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Application of microencapsulated *Lactobacillus rhamnosus* ATCC 7469 with milk protein concentrate in the production of functional orange juice and investigation of its qualitative characteristics

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ARTICLE INFO	ABSTRACT
Article History: Received: 2025/08/31 Review: 2025/11/17 Accepted: 2025/11/27 Keywords: Microencapsulation, <i>Lactobacillus rhamnosus</i>, Milk Protein Concentrate, Viability, Orange Juice DOI: 10.48311/fsct.2026.84092.0 *Corresponding Author E-	Abstract Microencapsulation technology is a novel approach to enhancing bacterial resistance against environmental, chemical, and gastrointestinal stresses. In this study, milk protein concentrate (MPC) was used as a wall material due to its strong biological and protective properties. The aim was to evaluate the effect of this material, combined with transglutaminase enzyme, on the survival of <i>Lactobacillus rhamnosus</i> in the acidic environment of orange juice. Four samples were prepared: S1 (plain orange juice), S2 (protein + bacteria), S3 (protein + bacteria + enzyme), and S4 (protein + enzyme). During storage, samples were analysed for bacterial viability, antioxidant activity, pH, titratable acidity, vitamin C, reducing sugar, total phenolic content, color, FTIR spectra, and molecular weight. The results showed that sample S3 exhibited the best performance with a viability of 5.5×10^7 CFU/mL, antioxidant activity of 68.5%, and acidity of 4.4%, along with a pH of 3.6. In contrast, sample S4 showed the lowest viability (3.8×10^7 CFU/mL) and vitamin C content (32.3 mg/L). Sample S2 had the highest total phenolic content (14.7 mg GAE/mL) and the lowest reducing sugar (9.07 mg/mL). During storage, vitamin C, reducing sugars, and phenolics decreased, whereas acidity and antioxidant activity increased. Samples containing bacteria showed higher vitamin C and acidity, while those with the enzyme exhibited higher reducing sugar content. FTIR analysis confirmed the presence of carbonyl, methyl, and amine functional groups. Sample S2 showed decreased molecular weight and increased solubility, indicating effective interactions between bacteria and protein.

1- INTRODUCTION

In recent years, consumer awareness regarding the link between food and health has increased significantly, and health-oriented food products, particularly functional foods and probiotics, have garnered special attention (١). As live microorganisms, probiotics exert beneficial effects on human health, including improving intestinal microbial flora, strengthening the immune system, reducing blood cholesterol levels, and preventing diseases (٢). However, unfavorable environmental and gastrointestinal conditions pose a challenge to the viability of these bacteria. The use of novel methods, such as microencapsulation, can improve the stability and efficacy of these microorganisms (٣). Previous research has shown that *Lactobacillus* species are among the most important probiotics used due to their resistance to harsh gastric conditions and bile salts (٤, ٥). However, the stability of these bacteria in food products, particularly in acidic environments such as fruit juices, is low. Numerous studies have investigated the use of various materials for the microencapsulation of probiotics, including plant gums and hydrocolloids (٦). Milk protein concentrates have been introduced as a suitable option for microencapsulation due to their favorable rheological properties and their ability to protect bacteria (٧). Furthermore, the enzyme transglutaminase can improve the properties of protein matrices and provide enhanced protection (٨). The need to replace dairy products with non-dairy alternatives for sensitive groups, such as vegetarians, individuals with lactose intolerance, or those sensitive to milk proteins, has increased the importance of utilizing new matrices for the microencapsulation of probiotics. Moreover, the increased consumption of fruit juices as a natural source of vitamins and antioxidant compounds has made these products potential carriers for the delivery of probiotics. The application of novel technologies for the extraction of pigments and bioactive plant compounds has provided new prospects for the enrichment and improvement of the nutritional value of fruit juices in the food industry (٩). In this regard, investigating the effect of various wall materials on the viability of probiotics in fruit juices can be an effective step toward the

production of health-oriented products. Previous studies have also investigated the microencapsulation of various *Lactobacillus* species in protein matrices. For example, Maleki et al. (2023), in the production of probiotic ice cream, showed that the microencapsulation of *Lactobacillus rhamnosus* in a combined matrix of whey protein and bacterial cellulose significantly increased viability during storage (١٠). Afzal et al. (2024) also reported that using milk protein concentrate as a wall material for the microencapsulation of probiotics results in a high encapsulation efficiency (over 90%) and enhances bacterial stability under acidic conditions (١١). In another study, Dikmetas et al. (2025) demonstrated that the incorporation of probiotic bacteria into fermented fruit juices using protein matrices and cross-linking enzymes, such as transglutaminase, can improve the sensory quality and antioxidant activity of the product (١٢). Additionally, Randazzo et al. (2013) investigated the survival of *L. rhamnosus* in peach jam and showed that microencapsulation significantly increases viability during storage (١٣). Collectively, these findings suggest that the combination of milk proteins with cross-linking enzymes can provide an efficient medium for protecting probiotic bacteria in non-dairy products such as fruit juices. The objectives of this study include investigating the microencapsulation of *Lactobacillus rhamnosus* using milk protein concentrate and the enzyme transglutaminase to improve the viability of this bacterium in the acidic environment of orange juice, as well as evaluating the effect of different wall materials on the physicochemical properties of the final product. Furthermore, this research aims to propose an approach to enhance the stability and application of probiotics in non-dairy products, which could lead to the development of health-promoting products with broader consumer appeal.

2-Materials and Methods

2.1. Materials, equipment, and microorganisms

The chemicals used in this study included skim milk powder (Sigma-Aldrich), MRS agar medium (Merck), Tween 80 (Scharlau), buffered peptone water (Micro Media), and

canola oil (Ladan). Laboratory equipment included an autoclave (Rayhan Teb, Iran), a digital scale model FEW (Japan), a magnetic stirrer model L-81 (Stuart), a centrifuge (Shimifan, Iran), a vortex mixer model L-81 (LABINCO, Netherlands), an incubator (Shimaz Co.), a benchtop refractometer model 2WAJ (China), various laboratory glassware (Simax), micropipettes (Dragon, Germany), and a digital pH meter model pH Lab 827 (Metrohm, Switzerland), which were used for the various experimental procedures. The bacterial strain *Lactobacillus rhamnosus* was obtained from Pardis Roshd Mehregan Products Co. and was stored under sterile conditions in a refrigerator at 4°C until use.

2.2. Bacterial preparation, activation, and purification

To prepare the skim milk medium, 10 g of skim milk powder was dissolved in 100 mL of distilled water, and the resulting solution was sterilized in an autoclave at 121°C for 15 min. After cooling the solution to room temperature, *Lactobacillus rhamnosus* was added at a concentration of 2% (w/w) and mixed thoroughly. The prepared mixture was incubated at 37°C for 72 h to activate the bacteria. Following the incubation period, the activated bacteria were subcultured for purification using the pour-plate method on MRS agar medium. The colonies formed after 72 h of incubation at 37°C were observed to be free of any contamination and were subsequently used for further experimental steps (13).

2.3. Production and evaluation of microencapsulation efficiency

To produce the microcapsules, 5 g of milk protein concentrate (MPC) was dissolved in 100 mL of distilled water, and the resulting solution was heated to 60°C. After sterilization and cooling of the solution, 10 mL of the bacterial suspension was added to 50 mL of the MPC solution and mixed gently. The obtained mixture was gradually added to 250 mL of canola oil containing 2% (w/v) Tween 80 and emulsified under constant stirring. The emulsified sample was stored in a refrigerator (at 4°C) for 24 h. Subsequently, 250 mL of 0.1

M calcium chloride solution (at 4°C) was added to the system. Following sedimentation, the microcapsules were separated and purified via centrifugation and washing with physiological saline. Encapsulation Efficiency (EE%) was determined by comparing the number of unencapsulated viable cells to the total number of viable cells after the microencapsulation process. Cell enumeration was performed directly from the emulsion (9).

2.4. Measurement of phenolic compounds and antioxidant activity

To determine the total phenolic content, 0.1 mL of the sample was mixed with 0.5 mL of the Folin-Ciocalteu reagent and 0.4 mL of 7.5% sodium carbonate solution. The mixtures were incubated in the dark at 30°C for 30 min. Subsequently, the absorbance of the samples was measured at 765 nm using a spectrophotometer. The total phenol content was expressed as milligrams of gallic acid equivalents per milliliter of the sample (mg GAE/mL), based on a gallic acid standard curve (14). The antioxidant activity of the samples was also assessed using the DPPH radical scavenging assay. To this end, 2.5 mL of the sample was mixed with 1 mL of the DPPH solution, and after 30 min of reaction in the dark, the absorbance of the solution was measured at 518 nm. A tube containing DPPH without the sample was used as a control (15). The antioxidant activity was calculated as the percentage of DPPH absorbance inhibition using the following equation:

$$100 \times \frac{A - B}{A} = (\%) \text{Antioxidant activity}$$

Where A is the absorbance of the control and B is the absorbance of the sample.

2.5. Measurement of reducing sugars

To determine the reducing sugar content, 25 mL of the sample was initially taken and diluted to a final volume of 100 mL with distilled water. The solution was then filtered using activated carbon. Into a 100 mL Erlenmeyer flask, 5 mL of Fehling's solution A, 5 mL of Fehling's solution B, and a few drops of methylene blue indicator were added. The resulting mixture was heated gently on a hot plate, and several

glass beads were added to prevent bumping (sudden boiling). The filtered solution was then titrated slowly into the boiling mixture using a pipette until the color changed to a stable brick-red (۱۶). If the color reverted to blue after the addition of the indicator, the titration was repeated.

$$\frac{\text{Fehling factor} \times 100 \times 100}{\text{volume of sample filtrate used} \times 25 \times 100} = (\%) \text{Reducing sugar}$$

This reaction is based on the reduction of cupric ions (Cu^{2+}) to cuprous ions (Cu^{+}) in the solution.

2.6. Determination of Vitamin C content

To determine the Vitamin C content, 10 mL of the sample was placed into a 100 mL volumetric flask, and 100 mL of a 10% trichloroacetic acid (TCA) solution was added. The flask was then filled to the 100 mL mark with distilled water. Subsequently, 10 mL of this solution was transferred into a clean Erlenmeyer flask, and 2.5 mL of acetone was added. The mixture was titrated with 2,6-dichlorophenolindophenol until a light pink (or soft red) color appeared.

The Vitamin C content was calculated using the following equation:

$$\begin{aligned} & \text{Titrant (consumption) volume} \times \frac{100}{10} \\ & \times \left(\frac{\text{Sample volume}}{X} \right) \\ & = \text{Vitamin C (mg)} \end{aligned}$$

2.7. Viability assessment

To evaluate the viability and stability of *Lactobacillus rhamnosus*, a comparison was performed on the bacterial viability results over a 30-day storage period at 4°C and 25°C in an orange juice medium. The results indicated that free (unencapsulated) bacteria experienced a significant reduction in their colony-forming unit (CFU) counts throughout the storage period. In contrast, the microencapsulated samples, which were protected by the coating, exhibited a lower reduction in bacterial counts. These results demonstrate the protective effect of the microencapsulation coatings on bacterial

viability during storage at different temperatures.

2.8. pH and total acidity measurement

To measure pH and total acidity, 5 mL of the filtered sample (or diluted and filtered sample in the case of concentrates) was transferred to an Erlenmeyer flask using a pipette. The pH was measured directly using a digital pH meter, which had been calibrated at room temperature prior to use. For total acidity, the sample was titrated with 0.1 N sodium hydroxide (NaOH) (۱۷). The endpoint of the titration was determined based on the indicator used: the appearance of a stable pink color for phenolphthalein, or a stable blue color for methylene blue.

The total acidity was calculated as citric acid equivalents using the following equation:

$$100 \times 0.0064 \times N \\ = \text{Total acidity (g citric acid / 100 mL)}$$

Where:

N is the volume of NaOH used (mL).

0.0064 is the milliequivalent factor for citric acid in titration with NaOH.

V is the volume of the sample (mL).

2.9. Fourier-Transform Infrared (FTIR) spectroscopy

For FTIR analysis, transparent pellets containing the samples were prepared. Specifically, a specified amount of the dried sample was thoroughly mixed with dry, pure potassium bromide (KBr) at a weight ratio of 1:20 (w/w). The resulting mixture was pressed in a pellet-forming die under a pressure of approximately 60 kPa for 10 min. The transmittance spectra of the samples were then recorded and analyzed in the wavenumber range of 4000 to 400 cm^{-1} with a resolution of 0.15 cm^{-1} using an FTIR spectrophotometer (Model Spectrum Two, PerkinElmer, USA) (۱۸).

2.10. Measurement of molecular weight and particle diameter

To determine the molecular weight of the sample, a stock solution with a concentration of 0.05 g/mL was prepared and subsequently diluted to three lower concentrations: 0.01, 0.005, and 0.0025 g/mL. The light scattering intensity of the samples was measured using a Zetasizer Nano instrument. Based on the obtained data and the Rayleigh plot, the molecular weight of the sample was estimated as a function of concentration from the slope of the curve. To measure the average particle diameter (size distribution), dynamic light scattering (DLS) was performed using the same instrument, and the results were reported based on the number- and volume-weighted averages (¹⁸).

2.11. Color measurement

To evaluate the color characteristics of the samples, a portable Minolta CR-400 colorimeter (Japan) was used based on the CIE Lab color space. Prior to testing, the device was calibrated against a standard white plate with reference values of $L^* = 84.59$, $a^* = 1.11$, and $b^* = 3.32$. After calibration, the three color coordinates, including lightness (L^*), redness-greenness (a^*), and yellowness-blueness (b^*), were recorded for each sample (¹⁹). To determine the overall color difference of the sample relative to the reference color, the ΔE index was calculated using the following equation:

$$\sqrt{2(b^* - b)^2 + 2(a^* - a)^2 + 2(L^* - L)^2} = \Delta E$$

Also, the whiteness index (WI) was calculated using the following formula:

$$\sqrt{2a^2 + b^2 + (L - 100)^2} - 100 = WI$$

2.12. Statistical analysis

All experiments were conducted in three independent replicates. Data analysis was performed using SPSS software based on a factorial experiment in a completely randomized design (CRD). Prior to the analysis of variance, the normality of the data was assessed using the Kolmogorov-Smirnov test. Then, one-way analysis of variance (ANOVA)

was performed to compare the means, and when the treatment effects were significant ($P < 0.05$), Tukey's post hoc test was used for pairwise comparison of the means.

3- Results and Discussion

3.1. pH and total acidity of the samples

The pH values of the samples ranged from 3.61 to 4.03, and a significant difference was observed among them ($p \leq 0.05$). As shown in Figure 1(a), the highest pH value belonged to sample S4 (containing MPC protein and TG enzyme without bacteria), whereas the samples containing *Lactobacillus rhamnosus* (S2 and S3) exhibited lower pH values. This reduction can be attributed to the metabolic activity of the bacterium and the production of organic acids. Comparison of the mean values showed that the pH difference between S3 and S2 was only 0.01 units, while the greatest difference was observed between S2 and S1 (equivalent to 0.38 units). A decrease in pH was also observed during storage, which is consistent with the findings reported by Shirpour et al. (2016) (²⁰). Tukey's test, as presented in Figure 1(b), also confirmed significant differences among some samples.

The acidity of the samples ranged from 3.96% to 4.33%. Sample S4 (without bacteria) showed the lowest acidity, whereas the bacterial samples exhibited higher acidity values. A significant difference was recorded among the treatments ($p \leq 0.05$). The greatest difference was observed between samples S2 and S3, with a value of 0.10%, while the smallest difference was found between S4 and S1, with a value of 0.04%. As shown in Figure 1(b), the increase in acidity was mainly related to the metabolic activity of *L. rhamnosus* and the production of organic acids during storage. On the other hand, the combination of MPC protein and TG enzyme played an effective role in maintaining stability and controlling acidity. Similar results were reported by Maleki et al. (2023) in probiotic ice cream and Randazzo et al. (2013) in peach jam, where the reduction in pH was attributed to lactic acid production by *Lactobacillus rhamnosus* (^{21, 22}). These findings may have potential applications in the development of stable and health-oriented probiotic beverages.

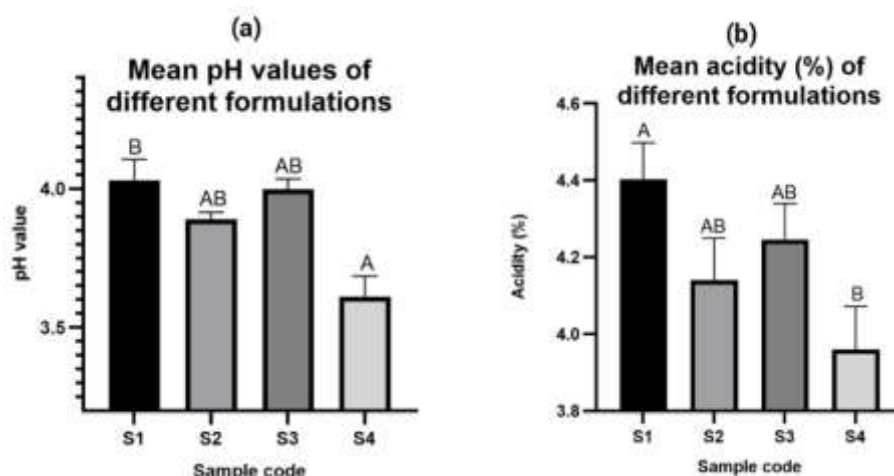


Figure 1. (a) pH and (b) Acidity of the Samples

3.2. Vitamin C

The results presented in Figure 2(a), together with the one-way analysis of variance, showed that there was a significant difference in vitamin C content among the studied samples ($p \leq 0.05$). The mean values of vitamin C in the samples ranged from 32.280 to 45.480 mg/L. The greatest difference in mean value was observed between samples S4 and S1 (1.32%), while the smallest difference was found between S4 and S3 (0.22%). Sample S4 (without probiotic bacteria) had the lowest vitamin C content, whereas the samples containing *Lactobacillus rhamnosus* (S1, S2, and S3) showed significantly higher values.

These findings confirm that the presence of probiotic bacteria contributes to the preservation of vitamin C, which may be related to the antioxidant properties of this vitamin and the metabolic requirements of the bacteria during the early stages of growth. This finding is consistent with the reports of Silalahi et al. (2018) in yogurt drink and Dikmetas et al. (2025) in fermented fruit juices; both studies showed that probiotic bacteria, during the initial stages of growth, enhanced antioxidant capacity by preserving vitamin C (21, 11). However, a gradual decrease in vitamin C was observed in some samples over time, which is similar to reports in other functional products, including probiotic ice cream (10), and may be attributed to its uptake and utilization by bacterial cells. This suggests that the presence of probiotics initially helps preserve the vitamin, but may

subsequently contribute to its reduction over time.

3.3. Reducing sugars

Based on the results shown in Figure 2(b) and the analysis of variance, there was a significant difference among the different treatments in terms of reducing sugar content ($p \leq 0.05$). The mean values of reducing sugars in the samples ranged from 9.067 to 16.333 mg/mL. The greatest difference in mean values was observed between samples S3 and S2 (3.2%), whereas the smallest difference was found between S2 and S1 (0.72%). The lowest sugar content was recorded in sample S2 (without transglutaminase enzyme), while the highest value was observed in sample S4.

The use of transglutaminase enzyme significantly increased the reducing sugar content, which may contribute to improving the functional properties of probiotic products. In contrast, the gradual decrease in sugars during the 14 days of storage was most likely due to their utilization by *Lactobacillus rhamnosus*. This finding is in agreement with the report of Shirpour et al. (2016), who stated that an increase in bacterial density leads to a greater reduction in reducing sugars (90). In addition, our results are consistent with the findings of Afzal et al. (2024), who reported a decrease in reducing sugars as a result of metabolic consumption by *lactobacilli* (9). The regression model, with a high coefficient of determination ($R^2 = 0.906$ and Adjusted $R^2 = 0.872$), also showed that the changes in reducing sugars

were highly predictable. Therefore, simultaneous control of enzyme composition and bacterial activity can greatly contribute to

optimizing the formulation of probiotic products.

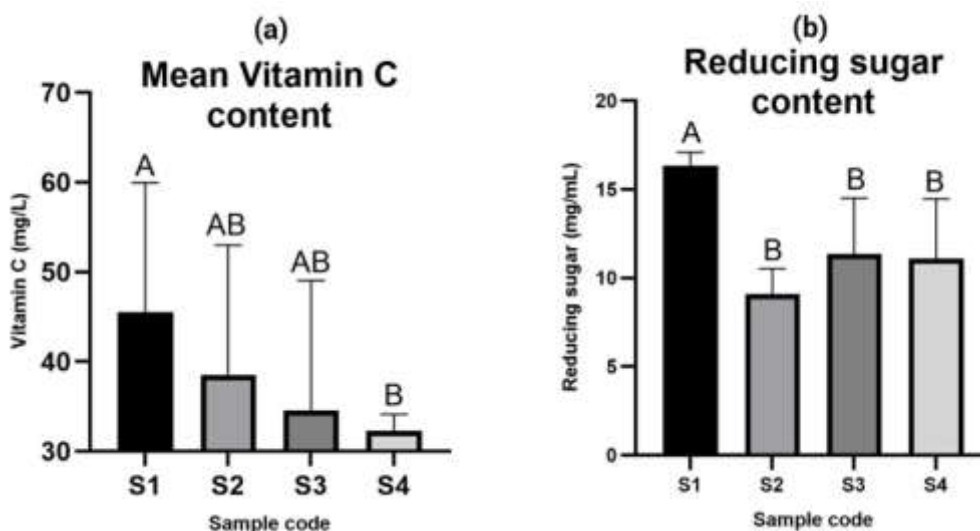


Figure 2. (a) Vitamin C Content and (b) Reducing Sugars Content of the Samples

3.4. Antioxidant activity and total phenolic content

The analysis of variance for both free radical scavenging activity and total phenolic content showed significant differences among the studied samples ($p \leq 0.05$). As illustrated in Figures 3(a) and 3(b), the greatest mean difference for both parameters was observed between samples S4 and S3, with this pair showing the highest variation in antioxidant activity and total phenolic content, respectively. In contrast, the smallest difference in both parameters was found between samples S3 and S1, while no significant differences were observed among the other samples.

With regard to antioxidant activity, the mean values ranged from 32.02% to 68.50%. The results indicated that the combined effect of the bacterium, transglutaminase enzyme, and milk protein concentrate significantly enhanced antioxidant activity in sample S3. However, this antioxidant activity decreased over time, which is consistent with previous reports describing the gradual decline in antioxidant capacity of probiotic products during storage (۳۱).

Moreover, the prediction equation for antioxidant activity, with R^2 and adjusted R^2 values of 0.974 and 0.965, respectively, indicated the high accuracy of the model in predicting this response.

For total phenolic content, the mean values ranged from 9.867 to 14.700 $\mu\text{g/mL}$. In the sample without bacteria (S4), the total phenolic content decreased, whereas the simultaneous presence of the bacterium and milk protein concentrate increased the total phenolic content, thereby improving antioxidant activity. This increase in total phenolics declined over time, a trend that has also been reported previously in similar products (۳۲). Similar findings were reported by Lathoura et al. (2021) in kombucha and by Maleki et al. (2023) in probiotic synthetic products, where the presence of probiotics increased free radical scavenging capacity at the beginning of storage, followed by a gradual decline over time (۳۲, ۳۳). The prediction equation for total phenolic content, with R^2 and adjusted R^2 values of 0.858 and 0.805, respectively, also demonstrated the high accuracy of the model in predicting total phenolic content.

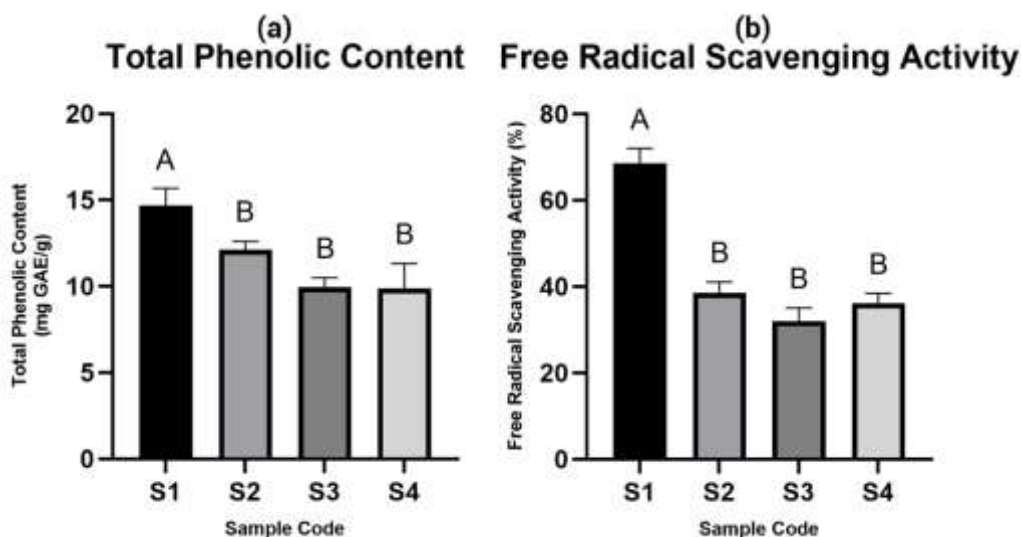


Figure 3. (a) Total Phenolic Content (TPC) and (b) Free Radical Scavenging Activity (FRSA) of the samples (S1–S4).

3.5. Viability

According to the results of the analysis of variance, a significant difference was observed in viability among the different samples ($p \leq 0.05$). The results showed that the mean difference between samples S3 and S2 was greater than that of the other samples, with a difference of 0.2%. In addition, samples S4 and S3 showed the smallest mean difference (0.16%), and no significant difference was observed among the other samples. The mean viability of the samples ranged from 3.867% to 5.500%. The highest viability was observed in sample S3, whereas sample S4 showed the lowest viability compared with the other samples. This finding is consistent with similar reports on improved survival in microencapsulated dairy products (۲۳, ۱۰). The results obtained in the present study are also in agreement with those reported by Randazzo et al. (2013), who investigated the survival of the probiotic bacterium *Lactobacillus rhamnosus* in peach jam during the storage period (۱۲).

3.6. Fourier-transform infrared spectroscopy (FTIR)

The FTIR spectra revealed the presence of several functional groups in the structure of the samples. The absorption bands related to amide (N-H) bonds were observed in the region around 3300 cm^{-1} , while the amide I band appeared at approximately 1650 cm^{-1} , indicating the proteinaceous structure of the samples (۲۴). H-C stretching vibrations were identified in the range of 2909.91 to 2931.99 cm^{-1} , and methyl (CH₃) groups were detected at 2870.95 and 2875.00 cm^{-1} . In addition, the peaks observed at 1653.87 , 1658.36 , and 1648.72 cm^{-1} were assigned to carbonyl (C=O) groups. Functional groups containing carbon-carbon double bonds (C=C) were also identified at 1655.00 , 1538.21 , 1538.03 , 2076.87 , and 1430.71 cm^{-1} . Furthermore, ether (C-O-C) groups were detected at 1109.53 , 1109.00 , and 1057.61 cm^{-1} , while the amine (NH₂) group was observed at 3357.67 cm^{-1} . These findings confirm that the protein and bacterial matrix used in this study contained a variety of functional groups and indicate the presence of intermolecular chemical interactions within the structure of the samples (۱۷).

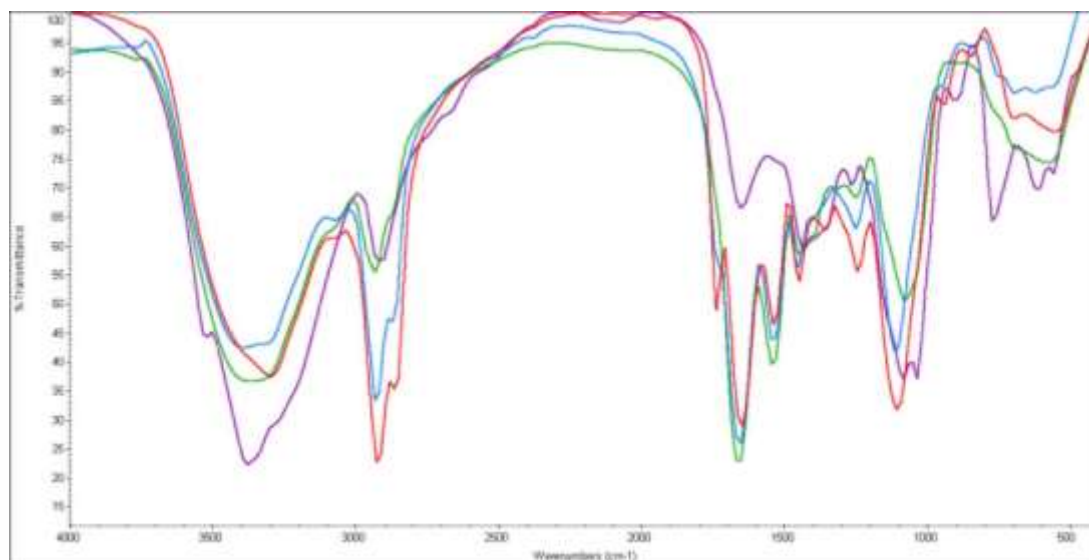


Figure 5. FTIR spectra of four different formulations overlaid for comparison. The red spectrum corresponds to *Lactobacillus rhamnosus* combined with milk protein concentrate. The blue spectrum represents *Lactobacillus rhamnosus* with milk protein concentrate and transglutaminase enzyme. The green spectrum shows the milk protein concentrate alone, while the purple spectrum corresponds to the transglutaminase enzyme. Differences in spectral bands reflect molecular interactions and structural modifications among the formulations.

3.7. Particle size and molecular weight

Evaluation of the results presented in Figures 6(a) and 6(b) showed that the particle diameter at the 80% frequency range was slightly greater than 100 nm. The small particle size may play an important role in improving emulsion stability against sedimentation or creaming (۲۵). The molecular weights of the samples, measured by DLS at 25°C, were 4.787×10^4 and 4.286×10^4 kDa, respectively. A high molecular weight is generally associated with the formation of a thicker layer around the particles, which contributes to greater structural stability and improved thermal resistance (۲۶). On the other hand, a reduction in molecular weight may enhance solubility, which can be advantageous in certain applications (۲۷).

The Z-average, as an indicator of the hydrodynamic particle size, was 324.1 and 717.52 nm for the samples, respectively. This parameter is interpretable only for dispersed particles or soluble molecules. In some formulations, an excessive amount of whey protein isolate led to the formation of smaller and more fragile microcapsules. In agreement with the findings of Manga et al. (2023), a decrease in molecular weight may improve dispersibility and enhance the solubility of milk proteins, which is consistent with the results of the present study (۲۶). Overall, these findings emphasize that particle size and molecular weight are critical parameters affecting the quality, stability, solubility, and performance of probiotic and emulsion-based products. Careful selection of formulation components and processing conditions can help optimize these properties and improve the nutritional and functional value of the final product.

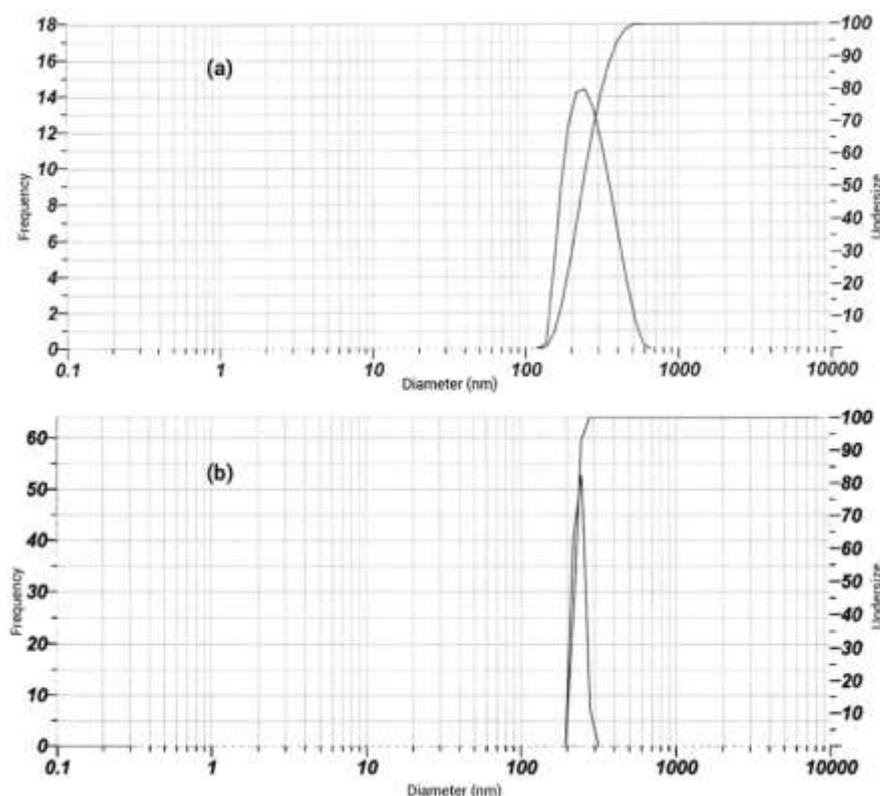


Figure 6. (a) Particle Size of *Lactobacillus rhamnosus* and Milk Protein Concentrate, (b) Particle Size of *Lactobacillus rhamnosus*, Milk Protein Concentrate, and Transglutaminase Enzyme

3.8. Color measurement

The colorimetric results of the samples (Table 1) showed that the color parameters L^* (lightness), a^* (redness-greenness), and b^* (yellowness-blueness) differed significantly among the various treatments. The lightness (L^*) values ranged from 57.8 to 73.5. Sample S2 (containing bacteria and milk protein concentrate) showed the lowest lightness value (57.8), whereas sample S1 (control) exhibited the highest value (73.5). The reduction in lightness in S2 was probably due to the metabolic activity of *Lactobacillus rhamnosus* and its effect on the matrix components ($\Upsilon^{\wedge}$). A decrease in sample transparency was also observed over time, which may be related to structural changes, particle aggregation, or associated intermolecular interactions (Υ°).

For the a^* parameter (redness to greenness), the values ranged from -8.7 to 1.7. The highest value was recorded for S2 (1.7), while the lowest value belonged to S1 (-8.7). These changes may have been influenced by enzymatic reactions or bacterial metabolism

(Υ°). The b^* value (yellowness) ranged from 11.3 to 15.4. The highest value was observed in sample S1 (15.4), while the lowest value was found in S2 (11.3). Sample S3, which contained all three components (bacteria, protein, and transglutaminase enzyme), showed a higher b value than S2 and S4, which may be related to the formation of yellow-colored compounds resulting from enzyme activity or bacterial metabolism ($\Upsilon^{\wedge}$).

Overall, the data indicate that the presence of *Lactobacillus rhamnosus* and transglutaminase enzyme affected the color characteristics of the samples. The reduction in lightness and the changes in the a^* and b^* color indices were likely due to chemical and physical reactions within the matrix structure. Similar changes in color parameters were reported by Ababaf et al. (2020) in soy yogurt and Masia et al. (2021) in fermented plant-based materials, where the presence of *lactobacilli* and transglutaminase led to reduced lightness and increased yellowness in fermented products ($\Upsilon^{\wedge}$, Υ°).

Table 1. Color Measurement of the Samples

Sample	b* (Yellow to Blue)	a* (Red to Green)	L* (Lightness)
S1	15.4 ^a ± 0.6	-8.7 ^b ±0.3	73.5 ^a ± 0.8
S2	11.3 ^c ± 0.7	1.7 ^a ± 0.4	57.8 ^c ± 1.0
S3	14.5 ^a ± 0.8	0.9 ^a ± 0.5	60.8 ^b ± 0.9
S4	12.4 ^b ± 0.5	-0.8 ^a ±0.4	62.3 ^b ± 1.1

Values are expressed as mean ± standard deviation (n = 3). Different letters within each column indicate significant differences ($P < 0.05$) according to Tukey's test.

4-Conclusion

The results of this study showed that the combination of milk protein concentrate, transglutaminase enzyme, and *Lactobacillus rhamnosus* had a significant effect on the physicochemical and functional properties of the beverage. Among the treatments, sample S3, which contained all three components, showed superior performance in terms of bacterial viability, antioxidant activity, and vitamin C retention, making it a promising candidate for the development of probiotic products. Although this sample exhibited a lower pH than the other treatments, the decrease in pH and the increase in acidity were correlated with enhanced bacterial viability.

Sample S4 (protein + enzyme) showed the highest pH, lightness, and reducing sugar content, but exhibited the lowest viability and vitamin C content. In contrast, sample S2 (protein + bacteria) had the highest total phenolic content and the lowest reducing sugar content and lightness. During the storage period, decreasing trends were observed in vitamin C, pH, phenolic compounds, and reducing sugars, whereas acidity and antioxidant activity increased. These changes were mainly attributed to the metabolic activity of the bacteria and the interactions among matrix components.

Furthermore, FTIR analysis confirmed the presence of important functional groups, including carbonyl, methyl, and amine groups, which play a role in structural stability and functional properties. The particle size was in the nanometric range, and the samples showed differences in

molecular weight; in particular, S2 had a lower molecular weight, which may contribute to improved solubility. Regarding color characteristics, sample S2 showed the lowest lightness and the highest redness value, while S4 exhibited the highest lightness. An increase in yellowness was also notable in sample S3 over time.

Overall, the findings of this study confirm that selecting an appropriate combination of biological and functional components can improve the nutritional properties, stability, and sensory quality of probiotic products. Sample S3, with the highest viability, antioxidant activity, and vitamin C content, was identified as the optimal formulation in this study and may serve as a suitable basis for the development of functional beverages.

Data Availability

The data that support the findings of this study are available on request from the corresponding author.

Conflict of Interest

The authors have no conflicts of interest to declare.

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Author Contributions

Conceptualization: Fereydoun Nabizadeh, Mahmoud Rezazadeh Bari, Saber Amiri; Methodology: Fereydoun Nabizadeh, Saber Amiri; Data collection and analysis: Fereydoun Nabizadeh, Hossein Abdollahi; Writing – original draft: Fereydoun Nabizadeh; Writing – Review and editing: Saber Amiri, Hossein Abdollahi, Seyed Alireza Banihashemi; Supervision: Mahmoud Rezazadeh Bari, Saber Amiri, Seyed Alireza Banihashemi.

AI statement

AI tools were used solely for language editing and grammar improvement. All authors reviewed and approved the final manuscript, taking full responsibility for its content.

5-Resources

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کاربرد لاکتوباسیلوس رامنوسوس ATCC 7469 ریزپوشانی شده با پروتئین تغلیظ شده شیر در تولید آب پرتقال فراسودمند و بررسی ویژگی های کیفی آن

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فناوری ریزپوشانی روشی نوین برای افزایش مقاومت باکتری ها در برابر عوامل محیطی، شیمیایی و گوارشی است. در این پژوهش از پروتئین کنسانتره شیر به دلیل ویژگی های زیستی و حفاظتی بالا به عنوان ماده دیواره ای استفاده شد. هدف، بررسی تأثیر این ترکیب به همراه آنزیم ترانس گلوتامیناز بر زنده مان *Lactobacillus rhamnosus* در محیط اسیدی آب پرتقال بود. چهار نمونه شامل S1 (آب پرتقال خالص)، S2 (پروتئین + باکتری)، S3 (پروتئین + باکتری + آنزیم) و S4 (پروتئین + آنزیم) تهیه شد و طی دوره نگهداری از نظر زنده مان باکتری، فعالیت آنتی اکسیدانی، pH، اسیدیته، ویتامین C، قند احیاکننده، ترکیبات فنلی، رنگ، طیف سنجی FTIR و وزن مولکولی بررسی شدند. نتایج نشان داد نمونه S3 با زنده مان 1.0×10^7 CFU/ml، فعالیت آنتی اکسیدانی ۶۸/۵٪ و اسیدیته ۴/۴٪ بهترین عملکرد را داشت و در مقایسه با سایر تیمارها pH برابر ۳/۶ داشت. در مقابل، نمونه S4 کمترین زنده مان 1.0×10^3 CFU/ml و کمترین مقدار ویتامین C (۳۲/۳ mg/L) را نشان داد. نمونه S2 دارای بیشترین ترکیبات فنلی (۱۴/۷ mg GAE/ml) و کمترین قند احیاکننده (۹/۰۷ mg/ml) بود. در طول نگهداری، ویتامین C، قندهای احیاء و فنول ها کاهش، در حالی که اسیدیته و فعالیت آنتی اکسیدانی افزایش یافتند. نمونه های حاوی باکتری، ویتامین C و اسیدیته بیشتری داشتند و نمونه های حاوی آنزیم، قند احیاکننده بیشتری نشان دادند. طیف سنجی FTIR حضور گروه های عاملی کربونیل، متیل و آمین را تأیید کرد. نمونه S2 با کاهش وزن مولکولی و افزایش انحلال پذیری، نشان دهنده تعامل مؤثر باکتری و پروتئین بود.