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Viability of Free and Alginate–Carrageenan Gum Coated *Lactobacillus acidophilus* and *Lacticaseibacillus casei* in Functional Cottage Cheese

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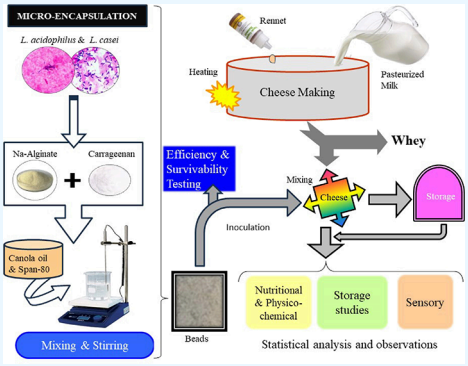
ABSTRACT: The survivability of encapsulated and nonencapsulated probiotics consisting of *Lactobacillus acidophilus* and *Lacticaseibacillus casei* and the nutritional, physicochemical, and sensorial features of cottage cheese were investigated under refrigeration storage at 4 °C for 28 days. Microbeads of *L. acidophilus* and *L. casei* were developed using 2% sodium alginate, 1.5% sodium alginate and 0.5% carrageenan, and 1% sodium alginate and 1% carrageenan using an encapsulation technique to assess the probiotic viability in cottage cheese under different gastrointestinal conditions (SGF (simulated gastric juice), SIF (simulated intestinal fluid)), and bile salt) and storage conditions. Scanning electron microscopy (SEM) elucidated the stable structure of microbeads, Fourier transform infrared spectroscopy (FTIR) confirmed the presence probiotics in the microcapsules, and X-ray diffraction (XRD) demonstrated the amorphous state of microbeads. Furthermore, the highest encapsulation efficiency was observed for alginate 1% and carrageenan 1% microbeads (T_3), i.e., 95%. Likewise, viability was recorded in T_3 against SGF, SIF, and bile salt solution, i.e., 8.5, 8.8, and 8.9 log CFU/g at 80 min of exposure, compared to the control. The results of pH showed a significant ($p < 0.05$) decline that ultimately increased the titratable acidity. Nutritional analysis of cottage cheese revealed the highest levels of ash, protein, and total solids in T_3, exhibiting mean values of 3.2, 22, and 43.2 g/100 g, respectively, after 28 days of storage. The sensory evaluation of cottage cheese demonstrated better color, flavor, and textural attributes in T_3. Conclusively, synergistic addition of *L. acidophilus* and *L. casei* encapsulated with alginate–carrageenan gums was found to be more effective in improving the viability of probiotics in cottage cheese than noncapsulated cells while carrying better magnitudes of ash and protein, lower acidity, and pleasant taste.



1. INTRODUCTION

Probiotics are health-promoting beneficial microbes exploited for their promising health significance. Probiotics are natural therapeutic agents used to improve human health against various health maladies such as cancer, diabetes, and cardiovascular health challenges.[1] Retrospective studies have clarified the therapeutic applications of *B. longum*, *B. lactis*, *L. plantarum*, *L. casei*, and *L. acidophilus* in various sectors of the food and nutraceutical industries.[2] Specifically, these studies highlight their utility in the dairy sector, wherein they serve as nutritional supplements incorporated into products such as cheese, yogurt, and acidified milks.[2] Furthermore, the combined use of probiotics with prebiotics has been acknowledged to enhance various health attributes. Renowned for their role in improving immunity, reducing serum cholesterol, alleviating lactose intolerance, promoting colonic health, and serving as dietary ingredients, probiotics demonstrate a positive correlation with prebiotics in retrospective studies.[3] These studies highlight the association of probiotic and prebiotic consumption with elevated immune response, prevention of certain malignancies, reduced intestinal inflammation, and improved hypertension.[2] Probiotics play a significant role in converting phenolics into biologically active metabolites, suggesting their potential as antibiotics to address health conditions such as diabetes, obesity, atherosclerosis, and bone density loss.[4]

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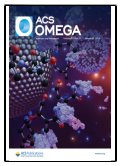

Encapsulation is a viable technique that is helpful in improving the growth of probiotics and extension of shelf life of food products. Textural and sensorial attributes of finished goods containing probiotics are dependent on the nature of encapsulation and size of microbeads.[5] Emulsion, a type of encapsulation, is considered as a viable, economical, and adaptable technique used to produce encapsulated probiotics.[6] Alginate is a consumable and digestible polymer that is widely exploited for the encapsulation of probiotics including LAB such as *L. rhamnosus*, *L. acidophilus*, *L. lactis*, *L. casei*, and *L. plantarum*.[7] Alginate is capable to withstand severe acidic conditions and even pH lower than 2, disintegrate the matrix, and release its constituents.[8] Previous studies have demonstrated the combined effect of alginate with other food polymers as viable encapsulation materials vs alginate alone due to its ability to withstand highly acidic conditions in human stomach.[9] Previous studies have identified the combined use of sodium alginate and carrageenan gums for probiotic encapsulation, leveraging their broad bioadaptability, versatility, heat resistance, and effectiveness in low pH conditions.[10] These coating materials, known for their nontoxicity, affordability, and ease of handling, are particularly suitable for encapsulation. Sodium alginate is crucial for bead or capsule development when using carrageenan gum alone proves challenging, as the latter cannot bond with calcium ions.[7] Therefore, sodium alginate is necessary to form bonds with the hardening solution of calcium chloride. The gums, including sodium alginate and carrageenan, primarily enhance bead stability.[11] The scientific literature has also documented the use of various natural encapsulating plant materials, such as sodium alginate, gum arabic, carrageenan, xanthan gum, proteins like albumin and casein, maltodextrin, chitosan, zein, dextran, and cellulose, as viable ingredients for encapsulation.[11]

Cottage cheese is a fermented, unripened, and semisoft dairy product known for its enriched nutritional significance for all age groups and is prepared from pasteurized milk.[11] This traditional product has been in use for ages, as earlier studies have reported that it is the most customary cheese type manufactured in households in various European regions.[12] It contains high water level, low milk fat, and higher acidity along with unique consistency, taste, smell, and color.[13] Value-added products like cheese are preferred for their good profile of bioactive compounds such as phenolic substances and flavonoids that undergo metabolism by correlated actions of probiotics and enzymatic activities.[14] Because of a wide array of nutritional significance, the product is well consumed worldwide including in South Asia, Indo-Pak, Egypt, and European countries.[15] Probiotics such as *Lactobacillus acidophilus* and *Lacticaseibacillus casei* have been employed in the development of value-added dairy products such as cheese, yogurt, and whey-protein-based goods as a viable carrier of health-promoting nutrients including proteins, fats, and minerals.[15] Earlier studies have reported the extension of shelf life of cottage cheese by using natural antimicrobial compounds, plant derivatives, microbial flora, and its metabolites.[16,17,18]

However, a meager amount of data is available on hand; therefore, the present research study was designed to evaluate the impact of free and encapsulated probiotics consisting of *L. acidophilus* and *L. casei* on the nutritional, physicochemical, and sensorial attributes of functional cottage cheese.

2. MATERIALS AND METHODS

2.1. Raw Materials, Chemicals, and Reagents. Pasteurized milk (at 72−75 °C for 12−15 s) and canola oil were procured from a local market in Faisalabad, Pakistan. Analytical-grade reagents and chemicals including growth media (De Man, Rogosa, and Sharpe agar), sodium alginate (E-401), carrageenan gum (E-407), and calcium chloride were purchased from Thermo Fisher Scientific (Thermo-Fisher Scientific Inc., MA, USA), Sigma-Aldrich (Sigma-Aldrich, MO, USA) and Carlo-Erba (Milano, Italy), respectively. All glassware and media were sterilized at 171 °C for 30 min in a hot air oven (Memmert GmbH + Co., Büchenbach, Germany) and stored under refrigerated conditions for further appraisal in the study.

2.2. *Lactobacillus acidophilus* and *Lacticaseibacillus casei* Cultures and Inoculum Preparation. Lyophilized cultures of *L. acidophilus* and *L. casei* were obtained from the Institute of Microbiology and Molecular Genetics, University of the Punjab, Lahore. The growth medium was prepared using MRS agar and sterilized in a vertical autoclave (3870ELV-D, Tuttnauer, Breda, Netherlands), and bacterial strains were inoculated on a cell culture dish using quadrant streaking and an incubator at 37 °C for 48 h.[10] The purity of cultures was observed by performing a catalase test and gram staining. Both strains were tested for microbial growth in MRS broth and incubated before subsequent analysis. The strains were garnered by centrifugation (Thermo Fisher Scientific Inc., USA) at 3000*g* for 20 min and flushed with clean distilled water. After washing, final inocula were obtained with microbial cell concentration of 10^{8-9} CFU/g.

2.3. Bacterial Bead Preparation. Bacterial cells were encapsulated to form microbeads in accordance with the method as adopted by Afzaal et al.[20] with slight modifications. The hydrogels of sodium alginate and sodium alginate−carrageenan were prepared at different concentrations to encapsulate *L. acidophilus* and *L. casei*. Hydrogels were prepared well before encapsulation for obtaining better efficiency. Microbeads were prepared using an emulsion-based technique. Sodium alginate and sodium alginate−carrageenan gum solutions were sterilized at 121 °C for 15 min. Afterward, precentrifuged/purified cell solution (∼1 mL) was added in 20 mL of all sterilized sodium alginate and sodium alginate−carrageenan solutions.[10] Thereafter, about 100 mL of canola oil accompanied by an emulsifier (i.e., Span-80) was amalgamated with the solutions followed by continuous stirring using a magnetic stirrer (MSH-D, DAIHAN Scientific, South Korea). The emulsion was prepared using sodium alginate and sodium alginate−carrageenan solutions, centrifuged cell solutions, and canola oil (i.e., 100 mL) by stirring the admixture at 200 rpm for 15 min.[19] Bead firmness was obtained by mixing the stable emulsion with calcium chloride solution (i.e., 0.1 M, 100 mL). Subsequently, alginate beads (T_1) and alginate−carrageenan beads (i.e., T_2 and T_3) were collected from the stable emulsions using Whatman No. 4 filter paper and stored at 4−7 °C for further assessment.

2.4. Characterization of Microcapsules. Fourier transform infrared (FTIR) analysis was carried out using the methodology of Afzaal et al. (2020)[20] for encapsulated bacterial beads of *L. acidophilus* and *L. casei* coated with selected treatments. For this, samples were analyzed by FTIR (Thermo Scientific Nicolet 6700, USA) with transmittance

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mode 4000:400, resolution 4 cm^{-1}, and signal-to-noise ratio 8000:1, and various peaks were interpreted. Scanning electron microscopy (SEM, CAMBRIDGE S 360) was used to visualize the structural characterization of microbeads. Freeze-dried microbeads were treated with 2% glutaraldehyde at pH 7.2 and temperature 4 °C overnight. The phosphate buffer (0.1 M) was used to wash samples, and a series of ethanol concentrations (50−100%) were used for dehydration. Samples were fixed (180 s at 40 mA) in stubs coated with a gold layer, and micrograph images were processed with Adobe Photoshop. X-ray diffraction (XRD) was also caried out to evaluate the amorphous/crystalline state of the microbeads using the methodology of Afzaal et al. (2020).[20]

2.5. Encapsulation Efficiency. Total viable counts of *L. acidophilus* and *L. casei* were enumerated before and after production of microcapsules in accordance with the protocol as delineated by Azam et al.[21] Encapsulation efficiency was estimated by dissolving microcapsules into a sodium citrate solution (i.e., 9 mL, 2% w/v), and the final pH was adjusted to 7.0. The probiotic strains were released, serially diluted up to 10 times, inoculated over MRS agar plates, and incubated at 37 °C for 48 h in an anaerobic chamber (Bactron SHEL LAB Anaerobic Chamber, USA). Bacterial enumeration was performed at a colony counter (Sorcerer, Philadelphia, USA). Encapsulation efficiency was recorded using the following equation;

$$\text{Encapsulation efficiency(EE)} = \frac{\log 10N}{\log 10N_0} \times 100$$

N = the number of cells released from microspheres
N_0 = the number of free cells used before encapsulation

2.6. Bead Size. Bead size/diameter of microbeads was estimated using a fluorescence microscope (SWIFT M7000D and 4000D) following the method as adopted by Azam et al.[22] Randomly selected alginate and carrageenan beads were vortexed and placed on a stage micrometer.

2.7. Survivability of Free and Microencapsulated *L. acidophilus* and *L. casei* in Simulated Gastric Fluid, Simulated Intestinal Fluid, and Bile Salt Solution. Encapsulated *L. acidophilus* and *L. casei* were analyzed for their viability following the method adopted by Yasmin et al.[23] with slight modifications. Simulated gastric fluid (SGF) was prepared using 3.0 g/L pepsin, NaCl (2.0 g/L), and salt water, and pH was adjusted at 1.2 using 1 M HCL. Encapsulated bacterial strains (0.50 g) were poured onto the SGF, grown on MRS broth, and incubated at 37 °C for 0, 20, 40, 60, and 80 min. The enumeration was performed following the washing and harvesting of beads using a centrifuge (Thermo Fisher Scientific Inc., USA) at 4000 rpm for 10 min. Free cells (0.50 mL) were diluted using SGF (i.e., 4.5 mL) and tested for 80 min followed by inoculation of free *L. acidophilus* and *L. casei* on MRS agar and incubation. The results were estimated as log CFU/mL.

L. acidophilus and *L. casei* were evaluated using simulated intestinal fluid (SIF) that constituted of pancreatin based on trypsin activity at 100 U/mL, $CaCl_2$ (i.e., 0.2 g/L), KCl (i.e., 0.84 g/L), $NaHCO_3$ (i.e., 1.39 g/L), NaCl (6.5 g/L), and KH_2PO_4 (50 mM, 3.0 g/L) at 6.8 pH. Free (i.e., 0.5 mL) and encapsulated cells (i.e., 0.5 g) were amalgamated with SIF (i.e., 4.5 mL) with continuous stirring at 100 rpm and incubated at 37 °C. Thereafter, about 80 μL of the prepared probiotic admixture was taken for enumeration at 0, 20, 40, 60, and 80 min using the pour plate method.

L. acidophilus and *L. casei* were analyzed using bile salt solution (i.e., 2.5% w/v) by following the protocol as outlined by Xiao et al.[24] Accurately measured 4.5 mL of bile salt solution was mixed with free (i.e., 0.5 mL) and encapsulated strains (i.e., 0.5 g) with continuous stirring at 100 rpm and incubated at 37 °C. The samples were serially diluted with sodium chloride (0.8%). Subsequently, precisely measured 80 μL aliquots of the mixture were poured on the MRS agar plates for enumeration.

2.8. Storage Stability of Free and Encapsulated *L. acidophilus* and *L. casei*. The storage stability of free and microencapsulated *L. acidophilus* and *L. casei* was assessed as described by Yasmin et al.[23] Serially diluted free cells of *L. acidophilus* and *L. casei* were inoculated on MRS agar at 80 μL for the determination of viable counts at 0, 7, 14, 21, and 28 days, whereas the encapsulated cells of *L. acidophilus* and *L. casei* were released from the microcapsules by dissolving in sodium citrate solution (i.e., 4.5 mL, 50 mM) and inoculated.

2.9. Preparation of Probiotic Cottage Cheese. Cottage cheese was prepared in accordance with the procedure elucidated by Chew et al.[2] with slight modification. Pasteurized milk with low fat (i.e., 1−2% fat) was used to manufacture the cottage cheese. Pasteurized milk was preheated at 37 °C followed by addition of rennet at 100 μL/L and starter culture (0.2%) of treatments (T_0−T_3), i.e., free and microencapsulated *L. acidophilus* and *L. casei* with different combinations of sodium alginate and carrageenan. The pH of cheese milk was adjusted to 4.7−4.5 for formation of curd by incubating the milk at 37 °C. The curd was cut into 1 cm^3 pieces manually with a cheese wire knife, and whey was released by two to three washings with cold water (i.e., 4 °C). The cottage cheese was prepared as cottage cheese with free *L. acidophilus* and *L. casei* (control, T_0), cottage cheese coated with 2% sodium alginate (T_1), and cottage cheese coated with 1.5% sodium alginate and 0.5% carrageenan (T_2), and cottage cheese coated with 1% sodium alginate and 1% carrageenan (T_3). All cottage cheese treatments were stored at 4−6 °C for 28 days for further experimentation on different intervals.

2.10. Nutritional and Physicochemical Analysis of Probiotic Cottage Cheese. Cottage cheese was estimated for its nutritional composition following the methods as outlined by Latimer,[25] i.e., moisture (Method No. 925.10), crude ash (Method No. 923.03), crude fat (Method No. 920.85), and crude protein (Method No. 920.87), lactose content (Method No. 984.15), pH, and titratable acidity (Method No. 947.05). The hardness of the cottage cheese samples was estimated in accordance with the protocols as delineated in Chakraborty et al.[26] using the TA-XT Plus texture analyzer. Seventeen millimeter cheeses were used for the test. The speed of the penetration of a 4 mm cylindrical probe was adjusted during the pretest and penetration at 0.5 mm s^{-1}, whereas for the post-test, the speed was 10 mm s^{-1}. The meltability of cottage cheese samples was determined as mentioned by Komansilan et al.[27]

2.11. Sensory Evaluation of Probiotic Cottage Cheese. Sensory evaluation of probiotic cottage cheese was carried by the 10 sensory panelists (aged between 35 and 40 years) on the nine-point hedonic scale for the sensory parameters consisting of flavor, color, texture, aroma, taste, and overall acceptability by trained panelists (age group ∼35−45). The sensory evaluation of cottage cheese was conducted

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unbiasedly with clear day light and clean drinking water for proper organoleptic evaluation of the final product. Half scores and weighting factors were used to provide a more nuanced and precise evaluation of these attributes. Citrus slices were utilized for palate cleansing during the sensory evaluation. The sensory acceptability scale was 1: disliked extremely to 9: liked extremely.[10]

2.12. Enumeration of *L. acidophilus* and *L. casei* of Cottage Cheese. Probiotic counts of free and microencapsulated *L. acidophilus* and *L. casei* were carried out by following the protocol as outlined by Afzaal et al.[28] Probiotics were released from the alginate and alginate−carrageenan microbeads and inoculated on MRS agar plates at 37 °C for 48 h. The probiotic colonies were enumerated using a colony counter (Sorcerer, Philadelphia, USA) and calculated as CFU/g.

2.13. Statistical Analysis. All experiments were performed in triplicates, and the results were expressed as ± standard deviation (S.D.). The data including the encapsulation, efficiency, and survivability and nutritional, textural, and sensorial attributes of cottage cheese were analyzed using two-way analysis of variance (ANOVA) and the least significant difference (LSD) test at $p < 0.05$ (Analytical Software, Statistix 8.1, Chicago, USA).[29]

3. RESULTS AND DISCUSSION

3.1. Probiotic Inoculum Preparation and Characterization of Microcapsules. *Lactobacillus acidophilus* and *Lacticaseibacillus casei* were cultured on MRS agar plates for 48 h at 37 °C (Figure 1). Isolated colonies of both strains were recultured on MRS broth to obtained 10^{8-9} CFU/mL of purified culture. After centrifugation, *L. acidophilus* and *L. casei* cells were encapsulated with different combinations (T_0−T_3) of sodium alginate and sodium alginate−carrageenan. Cultures were carefully maintained under controlled conditions until reaching the logarithmic growth phase, and subsequent cell enumeration through serial dilution and plating on selective agar revealed viable cell concentrations. Standardization of the inocula for encapsulation was achieved through optical density measurements at 600 nm and confirmed by plate counts, guaranteeing consistency and reproducibility in subsequent assays. The optical density of the probiotic free cells and encapsulated with different treatments is presented in Figure 1c.

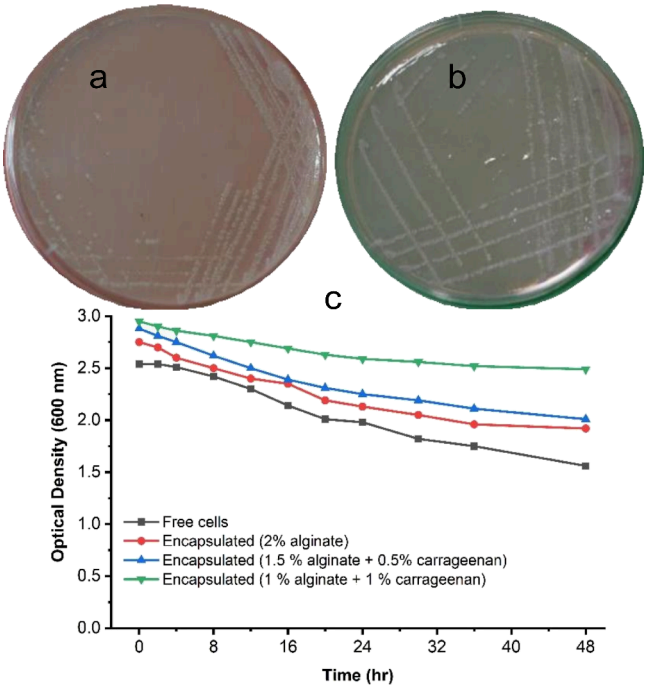

Figure 1. Culture of (a) *Lactobacillus acidophilus* and (b) *Lacticaseibacillus casei* on MRS agar plate for 48 h at 37 °C. (c) Optical density of *L. acidophilus* and *L. casei* free cells encapsulated with different alginate−carrageenan combinations.

The results for the diameter of beads prepared from alginate and alginate−carrageenan gum were recorded as 1.2 and 1.6 mm, respectively (Table 1). The findings for alginate-coated beads elucidated smaller diameters when compared to the alginate−carrageenan-coated beads. The diameter of the microcapsules is largely dependent upon the encapsulating materials and method of microencapsulation. Earlier studies by Azam et al.[30] have reported a similar justification for the diameter of beads wherein the scientists delineated a significant increment in bead diameter upon addition of the carrageenan gum that could be attributed to the viscous nature of carrageenan. Retrospective studies have exhibited lower diameters of the beads ranging from 0.01 to 0.04 mm, which could be linked with the nature and concentration of the polymers and encapsulation method.[31] Previously, studies have demonstrated the impact of alginate with whey protein concentrate (WPC) on the bead diameter when compared to alginate alone. Alginate beads possess a diameter of 716 μm, whereas alginate combined with WPC beads has an average diameter of 727 μm.[32]

3.2. Encapsulation Efficiency. The results for encapsulation efficiency of microbeads exhibited the significantly ($p < 0.05$) improved encapsulation efficiency for the alginate−carrageenan coated microbeads as compared to alginate alone. The results elucidated an increase in encapsulation efficiency upon increasing the concentration of carrageenan gum (Table 1). The results of encapsulation efficiency in our study are in close agreement with those of earlier studies, wherein the encapsulation efficiency of encapsulated *Lactobacillus acidophilus* and *Lacticaseibacillus casei* was increased without influencing the survival of probiotic cells during microencapsulation. The alginate−carrageenan gum coated beads showed the highest encapsulation efficiency, i.e., 93%, in contrast with the alginate coated beads, which had a lower encapsulation efficiency, i.e., 90. The significant improvement in encapsulation efficiency could be attributed to the combined impact of encapsulation ability of alginate and carrageenan gums, thereby protecting the probiotic growth under harsh environmental conditions and enhancing their growth in the intestine. Comparable findings were depicted by Damodharan et al.[33] wherein the use of alginate, locust bean gum, and fenugreek gums as encapsulating materials demonstrated an encapsulation efficiency of about 97%. The study reported better survivability of beads encapsulated with alginate 1%, locust bean gum 0.5%, and fenugreek gum 0.5% in simulated gastrointestinal fluid and exhibited the highest encapsulation efficiency of 97%. Nag et al.[34] reported a significant ($p < 0.05$) increase in the encapsulation efficiency of free cells and encapsulated beads in synergy from 42 to 90% on using the sodium caseinate and gellan gums. Similarly, Yasmin et al.[23] showed that alginate, pectin and whey protein concentrate based beads of *B. longum* had an appreciable encapsulation

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Table 1. Encapsulation Efficiency and Diameter of Alginate and Carrageenan Gum Coated Microbeads[†]

treatments	before encapsulation (N_0)	after encapsulation (N)	efficiency (%)	diameter
T_1	9.40 ± 0.05^a	8.50 ± 0.03^a	90.40 ± 1.05^b	1.14 ± 0.02^c
T_2	8.60 ± 0.04^b	7.90 ± 0.02^b	91.70 ± 0.58^{ab}	1.40 ± 0.04^b
T_3	8.50 ± 0.06^b	7.88 ± 0.03^b	92.70 ± 0.30^a	1.65 ± 0.03^a

[†]T_1 = cottage cheese coated with 2% sodium alginate; T_2 = cottage cheese coated with 1.5% sodium alginate and 0.5% carrageenan; T_3 = cottage cheese coated with 1% sodium alginate and 1% carrageenan. The values with same superscript letters are non-significant while different superscript letters in a column are significantly different ($p < 0.05$).

efficiency, i.e., 85%. The higher encapsulation efficiency of the sodium caseinate and gellan gums could be attributed to the ability of the walls of these polymers to act as insulation. Encapsulation efficiency exhibits a direct correlation with the bead size.[34] Round-shaped beads tend to have better encapsulation efficiencies when compared with the other microbeads as these exhibit good association with bacterial cells.

3.3. Structural Characterization of Microbeads. The results of FTIR spectroscopy are presented in Figure 2. The

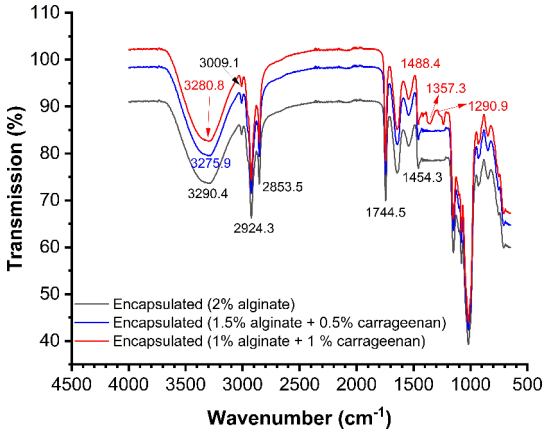

Figure 2. Fourier transform infrared (FTIR) spectra of encapsulated probiotics with different combinations of sodium alginate and carrageenan.

FTIR spectrum of the tested sample had prominent peaks at different wavenumbers, providing insights into its chemical composition and functional groups. The prominent peak at 3290.4 cm^{-1} for T_1, which represents the stretching vibrations of the O−H bonds, had little change to 3280.8 cm^{-1} in T_3. This indicates possible alterations in hydrogen bonding or modifications in the surroundings of hydroxyl groups. The existence of aromatic structures was revealed by a distinct peak at 3009.1 cm^{-1}, which corresponds to the stretching vibrations of C−H bonds in the aromatic compounds. Another significant signal seen at 2924.3 cm^{-1} represented asymmetric stretching vibrations of C−H bonds in aliphatic hydrocarbons, indicating the likely presence of alkanes or related compounds. The peak at 2853.5 cm^{-1} indicated the presence of C−H bonds in the aliphatic molecules. The prominent signal at 1744.5 cm^{-1} signifies C═O stretching vibrations, which suggests the existence of carbonyl groups. In addition, the peak observed at 1454.3 cm^{-1} in T_1, which is related to the bending of C−H bonds in aliphatic compounds, saw a shift to 1488.4 cm^{-1} in T_3. This shift could indicate changes in the molecular structure or arrangement of aliphatic groups due to the presence of carrageenan. The peaks observed at 1357.3 and 1290.9 cm^{-1} were determined to be C−H bending vibrations in methyl groups and unsaturated hydrocarbons, respectively. The results, encompassing both shifts and intensities, indicated changes in the chemical composition and structural properties of microbeads coated with different combinations of alginate and carrageenan.

The SEM micrograph of microencapsulated probiotics *L. acidophilus* and *L. casei* revealed distinctive morphological features (Figure 3). Sodium alginate (2%, T_1) coated microcapsules exhibited a textured surface with occasional irregularities, suggesting a slightly rougher encapsulation matrix compared to that of others (Figure 3a). On the other hand, sodium alginate (1%) and carrageenan (1%) coated microcapsules (T_3) displayed a smooth surface morphology characterized by uniform coatings around probiotic cells (Figure 3c). Cross-sectional analysis demonstrated a homogeneous distribution of probiotic cells within the T3 hydrogel matrix, emphasizing the well-defined structure of the microcapsules. Despite these differences, both matrices containing carrageenan (T_2 and T_3) maintained well-defined encapsulation structures in cross-sectional views. X-ray diffraction analysis (Figure 3d,e) revealed intriguing structural features, particularly when examining the possibility of an amorphous structure of microbeads prepared with different combinations of sodium alginate and carrageenan (T_1−T_3). In case of higher sodium alginate concentration (T_1 and T_2), slight diffraction peaks were observed at 2θ = 6.4, 21.5, and 25.8° that are typically associated with crystalline structures. However, a wide dispersion pattern that suggests a structure lacking a definite shape was identified. These findings indicate that a combination of 1% sodium alginate and 1% carrageenan, when used in microencapsulation, has the potential to take on an amorphous structure. The absence of clearly identifiable peaks indicates a chaotic chemical structure within the encapsulation matrix. The presence of *L. acidophilus* and *L. casei* did not modify the amorphous characteristic of microbeads, suggesting that the probiotics are integrated into the disordered framework of the substance. These results confirmed the findings of the FTIR and SEM analysis.

3.4. Survivability of Free and Encapsulated *L. acidophilus* and *L. casei* in Simulated Gastric Fluid, Simulated intestinal Fluid, and Bile Salt Solutions. The survivability of encapsulated probiotics was analyzed under simulated gastric conditions of different time conditions, i.e., 20−80 min. The results for survivability under SGF conditions elucidated the highest survivability of 10.9 CFU/g for the microencapsulated cells for alginate 1% and carrageenan gum 1% (i.e., T_3) when compared to the free cells (i.e., T_0) that showed lower survivability values, i.e., 4.4 CFU/g at 80 min. The results showed significant ($p < 0.05$) log reduction in all treatments on increasing the time from 20 to 80 min from 9.5 to 4.4 (i.e., T_0), 9.3 to 7.7 CFU/g (i.e., T_1), 9.4 to 8.0 CFU/g (i.e., T_2), and 9.6 to 8.5 CFU/g (i.e., T_3), respectively (Figure 4a). The higher survivability of alginate and carrageenan gum

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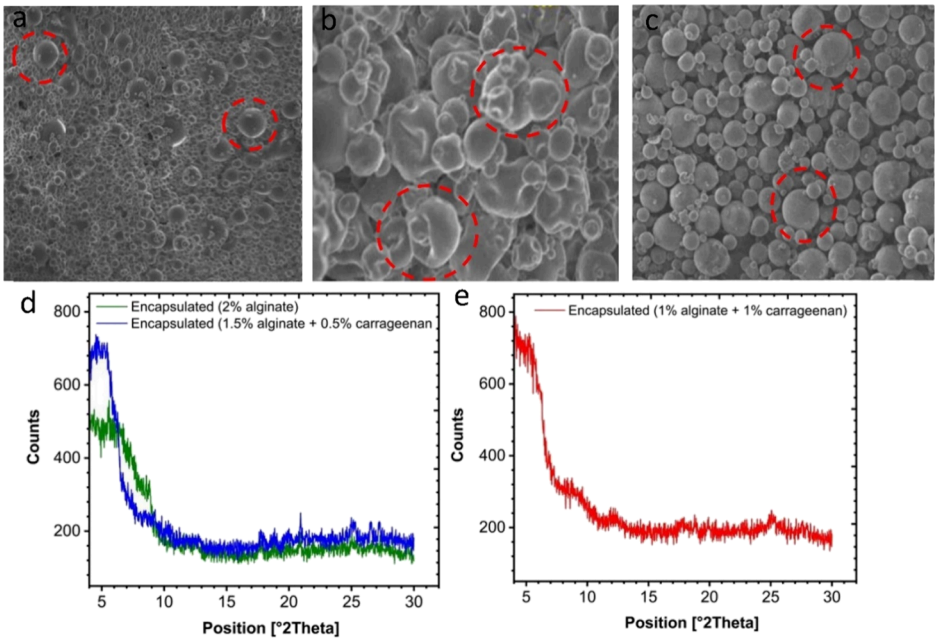

Figure 3. Scanning electron micrographs of microencapsulated *L. acidophilus* and *L. casei* with 2% sodium alginate (a), 1.5% sodium alginate and 0.5% carrageenan (b), and 1% sodium alginate and 1% carrageenan (c). X-ray diffraction spectra (d) of 2% sodium alginate (green) and 1.5% sodium alginate + 0.5% carrageenan (blue). X-ray diffraction spectra (e) of 1% sodium alginate and 1% carrageenan.

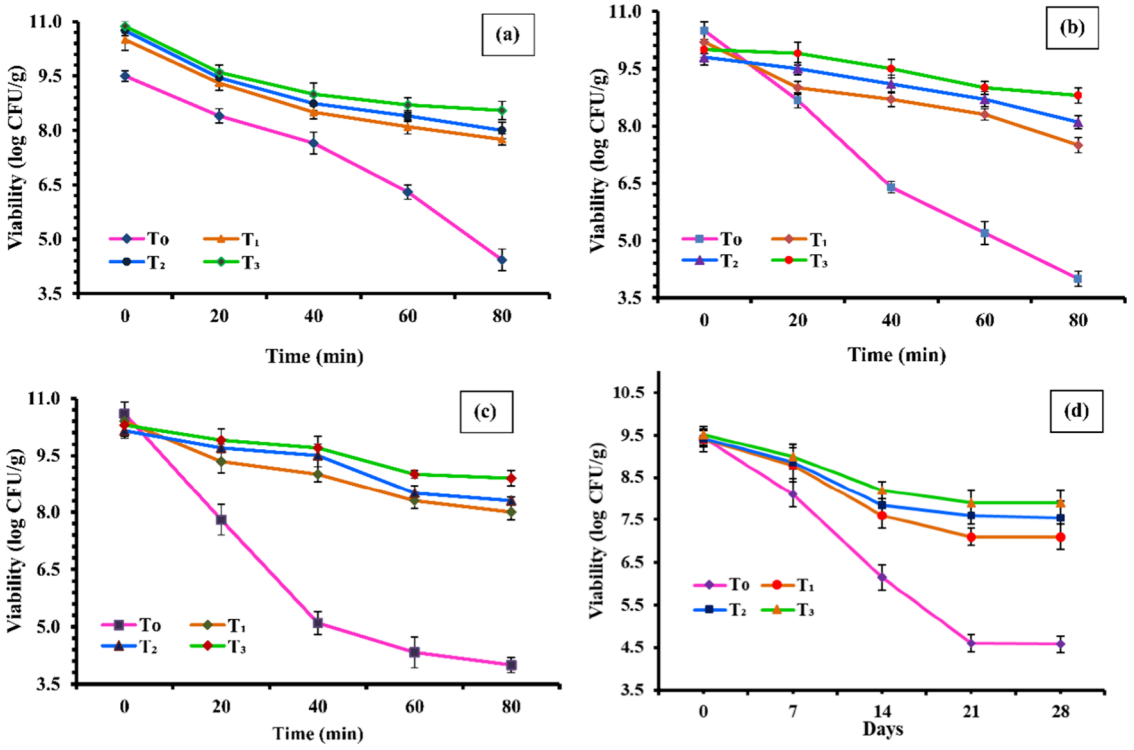

Figure 4. Survivability of free and encapsulated *L. acidophilus* and *L. casei* in (a) stimulated gastric fluid (SGF), (b) stimulated intestinal fluid (SIF), and (c) bile salt solution and (d) storage stability of microbeads after fortification in cottage cheese. T_0 = free *L. acidophilus* and *L. casei* (control), T_1 = coated with 2% sodium alginate, T_2 = coated with 1.5% sodium alginate and 0.5% carrageenan, and T_3 = coated with 1% sodium alginate and 1% carrageenan.

(i.e., T_3) could be attributed to the lower porosity and better solidification of alginate.[35,36] Another study by Silva et al.[35] studied the impact of alginate−gelatin fructooligosaccharide encapsulation for *L. acidophilus* in yogurt under SGF conditions and reported appreciable survivability up to 2 h that could be attributed to the spongy porous nature of the gel. Likewise, Jin et al.[37] reported the reduced viability of probiotics under gastric juice due to pepsin and muriatic acid of the stomach. Likewise, the reduction in mortality rates of encapsulated probiotics could be attributed to the gum containing coating. A group of researchers consisting of Chen et al.[38] reported the increase in survivability of probiotics in

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Table 2. Physicochemical Attributes of *L. acidophilus* and *L. casei* Cottage Cheese[†]

parameters	treatments	days				
		0	7	14	21	28
pH	T_0	5.80 ± 0.01^{a}	$5.71 \pm 0.02^{c-e}$	$5.67 \pm 0.02^{d-i}$	$5.66 \pm 0.02^{e-j}$	$5.63 \pm 0.03^{h-j}$
	T_1	5.79 ± 0.03^{ab}	$5.70 \pm 0.01^{c-f}$	$5.66 \pm 0.02^{e-j}$	$5.64 \pm 0.03^{f-j}$	$5.62 \pm 0.02^{h-j}$
	T_2	$5.76 \pm 0.01^{a-c}$	$5.69 \pm 0.03^{d-g}$	$5.65 \pm 0.02^{e-j}$	$5.63 \pm 0.02^{g-j}$	5.61 ± 0.02^{ij}
	T_3	$5.73 \pm 0.03^{b-d}$	$5.69 \pm 0.03^{d-g}$	$5.68 \pm 0.01^{d-h}$	$5.65 \pm 0.04^{e-j}$	5.60 ± 0.03^{j}
titratable acidity (%)	T_0	0.24 ± 0.00^{i}	$0.26 \pm 0.01^{g-i}$	0.39 ± 0.01^{de}	0.43 ± 0.02^{cd}	0.57 ± 0.01^{a}
	T_1	0.26 ± 0.01^{hi}	$0.29 \pm 0.00^{f-h}$	0.41 ± 0.01^{de}	0.46 ± 0.01^{bc}	0.48 ± 0.01^{b}
	T_2	0.28 ± 0.01^{fgh}	0.29 ± 0.01^{fgh}	0.40 ± 0.02^{de}	0.46 ± 0.01^{bc}	0.49 ± 0.02^{b}
	T_3	$0.28 \pm 0.01^{f-i}$	0.30 ± 0.01^{fg}	0.32 ± 0.02^{f}	0.38 ± 0.02^{e}	0.43 ± 0.01^{cd}

[†]T_0 = cottage cheese with free *L. acidophilus* and *L. casei* (control); T_1 = cottage cheese coated with 2% sodium alginate; T_2 = cottage cheese coated with 1.5% sodium alginate and 0.5% carrageenan; T_3 = cottage cheese coated with 1% sodium alginate and 1% carrageenan. The values with same superscript letters are non-significant while different superscript letters in a column are significantly different ($p < 0.05$).

simulated gastric juice due to whey protein isolates used as coating material. Another research work by Qi et al.[39] showed the survivability of *S. boulardii* to be enhanced by 90% on microencapsulation under simulated gastric conditions when compared to the free cells at 3 h.

Findings for the survivability of probiotics under SIF conditions showed the maximum survivability of probiotics for the control (T_0), i.e., 10.5 CFU/g at 0 min, whereas among the treatments, the maximum survivability of probiotics was recorded for alginate 1% and carrageenan gum 1% (T_1), i.e., 10.2 CFU/g at 0 min, followed by the minimum probiotic survivability revealed for alginate 1% and carrageenan gum 1% (T_1), i.e., 7.5 CFU/g at 80 min. The data for survivability of probiotics at different intervals of time exhibited the highest survivability at 80 min for alginate 1% and carrageenan gum 1% (T_3), i.e., 8.8 CFU/g. The findings portrayed a gradual decline in survival rates of the probiotics on increasing the time from 0 to 80 min (Figure 4b). The least survival of free cells could be linked to the free penetration of SIF into the bacterial cells. A study by Jin et al.[37] reported the lowest survivability of probiotics for free cells on exposure of free cells to SIF conditions due to lower permeability and noncoating. However, a study by Mahmoud et al.[40] portrayed an increase in survivability of probiotics on encapsulation using alginate−chitosan, alginate−skim milk, alginate−dextrin, and alginate−denatured whey proteins at 2 h that could be associated with the lower porosity and nonpermeability. Likewise, Shu et al.[41] revealed only 0.64 log reductions in *L. acidophilus* encapsulated with xanthan−chitosan coating and 2 h exposure when compared with the control. The viability of encapsulated probiotics could be due to the ion exchange reduction between the SIF and the microbeads.

The results for free and encapsulated probiotics in bile salt solution showed a significant ($p < 0.05$) decline in the survival rate of probiotics from 10.6 to 4.0 CFU/g on enhancing the time from 0 to 80 min for the control. Meanwhile, among the treatment groups, data revealed a significant ($p < 0.05$) increase in the survivability of probiotics on increasing the carrageenan gums. The survivability significantly ($p < 0.05$) decreased from 9.4 to 8.0, 9.7 to 8.3, and 9.9 to 8.9 CFU/g for alginate 2% (T_1), alginate 1.5% and carrageenan 0.5% (T_2), and alginate 1% and carrageenan 1% (T_3), respectively (Figure 4c). Beads containing carrageenan in their coating possessed a higher survival rate as compared to alginate beads because of the properties that hinder the diffusion of bile solution. Because of the low diffusion rate, bile solution in carrageenan gum coated alginate microcapsules (T_3) enabled the maximum bacterial count after 80 min of exposure to bile salt solution.[41] Comparable findings were elucidated in the study by Eckert et al.[42] wherein the researchers depicted 0.67 log reductions of probiotics on encapsulation with carrageenan gums and their exposure to bile salt solutions. Likewise, earlier studies by Yao et al.[43] depicted the significant role of alginate and gelatin microencapsulated cells on log reduction of the probiotic free cells in 2−3% bile salt solution when compared with the control (i.e., free nonencapsulated probiotic cells) exhibiting the 85% survivability.

3.5. Storage Stability of Free and Encapsulated *L. acidophilus* and *L. casei*. The results for storage stability at 0−28 days of free and encapsulated *L. acidophilus* and *L. casei* elucidated a significant ($p < 0.05$) decrease of probiotics' viability (Figure 4). The data on the storage stability of probiotics showed a significant ($p < 0.05$) decrease in the viability of probiotics for a storage period of 0−28 days from 9.5 to 4.5 (i.e., T_0), 9.4 to 7.1 (i.e., T_1), 9.4 to 7.5 (i.e., T_2), and 9.5 to 8.0 CFU/g (i.e., T_3) (Figure 4). The results exhibited the significant outcome of the synergistic effect of using alginate and carrageenan gum for encapsulation that improved the beads' structure and survivability when compared to using alginate alone. Yao et al.[43] reported better survival of the probiotics of encapsulated probiotics observed under 4 weeks of refrigeration storage in comparison to the free cells. Comparable findings were reported in the study by Riaz et al.[9] wherein encapsulation of *B. bifidum* with alginate−zein coating resulted in 1.8 log cfu/mL reductions in encapsulated bacteria antagonistic to free cells that showed about 7.7 log cfu/mL reductions at 32 days of storage. Previously, studies have indicated the positive correlation of using the carrageenan gum in synergy with the alginate to encapsulate the probiotics. The results showed improved bead structure and weakened penetration into the beads and enhanced survivability during the storage.[30]

3.6. Physicochemical Attributes of *L. acidophilus* and *L. casei* Cottage Cheese. A physicochemical analysis of *L. acidophilus* and *L. casei* cottage cheese was performed (Table 2). The results for pH contents of the cottage cheese samples and control indicated a significant ($p < 0.05$) decline on storage of 0−28 days. The pH values of control ranged between 5.8 and 5.6 from 0 to 28 days of storage. Among the treatment groups, the pH values of different treatments varied: T_1 (5.8−5.6), T_2 (5.7−5.6), and T_3 (5.7−5.6). The results showed the highest pH values on zeroth day, whereas the lowest pH values were observed on the 28th day. The variation in the pH values of cottage cheese at maximum storage, i.e.,

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Table 3. Nutritional Composition of *L. acidophilus* and *L. casei* Cottage Cheese (g/100 g)[†]

parameters	treatments	days				
		0	7	14	21	28
ash	T_0	3.06 ± 0.01^{f}	$3.08 \pm 0.0^{d-f}$	$3.08 \pm 0.03^{c-f}$	$3.12 \pm 0.03^{a-e}$	$3.13 \pm 0.02^{a-d}$
	T_1	3.07 ± 0.01^{ef}	$3.08 \pm 0.01^{c-f}$	$3.11 \pm 0.01^{a-f}$	3.13 ± 0.01^{abcd}	3.14 ± 0.01^{ab}
	T_2	3.06 ± 0.01^{f}	$3.08 \pm 0.01^{c-f}$	$3.10 \pm 0.01^{b-f}$	3.14 ± 0.01^{ab}	3.15 ± 0.00^{ab}
	T_3	$3.08 \pm 0.01^{c-f}$	3.13 ± 0.01^{abc}	$3.11 \pm 0.02^{a-f}$	3.15 ± 0.01^{ab}	3.16 ± 0.01^{a}
fat	T_0	21.97 ± 0.02^{a}	21.86 ± 0.03^{b}	21.66 ± 0.01^{c}	21.55 ± 0.03^{d}	21.42 ± 0.02^{e}
	T_1	21.05 ± 0.03^{g}	20.80 ± 0.02^{i}	20.74 ± 0.03^{ij}	20.56 ± 0.02^{k}	20.47 ± 0.01^{l}
	T_2	20.10 ± 0.01^{m}	19.94 ± 0.01^{n}	19.90 ± 0.02^{no}	19.85 ± 0.01^{o}	19.63 ± 0.02^{p}
	T_3	21.21 ± 0.02^{f}	21.05 ± 0.03^{g}	20.91 ± 0.01^{h}	20.70 ± 0.01^{j}	20.49 ± 0.03^{l}
lactose	T_0	1.59 ± 0.02^{a}	$1.55 \pm 0.01^{a-d}$	$1.52 \pm 0.02^{b-e}$	$1.52 \pm 0.01^{b-e}$	1.46 ± 0.01^{ef}
	T_1	1.58 ± 0.01^{ab}	$1.54 \pm 0.02^{a-d}$	$1.51 \pm 0.03^{c-e}$	1.51 ± 0.02^{cde}	1.43 ± 0.02^{fg}
	T_2	1.57 ± 0.03^{abc}	$1.54 \pm 0.01^{a-d}$	1.50 ± 0.01^{de}	$1.51 \pm 0.03^{c-e}$	1.40 ± 0.03^{fg}
	T_3	$1.56 \pm 0.02^{a-d}$	$1.53 \pm 0.03^{a-d}$	1.50 ± 0.03^{de}	1.50 ± 0.02^{de}	1.38 ± 0.01^{g}
protein	T_0	20.02 ± 0.01^{p}	20.09 ± 0.03^{o}	20.12 ± 0.03^{o}	20.56 ± 0.02^{h}	20.96 ± 0.04^{d}
	T_1	20.23 ± 0.01^{n}	20.29 ± 0.01^{m}	20.39 ± 0.01^{k}	20.66 ± 0.02^{g}	21.01 ± 0.02^{c}
	T_2	20.27 ± 0.01^{m}	20.31 ± 0.01^{m}	20.43 ± 0.01^{j}	20.79 ± 0.02^{f}	21.23 ± 0.02^{b}
	T_3	20.35 ± 0.02^{l}	20.41 ± 0.01^{jk}	20.480 ± 0.01^{i}	20.91 ± 0.03^{e}	21.48 ± 0.02^{a}
total solids	T_0	40.78 ± 0.01^{h}	40.34 ± 0.01^{j}	40.20 ± 0.03^{k}	40.25 ± 0.01^{k}	40.52 ± 0.02^{i}
	T_1	42.92 ± 0.02^{c}	$42.99 \pm 0.02_{b}$	42.10 ± 0.01^{d}	42.13 ± 0.02^{d}	42.07 ± 0.03^{d}
	T_2	40.92 ± 0.03^{g}	40.99 ± 0.03^{f}	41.10 ± 0.03^{e}	41.16 ± 0.03^{e}	41.10 ± 0.01^{e}
	T_3	42.89 ± 0.02^{c}	42.92 ± 0.01^{c}	43.05 ± 0.03^{b}	43.15 ± 0.01^{a}	43.16 ± 0.02^{a}

[†]T_0 = cottage cheese with free *L. acidophilus* and *L. casei* (control); T_1 = cottage cheese coated with 2% sodium alginate; T_2 = cottage cheese coated with 1.5% sodium alginate and 0.5% carrageenan; T_3 = cottage cheese coated with 1% sodium alginate and 1% carrageenan. The values with same superscript letters are non-significant while different superscript letters in a column are significantly different ($p < 0.05$).

Table 4. Probiotic Count (log cfu/mL) of Free and Encapsulated *L. acidophilus* and *L. casei* Cottage Cheese[†]

treatments	days				
	0	7	14	21	28
T_0	7.87 ± 0.01^{h}	8.71 ± 0.03^{b}	8.10 ± 0.03^{f}	7.21 ± 0.02^{k}	6.40 ± 0.03^{m}
T_1	7.00 ± 0.02^{l}	8.00 ± 0.02^{g}	8.00 ± 0.02^{g}	7.55 ± 0.01^{j}	7.18 ± 0.02^{k}
T_2	8.25 ± 0.03^{de}	8.69 ± 0.01^{bc}	8.23 ± 0.02^{e}	7.64 ± 0.02^{j}	7.77 ± 0.01^{i}
T_3	8.33 ± 0.02^{d}	9.69 ± 0.02^{a}	9.60 ± 0.01^{a}	8.75 ± 0.01^{b}	8.60 ± 0.02^{c}

[†]T_0 = cottage cheese with free *L. acidophilus* and *L. casei* (control); T_1 = cottage cheese coated with 2% sodium alginate; T_2 = cottage cheese coated with 1.5% sodium alginate and 0.5% carrageenan; T_3 = cottage cheese coated with 1% sodium alginate and 1% carrageenan. The values with same superscript letters are non-significant while different superscript letters in a column are significantly different ($p < 0.05$).

28th day, was recorded as 0.2, which is in close corroboration with the earlier studies.[44] Comparable findings were revealed in earlier studies wherein the scientists reported a significant decrease in pH values from 5.7 to 5.6 for T_3 on storage for 28 days. The resultant decrease in pH could be linked with the conversion of lactose into lactic acid because of the probiotic activities of probiotics during the storage.[45]

The cottage cheese testing exhibited a significant ($p < 0.05$) increase in the mean concentrations of the acidity on 0−28 days of storage (Table 2). The data on comparative assessment of cottage cheese elucidated a significant ($p < 0.05$) increment in mean values of titratable acidity, i.e., T_1 (0.2−0.5%), T_2 (0.3−0.5%), and T_3 (0.3−0.4%), as compared to the control that ranged between 0.24 and 0.57 at 0−28 days of storage. Previous studies have reported that lactic-acid-producing bacteria have a significant impact on titratable acidity and pH levels. The rapid increase in the number of these bacteria causes the decomposition of biological substances, such as lactose, transforming them into lactic acid. As a result, the pH level decreased from 4.65 ± 0.9 to 3.82 ± 0.8 and the titratable acidity in cottage cheese increased from 0.76 ± 0.3 to $0.97 \pm 0.3\%$.[46]

3.7. Nutritional Composition of Cottage Cheese Supplemented with *L. acidophilus* and *L. casei*. Cottage cheese enriched with *L. acidophilus* and *L. casei* exhibited considerable magnitudes of nutrients (Table 3). The results for protein, ash, and total solids of cottage cheese samples and control indicated a significant ($p < 0.05$) increase on storage of 0−28 days wherein the mean values of control ranged from 20 to 21, 3.0 to 3.2, and 40 to 41 g/100 g, respectively. This decrease in protein content during storage time might be due to proteolytic enzymes and bacteria present in cottage cheese. Bacteria contribute to proteolysis through metabolic processes and break down proteins into smaller peptides and amino acids. The decrease in moisture content might also have contributed to the decrease in protein content in the cottage cheese. Among the treatment groups, mean values of protein, ash, and total solids were recorded as T_3 (i.e., 20.3−21.5 g/100 g), ash (3.08−3.16 g/100 g), and total solids (42.9−43.1 g/100 g). Results for lactose and fat content indicated a significant ($p < 0.05$) decline on storage of 0−28 days. The lowest mean values of lactose content were noticed in T_3 (i.e., 1.56−1.38 g/100 g) in comparison with the control (i.e., 1.6−1.5 g/100 g) (Table 3). During storage time, oxidation was a significant contributor to decreasing the fat content of cottage

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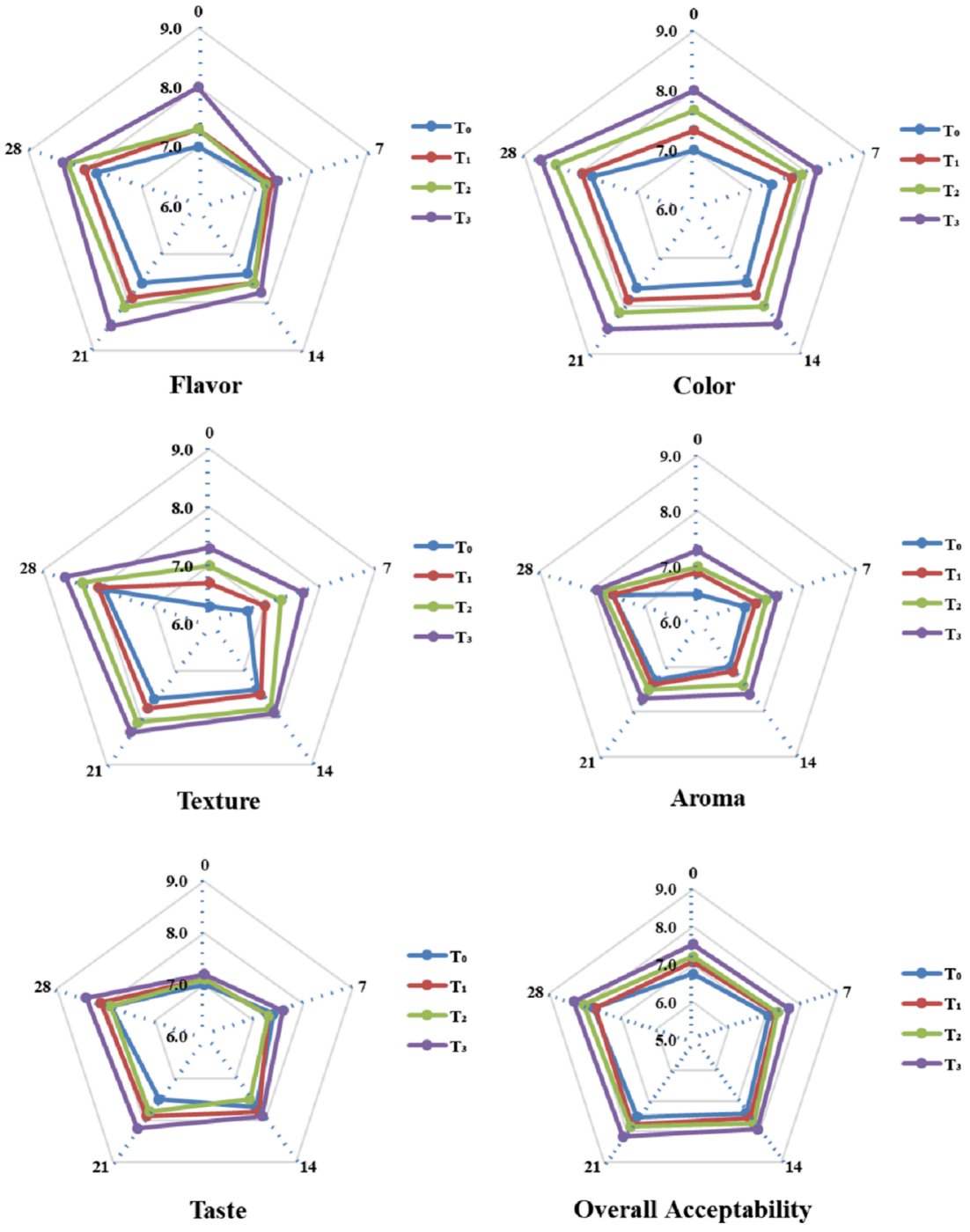

Figure 5. Sensory evaluation of free and encapsulated *L. acidophilus* and *L casei* treated probiotic cottage cheese at 0, 7, 14, 21, and 28 days. T_0 = cottage cheese with free *L. acidophilus* and *L casei* (control), T_1 = cottage cheese coated with 2% sodium alginate, T_2 = cottage cheese coated with 1.5% sodium alginate and 0.5% carrageenan, and T_3 = cottage cheese coated with 1% sodium alginate and 1% carrageenan.

cheese. Bacterial metabolism and lipase enzymes might also contribute to the degradation of fats in cottage cheese. A similar linearity was observed by Hussain and Kanwar,[47] who revealed the dried cheese product (i.e., Ladakhi *churpe*) to exhibit a significant decline in lactose contents that could be linked with the lactic acid breakdown during storage. Earlier studies by El-Sayed and El-Sayed[48] and Dafalla et al.[49] delineated the increase in protein (9.4−9.9 g/100 g) and total solid (28.1−29.1 g/100 g) contents of soft cottage cheese prepared using the prebiotic aloe vera pulp on storage of 1−4 weeks. The variation in the protein and total solid magnitudes could be due to the decrease of moisture contents during storage.[50]

3.8. Enumeration of Free and Encapsulated *L. acidophilus* and *L. casei* in Cottage Cheese. The results for enumeration count of probiotics elucidated a significant ($p < 0.05$) decrease in all treatments of cottage cheese at 7−28 days (Table 4). The initial probiotic count for free probiotics (T_0) was recorded as 7.9 log cfu/mL, which gradually decreased to 6.4 log cfu/mL during 4 weeks of storage, and a lower reduction was observed for the encapsulated probiotic treatments. The viable count of free and encapsulation bacteria

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was increased in the first week of storage as a result of the favorable growth conditions. The viable count of encapsulated probiotics, i.e., alginate 2% (T_1), was revealed to be 7.2 log CFU/g, whereas the alginate 1.5% and carrageenan 0.5% (T_2) and alginate 1% and carrageenan 1% (T_3) showed 7.7 and 8.6 log CFU/g, respectively, at the maximum storage of 28 days. The alginate and carrageenan gum-based encapsulation plays a remarkable role in the protection of probiotics under acidic conditions. The lower survivability of free probiotics could be linked to the zero encapsulation/coating under lower pH and high acidity conditions. Comparable findings have been portrayed by earlier studies of Ahmed et al.[44] and Haghshenas et al.[51] wherein the researchers reported log reductions in free cells when compared with the encapsulated ones. Another study by Kataria et al.[52] reported the free cells of *Enterococcus durans* to be reduced from 9.5 to 2.8 log CFU/g on storage of cheese at 3−9 °C for 4 weeks.

3.9. Sensory Evaluation of Probiotic Cottage Cheese.

The findings for organoleptic evaluation of the cottage cheese on 0−28 days of study revealed a significant ($p < 0.05$) increase in overall organoleptic scores of each parameter (Figure 5). The maximum color values of cottage cheese were recorded for T_3 (i.e., 8.7) on the 28th day, whereas the minimum score was given by T_1 (i.e., 7.3) on zeroth day in comparison with the control (T_0), i.e., 7.0 and 7.8, respectively. The significant ($p < 0.05$) increase in sensory scores for color of cottage cheese on storage of 0−28 days might be associated with the ripening. The highest sensory score for the flavor of cottage cheese was given to T_3 (i.e., 8.4) on the 28th day, whereas the minimum score was given to T_2 (i.e., 7.2) on the seventh day in comparison with the control (T_0), i.e., 7.02 and 7.8 on 0 and 28 days, respectively. Cottage cheese showed the highest sensory scores for overall acceptability, i.e., 7.7−8.3 on the 28th day of storage, which is the highest for all treatments and control. The study revealed higher sensory scores for overall acceptability with increased storage time. It is evident from earlier studies by Mushtaq et al.[53] and Ali et al.[46] wherein the use of mixed probiotics for the development of cheese resulted in improved sensorial scores of the cheese, i.e., aroma (i.e., 6.3−7.3), flavor (i.e., 6.1−6.6), and overall acceptability (i.e., 8.0). Conclusively, based on opinion and sensory scores assigned by the panel of sensory experts, the cottage cheese prepared with the sodium alginate 1% and carrageenan 1% (i.e., T_3) was found to be the best among all other treatments for all aspects of sensory characteristics.

4. CONCLUSIONS

This study comprehensively investigated the survivability of probiotics, specifically *Lactobacillus acidophilus* and *Lacticaseibacillus casei* in both encapsulated and nonencapsulated forms, when added to cottage cheese and stored for 28 days at a temperature of 4 °C. Through the application of an encapsulation method that combines varying ratios of sodium alginate and carrageenan, it became feasible to fabricate and thoroughly examine microbeads. FTIR spectroscopy confirmed the presence of probiotics in the microcapsules, whereas SEM verified the stable structure of the microbeads. X-ray diffraction (XRD) tests confirmed the presence of the microbeads under amorphous conditions. The microbeads (T_3) composed of 1% sodium alginate and 1% carrageenan demonstrated the maximum encapsulation efficiency, achieving a notable 95%. T_3 exhibited remarkable probiotic vitality, with a count of 8.5, 8.8, and 8.9 log CFU/g after 80 min of exposure to simulated intestinal fluid, simulated gastric juice, and biliary salt solution, respectively. The pH measurements demonstrated a statistically significant reduction ($p < 0.05$), leading to an increase in titratable acidity. After 28 days of storage, nutritional analysis showed that T_3 had the highest levels of ash, protein, and total solids. The formulation of T_3 was repeatedly praised for its excellent color, flavor, and texture, leading to good sensory assessments. Furthermore, this approach resulted in improved nutritional content, reduced acidity, and an overall more enjoyable flavor profile.

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Notes

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