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Potential of Incorporating a Functional Probiotic Encapsulant in Whipped Cream

Kritika Gaba [1,2] and Sanjeev Anand [1,2,*]

[1] Midwest Dairy Foods Research Centre, South Dakota State University, Brookings, SD 57007, USA
[2] Dairy and Food Science Department, South Dakota State University, Brookings, SD 57007, USA
* Correspondence: sanjeev.anand@sdstate.edu; Tel.: +1-(605)-688-6648

Abstract: The probiotic foods market is expanding; however, maintaining probiotics viability is challenging during manufacturing and storage conditions. In this study, a functional ingredient containing whey protein hydrolysate-encapsulated probiotics was standardized into whipped cream, followed by its characterization and storage stability study. The whipped cream was prepared under standard laboratory conditions, and the encapsulant was added at 0.1% and 1% w/w levels. The samples were further characterized through viable probiotic counts, physicochemical and microstructural analysis. Analyses were conducted in triplicates, and ANOVA was applied to differentiate between the mean values ($p < 0.05$). The whipped cream variant with 1% w/w encapsulant addition exhibited higher viability of *Lactobacillus acidophilus* ATCC4356 (LA5) (7.38 $\pm$ 0.26 $\log_{10}$CFU/g) and *Bifidobacterium animalis* ssp. *Lactis* ATCC27536 (BB12) (7.25 $\pm$ 0.56 $\log_{10}$CFU/g) along with enhanced physicochemical properties as compared to the LA5 (6.53 $\pm$ 0.45 $\log_{10}$CFU/g) and BB12 (6.41 $\pm$ 0.39 $\log_{10}$CFU/g) counts in the 0.1% variant. This was attributed to the thicker and uniform encapsulant deposition at the O/W interface observed in micro-images. The storage stability results did not show a substantial difference in viability for encapsulated probiotics compared to the control. The encapsulant also maintained the 1:1 ratio of LA5 and BB12. Thus, a value-added range of dairy products could be introduced with enhanced physicochemical attributes.

Keywords: functional foods; whipped cream; whey protein hydrolysate; probiotics; functionality; physicochemical

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1. Introduction

With the change in consumer preferences towards convenient, wholesome, and functional food products, milk and milk products offer numerous opportunities to develop a value-added range of products with their functional and bioactive properties. The functional foods global market will grow at a compound annual growth rate (CAGR) of 7.9% by 2026, of which dairy products hold a significant share being excellent delivery systems for bioactive ingredients [1]. Functional ingredients like probiotics pose challenges in maintaining their viability during product processing and storage conditions. Several extrinsic and intrinsic factors, including probiotic strain selection, inoculum preparation, oxygen stress, moisture content, pH, storage temperature, osmotic pressure, packaging material, food composition, and processing conditions like heat treatments, can decrease their viability and even cell death [2]. Encapsulation is an effective delivery system to improve the viability of the probiotics during these conditions [3]. These functional ingredients also tend to interfere with the physicochemical attributes of the matrix altering the rheological and textural properties.

A previous study of our lab developed a functional spray-dried conjugated whey protein hydrolysate-maltodextrin (WPH-MD) probiotic encapsulant using the common probiotic strains *Bifidobacterium animalis* ssp. *lactis* ATCC27536 (BB12) and *Lactobacillus acidophilus* ATCC4356 (LA5). This encapsulant showed high viable probiotic counts

(8.98 ± 0.02 $\log_{10}$CFU/g) and enhanced bioactive properties such as antioxidant, antihypertensive and antimicrobial properties with storage stability of 16 weeks under refrigerated conditions [4,5]. A novel range of value-added functional food products could thus be developed by utilizing the nutritional and bioactive properties of WPH-MD probiotic encapsulant. Based on the encapsulant stability, we hypothesized that the viscous matrix, like whipped cream, would be a suitable carrier without interfering with the product attributes.

Whipped cream is a complex oil-in-water food emulsion composed of a partial crystal network of fat globules, plasma serum proteins, and air bubbles entrapped by mechanical agitation [6–8]. Many previous studies have reported incorporation of probiotics in dairy fat-rich matrices. However, the addition of unencapsulated or free probiotics declined over the storage time and interfered with the product attributes [9–14].

In view of this, the current study was undertaken to develop whipped cream with the addition of a functional ingredient, WPH-MD-encapsulated probiotic formulation, followed by its characterization through physicochemical and microstructural analysis. The second objective focused on the storage stability studies of the WPH-MD-encapsulated probiotic whipped cream product under refrigerated conditions (4 °C) for 12 days.

2. Materials and Methods

2.1. Preparation of the Spray-Dried Conjugated WPH-MD-Encapsulated Probiotic Formulation

The spray-dried conjugated WPH-MD-encapsulated probiotics formulation was prepared according to the protocol described by [15]. The commercial strains BB12 and LA5 were obtained from ATCC and were cultured in MRS (De Man, Rogosa, and Sharpe) broth (BD Difco™, Fisher Scientific, Waltham, MA, USA). The cell suspensions were prepared at a concentration of 9–10 logs CFU/mL. Then, the conjugated WPH-MD solution was prepared as per the protocol described by [4,5,15]. The WPH10 and maltodextrin (DE10) (Sigma Aldrich, St. Louis, MO, USA; Fisher Scientific, Waltham, USA) solution mixture was prepared in three stages: solubilization (25 °C, 2 h), hydration (4 °C, 18 h) and heating (90 °C, 24 h). The prepared solution was then adjusted to pH 8.2 followed by inoculation with the prepared BB12 and LA5 cell suspensions. The conjugated mixture was spray dried using a NIRO spray dryer in Davis Dairy Plant, SDSU. The spray-dried formulation was stored at 4 °C for further use.

2.2. Preparation of WPH-MD-Encapsulated Probiotic Whipped Cream Variants

Raw cream was sourced from the Davis Dairy Plant, SDSU with a fat content of 38%. It was divided into 100 g portions to prepare the whipped cream variants by incorporating the WPH-MD-encapsulated probiotic formulation. It was then subjected to batch pasteurization (66 °C, 30 min) as per the PMO guidelines (Grade "A" Pasteurized Milk Ordinance, 2017 revision) in a shaking water bath (BS-06 Lab Companion, Billerica, MA, USA). The pasteurized cream was then homogenized at 50 °C and $1000\times$ *g* rpm for 1 min. The homogenized cream was further stored at refrigerated conditions for 24 h to increase the fat globules' partial coalescence before the whipping process [16,17].

After cooling, the hydrated cream samples were whipped in a manual churner. Firstly, a portion of the cream was whipped for different time durations to obtain the maximum overrun, and then, the remaining sample was whipped at the maximum overrun. The formation of high peaks indicated the endpoint of whipped cream. After the whipping process, the samples were spiked at 0.1% and 1% *w*/*w*. Control whipped cream was prepared with the same whipping conditions without the addition of the WPH-MD-encapsulated probiotic formulation. The formulation was gently mixed, then stored at refrigerated conditions (4 °C) for further analysis.

2.3. Viable Probiotic Counts of 0.1% and 1% Whipped Cream Variants

2.3.1. Experimental Design

Both variants were characterized through viable probiotic counts enumeration, physicochemical and microstructural analysis. Three independent trials were conducted. Each

trial was performed in triplicate. ANOVA were applied to differentiate between mean values. The analysis was performed using MS Excel and R-studio version 2022.02.3 (Build 492).

2.3.2. Enumeration of the Viable Probiotic Counts

The viability of the probiotic organisms LA5 and BB12 were enumerated by standard pour plate technique. Firstly, the viable probiotic counts of the spiked conjugated solution and the probiotic encapsulant powder before and after spray drying were enumerated. Then, viable probiotic counts of 0.1% and 1% w/w levels in whipped cream variants were enumerated as per the protocol described by [15]. Dilutions were prepared using PBS (phosphate buffer saline). L-cysteine (0.05% w/v) was added for the BB12 strain whereas LA5 was directly plated with MRS agar using the pour plate technique. The plates were incubated anaerobically at 37 °C for 72 h using a gaspak system [18]. From the MRS (LA5) and MRS with L-cysteine (BB12) plates, representative colonies were isolated and characterized through matrix-assisted laser desorption ionization time of flight (MALDI-TOF).

2.4. Physicochemical Analysis to Assess the Influence of the WPH-MD-Encapsulated Probiotics Formulation on the Whipped Cream Functionality

2.4.1. Effect of the WPH-MD-Encapsulated Probiotics Formulation on the Overrun of Whipped Cream Variants

Overrun is the weight of air entrapped per weight of whipped cream. Overrun was calculated by relating the volume and density of the cream to the weight before and after whipping. The analysis was performed by filling a tube to the set volume of 50 g followed by measuring the weight of the cream before and after whipping, and was expressed as [7,17]:

$$Overrun = \frac{M_1 - M_2}{M_2} \times 100\%$$

where
M_1 is the weight of cream (g) before whipping, and
M_2 is the weight of whipped cream (g).

2.4.2. Effect of the WPH-MD-Encapsulated Probiotics Formulation on the Foam Stability of Whipped Cream Variants

Foam stability is a quantitative index of whipped cream stability regarding drainage percentage under gravity. For this, 20 g of whipped cream sample was taken on a Buchner funnel, followed by incubation at 25 °C for 3 h with a calibrated beaker to collect the serum. The weight of the serum was calculated with the following expression [7,17].

$$Foam\ Stability(\%) = \frac{Wt.\ of\ serum\ collected}{Wt.\ of\ whipped\ cream} \times 100$$

2.4.3. Effect of the WPH-MD-Encapsulated Probiotics Formulation on the Partial Coalescence of Whipped Cream Variants

The amount of non-emulsified fat in the whipped cream samples was determined according to the methodology by [7]. The solvent dye technique was used to determine the partial coalescence of fat. Oil Red O solution was prepared with soybean oil at 0.15 mg/g. The solution was stirred overnight for homogeneous mixing of the solution. The solution's absorbance was measured at 520 nm in a UV–visible spectrophotometer. Soybean oil was used as the blank. The solution was mixed at a ratio of 1:2 with the whipped cream sample, followed by gentle centrifugation at 3500 rpm for 30 min. The free fat is dissolved in the colored oil layer floating above the cream surface while other fat remains in the droplet form. This is known as the diluted dye solution, which was transferred to a Petri dish to

calculate the mass fraction of oil in the cream. The absorbance of the diluted dye solution was measured at 520 nm. The amount of free fat was calculated using the below equation:

$$\varnothing_d = \frac{m_0(\alpha - 1)}{m_e \varnothing},$$

where
$\varnothing_d$ is the mass fraction of non-emulsified fat,
m_0 is the amount of Oil Red O solution (g),
m_e is the amount of cream (g),
α is the ratio of absorbance of Oil Red O solution before and after centrifugation, and
$\varnothing$ is the mass fraction of oil in the cream.

2.4.4. Effect of the WPH-MD-Encapsulated Probiotics Formulation on the Rheological Properties of Whipped Cream Variants

The rheology was performed using Anton Par Rheometer (MSR 92) with the following conditions: 40 mm parallel plate system at a frequency range of 0.1–100 Hz, shear stress of 10 Pa, and 0.5% strain at a temperature of 25 °C. The samples were equilibrated to room temperature. The loss and storage modulus values and graphs were obtained for the 0.1% and 1% whipped cream variants [7].

2.4.5. Effect of the WPH-MD-Encapsulated Probiotics Formulation on the Color Parameters of Whipped Cream Variants

The color parameters of the whipped cream variants were measured using a colorimeter device (Minolta Calorimeter). Hunter L* (100 = perfect white;0 = black) for lightness, a* (+ = redness; − = greenness), and b* (+ = yellowness; − = blueness) values were measured. The sample was poured into a small dish, and the flashlight recorded the readings. The average values of L*, a*, and b* were obtained for 0.1% and 1% whipped cream variants and the control sample with no encapsulant addition [19].

2.4.6. Interaction of WPH-MD Probiotic Encapsulant with the Whipped Cream Matrix through Confocal Laser Scanning Microscopy (CLSM)

Mounting Protocol

The confocal laser scanning microscopy (CLSM) (Olympus FV1200 Scanning Confocal Microscope, Functional Genomics Core Facility, SDSU) dual-stain technique was performed to study the interaction of fats in whipped cream and proteins in the encapsulant. Nile Red (NR) dye was used to stain the fat globules in the whipped cream, and fluorescein isothiocyanate (FITC) was used to stain the protein bodies in the whipped cream. NR (0.02%) and FITC (0.02%) were prepared in PEG-200 and acetone solvents, respectively. The whipped cream samples were stained with 1 mL of NR and FITC before whipping, and the stained cream was whipped with the maximum overrun. The whipped cream was then spiked with 0.1% and 1% w/w probiotic encapsulant, and the stained samples were placed on a microscopic slide using sterile inoculation loops. The samples were covered with glass slides and left at room temperature for 30 min for equilibrium. The control slide contained no probiotic encapsulant addition [20].

Imaging Protocol

The confocal micro-images were obtained with the following conditions. 60× magnification was used using an Ar laser. The fluorescence light emitted by NR and FITC was detected at 595–648 nm and 500–536 nm, respectively. The microscopic slides were adjusted in white light, followed by changing the settings to live images with the Ar fluorescence laser. Images were captured using fine and coarse focus adjustments for all microscopic slides [20].

2.5. Storage Studies of the WPH-MD-Encapsulated Probiotics Whipped Cream at Refrigerated Conditions

2.5.1. Experimental Design

For the storage stability studies, 1% whipped cream samples were prepared. For microbiological analysis, the controls were prepared with unencapsulated or free probiotic cells of BB12 and LA5. All the samples were stored at refrigerated conditions (4 °C). Samples were pulled out at a frequency of 0, 4, 8, and 12 days to enumerate the viability. The experiment was performed in triplicate. ANOVA was applied to differentiate between mean values. The analysis was performed using MS Excel and R-studio version 2022.02.3 (Build 492).

2.5.2. Enumeration of Viable Probiotic Counts

The viable probiotic counts were enumerated using the standard pour plate technique. The diluted samples were plated on MRS Agar (LA5) and MRS agar with 0.05% L-cysteine for BB12 strain under the anaerobic incubation conditions (37 °C, 72 h) using gaspak sachets.

2.6. Statistical Analysis

Three independent trials were conducted. Means were compared using one-way and two-way analysis of variance (ANOVA) using MS Excel and R-studio version 2022.02.3 (Build 492). Differences were considered significant at $p < 0.05$.

3. Results and Discussion

3.1. Viable Probiotic Counts in 0.1% and 1% Whipped Cream Variants with the Incorporation of the WPH-MD-Encapsulated Probiotic Formulation

Incorporating functional ingredients such as probiotics in a food matrix is challenging due to their interference with the physicochemical characteristics of the product matrix. Key strategies such as microencapsulation are gaining attention to protect the ingredients from extrinsic and intrinsic factors [21].

This study prepared 0.1% and 1% whipped cream variants by incorporating the WPH-MD-encapsulated probiotic formulation. Table 1 presents the viability of probiotic organisms (LA5 on MRS agar and BB12 on MRS agar with L-cysteine) in 0.1% and 1% whipped cream variants.

Table 1. Viable probiotic counts (log_{10}CFU/g) of whipped cream variants on MRS (LA5) and MRS with L-cysteine (BB12) agar.

Whipped Cream Variants	Viability of LA5 (MRS Agar)	Viability of BB12 (MRS + L-Cysteine Agar)
0.1% whipped cream variant	6.53 ± 0.45^{aA}	6.41 ± 0.39^{aA}
1% whipped cream variant	7.38 ± 0.26^{bB}	7.25 ± 0.56^{bB}

Means ± SE accompanied by the lowercase (a, b) represents column-wise and uppercase; (A, B) represent row-wise comparison using ANOVA analysis ($p \leq 0.05$).

The 1% whipped cream variant showed significantly higher viability of probiotic organisms LA5 (7.38 ± 0.26 log_{10}CFU/g) and BB12 (7.25 ± 0.56 log_{10}CFU/g). In comparison, the 0.1% whipped cream variant showed the viability of 6.53 ± 0.45 log_{10}CFU/g and 6.41 ± 0.39 log_{10}CFU/g for LA5 and BB12, respectively. Both the variants showed a minimum of 6 logs incorporation in the final product thus meeting the FAO/WHO recommendations for the probiotic concentration [22]. This could be due to the enhanced protection provided through encapsulation and the suitability of whipped cream matrix. Other factors could be the complex colloidal structure of whipped cream, high total solids, and multiphase composition providing further protection to the bacteria [23].

Many previous studies have reported the suitability of viscous matrices, including that of cheeses such as cheddar, cottage, pasta filata, minas fresh, and others, for incorporating some probiotic organisms [9–14]. Other fat-rich matrices, such as ice cream, have also

been reported as a potential delivery system of probiotics maintaining satisfactory cell viability of 6–7 logs CFU/g [24]. An earlier study from our lab developed a probiotic butter using the whey protein hydrolysate-maltodextrin-encapsulated probiotics formulation. The study provided a proof of concept of utilizing fat-rich matrices like that of butter for the probiotic's incorporation. The probiotics LA5 and BB12 retained their viability of over 5 and 4 logs CFU/g during storage conditions in unsalted and salted butter variants, respectively [15].

In our study, LA5 and BB12 were added at the ratio of 1:1 during the probiotic encapsulant preparation and results also demonstrated insignificant differences between the LA5, and BB12 counts in the 0.1% and 1% variants. This further demonstrated that the encapsulation protected both strains regardless of some differences in their sensitivities towards extrinsic and intrinsic factors.

Overall, the whipped cream matrix was identified as a suitable carrier for the WPH-MD-encapsulated probiotics formulation, maintaining the probiotic viability of a minimum of 6 logs CFU/g in the final product. Moreover, the 1% whipped cream variant showed significantly higher viability of the organisms LA5 and BB12 than the 0.1% variant.

3.2. Effect of the WPH-MD-Encapsulated Probiotics Formulation on the Overrun of Whipped Cream Variants

Overrun is one of the most common parameters indicating the stability of whipped cream in terms of the gas holdup or the maximum air incorporated during the whipping process. A higher overrun indicates higher foam stability, and textural properties.

As higher overrun indicates higher foam stability, individual sample was whipped to their maximum overrun to attain the higher stability. As shown in Table 2, the 0.1% whipped cream variant showed an overrun of 145.43 ± 0.68%, and the 1% whipped cream variant had an overrun of 154.76 ± 0.29%. However, the control (with no encapsulant addition) exhibited a maximum overrun of 160.14 ± 0.16%. A higher overrun indicates higher stiffness, foam stability, and textural properties.

Table 2. Overrun (%) of 0.1% and 1% whipped cream variants.

Whipped Cream Variants	Overrun (%)
0.1% whipped cream variant	145.43 ± 0.68 [a]
1% whipped cream variant	154.76 ± 0.29 [a]
Control (with no WPH-MD-encapsulated probiotics formulation)	160.14 ± 0.16 [b]

[a,b] values followed by different superscripts represent column-wise comparison and are significantly different ($p < 0.05$).

The 0.1% and 1% whipped cream variants showed significantly decreased overrun compared to the control with no WPH-MD-encapsulated probiotics formulation addition.

The slightly decreased overrun values of 0.1% and 1% whipped cream variants could be due to the formation of a viscoelastic layer around the partially coalesced fat globules and air bubbles. These interfacial layers are known to provide stability and resistance to the colloidal structure of whipped cream. These layers also play an important role in incorporating air bubbles. This can further lead to increased viscosity, making the emulsion less sensitive to partial coalescence, resulting in a decline in overrun [16].

Some of the previous studies reported comparable findings in different whipped cream variants with protein-based ingredients. One of the studies focused on the effect of a protein-polysaccharide complex containing sodium caseinate and carboxymethyl cellulose on the physicochemical properties of low-fat whipped cream. The study reported a decrease in overrun values with an increase in CN (protein-polysaccharide complex) concentrations (>0.5%) [16]. Another study also reported similar results by incorporating sorbitan monostearate, an emulsifier, on whipped cream's physicochemical and whipping properties. It also indicated decreased overrun with the increase in viscosity due to the

presence of proteins (0.9%) in the serum phase. This formed a thick viscoelastic layer surrounding the fat globules and restricted air incorporation [8].

Both 0.1% and 1% addition showed a decline in the overrun, demonstrating the possibilities of enhanced interaction at the interface representing increased structural stability. These results were further analyzed with the confocal micro-images.

3.3. Effect of the WPH-MD-Encapsulated Probiotics Formulation on the Foam Stability of Whipped Cream Variants

Foam stability measures serum loss or cream drainage at ambient temperature. Several factors, including the viscoelastic properties of interfacial films, partial coalescence of fat globules, and a continuous aqueous phase, govern the foam stability.

As shown in Figure 1, the 1% whipped cream variant showed the lowest cream drainage of 12.07 ± 0.82% compared to the 0.1% variant, and the control (with no formulation addition) with a serum loss of 18.08 ± 0.57% and 14.57 ± 0.29%, respectively. Lower cream drainage or serum loss represents increased stability. The results indicated enhanced stability with the addition of the WPH-MD encapsulant.

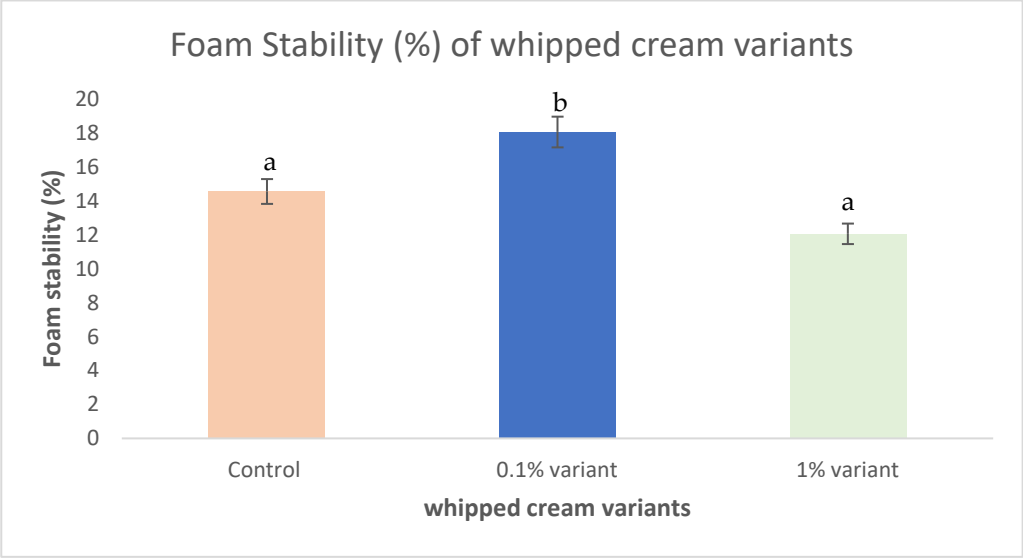

Figure 1. Foam stability (%) of whipped cream variants. [a,b] values followed by different superscripts are significantly different ($p < 0.05$).

Foam could collapse in different forms, such as serum loss from the colloidal structure, air bubbles coalescence, and large bubbles' presence leading to a disproportionate structure. Serum loss occurs due to the difference between the inside and outside air bubbles pressure. Air bubbles have a higher intensity of pressure than the external pressure of the liquid, leading to the drainage of this liquid into the air bubbles spaces. Surface-active compounds like whey proteins might create a viscoelastic layer at the O/W interface that prevents foam collapse. The proteins could result in an orderly arrangement of fat globules and air bubbles at the interface, preventing air cell migration. This further results in the appropriate partial coalescence of fat globules, emulsion stability, higher water-holding capacity, and reduced serum loss [16].

Based on the results, whey proteins present in the encapsulant with its surface-active properties might have resulted in an orderly arrangement of fat globules and air bubbles, further improving the partial coalescence of the fat globules. The increased viscosity resulted in decreased overrun further leading to increased foam stability, indicating the stabilized whipped cream structure.

Some of the previous studies also attempted to modify the whipped cream's physicochemical properties. The studies showed increased foam stability by adding surface-active molecules proteins, protein-polysaccharide complexes, and food-grade emulsifiers like

seed or guar gum. A study showed an increase in foam stability by incorporating milk fat globule membrane protein (MFGMP), illustrating an orderly arrangement of fat globules at the interface and higher water-holding capacity. It also reported a directly proportional relationship between foam stability and MFGMP concentration [7]. Another study revealed comparable results on foam stability with the addition of modified whey protein concentrate [17]. A study observed the impact of a protein-polysaccharide complex on the whipped cream attributes. The study reported the lowest serum loss by adding a food-grade seed gum representing the formation of viscoelastic layers around the fat globules, enhanced viscosity, and reduced partial coalescence [16]. Another study revealed that the enhanced interactions led to an increase in foam stability with reduced serum loss. Although a different study focused on the impact of ultrasound and cavitation on the whipping cream properties, the study revealed comparable interactions and increased foam stability results [25].

Overall, the results demonstrated increased foam stability of the 1% whipped cream variant with the addition of WPH-MD probiotic encapsulant owing to the enhanced interactions of whey proteins and fat globules at the interface.

3.4. Effect of the WPH-MD-Encapsulated Probiotics Formulation on the Rheological Properties of Whipped Cream Variants

Elastic or storage modulus (G′) indicates the structure stiffness depending on the 3-D colloidal network and bond strength formed by the air bubbles, partially coalesced fat globules, and proteins at the interface [25]. Elastic or storage modulus (G′) and loss modulus (G″) were calculated at a frequency range of 0.1–100 Hz with a shear rate of 0.5%.

Table 3 represents the frequency sweep measurement of 0.1% and 1% whipped cream variants.

Table 3. Storage (G′) and loss modulus (G″) of 0.1% and 1% whipped cream variants.

Whipped Cream Variants	G′ (Pa)	G″ (Pa)
0.1% whipped cream variant	$13,122.87 \pm 1.86^{a}$	2626.77 ± 1.75^{A}
1% whipped cream variant	$14,585.73 \pm 1.17^{b}$	3004.32 ± 1.25^{B}
Control (with no WPH-MD-encapsulated probiotics formulation)	9034 ± 1.92^{c}	2148.07 ± 1.27^{C}

$^{a–c, A–C}$ values followed by different superscripts represent column-wise comparison and are significantly different ($p < 0.05$).

The storage modulus was more significant than the loss modulus for all the whipped cream samples (0.1% and 1% variants and control with no formulation addition). The results indicated the behavior of a weak gel system. Both parameters (storage and loss modulus) increased with the frequency and concentration of the WPH-MD encapsulant.

The whipped cream variant with 1% *w*/*w* WPH-MD encapsulant showed significantly higher G′ and G″ than the 0.1% whipped cream variant. This observation demonstrated increased rigidity of the whipped cream structure with increased concentration of the WPH-MD encapsulant [7]. This could be attributed to the enhanced molecular interactions between the WPH-MD encapsulant and whipped cream structure, increasing serum viscosity and resisting further shearing of the whipped cream [6]. The whipping process includes the primary stabilization of air cells through the adsorption of native proteins, that is, whey proteins and beta-caseins forming a protein air-serum interface. Fat droplets are accumulated on the protein-stabilized interfacial surface. Lastly, the partial coalescence of fat globules leads to a three-dimensional network providing rigidity and stability to the whipped cream structure [26].

These results are consistent with the other studies that showed improved rheological behavior with the addition of fat milk globule membrane proteins in whipped cream [6]. The results indicated solid-like behavior with an increase in storage and loss modulus. Previous literature has also reported improved rheological behavior with the addition of

polysaccharides. The study showed an increase in G′ and G″ with adding xanthan gum at low concentrations (0–0.8%) in the ice cream mix [27].

Therefore, surface-active substances like whey protein hydrolysates could enhance interactions between fat globules and whey proteins, providing strength to the whipped cream. Hence, these interactions will likely increase the rheological parameters, including storage and loss modulus. The 1% whipped cream variant showed significantly higher rheological parameters as compared to the 0.1% variant.

3.5. Effect of the WPH-MD-Encapsulated Probiotics Formulation on the Partial Coalescence of Whipped Cream Variants

Partial coalescence determines the stability of foam in products like whipped cream. The data presented in Figure 2 showed that the 1% whipped cream variant had the highest partial coalescence, with 36% compared to the 0.1% whipped cream variant.

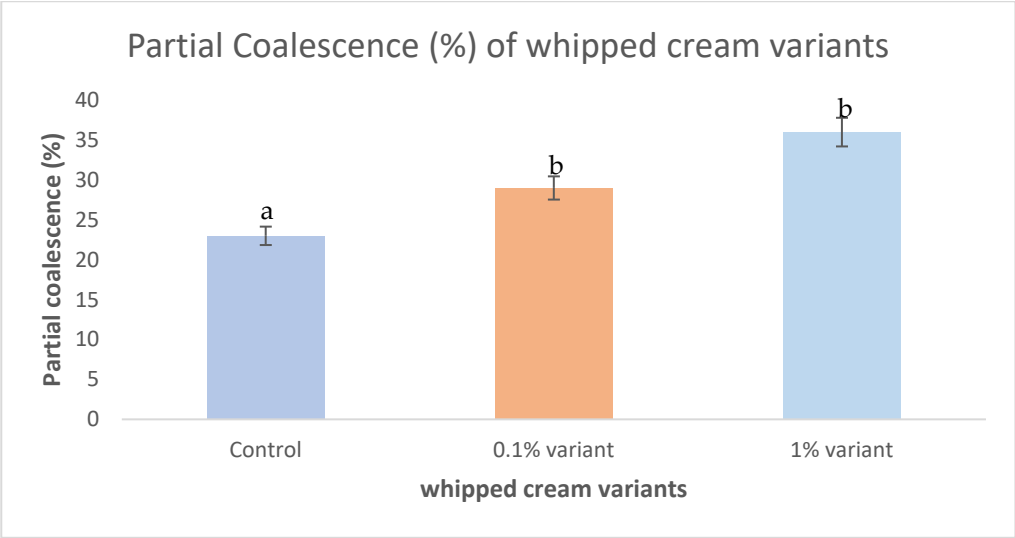

Figure 2. Effect of WPH-MD probiotic encapsulant on the partial coalescence of whipped cream variants. [a,b] values followed by different superscripts are significantly different ($p < 0.05$).

Higher values of partial coalescence represent higher stability of whipped cream. This could be due to whey proteins in the WPH-MD encapsulant, known for its functional and amphiphilic properties. Additionally, the results showed an increase in partial coalescence with the increase in the concentration of WPH-MD encapsulant. This can be further related to the results of foam stability. Microstructures of spiked whipped cream samples also exhibited enhanced stability due to the deposition of whey protein hydrolysate in the encapsulant. The protein-covered fat droplets incorporate more air into the whipped cream, allowing the aggregation of fat droplets to increase partial coalescence. Although the increase in partial coalescence provides stability to the whipped cream structure, an abrupt increase can also destabilize the structure [7].

The study found similar results for the partial coalescence trend with the addition of MFGMP levels in whipped cream. It showed the highest stability in the 5% spiked whipped cream variant compared to the 1% variant. This showed the positive correlation of the probiotic encapsulant with the whipped cream stability. Although we found a moderate increase in the values of partial coalescence, the 1% whipped cream variant showed higher partial coalescence as compared to the 0.1% whipped cream variant.

Overall, the 1% whipped cream variant showed the highest partial coalescence compared to the 0.1% variant and control with no encapsulant addition. The foam stability, rheological properties, and overrun results indicated a similar interpretation of increased partial coalescence resisting the air incorporation, resulting in improved interfacial interactions.

3.6. Effect of the WPH-MD-Encapsulated Probiotics Formulation on the Color Parameters of Whipped Cream Variants

The color parameters L*, a*, and b* of the whipped cream variants changed significantly, as presented in Table 4. The results revealed that the 1% whipped cream variant showed significantly higher values of a* and b* compared to the 0.1% whipped cream variant and control (with no encapsulant addition).

Table 4. Color parameters of 0.1% and 1% whipped cream variants.

Color Parameters	0.1% Whipped Cream Variant	1% Whipped Cream Variant	Control (with No Formulation)
L*	58.59 ± 1.59^{a}	63.90 ± 1.89^{a}	70.61 ± 1.95^{b}
a*	$0.77 \pm 0.04^{a'}$	$1.55 \pm 0.05^{b'}$	$0.51 \pm 0.03^{c'}$
b*	$9.6 \pm 0.2^{a''}$	$10.97 \pm 0.24^{b''}$	$8.97 \pm 0.28^{c''}$

$^{a,b, a'–c', a''–c''}$ values followed by different superscripts are significantly different between the columns ($p < 0.05$).

The increase in b* values indicated the yellowish color of the samples [28]. This could be due to the golden-brown color of the spray-dried encapsulant spiked at 1% in the whipped cream. On the other hand, the L value decreased for the 1% whipped cream variant. However, the difference between the 0.1% and 1% variants was insignificant. The decreasing trend can be due to the decrease in whiteness with the encapsulant incorporation. The significant decrease in L* values and increase in a* and b* values highlighted the light scattering phenomenon. The presence of fat globules contributes to the yellow-color wavelength.

In contrast, the protein part represents the blue-color range, and the combination of proteins in the fat matrix indicates a dark-yellow or green-tinge-colored wavelength. This alternation in color indicates the change in dimensions of light scattering due to a change in particle size. Whey proteins have amphiphilic properties and surface-active properties that act as a stabilizer and could alter the surface interactions of the air and fat bubbles at the interfacial layer. Incorporating WPH-MD probiotic encapsulant resulted in similar results as observed through other physicochemical properties, including overrun, foam stability, and rheological parameters. The second factor can be the color of the WPH-MD probiotic encapsulant due to the formation of intermediate and advanced products during the Maillard reaction.

The study observed similar variations in the color parameters by incorporating a protein-polysaccharide formulation in a low-fat whipped cream matrix [16]. Therefore, whipped cream variants depicted the light scattering phenomenon that showed variations in L*, a*, and b* values.

3.7. Imaging Protein and Fat Globule Distribution through Confocal Laser Scanning Microscopy

The confocal laser scanning microscopy (CLSM) observed the interaction of the WPH-MD probiotic encapsulant with the fat globules in whipped cream. These microstructural images depicted changes in appearance characterized by fats and protein-polysaccharide connectivity.

As shown in Figure 3, all whipped cream samples, including control, 0.1%, and 1% variants, showed uniform distribution of spherical air cells, indicating a well-developed network of milk fat globules and plasma proteins. Proteins existed only in the plasma phase for the control with no encapsulant addition. In contrast, 0.1% and 1% whipped cream variants showed proteins in the lamella phase and at the oil-water interface. However, the 1% whipped cream variant showed a more uniform and thicker protein deposition around the air cell than the 0.1% variant, as shown in Figure 3 (b1 and b2) and (c1, c2, and c3) sections.

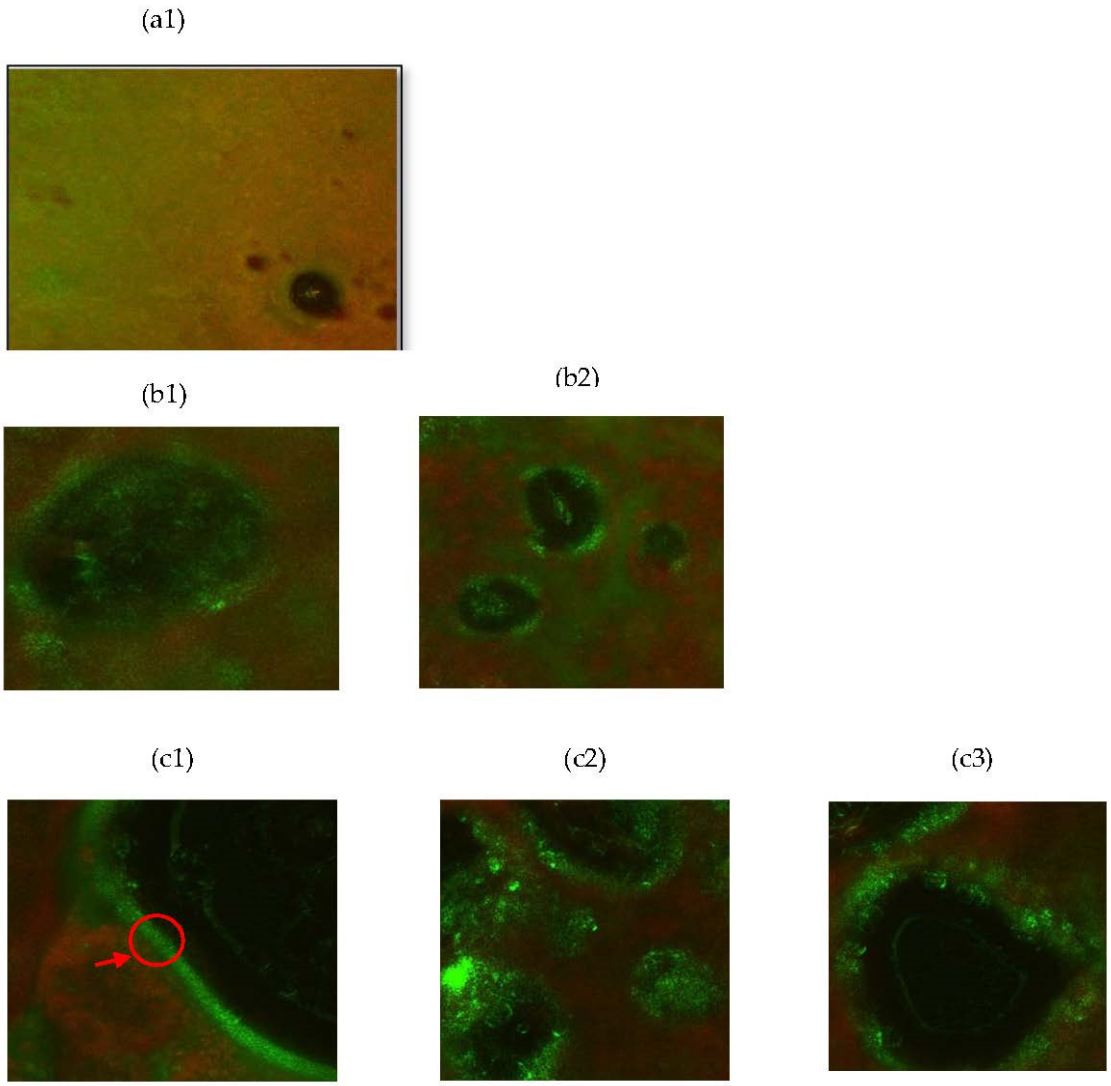

Figure 3. Confocal laser scanning micro-images of whipped cream variants at 60× magnification. (**a1**): control whipped cream with no WPH-MD encapsulant; (**b1**,**b2**): whipped cream with 0.1% WPH-MD probiotic encapsulant; (**c1**,**c2**,**c3**): whipped cream with 1% WPH-MD probiotic encapsulant. Black holes represent the air cells entrapped during whipping process. Red zone indicates the fat presence stained with NR dye and green zone indicates the whey proteins stained with FITC dye. Red arrow indicates the adsorbed WPH-MD layer at the interface surface of whipped cream.

Whipped cream contains many air cells with a diameter range of 20–50 μm. These air cells are unstable in a whipped cream structure and stabilized by a colloidal three-dimensional network of coalesced milk fat globules. Further partial coalescence results in irreversible aggregation that causes a continuous network of fat globules and air cells. However, this emulsion system is thermodynamically unstable, and hence emulsifiers can help stabilize this bubble mechanism by forming a viscoelastic layer of whey proteins around the partially coalesced fat globules [29].

Thus, 0.1% and 1% whipped cream variants showed improved foam stability, rheological properties, overrun values, and partial coalescence as compared to the control. This could be due to the deposition of protein bodies in the encapsulant at the O/W interface surrounding the air cells.

3.8. Viability of Probiotic Whipped Cream during Refrigerated Storage Conditions (4 °C)

Based on the data presented in Table 1, the 1% whipped cream variant exhibited significantly higher viability of probiotic organisms, LA5 and BB12, than the 0.1% variant.

Hence, storage stability studies were performed with a 1% whipped cream variant. Control was prepared with free or unencapsulated probiotics (LA5 and BB12).

As shown in Figure 4, whipped cream with encapsulated probiotics (LA5 and BB12) did not show a substantial decrease in their viability during storage. On the 12th day, the probiotic organisms (LA5 and BB12) retained approximately 6 logs CFU/g. On the other hand, control with unencapsulated probiotics showed a significant difference in the probiotic's viability (LA5 and BB12) at 0-, 4-, 8- and 12-day storage days. The probiotic counts dropped to approximately 5 logs CFU/g by the end of the storage time. The results demonstrated that the encapsulation protected the probiotics, LA5 and BB12, by retaining their viable cells during the storage.

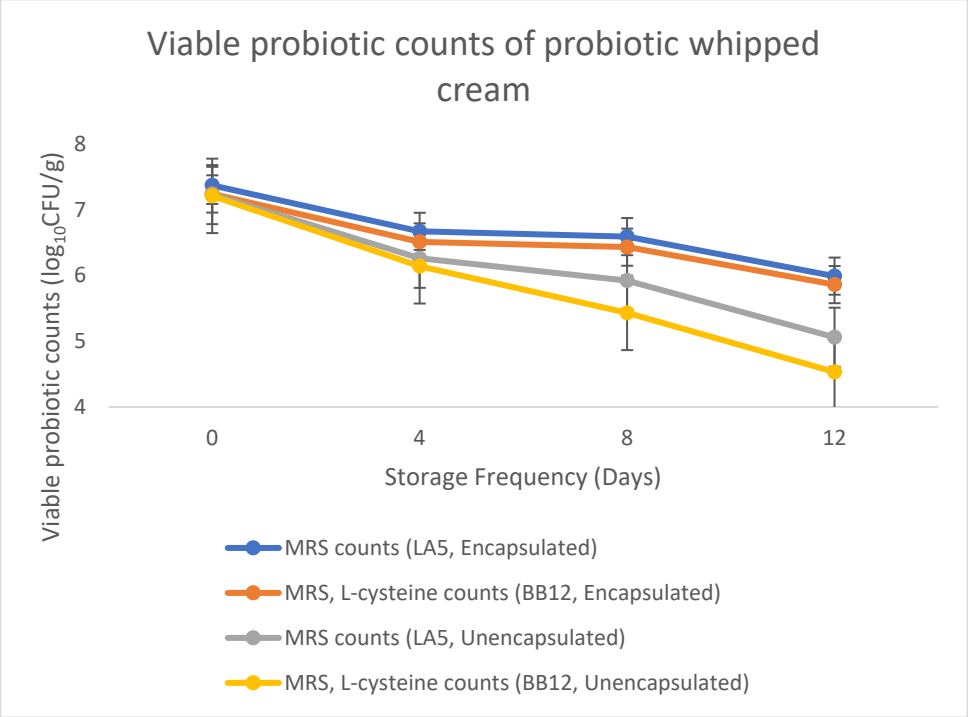

Figure 4. Viability (LA5 and BB12) of probiotic whipped cream during refrigerated storage conditions (4 °C).

Some of the previous studies have demonstrated comparable results in different food matrices with different experimental conditions. A few researchers have exploited whipped cream as a potential carrier for unencapsulated probiotic organisms. A study provided a proof of concept for using the cream as a potential probiotic carrier that showed probiotic counts higher than 6 logs CFU/g for 22 days. However, it impacted the chemical properties of the cream [30]. Others have reported ice cream, cream cheese, butter, and cheese as some of the dairy's fat-rich based potential carriers of free probiotic cells [31–34]. The study showed that dairy dessert supplementation with whey protein concentrate improved the *L. acidophilus* viability during storage [33]. This could be due to the presence of polysaccharide that further produces short-chain fatty acids with probiotics' metabolic activity resulting in a decrease in pH, creating a more favorable environment for the probiotic organisms [35].

Another important observation in our study was the ratio of LA5 and BB12 during the storage period. Encapsulated probiotics showed similar trend lines for the LA5 and BB12, retaining more than 6 logs CFU/g. On the other hand, control with unencapsulated probiotics showed a gap between their trend lines for both LA5 and BB12. This demonstrated that the encapsulation helped to maintain the LA5 and BB12 ratio close to 1:1, whereas the control showed a drop in their viability. While comparing LA5 with BB12, the latter showed significantly lower viability by the end of the storage period.

Overall, the 1% whipped cream variant retained probiotics viability of approximately 6 logs CFU/g for 12 days at refrigerated conditions. Therefore, whipped cream was observed to be a suitable carrier for the encapsulated probiotics with 12 days of storage stability, as evaluated in this study. To draw a broader conclusion, longer duration storage studies are recommended.

4. Conclusions

The rise in demand for bioactive and functional foods provides opportunities for the dairy industry to expand its product portfolio through value-addition by incorporating bioactive ingredients like probiotics and whey proteins in the dairy matrices. This study incorporated a previously developed conjugated spray-dried WPH-MD-encapsulated probiotics formulation into whipped cream. The encapsulant was spiked at 0.1% and 1% *w*/*w* levels, then characterized by viable probiotic counts and physicochemical and microstructural analyses. Of which, the 1% whipped cream variant exhibited significantly higher viability of LA5 and BB12 and improved physicochemical properties, including foam stability, storage, loss modulus, and partial coalescence, compared to the 0.1% variant. These observations were in-line with micro-images obtained through CLSM. Deposition of the WPH-MD-encapsulated probiotics formulation around the air cells at the O/W interface and partially coalesced fat globules indicated improved interfacial interactions. Hence, storage stability studies were performed with a 1% variant at refrigerated conditions for 12 days. The results showed probiotics viability of approximately 6 logs CFU/g over the storage time with no significant difference in the probiotic counts. However, future studies would be required to study the stability of the WPH-MD-encapsulated probiotics formulation in whipped cream matrix along with the physicochemical characteristics during extended storage period. To conclude, fat-rich matrices like whipped cream proved to be potential carriers for the encapsulated probiotics formulation like WPH-MD probiotic encapsulant. This would also provide bioactive benefits of probiotics and whey proteins and improved product attributes.

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