w), along with the bacterial mixture (Maleki et al., 2023), as depicted in Table 1.

Determination of microencapsulation efficiency (ME) and size

To determine microencapsulation efficiency (ME), the powder was treated with a solvent such as hexane to extract free oil. The mixture was then filtered using a Whatman no. 1 filter paper. The sample was washed several times with hexane and sequentially dried at 25°C and 60°C to evaporate residual solvent (Choudhury et al., 2021).

Microcapsule size distribution was measured using a laser diffraction technique with a Hydro 2000 MU (A) wet cell and 0.05 M $AlCl_3$ as a suspending solution (Mastersizer 2000, Malvern Instruments, Malvern, UK) (Singh and Kaur, 2012).

Release profile

To evaluate probiotic release, 0.75 g of each treatment was added to 37.5 mL of 0.85% saline solution, mixed at 150 rpm at 30°C, and sampled at time intervals of 1, 3, 10, 24 and 48 h. Colony-forming units (CFUs) were determined using the plate count method. The release percentage at each time point was calculated based on initial microcapsule viability (Lopes et al., 2023).

Fourier transform infrared spectroscopy (FTIR)

FTIR analysis of powdered samples was conducted using a Spectrum RX1 spectrometer (PerkinElmer, USA) with the KBr disk method over the 400–4000 cm^{-1} range (Fracasso et al., 2017).

X-ray diffraction (XRD) and morphological assessments

XRD analysis of ACMC, BOPP, and microcapsules was conducted using a Lab X XDR-6000 diffractometer (Shimadzu Japan) with Cu- Kα radiation (λ = 1.54056 Å), operating at 30 kV and 30 mA. Data were collected over a 2θ range of 5–70º at a continuous scanning rate of $2°/min^{-1}$ (Khorasani and Shojasadati, 2017).

Morphological characteristics of the samples were examined using scanning electron microscopy (SEM, JSM-6510, JEOL) (Lopes et al., 2023).

Preparation of probiotic ice cream

Ice cream was prepared (based on 1 kg total weight) using a base formulation of 58% skimmed milk, 17% sugar, 20% cream, and 0.5% stabilizer. The optimal treatment (10%, 20%, or 30%) from the first stage was added to the base mixture, which was then pasteurized at 85°C for 30 seconds. After cooling, *B. lactis*, *L. rhamnosus* or a suspension containing both strains was added at a concentration of approximately 10^8 CFU/mL. Ice cream was then produced and overrun performed using a domestic ice cream maker. The resulting samples, containing various concentrations of BOP, were stored at –18ºC (Tab. 2).

Physicochemical, total phenolic content, and antioxidant activity of ice cream

pH was measured using a pH meter (Metrohm, Switzerland). Ash and total dry matter contents were determined by heating the samples in a furnace at 600°C and oven. Total phenolic content was assessed using the Folin-Ciocalteu reagent. In this procedure, 0.2 mL of ice cream sample was blended with 1 mL of Folin-Ciocalteu's reagent, 2 mL of 20% sodium carbonate (w/v), and 1.8 mL of distilled water. The mixture was incubated in the dark at room temperature for 20 min, and absorbance was measured at 735 nm (Sagdic et al., 2012). For the antioxidant assay, 25 mL of a 50% methanol solution was used to extract the sample for 12 h, followed by filtration using Whatman filters No. 4. Next, 0.3 mL of the extract was mixed with 1.2 mL ethanol and 1.5 mL of 1 mmol/L DPPH. The solution was kept in the dark for 30 min, and absorbance was measured at 517 nm (Abdel-Hamid et al., 2020).

Folate level assessment

High-performance liquid chromatography (HPLC) was used to measure folate compounds in the ice cream samples. Detection was carried out at a wavelength of 295 nm with a flow rate of 0.4 mL/min. Prior to injection, the samples were filtered through 0.45 nm filters and introduced into the HPLC device using a special syringe. Three folate standards (100, 50, and 25 ppm) were prepared under the same conditions. Folate content in the samples was evaluated by measuring the area under the curve (Rossi et al., 2011).

Viability of probiotic bacteria

The viability of probiotic bacteria was determined using the standard plate count method. Ice cream samples (10 g) were serially diluted in sterile peptone water (0.1%), with dilutions prepared up to 10^{-7}. Viable counts of *B. lactis* and *L. rhamnosus* were determined using MRS agar. The inoculated plates were incubated at 37°C for 48 hours, and colony counts were recorded for each sample (Abtahi and Nouri, 2025).

Statistical analysis

Data were statistically analyzed using one-way analysis of variance (ANOVA) under a completely randomized design with SPSS v. 22 software. All analyses were performed in triplicate. Duncan's multiple range test at the 0.05 significance level was used to determine significant differences between means.

RESULTS AND DISCUSSION

Characteristics of microencapsulated samples

The microencapsulation efficiency (ME) results for the samples are presented in Table 1, ranging from 53.2 ±1% for the 1:0 BL sample to 93.3 ±1% for the 1:1 BLLP sample. The lowest ME percentage was observed in the BOPP-free samples, indicating a significant effect of BOPP on encapsulation efficiency. It is likely that the cells were either damaged or not completely released from the matrix during incubation at high BOPP concentrations due to extended homogenization times, which resulted in reduced ME (Choudhury et al., 2021). Survivability ranges of 78.87% to 88.06% and 60% to 95% have been reported for the co-microencapsulation of *L. rhamnosus* and DHA fatty acid in alginate-pectin-gelatin biocomposites and starch-carboxymethyl cellulose-coated bacterial nanocellulose-pectin bionanocomposites, respectively. These biocomposites act as protective prebiotic matrices for ME (Khorasani and Shojasadati, 2017). Similar to the present findings, pectin had a more significant effect on improving ME in systems composed of alginate-pectin-gelatin (De Freitas et al., 2024; Lopes et al., 2023).

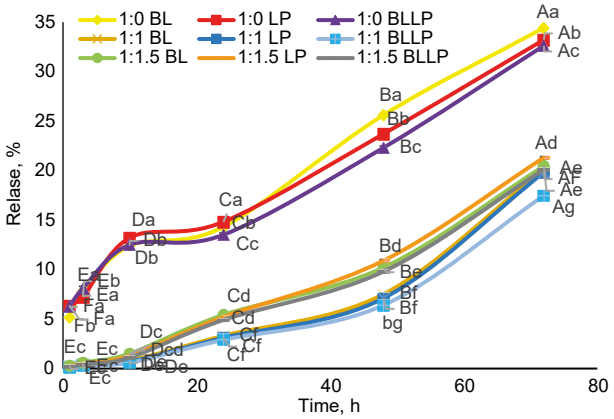
The D_{50} values of encapsulated samples presented in Table 1 ranged from 5.9 ±0.5 to 11.2 ±0.8 µm. The smallest particle sizes were observed in samples without BOPP, and particle size increased with higher BOPP content. Smaller droplets offered a wider surface area, allowing more rapid penetration of active substances (Singh and Kaur, 2012). A higher hydrogen ion concentration improved chain structure, resulting in a semi-spherical framework for microcapsules, consistent with observations in the present study (Abtahi and Nouri, 2025).

As shown in Figure 1, samples containing a 1:1 formulation of ACMC-BOPP (1:1 LP, BL, and BLLP) exhibited the lowest release levels. In contrast, samples with BOPP-free formulations (1:0 LP, BL, and BLLP) demonstrated the highest release over 72 h. Overall, the release process remained slow during the first 10 h and after that, followed by a significant increase. This suggests an initially controlled release of encapsulated substances that becomes more pronounced over time. ACMC can serve as a functional additive in biocomposites, enhancing their mechanical and barrier properties due to polymeric structure and high molecular weight (Manzoor et al., 2019). Additionally, the carboxyl groups in ACMC can interact with the hydroxyl

Table 1. The samples, bacterial strain, microencapsulation efficiency (%) and mean particle size (D_{50}, µm)

Samples	ACMC:BOPP ratio	Bacterial strain	Microencapsulation efficiency, %	Mean particle size D_{50}, µm
1:0 BL	1:0	*Bifidobacterium lactis*	56.5 ±3^{e}	5.9 ±0.5^{c}
1:0 LP	1:0	*Lacticaseibacillus rhamnosus*	53.2 ±1^{e}	6.3 ±0.4^{c}
1:0 BLLP	1:0	*Bifidobacterium lactis* and *Lacticaseibacillus rhamnosus*	55.1 ±2^{e}	6.2 ±0.5^{c}
1:1 BL	1:1	*Bifidobacterium lactis*	86.8 ±1^{b}	9.4 ±0.7^{b}
1:1 LP	1:1	*Lacticaseibacillus rhamnosus*	87.3 ±2^{b}	9.6 ±0.2^{b}
1:1 BLLP	1:1	*Bifidobacterium lactis* and *Lacticaseibacillus rhamnosus*	93.3 ±1^{a}	9.9 ±0.5^{b}
1:1.5 BL	1:1.5	*Bifidobacterium lactis*	78.3 ±1^{d}	11.8 ±0.3^{a}
1:1.5 LP	1:1.5	*Lacticaseibacillus rhamnosus*	80.6 ±2^{c}	11.1 ±0.6^{a}
1:1.5 BLLP	1:1.5	*Bifidobacterium lactis* and *Lacticaseibacillus rhamnosus*	84.9 ±1^{c}	11.2 ±0.8^{a}

Lowercase letters indicate significant differences between distinct samples in each column.



Uppercase and lowercase letters indicate significant differences between distinct days and samples.

Fig. 1. The cumulative release curve of microcapsules during shelf life

structures of BOPP through hydrogen bonding (Khorasani and Shojasadati, 2017).

In a previous study, nanocapsules containing *Bacillus subtilis* with pectin/starch/PEG formulations showed a release rate of 7.5% to 25% over 24 h (Lopes et al., 2023). ACMC–rice bran composites were found to increase the survivability of encapsulated probiotics under heat stress, which could be due to the higher porosity of CMC (Chitprasert et al., 2012). Other studies have evaluated the effectiveness of different matrices for probiotic microencapsulation in ice cream. For instance, *Lactobacillus acidophilus* encapsulated in sodium alginate and carrageenan demonstrated improved survival under simulated gastrointestinal conditions (Afzaal et al., 2019). Similarly, *Bifidobacterium bifidum BB-12* and *Lactobacillus acidophilus La-5* encapsulated in *Stevia rebaudiana* aqueous extract exhibited higher viability and sensory attributes in functional ice cream (Banuree et al., 2022). Encapsulation of *Bifidobacterium BB-12* with whey proteins, inulin and omega-3 fatty acids enhanced probiotic survival and nutritional value (Lai et al., 2024). Additionally, *Lactobacillus rhamnosus* encapsulated in whey protein, bio-cellulose and inulin components maintained high viability during storage (Maleki et al., 2023). Furthermore, *Lactobacillus reuteri* encapsulated in aluminum carboxymethyl cellulose-rice bran microcapsules demonstrated enhanced thermal stability and survivability, attributed to the porosity of the protective matrix (Chitprasert et al., 2012).

In the present study, ACMC and BOPP were used as a co-encapsulation matrix for *B. lactis* and *L. rhamnosus*. This combination not only enhanced physicochemical and sensory properties but also improved folate production and antioxidant activity in probiotic ice cream during storage. These findings are in line with earlier research and introduce a novel prebiotic source derived from citrus by-products.

From an industrial perspective, the 1:1 ACMC:BOPP ratio – which yielded the highest microencapsulation efficiency (93.3 ±1%) and controlled release profile – offers a cost-effective and scalable formulation. Bitter orange peel, a low-cost by-product of citrus processing, serves as an economical and sustainable source of pectin, reducing raw material costs. While ACMC is more expensive than basic encapsulants like alginate, its superior functional properties – especially in improving probiotic viability and minimizing premature release – can reduce losses during processing and storage. This trade-off may be justified in commercial production, especially when targeting premium functional food markets. Furthermore, valorizing bitter orange peel supports circular economy principles and may appeal to environmentally conscious consumers and investors. Therefore, the 1:1 formulation provides an optimal balance between performance and economic viability, making it suitable for large-scale probiotic ice cream manufacturing.

Assessment of microcapsule structure

Carboxylic groups related to ACMC and BOPP were visible in the 1000 cm^{-1} to 1050 cm^{-1} range across all spectra, though peaks differed slightly in samples containing pure components (Fig. 2a). A small peak observed between 1200 cm^{-1} and 1300 cm^{-1} corresponded to C-O group stretching in carbohydrates. Peaks from 1320 cm^{-1} to 1450 cm^{-1} were attributed to asymmetric and symmetric stretching vibrations for C-H bending in all spectra, respectively. The intensity of these peaks was higher in ACMC and BOPP samples than in others, due to encapsulation and new band formation. Peaks in the 1500 cm^{-1} to 1800 cm^{-1} region were associated with amide, carboxyl ester, and carboxylate groups present in BOPP, ACMC, and probiotics. The bonds in regions from 1740 cm^{-1} to 1755 cm^{-1} and 1690

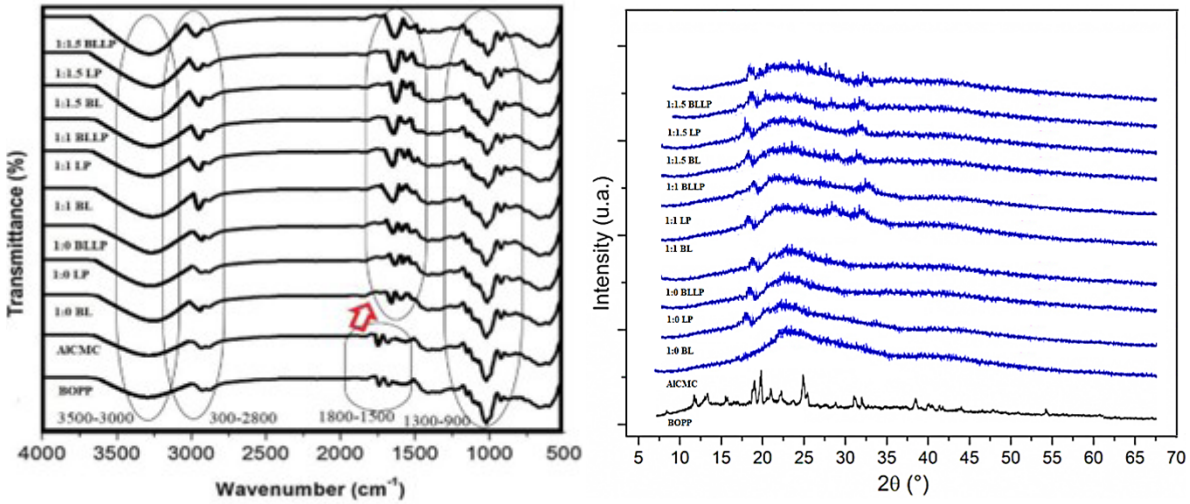


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Fig. 2. The Fourier transform infrared spectroscopy and X-ray diffraction of ACMC, BOPP, and microcapsule samples

cm^{-1} to 1660 cm^{-1} related to C=O stretching vibrations of methyl ester of carboxylic acid and free carboxyl groups in the BOPP and ACMC spectrum, respectively. However, in the probiotic samples, peaks in this range shifted and overlapped due to cross-linking and microcapsule formation (Khorasani and ShojaSadati, 2017). The amide protein region of probiotic bacteria was in the 1500 cm^{-1} to 1700 cm^{-1} range, with bands from 1620 cm^{-1} to 1570 cm^{-1} showing C=O (amide I) and C(=O)-O- asymmetric stretching vibrations ($COOCH_3$ in esterified carboxyl groups), respectively. The bands between 1500 cm^{-1} and 1550 cm^{-1} were related to N-H bending vibrations of amide II; therefore, a shift was observed from a peak at approximately 1750 cm^{-1} for ACMC and BOPP samples to 1690 cm^{-1} in microcapsules, indicating that cross-linking facilitated formation (Manzoor et al., 2019). The intensity of peaks from 1500–1800 cm^{-1} was slightly higher in the spectrum for 1:1.5 BL, LP, and BLLP (ACMC:BOPP) samples than in others, suggesting a greater presence of functional groups (Fracasso et al., 2017). The absorption peaks at 2910 cm^{-1} and 2870 cm^{-1} were assigned to C-H (CH, CH_2, and CH_3) asymmetric and symmetric stretching vibrations, respectively, in all samples, and the intensity in treatment containing 1.5% BOPP was higher than in others (Choudhury et al., 2021). No significant changes were observed in FTIR peaks for samples containing probiotics; however, variations in intensity at characteristic peak positions and slight shifts indicated composite interactions. The coating of synbiotic matrices relied on non-covalent intermolecular interactions (hydrogen and van der Waals bonds) between functional groups of ACMC and BOPP (Khorasani and ShojaSadati, 2017).

In Figure 2b, XRD spectra exhibit typical diffraction peaks for BOPP at around 2θ = 11, 13, 18, 20, 21, 25, 32, and 34. Generally, BOPP crystallinity is indicated by sharper and narrower diffraction peaks, which correspond to systematic polygalacturonic structures containing numerous carboxylic groups (Fracasso et al., 2017). ACMC displays characteristic function around 2θ = 20, indicating its amorphous nature (Manzoor et al., 2019). Previous research has shown that BOPP and ACMC exhibit crystalline and amorphous structures, respectively (Khorasani and ShojaSadati, 2017; Lopes et al., 2023). The diffraction peak of encapsulated samples showed a larger peak at 2θ = 27, while probiotic bacteria resulted in a new peak at 2θ = 18. The presence of pectin in the obtained microencapsulated samples caused a peak at 2θ = 32. The lower intensity of diffraction peaks in encapsulated samples compared to pure materials suggests a crystalline structure loss due to water absorption during blend preparation and the formation of inter- and intramolecular hydrogen bonds between polymers (Choudhury et al., 2021). Similar to the present results, the coated symbiotic matrix was detected by a peak

at 2θ = 16.91 after loading *Bacillus coagulans*, and bacteria entrapment in the starch-coated formation showed that the crystalline structure had transformed into an amorphous one (Khorasani and ShojaSadati, 2017).

Figure 3 illustrates the surface morphology of freeze-dried samples of BOPP, ACMC, and microcapsules containing bacteria. In the labeled BOPP image (Fig. 3j), the structure shows compact, nearly spherical granules, indicating a semi-crystalline morphology with minimal roughness, supporting its potential as a prebiotic filler (Lopes et al., 2023). The ACMC image (Fig. 3k) revealed a fibrous, irregular texture, consistent with its amorphous and water-soluble nature (Chitprasert et al., 2012). In microcapsule samples: 1:0 BL, 1:0 LP, and 1:0 BLLP (Fig. 3a–c), the surfaces appeared rough and irregular, with visible filaments or bacterial protrusions, suggesting limited encapsulation efficiency in the absence of BOPP. In the 1:1 BL, 1:1 LP and 1:1 BLLP images (Fig. 3d–f), surfaces appeared smoother and more continuous, indicating improved microcapsule integrity and effective crosslinking between ACMC and also BOPP. These samples showed minimal exposed bacterial structures, pointing to successful encapsulation. In the 1:1.5 BL, 1:1.5 LP and 1:1.5 BLLP images (Fig. 3g–i), although encapsulation appeared effective, some swelling or irregular layering was observed, possibly due to excess BOPP, which may have led to structural loosening or oversaturation.

These morphological differences were attributed to the solubility and interaction potential of BOPP and ACMC, where optimal ratios (such as 1:1) produced denser and smoother matrices. The absence of detectable bacteria on the surface, particularly in the

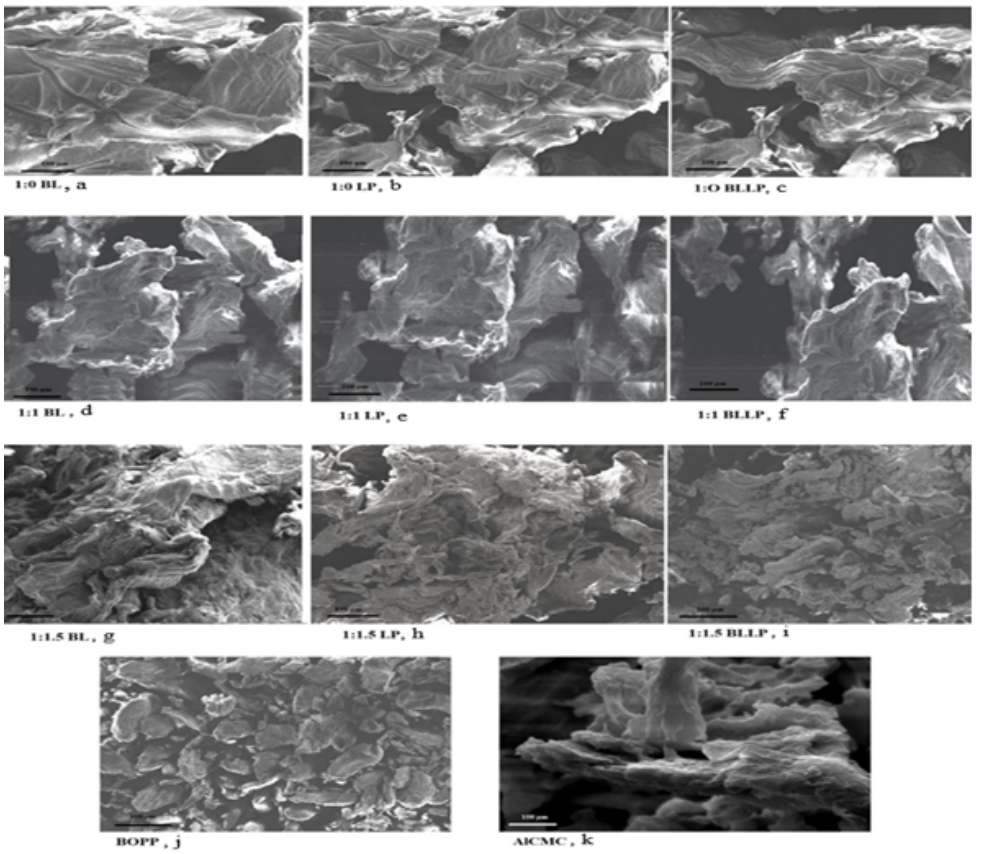

Fig. 3. The scanning electron microscopy images of microcapsule samples (a–i) and BOPP (j), ACMC (k)

1:1 samples, indicated successful entrapment, aligning with previous studies emphasizing the importance of matrix composition in shielding probiotics (Ashagrie et al., 2025; Khorasani and ShojaSadati, 2017; Maleki et al., 2023). The pectin/starch-based nanocomposite beads were successfully used to encapsulate *Bacillus subtilis*, resulting in well-enclosed structures as confirmed by morphological analysis (Lopes et al., 2023). Based on these findings, a 1:1 ratio of ACMC and BOPP was selected for incorporation into the ice cream.

Physicochemical characteristics of ice cream

The physiochemical attributes of probiotic samples containing *B. lactis* and *L. rhamnosus* are presented in Table 2. The results showed that pH was not significantly affected by increasing the percentage of microcapsules or by the type of probiotic strain used ($p > 0.05$). However, the dry matter and ash contents of the samples were significantly influenced by the varying levels of microcapsule incorporation ($p < 0.05$). The presence of citrus fruits – rich in sugar, fiber, and mineral compounds – appeared to enhance the effect of microcapsules (Hassan et al., 2024; Juan et al., 2025).

Biological assessment

Table 2 shows that phenolic components are highly dependent on the microcapsule percentage and probiotic bacteria strain in the ice cream formulations ($p < 0.05$). Therefore, increasing the microcapsule percentage led to a significant enhancement of phenolic compounds in the samples ($p < 0.05$). Probiotic samples containing a mixture of target bacteria had the lowest phenolic content compared to other treatments. Previous studies have shown that citrus peels contain a high concentration of phenolic compounds (De Freitas et al., 2024). Phenolic compounds can act as prebiotics and substrates for probiotic bacteria, leading to a reduction in their content, which is consistent with previous studies (Juan et al., 2025; Sagdic et al., 2012).

Variance analysis demonstrated that changes in antioxidant capacity (IC_{50}) of probiotic ice creams were significantly associated with microcapsule percentage and bacterial strain in the formulations ($p < 0.05$). Phenolic compounds function as hydrogen donors and regenerating agents in food systems and are capable of inhibiting free radicals activity (Khorasani and ShojaSadati, 2017). Phenolic content increased with the

Table 2. pH, dry matter (%), ash (%), phenolic compounds (mg GAE/100 g), and IC_{50} (mg/100 g) in ice cream samples, containing bitter orange peels and probiotic bacteria strains of *Bifidobacterium lactis* and *Lacticaseibacillus rhamnosus* (mean ±standard error)

Samples	Microcapsule percentage	Bacterial strain	pH	Dry matter %	Ash, %	Phenolic mg GAE/100 g	IC_{50} mg/100 g
Control	0	–	6.0 ±0.24^{a}	42.0 ±0.34^{d}	0.52 ±0.05^{d}	20.1 ±3.43^{g}	254.1 ±40.20^{a}
10% BL	10	*B. lactis*	6.0 ±0.62^{a}	44.0 ±0.55^{c}	1.06 ±0.02^{c}	41.1 ±1.21^{e}	180.1 ±2.30^{c}
10% LP	10	*L. rhamnosus*	6.0 ±1.05^{a}	44.0 ±0.62^{c}	1.07 ±0.02^{c}	42.1 ±1.18^{e}	180.1 ±2.27^{c}
10% BLLP	10	*B. lactis* and *L. rhamnosus*	6.0 ±0.44^{a}	44.0 ±0.80^{c}	1.06 ±0.03^{c}	38.1 ±1.75^{f}	185.1 ±2.22^{b}
20% BL	20	*B. lactis*	6.0 ±0.85^{a}	46.0 ±0.70^{b}	1.12 ±0.02^{b}	71.1 ±1.60^{c}	138.1 ±1.33^{e}
20% LP	20	*L. rhamnosus*	6.0 ±1.02^{a}	46.0 ±1.02^{b}	1.12 ±0.03^{b}	72.1 ±1.52^{c}	137.1 ±1.25^{e}
20% BLLP	20	*B. lactis* and *L. rhamnosus*	6.0 ±0.62^{a}	46.0 ±1.05^{b}	1.15 ±0.01^{b}	69.1 ±0.41^{d}	141.1 ±1.65^{d}
30% BL	30	*B. lactis*	6.0 ±0.52^{a}	51.0 ±3.36^{a}	1.20 ±0.03^{a}	103.1 ±1.04^{a}	100.1± 2.85^{g}
30% LP	30	*L. rhamnosus*	6.0 ±0.44^{a}	50.0 ±2.40^{a}	1.20 ±0.02^{a}	103.2 ±1.30^{a}	102.1 ±2.37^{g}
30% BLLP	30	*B. lactis* and *L. rhamnosus*	6.0 ±0.23^{a}	50.0 ±2.72^{a}	1.23 ±0.01^{a}	101.1 ±0.66^{b}	110.1 ±4.45^{f}

*Different lowercase letters show a significant difference ($p < 0.05$) in each column.

addition of target peels, and consequently, antioxidant capacity was expected to be higher in probiotic ice cream samples with greater microcapsule percentages. Ginger extract, recognized for its antioxidant and prebiotic properties, enhanced the growth of probiotic bacteria (Singh and Kaur, 2012), supporting the findings of this study.

From an economic and industrial standpoint, incorporating bitter orange peel as a pectin source not only improves the phenolic and antioxidant capacity of probiotic ice cream but also enhances cost-efficiency. Bitter orange peels are abundant agro-industrial by-products that are typically discarded as waste. Their use in food formulations promotes waste valorization and provides a low-cost alternative to synthetic antioxidants and prebiotic compounds (De Freitas et al., 2024; Juan et al., 2025). This approach reduces raw material costs, aligning with sustainable production goals. Moreover, functional foods enriched with natural bioactives such as phenolics from orange peels have increasing market demand and are often positioned as premium products, enabling higher retail pricing and profitability (Hassan et al., 2024). Compared to purchasing purified antioxidants or commercial encapsulants, using pectin extracted in-house offers significant economic advantages, especially for manufacturers seeking to improve the health profile of their products without substantially raising costs. Therefore, developing microcapsule formulations not only enhances nutritional value but also supports cost-effective and sustainable production practices.

Bacterial viability assessments

Figure 4 shows the survival counts of probiotic bacteria (*B. lactis* and *L. rhamnosus*) in probiotic ice creams during 20 days of shelf life. Analysis of variance indicated that viability was significantly influenced by bacterial species, microcapsule percentage, and storage duration in the formulations ($p < 0.05$). A population of 10^6 to 10^7 CFU/g in the diet is considered a therapeutic dose for probiotic cultures in processed foods (Ghorbani et al., 2023). The present results demonstrated that probiotic ice cream prepared with a 30% microcapsule content exhibited significantly higher viability, exceeding 10^6 CFU/g. Phenolic and dietary fiber contents increased with the addition of fruit pulps in ice cream formulations, which may have contributed to enhanced bacterial survival (Afzaal et al., 2019). However, bacterial populations in all samples declined significantly by the end of the storage period ($p < 0.05$). The bacteria population decreased at freezing temperatures due to mechanical damage caused by ice crystal formulation. Ice can rupture cells by forming internally and by increasing solute concentration

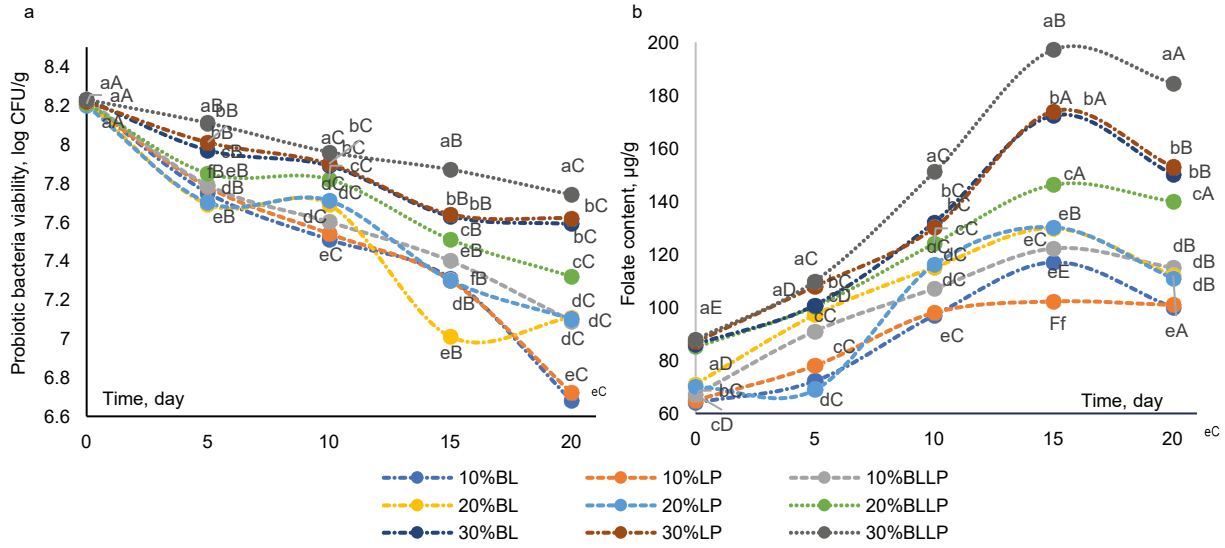

Fig. 4. The probiotic bacteria viability and folate in ice cream samples during storage (mean ±standard error)

in the extracellular medium (Ashagrie et al., 2025). These observations are consistent with previous studies on the viability of probiotic bacteria (Abdel-Hamid et al., 2020; Abtahi and Nouri, 2025).

Maintaining probiotic viability above the therapeutic threshold (≥ 10 CFU/g) during storage is essential not only for deriving health benefits but also for its economic value in the functional food sector. Ice cream formulations that support high probiotic survival, particularly those with 30% microcapsule inclusion, offer improved product stability and longer shelf life, reducing waste due to spoilage or subtherapeutic quality (Ghorbani et al., 2023). Moreover, viable probiotic counts can serve as a value-added marketing feature, enhancing product differentiation in a competitive landscape and supporting premium pricing strategies (Afzaal et al., 2019). The use of encapsulation systems derived from low-cost materials, such as bitter orange peel, improved both bacterial protection and cost-efficiency by eliminating the need for expensive synthetic coatings or preservatives. This dual benefit – extended probiotic survival and economical raw material sourcing – highlights the commercial potential of the proposed formulation strategy (Ashagrie et al., 2025).

Folate status responses

Figure 4 shows that folate levels were significantly influenced by shelf life, microcapsule percentage, and probiotic bacteria species in the ice cream formulations ($p < 0.05$). In the current study, folate content increased significantly with extended storage time and higher microcapsule concentrations in the samples ($p < 0.05$). This trend can be explained by the contribution of phenolic and fibrous components from the microcapsules, which provided a sufficient substrate for microorganisms. Therefore, higher BOP levels facilitated greater microbial access to essential substrates, resulting in increased folate production in the products (Cruxen et al., 2017). Similar findings were reported in a study where folate production was enhanced in probiotic soy milk formulations enriched with passion fruit by-products and fructooligosaccharides (Ashagrie et al., 2025). The results also demonstrated that ice cream samples containing a mixture of *B. lactis* and *L. rhamnosus* exhibited the highest folate concentrations, compared to those formulated with individual target bacterial strains. Consistent with the current findings, previous research showed that an appropriate combination of probiotic strains can improve folate levels in ice cream (Rossi et al., 2011).

CONCLUSION

The structural and functional characterization of microencapsulated samples indicated that a 1:1 ratio of ACMC and BOPP was the most effective for incorporation into ice cream. Physicochemical analyses showed that increased microcapsule content was associated with higher dry matter and ash levels; however, no significant effect was observed on pH. The highest levels of antioxidant activity, phenolic compounds, and folate were found in the probiotic ice cream samples with higher microcapsule inclusion. The findings demonstrate that microencapsulation positively influenced the viability of probiotics, maintaining levels above the recommended therapeutic threshold. Additionally, BOPP, as a potential prebiotic, improved the viability of probiotic bacteria (*B. lactis* and *L. rhamnosus*), indicating its suitability for application in functional food production.

DECLARATIONS

Data statement

All data supporting this study has been included in this manuscript.

Ethical Approval

Not applicable.

Competing Interests

The authors declare that they have no conflicts of interest.

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