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Development and storage stability of probiotic-enriched processed paneer spread incorporated with microencapsulated *Lactiseibacillus rhamnosus* under refrigerated conditions

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Abstract

The incorporation of probiotics into dairy matrices has gained significant attention due to their established health benefits; however, maintaining the viability of probiotic cultures in foods during processing and storage remains a major challenge. This study aimed to enhance the stability and delivery of *Lactiseibacillus rhamnosus* through microencapsulation using maltodextrin as a wall material and to evaluate its incorporation in a processed paneer spread. The encapsulated probiotic was subsequently incorporated into processed paneer spread, and its physicochemical, microbiological, and sensory attributes were assessed during refrigerated storage. Parameters such as pH, moisture content, TBARS, total plate count, yeast and mold count and probiotic viability were assessed along with organoleptic properties. pH, DPPH, showed a decreasing trend along the storage days while as moisture, TBARS and microbiological counts showed increasing trend. Proximate analysis revealed significant differences among treatments along the storage days. Results indicated that encapsulation significantly improved the survival of *Lactiseibacillus rhamnosus* compared to free cells, maintaining viable counts above the recommended therapeutic level throughout the storage period upto 9 days. The addition of encapsulated probiotics had minimal adverse effects on the sensory characteristics of the paneer spread upto around 9 days, ensuring consumer acceptability. Furthermore, the product demonstrated enhanced functional properties, highlighting its potential as a value-added probiotic dairy food. In conclusion, maltodextrin-based microencapsulation is an effective strategy to improve probiotic stability in paneer spread, offering a promising approach for the development of functional dairy products with health benefits.

Keywords: Microencapsulation, probiotics, processed paneer spread, *Lactiseibacillus*, maltodextrin

Introduction

The concept of functional foods has shifted towards promoting gastrointestinal health and exploring its close connection with the gut microbial ecosystem [1]. The growing consumer interest in supplementary functional foods has significantly driven research and development in probiotics, leading to a deeper understanding of their positive effects on overall gut health. The Food and Agriculture Organization/World Health Organization [2] defined probiotics as “live microorganisms which when administered in adequate amounts (10^6 - 10^7 cfu/g) confer health benefits to the host”. Commonly, strains of *Bifidobacterium* and *Lactobacillus* are utilized in the production of probiotic-enriched foods [3]. These probiotic microorganisms have enormous positive health impacts, including balancing the gut microbiota and mucosal protection against pathogens, strengthening the immune system, reducing serum cholesterol, have anti-carcinogenic and antibacterial activity [4]. Multiple factors may affect the viability of probiotic bacteria in foods including the probiotic strain used, pH and storage temperature and processing conditions. These foods must maintain the viability of probiotic at ≥ 7 log CfU/mL at the time of consumption, so the probiotic benefits can be achieved [5].

To overcome this, microencapsulation technique has been utilized as an effective way to protect the bioactive compounds like probiotics from unfavourable processing conditions,

temperature, transportation and during the intestinal transit [6]. Microencapsulation is a protection technique in which probiotic strains as core are encapsulated in a selective secondary membrane called wall that encloses them inside. *Lactocaseibacillus rhamnosus* is one of the most widely recognized and clinically approved probiotic bacteria, frequently employed in the formulation of probiotic food products [7]. It is a Gram-positive, non-motile, non-sporulating rod-shaped facultative anaerobic lactic acid bacterium which is commonly used probiotic in the production of yogurts [8] commercial fruit drinks [9] and soy beverages [10].

The appropriate selection of wall materials plays a critical role in determining the encapsulation efficiency, particle morphology, and long-term stability of probiotics, as these materials influence the protection of microorganisms during processing, storage, and gastrointestinal transit. Maltodextrin, a polysaccharide derived from starch hydrolysis, offers low viscosity at high concentrations and enhances the physical stability and solubility of microcapsules, making it suitable for freeze-dried formulations (11). Furthermore, maltodextrin moderately acts as a prebiotic. Freeze drying is also known as "lyophilization," combines the critical freezing step and sublimation step, by first freezing the water and then convert the frozen water into vapor by sublimation under reduced pressure [12]. Microencapsulation by freeze drying involves dispersion of probiotic cells in an aqueous solution of wall material and freezing at low temperatures, followed by sublimation of the frozen water under vacuum [13].

Paneer spread is prepared from paneer by addition of different emulsifying salts and water. The product is blended to produce a thick, soft and smooth textured paste that can easily be applied to breads or as dressings and dips or can be relished as such. Also The addition of probiotics adds value to the nutritional and health aspects of the product as it provides readily available source of good bacterial flora so that probiotic benefits can be achieved. It rich in protein, calcium, and healthy fats. It supports muscle growth, bone health, and provides sustained energy, making it a good choice for children, vegetarians, and fitness-conscious individuals.

The present study was undertaken to evaluate the storage stability, microbial quality, and sensory attributes of processed paneer spread enriched with microencapsulated probiotic bacterial strain *Lactocaseibacillus rhamnosus* under refrigeration conditions.

Materials and Methods

Milk for preparation of paneer spread was procured from local market. Maltodextrin (with purity 99%) were purchased from Hi-Media Lab. Pvt. Ltd. (Mumbai, India). Lactobacilli de Man Rogosa Sharpe (MRS) agar and MRS broth, calcium chloride, sodium chloride, tween 80, glycerol (Mol. Wt.: 92.09, purity: 98%), lysozyme, phosphate buffer, trisodium citrate and phosphate were procured from Hi-Media Lab. Pvt. Ltd. (Mumbai, India). Distilled water was used to prepare the culture media and biopolymer dispersions. All chemicals and solvents used for research were of analytical grade.

Probiotic strain *Lactocaseibacillus rhamnosus* was acquired from National Dairy Research Institute, Karnal Haryana. Lyophilized cultures were activated in MRS broth. 2.2.2. Encapsulating material Maltodextrin (MD) was used for microencapsulation process. The concentration of wall material solution used as 2 g/100 mL (2%). The wall

material was added to the 100ml distilled water and stirred with a magnetic stirrer until it dissolved completely. Further, the obtained solution was heated to 80 °C for 30 min and then cooled to room temperature. Freshly harvested cell culture was added to a solution of wall material (20 mL of cell suspension was put into 30 mL of wall material solutions [14]. A magnetic stirrer was used to thoroughly mix the final mixture for 20 min at 1500 rpm. For freeze drying the solution, a single compartment freeze-drier (BUCHI, Lyovapor L-200, Switzerland) was used to produce lyophilized powder. Condenser temperature of – 85 °C and a chamber pressure of 0.074 Mbar was set for 28 h. The dehydrated probiotic powder obtained was put into glass vial with screw-on lid and kept at 4 °C until further use.

Preparation of probiotic processed paneer spread:

Paneer spread was prepared following standard method of sayar *et al.* (2016) [15] with slight modifications. The paneer was sliced and minced in a cheese mincer using 3.4mm diameter plate. The minced paneer was transferred into a blender and various ingredients as 1% Trisodium citrate and water @ 15-20% were added and blended completely. Probiotic powder was added @ 1g to the processed paneer spread. The whole mixture was uniformly mixed until a smooth consistency was achieved. The prepared processed probiotic paneer spread was subjected to further analysis.

Analytical procedures

The pH of the probiotic processed paneer spread samples was determined as per the method of Trout *et al.* (1992) [16] by using a digital pH meter (Model CP 901, Century Instruments Ltd., and India). The percentage moisture, protein, fat and ash of the product samples were evaluated as per the standard procedure of AOAC (2019) [17]. Antioxidant activity was assessed using DPPH-RSA assays as per Brand-Williams *et al.* (1995) [18]. The estimation of TBARS value was done by following the method of Witte *et al.* (1970) [19] with slight modifications. 10g aliquot of the sample was taken and triturated with 25 ml of pre-cooled 20% trichloroacetic acid (TCA). The contents were then quantitatively transferred into a beaker by rinsing with 25 ml of chilled distilled water, well mixed and filtered through ash less Whatman filter paper No. 1 (GE Healthcare, U.K). A quantity of 5 ml of TCA extract (filtrate) was mixed with 5 ml of TBA reagent (0.01M) in duplicate test tubes and placed in hot air oven for 30 minutes until colour develops. A blank sample was made by mixing 3 ml of 10% TCA and 3 ml of the TBA reagent. Absorbance (O. D.) was measured, at fixed wavelength of 532 nm using UV-VIS spectrophotometer (HITACHI, UV-Spectrophotometer U1800, Japan). The TBA value was calculated as mg malonaldehyde per kg of sample by multiplying O.D. value with k factor 5.2.

Microbiological analysis: The samples of paneer were subjected to microbiological analysis for standard plate count, coliform count and yeast and mold count and Probiotic cell count as per the method described by APHA (1984) [20]. The cfu/g was calculated by taking average number of colonies which was multiplied by reciprocal of the dilution factor and expressed as log10cfu/g of sample.

Sensory evaluation: The paneer samples were presented to a group of semi-trained panelists consisting of the scientists and post graduate students of the Division of Livestock Products Technology, FVSc & AH, SKUAST-Kashmir. The

samples were evaluated for various sensory parameters, viz. Appearance, Flavour, Body and texture and Overall acceptability as per the modified score card which was prepared on the basis of scores as adopted by Peryam and Pilgrim (1957) [21] based on a 9-point descriptive scale, where 9 = like extremely and 1 = dislike extremely. A score of six corresponding to desirable sensory characteristics of the products was taken as the minimum score for the acceptability of the product.

Statistical Analysis: The data generated was analysed statistically using the software of Statistical Package for Social Sciences (SPSS-Base 20.0). Analysis of variance was computed and significance of mean was tested at 5% level of significance.

Storage studies of probiotic processed paneer spread under refrigeration conditions:

The developed paneer spread was kept under refrigeration conditions and evaluated at 0, 3, 6, 9 and 12 days interval. The product along with the control was evaluated for the following parameters:

Results and Discussion

The proximate composition of processed probiotic paneer spread on the 0th day is presented in Table 1. Statistical analysis revealed slight significant differences ($p \leq 0.05$) among treatments for protein, fat, and ash contents.

Protein content

Protein content across treatments ranged from 18.77 to 18.81% with an overall mean value 18.80%. The Maltodextrin treatment recorded a slightly higher protein content (18.81%), whereas the control sample showed the lowest value (18.77%). The structural integrity of casein proteins remains unaffected by the addition of carriers such as maltodextrin. This aligns with previous findings that microencapsulation materials are typically inert in terms of protein contribution, especially when used at low concentrations (Krasakoopt *et al.*, 2003) [22]. Similar findings have been reported by Bhagwat *et al.* 2016 [23] who observed marginal increase in protein content in whey protein-maltodextrin encapsulated probiotic systems incorporated into dairy matrices. The encapsulating agents might also contribute to minor increase in total nitrogen due to microbial biomass addition hence marginally adding to total protein content (Anal and Singh 2007) [24]. However, the overall variation in protein content among treatments was minimal, suggesting that probiotic incorporation and encapsulation did not adversely affect the protein integrity of paneer spread.

Fat content

Fat content showed slightly significant variation among treatments, with values ranging from 19.07 to 19.22%. This might be attributed to dilution effect caused by incorporation of probiotic biomass and encapsulating agents and possible binding interactions between fat globules and encapsulating matrices, slightly altering extractable fat. Encapsulation with maltodextrin often leads to marginal compositional shifts due to solid content addition and water-binding capacity (Anal & Singh, 2007) [24]. The maintenance of fat content within an acceptable range is desirable, as fat contributes to sensory attributes such as mouthfeel and flavor in paneer spread. The findings are in line with Kailaspathy, 2006 [25] who reported that addition of probiotic

cultures may slightly alter proximate composition of yogurt. The negligible variation in protein and fat content observed among treatments is consistent with findings of Poopan *et al.* (2024), [26] who reported that maltodextrin-based encapsulation systems act as inert carriers and do not significantly alter macronutrient composition. Similarly, Gaba *et al.* (2023) [27] demonstrated that incorporation of encapsulated probiotics results in only minor compositional changes attributable to dilution effects rather than biochemical interactions.

Ash content

Ash content, which reflects the mineral composition of the product, also differed significantly among treatments. The MD treatment recorded the lowest ash content (1.707%), as compared to free cells and control sample. Increased ash in free-cell samples may result from mineral contribution of microbial biomass while as, Reduced ash in maltodextrin samples could be due to Dilution of mineral fraction as Maltodextrin being a low-mineral carbohydrate carrier. Such variations are consistent with reports that encapsulation materials can modulate mineral content depending on their composition and concentration (Heidebach *et al.*, 2012) [28]. These results suggest that encapsulation materials not only influence macronutrient composition but may also impact mineral retention in probiotic dairy products. Overall, the proximate analysis demonstrates that incorporation of probiotics using different encapsulation strategies did not compromise the nutritional quality of paneer spread.

Table 1: Proximate analysis of processed probiotic paneer spread under refrigeration conditions ($4 \pm 1^\circ\text{C}$) (0th Day):

Treatment	Protein	Fat	Ash
Control	18.81 $\pm$ 0.006 ^a	19.22 $\pm$ 0.004 ^a	1.715 $\pm$ 0.004 ^a
Free cells	18.78 $\pm$ 0.003 ^a	19.08 $\pm$ 0.024 ^b	1.748 $\pm$ 0.004 ^b
Maltodextrin	18.77 $\pm$ 0.006 ^a	19.07 $\pm$ 0.023 ^b	1.707 $\pm$ 0.003 ^c

All values are mean $\pm$ standard error of three replicates. Statistically significant ($p \leq 0.05$) difference between different treatments, within columns, are indicated with different lowercase letters (a-c).

pH

The pH values of processed probiotic paneer spread during refrigerated storage are presented in Table. A significant decline ($p \leq 0.05$) in pH was observed across all treatments as storage progressed from 0 to 12 days, indicating ongoing metabolic activity of probiotic microorganisms and gradual acid development in the product (Anwar *et al.*, 2025) [29]. The maltodextrin treatment showed a slower pH decline over progressive storage days from 6.32 on 0th day to 5.44 on 12th day while as the control sample exhibited a steady decrease in pH from 6.61 on the 0th day to 5.11 on the 12th day. This decline is consistent with normal post-processing biochemical changes in dairy products, including lactose fermentation and organic acid production (Wang *et al.*, 2025) [31]. The FC treatment demonstrated the most pronounced decrease in pH, reaching 4.67 by the 12th day. This rapid acidification suggests higher probiotic metabolic activity and possibly greater release of viable cells from the product matrix as no encapsulating agents were used to protect the probiotic cells during storage. While lower pH values can enhance microbial safety and shelf stability, excessive acidification may negatively impact sensory acceptability. The results are in line with Anwar *et al.* 2025 [29] and Yang *et al.* 2023 [30]. The combined results of

proximate composition and pH analysis demonstrate that probiotic encapsulation strategies significantly influence both the nutritional and storage characteristics of paneer

spread. These attributes are essential for developing probiotic dairy products with enhanced shelf life, stability, and consumer acceptability.

Table 2: pH values of processed probiotic paneer spread under refrigeration conditions (4±1 °C):

Treatment	0 th Day	3 rd Day	6 th Day	9 th Day	12 th Day
Control	6.613±0.005 ^{aA}	6.128±0.012 ^{bA}	5.80±0.004 ^{cA}	5.44±0.005 ^{dA}	5.11±0.004 ^{eA}
FC	5.678±0.005 ^{aB}	5.215±0.004 ^{bB}	5.06±0.004 ^{cB}	4.963±0.007 ^{dB}	4.67±0.004 ^{eB}
MD	6.320±0.006 ^{aC}	6.205±0.004 ^{bC}	6.02±0.006 ^{cC}	5.93±0.005 ^{dC}	5.44±0.004 ^{eC}

All values are mean ± standard error of three replicates. Statistically significant ($p \leq 0.05$) difference between different treatments, within rows, are indicated with different lowercase letters (a-e) and column wise upper case letters (A-C).

Moisture Content

The changes in moisture content of processed probiotic paneer spread during refrigerated storage are presented in Table 3. Moisture content is a critical quality parameter influencing texture, spreadability, microbial stability, and shelf life of dairy-based spreads. Statistical analysis revealed significant differences ($p \leq 0.05$) among treatments as well as across storage intervals, indicating that both encapsulation material and storage duration influenced moisture retention. At 0th day, the moisture content ranged from 56.97% to 59.63%, with MD treatments showing significantly higher moisture levels compared to control and FC. The higher moisture retention MD treatments can be attributed to the superior water-binding capacity of maltodextrin, which is known hydrocolloid capable of forming stable matrices that retain free and bound water within the food system. During storage, a gradual but significant reduction in moisture content was observed across all treatments up to the 12th

day. This decline may be attributed to moisture redistribution within the matrix, slight syneresis, and evaporation during refrigerated storage. However, MD-treated samples consistently maintained higher moisture levels throughout the storage period, suggesting improved structural integrity and reduced moisture loss. In contrast, FC-treated samples showed comparatively lower moisture content at all storage intervals, possibly due to weaker water-holding capacity of the encapsulation matrix. The findings are in agreement with Novelina *et al.* 2025^[32] who studied the effect of varying ratios of maltodextrin and gum Arabic on encapsulation of Dadih (traditional fermented milk) into instant probiotic milk and observed that higher gum Arabic concentration increased moisture content of instant milk. Also Higher moisture retention may result in a tough and rubbery texture, thereby improving the body, texture, and functional properties of cheese (Fox, 2017)^[33].

Table 3: Moisture content of processed probiotic paneer spread under refrigeration conditions (4±1 °C)

Treatment	0 th Day	3 rd Day	6 th Day	9 th Day	12 th Day
Control	59.11±0.003 ^{aA}	58.76±0.003 ^{bA}	59.83±0.09 ^{cA}	58.61±0.04 ^{dA}	57.63±0.007 ^{eA}
FC	56.97±0.003 ^{aB}	56.42±0.003 ^{bB}	56.71±0.10 ^{cB}	56.43±0.09 ^{dB}	55.85±0.003 ^{eB}
MD	59.63±0.007 ^{aC}	59.11±0.004 ^{bC}	59.01±0.11 ^{cC}	58.73±0.09 ^{dC}	58.15±0.003 ^{eC}

All values are mean ± standard error of three replicates. Statistically significant ($p \leq 0.05$) difference between different treatments, within columns, are indicated with different lowercase letters (a-d) and row wise upper case letters (A-D).

Lipid Oxidation

TBARS

Lipid oxidation, assessed by thiobarbituric acid reactive substances (TBARS), is an important indicator of oxidative stability and shelf life of dairy products. The TBARS values of processed probiotic paneer spread during refrigerated storage are presented in Table 4. A progressive and statistically significant ($p \leq 0.05$) increase in TBARS values was observed in all treatments with advancement of storage period, indicating gradual lipid oxidation.

At the 0th day, TBARS values were low across all treatments (0.310-0.323 mg MDA/kg), reflecting minimal oxidative deterioration immediately after processing. However, by the 12th day, a marked increase was observed, particularly in FC (1.45 mg MDA/kg) and control sample (1.26 mg MDA/kg) treatments. The higher TBARS values in these treatments suggest comparatively lower oxidative protection, possibly due to increased oxygen permeability or

reduced antioxidant activity within the matrix. Among all treatments, MD consistently exhibited significantly lower TBARS values throughout storage, reaching only 1.11mg MDA/kg on the 12th day. This enhanced oxidative stability may be attributed to the protective encapsulation which can act as an oxygen barrier and limit exposure of milk fat to pro-oxidative conditions. Additionally, the control sample showed intermediate TBARS values, indicating that probiotic incorporation without effective encapsulation may not sufficiently inhibit lipid oxidation during prolonged storage. Overall, TBARS values further confirmed the superior oxidative stability of MD-treated samples compared to other treatments. Similar results were observed by Moghadam *et al.* 2019^[34] who used nanoencapsulated fish oil with gum Arabic carrier in low fat probiotic fermented milk and showed lower peroxide value of the final product containing gum Arabic encapsulated fish oil.

Table 4: TBARS(mgMDA/kg) of processed probiotic paneer spread under refrigeration conditions (4±1 °C)

Treatment	0 th Day	3 rd Day	6 th Day	9 th Day	12 th Day
Control	0.314±0.003 ^{aA}	0.390±0.003 ^{bA}	0.501±0.003 ^{cA}	0.781±0.003 ^{dA}	1.26±0.003 ^{eA}
FC	0.323±0.004 ^{aB}	0.411±0.003 ^{bB}	0.612±0.003 ^{cB}	0.902±0.007 ^{dB}	1.45±0.003 ^{eB}
MD	0.310±0.004 ^{aA}	0.387±0.003 ^{cC}	0.431±0.003 ^{cC}	0.751±0.003 ^{dC}	1.11±0.003 ^{eC}

All values are mean ± standard error of three replicates. Statistically significant ($p \leq 0.05$) difference between different treatments, within rows, are indicated with different lowercase letters (a-e) and between column wise upper case letters (A-C).

DPPH-RSA (1, 1-diphenyl 2-picryl-hydrazyl radical scavenging activity)

Antioxidant activity is an important functional attribute of probiotic dairy products, as it reflects their potential to neutralize free radicals and reduce oxidative stress. In the present study, antioxidant capacity of processed probiotic paneer spread was evaluated using DPPH and ABTS radical scavenging assays, and the results are presented in Table 6. Both assays revealed statistically significant ($p \leq 0.05$) differences among treatments as well as across storage intervals, indicating the influence of encapsulation material and storage duration on antioxidant stability.

DPPH radical scavenging activity showed a gradual and significant decline in all treatments during refrigerated storage. On the 0th day, DPPH values ranged from 13.55 to 13.68%, with MD-treated samples exhibiting slightly higher antioxidant activity compared to control sample. The higher

initial DPPH activity in MD-samples may be attributed to the protective encapsulation effect which helps preserve bioactive compounds released by probiotic metabolism and limits oxidative degradation. Similar results were observed by Kumar *et al.*, 2025^[35] in probiotic processed cheese and Yang *et al.*, 2024^[36] in cheddar cheese.

As storage progressed from 0 to 12 days, a consistent reduction in DPPH scavenging activity was observed across all treatments. This decline can be attributed to degradation of antioxidant compounds, reduced metabolic activity of probiotics over time, and increased oxidative reactions within the dairy matrix. However, MD-treated samples maintained slightly higher DPPH values throughout storage, retaining 11.33% scavenging activity on the 12th day, compared to 11.06% in control samples. The results are well in line with Rao *et al.*, 2016^[37].

Table 5: DPPH-RSA (1, 1-diphenyl 2-picryl-hydrazyl radical scavenging activity) (4 ± 1 °C)

Treatment	0 th Day	3 rd Day	6 th Day	9 th Day	12 th Day
Control	13.55 $\pm$ 0.04 ^{aA}	12.66 $\pm$ 0.006 ^{bA}	12.02 $\pm$ 0.003 ^{cA}	11.67 $\pm$ 0.003 ^{dA}	11.06 $\pm$ 0.002 ^{eA}
MD	13.68 $\pm$ 0.03 ^{aA}	12.92 $\pm$ 0.003 ^{bB}	12.42 $\pm$ 0.003 ^{cB}	11.86 $\pm$ 0.004 ^{dB}	11.33 $\pm$ 0.003 ^{EB}

All values are mean $\pm$ standard error of three replicates. Statistically significant ($p \leq 0.05$) difference between different treatments, within rows, are indicated with different lowercase letters (a-e) and column wise upper case letters (A-B).

Sensory attributes

The sensory evaluation of processed probiotic paneer spread in terms of colour & appearance, flavour, body & texture, and overall acceptability during refrigerated storage is presented in Table 7. Statistical analysis revealed significant differences ($p \leq 0.05$) among treatments as well as across storage intervals, indicating that both probiotic incorporation method and storage duration influenced sensory quality.

Colour and Appearance

Colour and appearance scores showed a gradual and significant decline across all treatments with advancement of storage period. On the 0th day, MD-treated samples recorded the highest score (8.29), followed by control, and FC treatments. The superior appearance of MD-treated samples may be attributed to better moisture retention and uniform dispersion of microcapsules within the paneer matrix, resulting in a smoother and more appealing surface.

As storage progressed, a noticeable reduction in colour and appearance scores was observed in all samples, likely due to moisture loss and oxidative changes. However, MD-treated samples consistently retained higher scores throughout storage, maintaining a score of 5.28 even on the 12th day. In contrast, FC-treated samples showed the most pronounced decline, which may be related to higher acidification and structural instability. The results are in agreement with Nale *et al.*, 2018^[38].

Table 6: Colour and appearance scores of processed probiotic paneer spread under refrigeration conditions (4 ± 1 °C)

DAYS	Control	FC	MD
0	8.19 $\pm$ 0.09 ^{aA}	7.67 $\pm$ 0.11 ^{bA}	8.29 $\pm$ 0.10 ^{cA}
3 RD	7.38 $\pm$ 0.11 ^{aB}	6.90 $\pm$ 0.14 ^{bB}	7.62 $\pm$ 0.11 ^{cB}
6 TH	6.67 $\pm$ 0.10 ^{aC}	5.67 $\pm$ 0.10 ^{bC}	6.81 $\pm$ 0.08 ^{cC}
9 TH	6.05 $\pm$ 0.08 ^{aD}	5.27 $\pm$ 0.08 ^{bD}	6.29 $\pm$ 0.09 ^{cD}
12 TH	5.33 $\pm$ 0.10 ^{aE}	4.52 $\pm$ 0.11 ^{bE}	5.28 $\pm$ 0.12 ^{cE}

All values are mean $\pm$ standard error. Statistically significant ($p \leq 0.05$) difference between different treatments, within rows, are indicated with different lowercase letters (a-c) and column wise upper case letters (A-E).

Flavour

Flavour scores declined significantly ($p \leq 0.05$) during refrigerated storage in all treatments. Initially, MD-treated samples exhibited the highest flavour score (7.71), suggesting better preservation of desirable dairy flavours and minimal off-flavour development. This may be due to controlled probiotic metabolism and reduced lipid oxidation, as supported by lower TBARS values in MD-treated samples.

With increasing storage duration, flavour deterioration was evident, particularly in FC-treated samples, which showed the lowest scores throughout storage. Excessive acid production and accumulation of metabolic by-products may have contributed to sour or undesirable flavours in these samples. MD-treated samples maintained comparatively higher flavour scores even on the 12th day (5.19), indicating better flavour stability and consumer acceptability. The results are in agreement with Nale *et al.*, 2018^[38] who studied microbial viability, physicochemical and sensory properties of kefir microcapsules prepared using maltodextrin/Arabic gum mixes. Sensory evaluation indicated that reconstituted samples obtained from microencapsulated kefir samples had high scores for flavour, appearance and texture.

Table 7: Flavour scores of processed probiotic paneer spread under refrigeration conditions (4 ± 1 °C)

DAYS	Control	FC	MD
0	7.90 $\pm$ 0.11 ^{aA}	7.28 $\pm$ 0.10 ^{bA}	7.71 $\pm$ 0.10 ^{cA}
3 RD	7.23 $\pm$ 0.09 ^{aB}	6.76 $\pm$ 0.09 ^{bB}	7.52 $\pm$ 0.11 ^{cB}
6 TH	6.38 $\pm$ 0.10 ^{aC}	5.66 $\pm$ 0.10 ^{bC}	6.80 $\pm$ 0.08 ^{cC}
9 TH	5.61 $\pm$ 0.10 ^{aD}	4.66 $\pm$ 0.10 ^{bD}	6.28 $\pm$ 0.10 ^{cD}
12 TH	4.80 $\pm$ 0.11 ^{aE}	3.76 $\pm$ 0.09 ^{bE}	5.19 $\pm$ 0.08 ^{cE}

All values are mean $\pm$ standard error. Statistically significant ($p \leq 0.05$) difference between different treatments, within rows, are indicated with different lowercase letters (a-d) and column wise upper case letters (A-E).

Body and Texture

Body and texture scores exhibited a declining trend during

storage across all treatments, reflecting gradual structural changes within the paneer spread matrix. On the 0th day, MD-treated samples scored the highest (7.80), indicating superior spreadability, cohesiveness, and mouthfeel. This enhanced texture can be attributed to the water-holding capacity and stabilizing effect of maltodextrin, which helps maintain a soft and uniform structure.

During storage, MD-treated samples continued to demonstrate significantly better textural attributes compared to control and FC treatments. Even on the 12th day, MD-treated samples retained a relatively high score (6.43), whereas FC samples exhibited the lowest textural acceptability. Similar results were observed by Sandhya *et al.*, 2025^[39] who developed millet-based synbiotic paneer containing *Lactobacillus* and revealed the product had better texture and yield.

Table 8: Body and Texture scores of processed probiotic paneer spread under refrigeration conditions (4±1 °C)

DAYS	Control	FC	MD
0	7.38±0.10 ^{aA}	6.80±0.08 ^{bA}	7.80±0.13 ^{cA}
3 rd	6.71±0.10 ^{aB}	6.52±0.10 ^{bB}	7.47±0.09 ^{dB}
6 th	6.52±0.11 ^{aC}	5.76±0.09 ^{bC}	6.80±0.08 ^{dC}
9 th	6.23±0.09 ^{aD}	5.38±0.10 ^{bD}	6.66±0.10 ^{dD}
12 th	5.57±0.11 ^{aE}	4.67±0.10 ^{bE}	6.43±0.11 ^{dE}

All values are mean ± standard error. Statistically significant ($p \leq 0.05$) difference between different treatments, within rows, are indicated with different lowercase letters (a-c) and column wise upper case letters (A-E).

Overall Acceptability

Overall acceptability scores followed a pattern similar to individual sensory attributes. On the 0th day, MD-treated samples recorded the highest overall acceptability (8.19), followed by control samples, while FC samples showed the lowest score. The superior overall acceptability of MD-treated samples may be attributed to a balanced combination of favourable appearance, pleasant flavour, and desirable texture.

Although a gradual decline in acceptability was observed in all treatments during storage, MD-treated samples maintained significantly higher scores throughout the refrigerated period, retaining an overall acceptability score of 6.23 on the 12th day. In contrast, FC samples showed the most rapid decline, which may be associated with higher acidification, moisture loss, and oxidative changes. Similar results were observed by Novelina *et al.*, 2025^[41] & Saeed *et al.*, 2023^[40] who showed that the addition of encapsulated cells improved the sensory characteristics of probiotic instant milk and cheese respectively.

Table 9: Overall acceptability scores of processed probiotic paneer spread under refrigeration conditions (4±1 °C)

DAYS	Control	FC	MD
0	7.24±0.09 ^{aA}	6.76±0.09 ^{bA}	8.19±0.08 ^{cA}
3 rd	6.71±0.10 ^{aB}	6.42±0.11 ^{bB}	7.57±0.11 ^{cB}
6 th	6.28±0.10 ^{aC}	5.61±0.06 ^{bC}	7.18±0.09 ^{cC}
9 th	5.71±0.10 ^{aD}	5.28±0.10 ^{bD}	6.71±0.10 ^{cD}
12 th	4.85±0.11 ^{aE}	4.47±0.11 ^{bE}	6.23±0.09 ^{cE}

All values are mean ± standard error. Statistically significant ($p \leq 0.05$) difference between different treatments, within rows, are

indicated with different lowercase letters (a-c) and column wise upper case letters (A-E).

Microbiological Quality

Microbiological quality is a critical determinant of the safety, shelf life, and acceptability of dairy-based products. The microbiological parameters of processed probiotic paneer spread during refrigerated storage are presented in Table 8, including total plate count (TPC), coliform count, and yeast and mould count. Significant differences ($p \leq 0.05$) were observed among treatments and storage intervals, indicating the influence of encapsulation material on microbial stability.

Total Plate Count (TPC)

The total plate count increased significantly in all treatments with advancement of storage period. On the 0th day, TPC values were low across all treatments (1.32-1.58 log₁₀ cfu/g), reflecting good hygienic processing conditions. A gradual increase was observed during storage, reaching the highest values on the 12th day. Among treatments, MD-treated samples consistently exhibited significantly lower TPC values throughout storage, recording 4.86 log₁₀ cfu/g on the 12th day compared to 5.85 log₁₀ cfu/g in the control samples. The lower microbial load in MD-treated samples may be attributed to better control of moisture migration, lower acid stress, and improved structural stability of the product matrix, as previously evidenced by physicochemical parameters. The relatively higher TPC in control samples may be associated with the absence of effective encapsulation, leading to faster deterioration of product structure and favourable conditions for microbial proliferation. Both the control and MD-treated samples were well within the microbiological limits for total plate count in cheese spreads well upto 9th day as per FSSAI, 2011^[43].

Coliform Count

Coliforms were not detected in any treatment throughout the entire storage period. The absence of coliforms confirms good manufacturing practices, effective heat treatment, and hygienic handling during processing and storage. This result also indicates the microbiological safety of probiotic paneer spread up to 9 days of refrigerated storage in MD-treated samples. The results are well in agreement with Hossain *et al.*, 2024^[42].

Yeast and Mould Count

Yeast and mould growth was not detected up to the 3rd day of storage in any treatment. However, from the 6th day onwards, gradual growth was observed, increasing significantly with storage time. Control samples exhibited the highest yeast and mould counts during storage, reaching 2.97 log₁₀ cfu/g on the 12th day. In contrast, MD-treated samples showed significantly lower yeast and mould counts throughout storage, with values of 2.31 log₁₀ cfu/g on the 12th day. The lower fungal growth in MD-treated samples may be attributed to reduced oxygen permeability, lower lipid oxidation, and better maintenance of product structure. The resultant values are well within the standards for yeast and mold by FSSAI, 2011^[43] for milk and milk products and BIS^[44] (250 cfu/g).

Table 10: Microbiological parameters of processed probiotic paneer spread under refrigeration conditions (4±1 °C)

Treatments	0 th Day	3 rd Day	6 th Day	9 th Day	12 th Day
Total Plate Count (log ₁₀ cfu/g)					
Control	1.32±0.004 ^{aA}	2.03±0.005 ^{bA}	3.12±0.004 ^{cA}	4.11±0.004 ^{dA}	5.85±0.003 ^{eA}
MD	1.58±0.003 ^{aB}	2.13±0.005 ^{bB}	3.02±0.004 ^{cB}	3.97±0.003 ^{dB}	4.86±0.004 ^{eB}
Coliform Count (log ₁₀ cfu/g)					
Control	ND	ND	ND	ND	ND
MD	ND	ND	ND	ND	ND
Yeast and mould count (log ₁₀ cfu/g)					
Control	ND	ND	1.75±0.003 ^{aA}	2.12±0.003 ^{bA}	2.97±0.004 ^{cA}
MD	ND	ND	1.70±0.003 ^{aA}	1.92±0.004 ^{bB}	2.31±0.004 ^{cB}

Mean ± SE with different superscripts in a row wise (lower case alphabet) and column wise (upper case alphabet) differ significantly ($p < 0.05$). n = 6 for each treatment.

Probiotic cell count

The viability of probiotic cells, expressed as log₁₀ cfu/g, during refrigerated storage is presented in Table 5. Maintaining an adequate viable probiotic count throughout the product's shelf life is essential to ensure health benefits, with a minimum recommended level of 10⁶-10⁷ cfu/g at the time of consumption. At the 0th day, all treatments showed high probiotic counts, ranging from 7.91 to 8.14 log₁₀ cfu/g, indicating successful incorporation of probiotics into the paneer spread. MD-treated samples exhibited significantly higher initial counts compared to FC suggesting better protection of probiotic cells during processing. A gradual and statistically significant decline in probiotic cell counts was observed in all treatments over the 12-day storage period. This

reduction can be attributed to factors such as low storage temperature, decreasing pH, accumulation of organic acids, and limited nutrient availability. However, the rate of decline varied significantly among treatments. MD-treated samples demonstrated better probiotic viability throughout storage, retaining 5.97 log₁₀ cfu/g even on the 12th day, which remained slightly lower than the recommended therapeutic range (6 log₁₀ cfu/g). The results are in line with Kia *et al.*, 2018^[45] who evaluated effects of simultaneous supplementation of milk protein concentrate as texture modifier and microencapsulated *Lactobacillus paracasei*. The results showed that yogurt samples containing encapsulated *L. paracasei* showed lower post-acidification and higher viability. The results are also well in agreement with Sharifi *et al.*, 2021^[46].

Table 11: Probiotic cell count (log₁₀cfu/g) of processed probiotic paneer spread under refrigeration conditions (4±1 °C)

Treatment	0 th Day	3 rd Day	6 th Day	9 th Day	12 th Day
FC	7.912±0.002 ^{aA}	7.72±0.006 ^{Ba}	7.01±0.003 ^{cA}	6.26±0.003 ^{dA}	5.15±0.003 ^{eA}
MD	8.14±0.002 ^{aC}	8.07±0.002 ^{bC}	7.96±0.003 ^{cC}	6.91±0.003 ^{dC}	5.97±0.003 ^{Ec}

All values are mean ± standard error of three replicates. Statistically significant ($p \leq 0.05$) difference between different treatments, within columns, are indicated with different lowercase letters (a-c)

Conclusion

The present study demonstrates that microencapsulation of *Lactobacillus* spp. Using maltodextrin is an effective strategy to enhance probiotic stability and functionality in a paneer-based spread. Encapsulation provided a protective barrier against environmental and processing related stresses, significantly improving the survivability of probiotic cells during refrigerated storage compared to free cells. The incorporation of encapsulated *Lactobacillus* into paneer spread resulted in a functional product that maintained probiotic viability around the recommended therapeutic threshold ($\geq 10^6$ -10⁷ CFU/g) throughout the storage period upto 9 days. Importantly, the addition of microcapsules did not adversely affect key physicochemical parameters such as pH, moisture, and texture, nor did it compromise majorly to sensory attributes, indicating good compatibility with the dairy matrix. These findings highlight the potential of maltodextrin as a cost-effective and efficient encapsulating agent for delivering viable probiotics in fresh dairy products. The developed processed probiotic paneer spread can be considered a promising functional food with enhanced health benefits.

Abbreviations

MD; Maltodextrin, FC: Free cells, ND: Not detected, TBARS; thiobarbituric acid reactive substances, CFU: colony forming units.

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