Probiotic-fortified *Solanum lycopersicum* (tomato) juice: free or encapsulated *Lactobacillus plantarum and Lactobacillus delbrueckii*

Reyhane Zakian, Marjan Nouri

Department of Food Science and Technology, Ro.C., Islamic Azad University, Roudehen, Iran

Abstract

Recent consumer perception of a nutritious diet improves demand for functional and safety products such as probiotics. The present research aims to investigate enriching *Solanum lycopersicum* (tomato) juice, including free and encapsulated probiotic bacteria. Initially, physicochemical attributes of encapsulations were evaluated. Then tomato juice samples, including a control without bacteria, free or encapsulated *Lactobacillus plantarum* (*L. plantarum*, T1 and T2), *Lactobacillus delbrueckii* (*L. delbrueckii*, T3 and T4), and a mixture (T5 and T6), were prepared over 28 days of shelf life. Several assays were performed, such as pH, lycopene, turbidity, stability, antioxidant, probiotic viability, sensory, and structure. Physicochemical functions of encapsulation illustrated that the results were in the suitable range. The pH of all treatments declined, and free *L. plantarum* demonstrated a greater effect on reduction. The control and encapsulated *L. plantarum* samples exhibited the lowest lycopene, ranging from 0.64 to 0.35 µL/mL, while the highest ranged from 0.64 to 0.50 µL/mL during the shelf life. Encapsulated dual bacteria indicated higher turbidity, stability, and antioxidant features compared to the control throughout shelf life. The control maintained greater transparency than others, and microbial analysis indicated that probiotic populations were elevated until the 14th day and then reduced. The encapsulated dual-bacteria illustrated the maximum viability and sensory, while the control had the minimum ratings. Morphological analysis confirmed a homogeneous structure for encapsulated bacteria. Overall results depicted that treatments containing encapsulated bacteria are considered the preferred option to promote nutritious juice.

Correspondence: Marjan Nouri, Department of Food Science and Technology, Ro.C., Islamic Azad University, Roudehen, Iran.
Tel.: +2176505016. Fax: +2176507664.
E-mail: marjan.nouri@iau.ac.ir

Key words: encapsulation, *Lactobacillus delbrueckii, Lactobacillus plantarum*, tomato juice, probiotic.

Contributions: RZ, formal analysis, methodology, investigation and drafted manuscript, resources, software and visualization; MN, conceptualization, writing – review, editing supervision, conducted statistical and project administration.

Conflict of interest: no conflict of interest declared by authors.

Ethics approval and consent to participate: ethical clearance was obtained from the Internal Ethical Clearance in compliance with institutional research ethics policies or similar, based on their institutional norms.

Availability of data and materials: data are available on request.

Funding: none.

Acknowledgments: the authors noticeably appreciate all participants who helped in the present study.

Received: 28 April 2025.
Accepted: 25 August 2025.
Early access: 20 October 2025.

Italian Journal of Food Safety 2025; 14:13932
doi:10.4081/ijfs.2025.13932

Introduction

Solanum lycopersicum (tomato), as an important member of the Solanaceae family, is widely cultivated around the world (Pereira *et al*., 2023). Tomatoes are classified as beneficial and valuable vegetables, including strengthening components such as vitamins, carotenoids, and phenolics (Liu *et al*., 2018). Tomato juice is a nutrient-dense beverage rich in bioactive compounds such as lycopene and a potent antioxidant known for its role in reducing oxidative stress and potentially risk of chronic diseases like cardiovascular disorders and certain cancers (Giordano *et al*., 2022). Its naturally low pH (approximately 4.0 to 4.5) makes it an ideal medium for probiotic fortification, as it supports the survival of acid-tolerant bacteria (Kaur *et al*., 2016).

Functional edible and safety products include special vitamins, essential fatty acids, minerals, biologically active constituents such as probiotics and dietary fibers (Budiati *et al.,* 2022). Probiotic bacteria, as live microorganisms consumed in balanced levels, provide health benefits to the host (Ghafari and Ansari, 2018).

Probiotics indicate specific characteristics, including being non-pathogenic, resistant to digestive enzymes, anticancer and antitumor functions, and also the ability to reduce cholesterol and blood fats (Castello *et al*., 2023; Fernandes *et al*., 2024). *Lactiplantibacillus plantarum* is one of the most widespread and important lactic acid bacteria species with high adaptability to different environments (Liu *et al*., 2018). Another important probiotic bacterium with similar effects is *Lactobacillus delbrueckii* strains in humans and other organisms (Kaur *et al.,* 2016). This homofermentative bacterium is one of the most widely used dairy starters and plays a key role in fermented yogurt and other products, including cheeses (Goderska *et al*., 2021).

The demand for functional foods like probiotic-fortified tomato juice stems from consumer preference for natural and nutrient-rich alternatives to synthetic supplements (Pereira *et al*., 2023). Unlike probiotic supplements, which are often isolated and lack dietary synergy, tomato juice provides prebiotic fibers and bioactive compounds that enhance efficacy and consumer appeal (Yang *et al*., 2019). Fortifying tomato juice with probiotics aims to combine the inherent nutritional benefits with the health-promoting effects, such as improved gut health and antioxidant activity, offering a non-dairy option for lactose-intolerant or vegan consumers (Dzandu *et al*., 2022; Pereira *et al*., 2023). The low pH of tomato juice, while suitable for acid-tolerant probiotics, can reduce free cell viability during storage and gastrointestinal transit due to acid stress and oxygen exposure (Giordano *et al*., 2022). Encapsulation technology addresses this by protecting probiotics within a carrier matrix (*e.g*., calcium alginate), enhancing their stability in acidic environments, extending shelf life, and ensuring higher survival rates in the gut (Ephrem *et al*., 2018).

Encapsulation techniques are increasingly employed to enhance probiotic survival in fruit juices and the human digestive system, which involves physically or mechanically trapping probiotic cells within a protective carrier matrix, producing particles ranging from nanometers to millimeters in diameter (Ahmadmoradi *et al*., 2024). This approach protects cells from adverse environmental conditions, such as low pH, oxygen exposure, and temperature fluctuations, through controlled release mechanisms (Ephrem *et al*., 2018). Methods like extrusion, emulsification, coacervation, and spray drying are commonly used, with emulsification being particularly effective for maintaining cell viability during food processing and storage (Da Silva *et al*., 2023). Encapsulation improves probiotic stability in acidic environments like fruit juices, enhances shelf life, and ensures higher survival rates during gastrointestinal transit, thereby maximizing health benefits for consumers (Giordano *et al*., 2022). Additionally, encapsulation can mask off-flavors, improve sensory attributes, and allow for targeted delivery of probiotics to the gut, making it a critical technology for developing functional beverages (Naga *et al*., 2016). In previous research, free lactic acid bacteria were employed for the fermentation of various safety-enhanced tomato products (Dzandu *et al*., 2022; Giordano *et al*., 2022; Pereira *et al*., 2023). Furthermore, *L. plantarum*, *Lactobacillus acidophilus*, and *L. delbrueckii* were applied for fermentation in carrot, beetroot, and tomato juices (Goderska *et al*., 2021). Free and immobilized *Lactobacillus paracasei* K5 were used in the fermentation of Comelian cherry juice (Mantzourani *et al.,* 2019), while immobilized *L. acidophilus* was employed for tomato juice fermentation (Yang *et al*., 2019). Additionally, grape juices fortified with either free or immobilized *Lacticaseibacillus rhamnosus* OLXAL-1 (Nikolaou *et al*., 2023). The aim of the present study is to develop a functional tomato juice fortified with free or encapsulated *L. plantarum* and *L. delbrueckii* to significantly enhance probiotic viability, stability, and nutritional quality over a 28-day shelf life. These microorganisms were selected because of their robust adaptability to acidic environments and proven health benefits, including antioxidant activity, cholesterol reduction, and gut health promotion (Kaur *et al*., 2016; Liu *et al*., 2018). *L. plantarum* is known for high acid tolerance and the ability to produce lactic acid, which enhances the preservation and sensory attributes of fermented juices (Wang *et al*., 2022). *L. delbrueckii* complements *L. plantarum* by contributing to flavor development and stability in fermented products (Goderska *et al*., 2022). Tomato juice is chosen as a carrier due to its inherent nutritional profile, low pH, and prebiotic fiber content, which synergistically support probiotic survival and activity (Yang *et al*., 2019). Encapsulation was employed to protect these probiotics from degradation during storage and digestion, aiming to deliver a higher viable cell count to consumers and enhance the functional juice properties (Pereira *et al*., 2023). In the present research, tomato juice samples included control without bacteria (C), free or encapsulated *L. plantarum* (T_1 and T_2), *L. delbrueckii* (T_3 and T_4), and a mixture (T_5 and T_6). Afterwards, several assays, including physicochemical, microbial, sensory, and structural characteristics, were monitored over 28 days of shelf life.

Materials and Methods

Preparation of materials

De Man, Rogosa, and Sharpe (MRS) and Mueller Hinton Agar, 2,2-diphenyl-1-picrylhydrazyl (DPPH), calcium alginate, and sodium alginate purchased from Merck, Germany. *L. plantarum* ATCC 14917 and *L. delbrueckii* ATCC 9649 in lyophilized forms were prepared from the Science and Technology Research Organization (Iran). For culture preparation, lyophilized strains were revived by inoculating 0.1 g each strain into 10 mL MRS broth and incubating at 37°C for 24 hours under microaerophilic conditions (5% CO_2) in an incubator (Memmert INCO108, Schwabach, Germany). The cultures were subcultured twice to ensure viability, then harvested by centrifugation (6000 g, 10 minutes, 4°C, Hettich Universal 320R, Tuttlingen, Germany). The cell pellets were resuspended in 10 mL sterile phosphate-buffered saline (pH 7.2), adjusted to 10^8 CFU/mL concentration using a spectrophotometer (OD_{600}=0.8-1.0, PerkinElmer Lambda 25 UV/Vis, Waltham, USA). For lyophilization, the cell suspension was mixed with a cryoprotectant (10% w/v skim milk, Merck, Darmstadt, Germany), frozen at -80°C about 24 hours in a freezer (New Brunswick U410, Eppendorf, Hamburg, Germany) and lyophilized using a freeze dryer (Martin Christ Alpha 1-2 LD Plus, Osterode, Germany) at -50°C and 0.01 mbar for 48 hours.

Encapsulation of *Lactobacillus plantarum* and *Lactobacillus delbrueckii* bacteria

Encapsulation was performed using the emulsification technique with calcium alginate. The 2% (w/v) sodium alginate solution (20 g in 1 L sterile distilled water) was prepared, autoclaved at 121°C for 15 minutes (Tomy SX-500 Lab Autoclave, Nerima-ku, Tokyo, Japan), and stored at 4°C for about 24 hours. Though 2% alginate was relatively low to balance viscosity and encapsulation efficiency, higher concentrations (*e.g*., 3 to 4%) complicated emulsification and reduced cell release in the gut (Da Silva *et al*., 2023). The 1% (v/v) bacterial suspension (10^8 CFU/mL) was mixed with 100 mL of sodium alginate solution. This mixture was added dropwise to 500 mL sunflower oil (Ladan, Tehran, Iran) containing 0.2% (v/v) Tween 80 (Merck, Darmstadt, Germany) and stirred at 400 rpm for 15 minutes using a magnetic stirrer (IKA C-MAG HS7, Staufen, Germany). The 0.2 M calcium chloride solution was slowly added to form capsule walls. Microcapsules were collected by centrifugation (4000 g and 10 minutes), washed twice with 100 mL sterile saline (0.9% NaCl), and stored at 4°C for 12 hours to harden (Giordano *et al*., 2022).

Physicochemical attributes of encapsulations

Particle size and zeta potential of encapsulated alginate spheres were measured using a Zetasizer (Malvern Zetasizer Nano-ZS, Worcestershire, UK) *via* dynamic light scattering (DLS). For zeta

potential, solid microcapsules were suspended in deionized water (pH 7.0) to form a dilute dispersion, as DLS measures the electrophoretic mobility of particles in a liquid medium, indirectly assessing surface charge (Da Silva *et al.,* 2023). Microcapsules produced by bacteria that were collected and washed to remove any free form using a disruptive procedure, such as applying a calcium chelator (Ethylenediaminetetraacetic acid) for alginate to break them open. The free bacteria were separated from microcapsules, and their numbers were measured using serial dilution and plating on MRS (Mirzaei *et al.,* 2011). After incubating at 37°C for about 48 hours, colonies were numbered to calculate viable bacteria in colony-forming units (CFU), and encapsulation efficiency was determined using Eq. 1 (Ghafari and Ansari, 2018):

Encapsulation efficiency (%) = Encapsulated bacteria (CFU) / Total number (CFU) × 100 [Eq. 1]

Tomato juice preparation

Ripe tomatoes were washed, homogenized, juiced, and pasteurized at 75°C for 10 minutes to achieve a total soluble solids content of ~5° Brix, then stored at 4°C for up to 20 days. The 1% (v/v) suspension of free or encapsulated *L. plantarum* and *L. delbrueckii* (10^8 CFU/mL) was inoculated into 100 mL tomato juice and incubated at 37°C for 48 hours. Tomato pulp was retained as a prebiotic, and seven different samples were prepared, including a control without bacteria (C), free or encapsulated *L. plantarum* (T_1 and T_2), *L. delbrueckii* (T_3 and T_4), and a mixture (T_5 and T_6) (an equal 1:1 ratio of bacteria used in samples T_5 and T_6).

Performed assays on treated samples, pH, and lycopene determination

The pH level was measured at 25°C using the pH meter (Metrohm 827, Herisau, Switzerland) for tomato juice samples (Pereira *et al.,* 2023). A total of 2.5 mL of tomato juice was combined in a 125 mL conical flask with a solvent mixture including hexane, acetone, and ethanol in a 2:1:1 volume ratio. The mixture was shaken at 150 rpm for 30 minutes to dissolve the carotenoids. Then, 10 mL of distilled water was added, which led to the separation into polar and non-polar layers. The non-polar phase was then transferred to a spectrophotometer, where absorbance was measured at a 472 nm wavelength. The lycopene was calculated using an extinction coefficient of E=3450 at 472 nm. Pure hexane solvent served as a control, and absorption data were converted to lycopene content based on the extinction coefficient in hexane (Dzandu *et al*., 2022; Giordano *et al*., 2022; Pereira *et al*., 2023).

Turbidity, transparency, and stability measurement

The turbidity was also determined using a turbidimeter, AN 210 (HACH, Loveland, CO, USA), and the transparency of juice was measured by transmittance (T%) at 660 nm wavelength and a spectrophotometer (PerkinElmer Lambda 25 UV/Vis Spectrometer, Shelton, CT, USA). The stability determination is based on separating precipitate from juice by centrifugation with preheating in a water bath and mass precipitate. The 10 mL juice was centrifuged (20000 g, 30 minutes, and 25°C, (Hettich 420, Tuttlingen, Germany), and a graduated cylinder determined the sediment volume; stability was also calculated by Eq. 2 (Ephrem *et al*., 2018):

Stability (%) = Sediment volume / Primary complex volumes × 100 [Eq. 2]

Antioxidant activity assay

The DPPH used to measure free radical scavenging activity, commonly expressed as half-maximal inhibitory concentration (IC_{50}), and tomato juice extract was prepared by mixing 5 mL juice with 10 mL ethanol, vortexing for 5 minutes, and centrifuging (5000 g, 10 minutes, 4°C) to obtain supernatant. The 2 mL aliquot of 0.1 mM DPPH in ethanol was blended with 2 mL extract (10-100 µg/mL), incubated in darkness at 20°C for 30 minutes, and absorbance was measured at 517 nm against an ethanol blank; then, IC50 was calculated using GraphPad Prism (Ma *et al*., 2025).

Probiotic viability evaluation

Bacterial counts were conducted to assess *L. plantarum* and *L. delbrueckii* viability in tomato juice throughout shelf life. The tomato juice sample was diluted with Ringer's solution, and an appropriate concentration was cultured on MRS agar medium using the pour plate method. Incubation occurred under anaerobic conditions at 37°C for 48 hours, with each count performed in triplicate (Ahmadmoradi *et al*., 2024).

Sensory analysis investigation

Sensory evaluation was conducted by 20 trained panelists using a 9-point hedonic scale (1 = very dislike and 9 = extremely liked) on days 1 and 28. Samples (30 mL each) were served in randomized order at 10°C in clear, coded plastic cups under controlled lighting. Panelists evaluated aroma, flavor, color, and overall acceptance, rinsing with water between samples to avoid carryover effects (Liu *et al.,* 2018).

Scanning electron microscopy image

Scanning electron microscopy (SEM) imaging focused on tomato juice samples to assess the structural integration of probiotics (free and encapsulated) in the matrix, not alginate microcapsules alone, for evaluating their stability and interaction with pulp fibers. Imaging alginate microcapsules alone would have been redundant given the particle size and zeta potential data already provided by DLS, which confirmed their suitability for food applications (<80 µm) and stability. Additionally, SEM imaging of microcapsules is resource-intensive and would not directly address evaluating functional performance for probiotics in juice. Instead, the imaging matrix provided insights into real-world applications, such as how encapsulation affects probiotic survival and sensory attributes in a complex food system. On the 1st and 28th days of shelf life, dry sample powder was prepared using a freeze dryer (Martin Christ Alpha 1-2 LD Plus, Osterode am Harz, Germany). To preserve the cellular structure of juice, a fixative such as glutaraldehyde is used. The sample was then gradually dehydrated using ethanol or acetone to remove water, which could cause distortion during imaging. A thin layer of conductive material, such as gold or platinum, was applied to enhance conductivity and reduce charging effects during SEM imaging. The prepared sample was then placed in the SEM chamber (LEO 1455 VP, Oberkochen, Germany); finally, settings (*e.g*., voltage, magnification) were adjusted to achieve the desired resolution and detail (Kaur *et al*., 2016).

Statistical analysis

Data obtained from three replicates were calculated as mean values ± standard deviation by SPSS version 20 software (IBM, Armonk, NY, USA). Variance analysis was performed using analysis of variance, and means compared by Duncan's test at a significant level ($p<0.05$).

Results and Discussion

Encapsulation efficiency, particle size, and zeta potential of encapsulated bacteria

In the present study, encapsulation efficiency for *L. plantarum*, *L. delbrueckii*, and their mixture were found to be 90.93%, 90.324% and 89.966%, with no significant differences between groups, respectively (Table 1). Consistent with these findings, survival of *Bifidobacterium adolescentis* 13703T encapsulated in type A pig gelatin (13%) and coated with 1% alginate *via* calcium gelation indicated an improvement in efficiency by over 40% (Annan *et al.,* 2008). Previous studies indicated that factors such as wall functions, core types, emulsifying characteristics, and drying parameters could influence microencapsulation performance (Ahmadmoradi *et al.,* 2024). The emulsification method is most commonly recommended for improving probiotic protection during food production and shelf life (Da Silva *et al.,* 2023; Fernandes *et al.,* 2024).

The particle size results illustrate average levels for *L. plantarum*, *L. delbrueckii,* and their combination are 1.75, 1.74, and 1.76 µm, respectively (Table 1). The particle size should not exceed 80 µm for an acceptable level in food applications to avoid any negative sensory effects (Da Silva *et al.,* 2023). Similar to the present results, in *L. plantarum* production encapsulated with alginate and chitosan, large particle sizes were obtained with 2.87±0.51 µm average particle size (Ahmadmoradi *et al.,* 2024). In another study, research on *Lactobacillus fermentum* encapsulation in alginate and combined with modified starch using the oil/water emulsification method reported 30 to 60 µm particle sizes (Martin *et al.,* 2013). Larger particle sizes were found on encapsulated *Lactobacillus gasseri* in apple juice with a 2.599±0.122 mm mean diameter (Rengadu *et al.,* 2021).

According to Table 1, zeta potential values are -35.23, -34.37, and -34.68 mV for *L. plantarum*, *L. delbrueckii,* and both encapsulated bacteria with suitable colloidal stability, respectively. Studies outlined that a negative zeta potential greater than -30 mV was sufficient to prevent droplet fusion (Da Silva *et al.,* 2023; Fernandes *et al.,* 2024). In another study, the zeta potential of microcapsules manufactured using internal emulsion by alginate and chitosan was measured to be + 39.2 mV (Ahmadmoradi *et al.,* 2024). Zeta potential above +30 or below -30 mv is commonly seen to be more balanced (Annan *et al.,* 2008).

Comparative assays for tomato juice samples, pH, and lycopene evaluation

Figure 1a illustrates pH results over time, showing no significant differences from the 1st to the 14th day of shelf life across all samples with a general downward trend ($p<0.05$). On the 7th, 14th, 21st, and 28th days, the highest pH was observed in the control, and the lowest was found in T_1. The free bacteria caused a greater decrease in pH than the encapsulated form. Additionally, *L. plantarum* caused a slight, but not significant, pH reduction when compared to *L. delbrueckii,* indicating that free *L. plantarum* had a more pronounced effect consistent with previous studies (Giordano *et al*., 2022; Pereira *et al*., 2023). *L. plantarum* indicated effectively lower pH levels during fermentation processes. For instance, it can maintain viability at pH (2) and is capable of producing significant lactic acid, which contributes to reduction (Wang *et al*., 2022). In contrast, *L. delbrueckii* exhibited a lower tolerance to acidic conditions compared to *L. plantarum* (Othman *et al*., 2009). The presence of probiotics in vegetable waters such as tomato, carrot, and beetroot demonstrated that *L. plantarum* consumed sugars from tomato water more rapidly than other species, resulting in increased acid production and a significant drop in pH (Goderska *et al*., 2022). The observed pH reduction in tomato water as a probiotic beverage is attributed to higher levels of lactic acid bacteria, including *L. plantarum* and *L. delbrueckii*, which metabolize sugars and produce organic acids during fermentation (Kaur *et al*., 2016), aligning with current findings.

Lycopene levels decreased significantly in all samples during storage, primarily due to temperature, light, oxygen, and isomerization. In tomato juice fermented with *L. plantarum*, lycopene content decreased after 48 h (Liu *et al*., 2018). The control sample exhibited the lowest lycopene content, dropping from 0.64 to 0.35 µL/mL, while T_2 showed the highest content, decreasing from 0.64 to 0.50 µL/mL (Figure 1b). The results indicated no significant differences in lycopene content among the samples containing various lactic acid bacteria, whether in free or encapsulated forms. However, fermentation with these bacteria appeared to inhibit lycopene degradation. Lactic acid bacteria fermentation enhances the release and bioavailability of lycopene by lowering pH, oxidative stress, and enzymatic activity (Goderska *et al*., 2022).

One study found that fermented vegetable juices containing L. plantarum strains had higher lycopene levels compared to controls, suggesting that specific probiotics could enhance carotenoid levels through fermentation (Chung *et al*., 2020). Another study revealed that fermentation with lactic acid bacteria, particularly *Latilactobacillus sakei*, increased lycopene in tomato powder by approximately 50.2% to the control, indicating nutritional value (Özdemir, 2022). Furthermore, a different study displayed that probiotic fermented milk combined with tomato paste yielded a higher lycopene content than alone, demonstrating that lactic acid bacteria can stabilize and potentially enhance levels during fermentation (Isnaini and Trimulyono, 2024).

Turbidity and transparency analysis

According to results (Table 2), turbidity declined during shelf life, and for the control, it was lower than the others during shelf life. Among probiotics, turbidity for samples containing encapsulated bacteria was higher than for free ones, and T_6 was the highest, followed by T_5, T_4, T_3, T_2, and also T_1, respectively. Control had the highest transparency throughout shelf life and showed a trend

Table 1. Encapsulation efficiency, particle size and zeta potential of encapsulated bacteria.

Treatment	Encapsulated *L. plantarum*	Encapsulated *L. delbrueckii*	Encapsulated *L. plantarum* and *L. delbrueckii*
Encapsulation efficiency (%)	90.093±0.1[a]	90.324±0.2[a]	89.966±0.2[a]
Particle size (µm)	1.75±0.02[a]	1.74±0.03[a]	1.76±0.02[a]
Zeta potential (mV)	-35.23±0.64[a]	-34.37±0.58[a]	-34.68±0.47[a]

[a]indicate significant differences in each row ($p<0.05$).

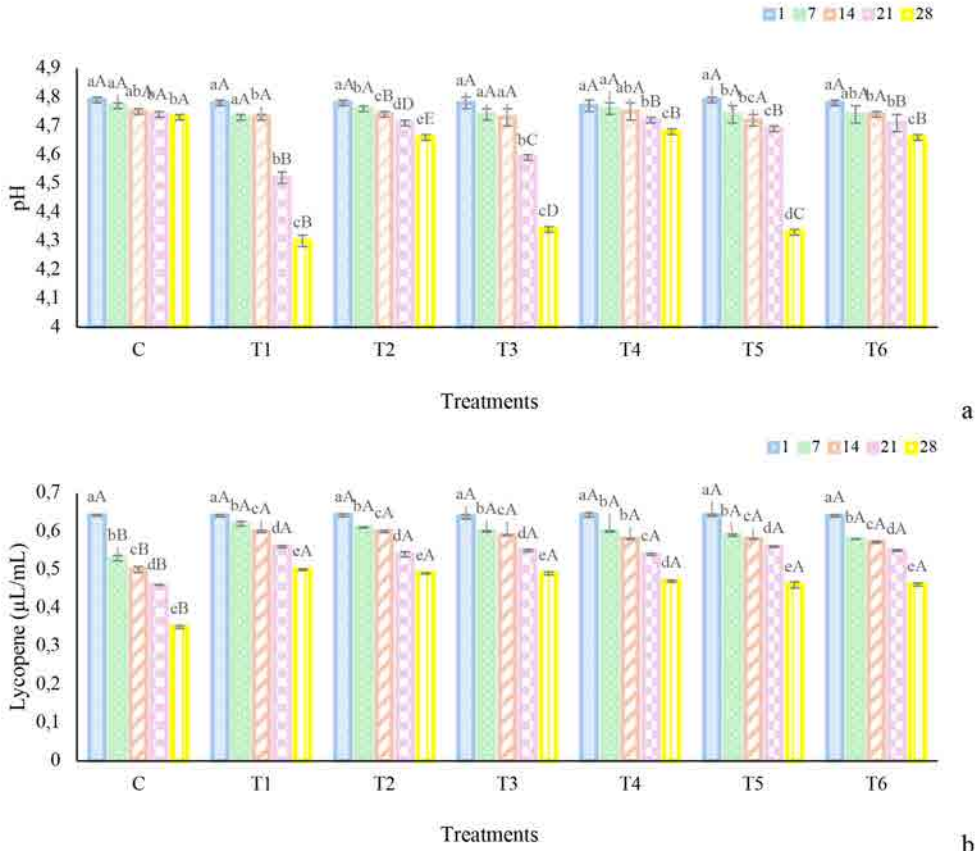

Figure 1. pH changes (a) and lycopene (b) for tomato juice samples during the shelf life. A-B and a-e as mean values with distinct letters among groups and sampling days are significantly different ($p<0.05$), respectively. Control without bacteria (C), free or encapsulated *L. plantarum* (T_1 and T_2), *L. delbrueckii* (T_3 and T_4) and a mixture (T_5 and T_6)

Table 2. Results related to turbidity, transparency and stability (%) for tomato juices during the 28 days of shelf life.

Day	C	T1	T2	T3	T4	T5	T6
Turbidity (NTU)							
1	0.340±0.002^{aC}	0.351±0.002^{aB}	0.350±0.002^{aB}	0.353±0.002^{aB}	0.350±0.002^{aB}	0.351±0.001^{aB}	0.358±0.002^{aA}
7	0.220±0.002^{bF}	0.260±0.001^{bE}	0.289±0.001^{bB}	0.284±0.001^{bC}	0.270±0.001^{bD}	0.292±0.001^{bA}	0.294±0.001^{bA}
14	0.170±0.002^{cF}	0.190±0.002^{cE}	0.220±0.001^{cC}	0.230±0.001^{cB}	0.200±0.001^{cD}	0.230±0.002^{cB}	0.250±0.001^{cA}
21	0.090±0.001^{dG}	0.130±0.005^{dF}	0.160±0.001^{dC}	0.150±0.002^{dD}	0.140±0.001^{dE}	0.190±0.005^{dB}	0.210±0.001^{dA}
28	0.080±0.001^{eG}	0.090±0.001^{eF}	0.100±0.001^{eE}	0.110±0.001^{eD}	0.130±0.001^{eC}	0.140±0.002^{eB}	0.160±0.001^{eA}
Transparency (%)							
1	49.26±0.15^{eA}	48.40±0.10^{eC}	48.20±0.34^{eC}	47.16±0.47^{eD}	49.23±0.11^{dB}	48.26±0.32^{eC}	48.23±0.20^{eC}
7	68.66±0.53^{dA}	68.30±0.26^{dA}	64.46±0.15^{dC}	65.00±0.26^{dB}	65.56±0.20^{cB}	63.06±0.20^{dD}	61.86±0.15^{dE}
14	78.13±0.20^{cA}	75.33±0.11^{cB}	72.76±0.15^{cE}	73.23±0.15^{cD}	74.33±0.15^{bC}	72.26±0.05^{cF}	71.43±0.20^{cG}
21	87.03±0.20^{bA}	82.93±0.05^{bB}	80.43±0.20^{bC}	79.06±0.20^{bE}	79.53±0.15^{aD}	77.30±0.40^{bF}	72.10±0.17^{bG}
28	88.40±0.10^{aA}	86.06±0.10^{aB}	81.16±0.15^{aC}	80.16±0.20^{aD}	79.23±0.15^{aE}	78.16±0.20^{aF}	73.13±0.15^{aG}
Stability (%)							
1	28.47±0.16^{aA}	28.57±0.28^{aA}	28.47±0.16^{aA}	28.38±0.16^{aA}	28.57±0.28^{aA}	28.47±0.15^{aA}	28.57±0.28^{aA}
7	26.76±0.16^{bB}	26.95±0.16^{bB}	27.04±0.16^{bA}	27.14±0.28^{bA}	27.14±0.28^{bA}	27.42±0.20^{bA}	27.42±0.28^{bA}
14	25.23±0.16^{cC}	25.71±0.28^{cB}	25.61±0.16^{cB}	25.90±0.16^{cB}	25.90±0.18^{cB}	26.57±0.28^{cA}	26.66±0.16^{cA}
21	24.66±0.16^{dB}	24.85±0.16^{dB}	25.04±0.16^{dB}	25.04±0.15^{dB}	24.76±0.16^{dA}	25.90±0.43^{dA}	26.09±0.32^{dA}
28	23.61±0.12^{eC}	23.80±0.13^{eB}	24.00±0.28^{eB}	23.90±0.16^{eB}	24.00±0.28^{eB}	25.04±0.43^{dA}	25.23±32^{eA}

Mean values with distinct letters among groups (A-D) and sampling days (a-c) are significantly different ($p<0.05$). Control without bacteria (C), free or encapsulated *L. plantarum* (T_1 and T_2), L. delbrueckii (T_3 and T_4) and a mixture (T_5 and T_6).

upward over time (Table 2). T_6 had the lowest transparency with an ordinal decreasing trend for T_5, T_4, T_3, T_2, and T_1 during shelf life. Probiotic presence improved turbidity and reduced transparency for samples, and bacteria combination had a greater effect compared to a single one. Samples containing *L. delbrueckii* caused less transparency and more turbidity than *L. plantarum*. Probiotic addition significantly affects vegetable juice due to the contrast of white color grains with deep purple tomato juice, which leads to improved turbidity from 6.8 to 7.6 NTU (Naga *et al*., 2016). Color changes in apple juice supplemented with free microorganisms and resistant starch were evaluated, and after adding microcapsules, the juice became slightly darker and more turbid (Rengadu *et al*., 2021). Similarly, probiotics caused improved turbidity and transparency in pineapple juice enriched by *Lactobacillus casei* (*L. casei*), *L. rhamnosus*, and inulin during refrigerated storage (Ghafari and Ansari, 2018).

Stability measurement

The stability results of probiotic tomato juice indicate no significant differences on the 1st day, but illustrate a significant downward trend throughout the shelf life ($p < 0.05$), as depicted in Table 2. The treatment containing encapsulated probiotic bacteria (T_6) exhibited higher stability compared to others, while the control indicated the lowest stability percentage. T_2, T_4, and T_6 (encapsulated form) demonstrated higher stability percentages than T_1, T_3, and T_5 (free). Studies highlighted that bioactive agent application is often limited due to their low solubility in water or physical and chemical instability, particularly in acidic products (Budiati *et al.,* 2022; Giordano *et al.,* 2022). In a study examining ultrasound as a strategy to reduce metabolic effects of *Limosilactobacillus reuteri* DSM 17938 in tomato juice, microencapsulation with sodium alginate was found to enhance probiotic stability, especially at 20 °C (Giordano *et al.,* 2022). The present results align with previous research that encapsulation of active compounds, enzymes, and probiotics applied for enrichment and preservation (Ephrem *et al.,* 2018) and extending the shelf life of tomato and carrot juice occurred through microencapsulation (Naga *et al.,* 2016). Studies on curcumin-enriched juices using extracellular vesicles as natural delivery systems for grape, tomato, and orange juices found that microcapsules indicated improved stability during storage compared to free (Naga *et al.,* 2016; Giordano *et al.,* 2022).

Antioxidant assessment

The antioxidant activity of samples was assessed using the DPPH radical scavenging method, which was preferred due to simplicity, rapid results, and reliability across various studies, establishing it as a standard technique in the field. The inhibition percentage at different concentrations was plotted using (GraphPad Software, San Diego, CA, USA), allowing for IC_{50} determination. The antioxidant function indicates an inverse correlation with IC_{50},

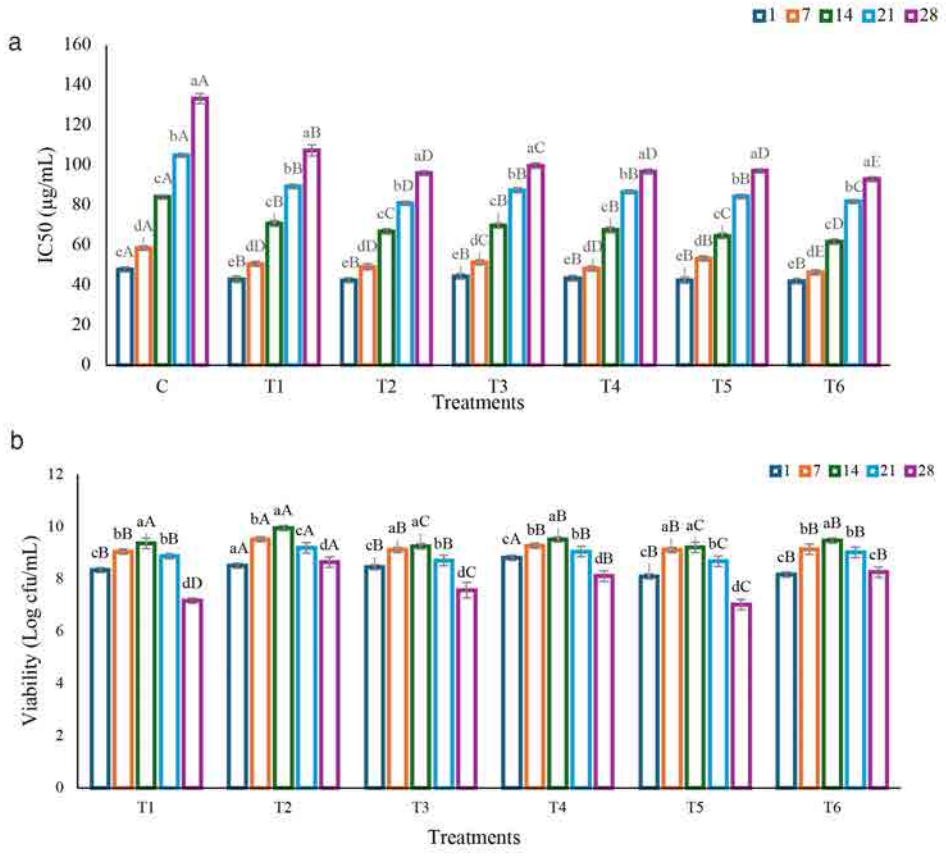

Figure 2. Antioxidant attributes (a, IC_{50}, µg/mL) and viability (b, Log CFU/g) for *Lactobacillus plantarum* and *Lactobacillus delbrueckii* in free and encapsulated forms in tomato juice. The mean values with distinct letters among groups (A-D) and sampling days (a-c) are significantly different ($p<0.05$). Control without bacteria (C), free or encapsulated *L. plantarum* (T_1 and T_2), *L. delbrueckii* (T_3 and T_4) and a mixture (T_5 and T_6).

as illustrated in Figure 2a, and this value increases over time for all samples. The highest and lowest IC_{50} were recorded for control (47.84 to 133.28 µg/mL) and T_6 (41.99 to 92.9 µg/mL) throughout the shelf life ($p < 0.05$), respectively. The sample without probiotics demonstrated the least antioxidant activity (highest IC_{50}), while the most (lowest IC_{50}) was observed in the encapsulated bacteria combination, attributed to a synergistic effect. The interaction between different bacterial strains can lead to synergistic effects, where the combined action for results in greater antioxidant activity than individual effects. This is noted in various juice formulations, where specific bacterial combinations improved antioxidant properties (Naga *et al*., 2016). Consistent with these findings, a study on fermented tomato juice with *L. delbrueckii* showed higher antioxidant activity compared to the control (Liu *et al.,* 2018). Another study reported 3% enhancement in antioxidants in fermented tomato juice, which was linked to microbial hydrolysis reactions that increased phenolic and flavonoid contents (Goderska *et al.,* 2022). Previous research indicated that certain lactic acid bacteria, including *L. delbrueckii, L. plantarum*, and *L. acidophilus*, significantly inhibited oxidative effects and improved antioxidant activity (Yang *et al.*, 2019; Goderska *et al.,* 2022).

Viability of probiotic bacteria

Figure 2b clearly demonstrates that probiotic viability initially increases until the 14th day, reaching a peak in T_2 (9.95 Log CFU/g); then it declines, with the lowest level recorded for T5 (7.02 Log CFU/g) on the 28th day. Encapsulated samples indicated greater viability than free ones, with *L. plantarum* exhibiting higher viability compared to *L. delbrueckii* and the combination. The initial increase in viability likely resulted from nutrient availability, such as sugars and prebiotic fibers in tomato juice, which support bacterial growth (Yang *et al*., 2019). The subsequent decline after day 14 may be attributed to nutrient depletion, accumulation of toxic metabolic by-products (*e.g*., lactic acid), and the acidic environment (pH 4.0-4.5), challenging bacterial survival, particularly for free cells (Kaur *et al*., 2016). Encapsulation mitigates these effects by providing a protective barrier, reducing exposure to low pH and oxidative stress (Giordano *et al*., 2022). The 28-day shelf life was chosen based on standard storage periods for refrigerated fruit juices, ensuring relevance to commercial applications where juices were typically consumed within 4 weeks (Pereira *et al*., 2023). Storage temperature (4°C) and pH were critical factors, with lower temperatures slowing metabolic activity and affecting viability, as evidenced by the negative correlation ($r=-0.72$, $p<0.05$). These findings aligned with studies showing that microencapsulated bacteria exhibited significantly higher survival rates compared to free cells during storage (Yang *et al*., 2019). Tomato juice at low pH (4.3-4.8) supported the growth of acid-tolerant bacteria like *L. plantarum*, which showed higher viability than *L. delbrueckii* (Kaur *et al*., 2016).

Combining antioxidant activity (IC50, µg/mL) and probiotic viability (Log CFU/g) in Figure 2 serves to illustrate their interre-

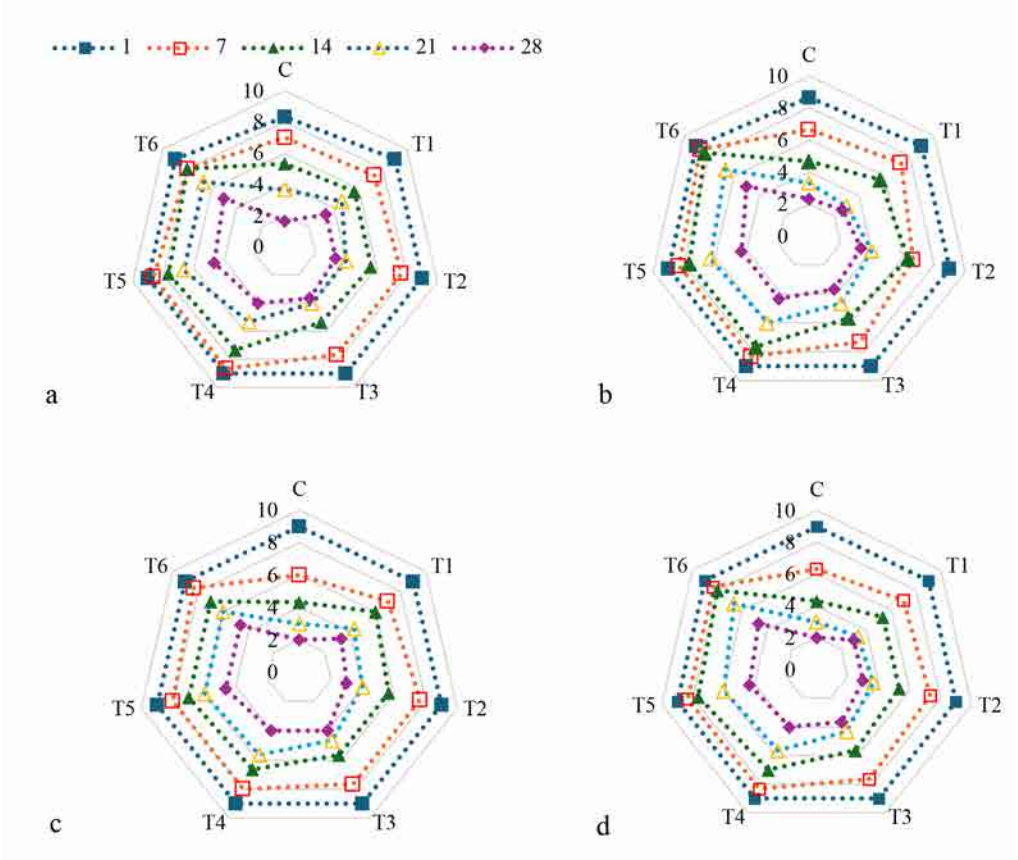

Figure 3. Results of (a) flavor, (b) aroma, (c) color and (d) overall acceptance for free and encapsulated *Lactobacillus plantarum* and *Lactobacillus delbrueckii* in tomato juice. Control without bacteria (C), free or encapsulated *L. plantarum* (T_1 and T_2), *L. delbrueckii* (T_3 and T_4) and a mixture (T_5 and T_6).

lated dynamics over the 28-day shelf life of tomato juice samples. Antioxidant activity and probiotic viability are closely linked because the metabolic function of viable probiotic bacteria, such as *L. plantarum* and *L. delbrueckii*, contributes to compounds, including organic acids and phenolic metabolites, which enhance free radical scavenging capacity (Goderska *et al*., 2022). By presenting these metrics together, Figure 2 highlights how higher probiotic viability (peaking at day 14 in T_2) correlates with lower IC50 values (indicating stronger antioxidant activity), particularly in encapsulated samples like T_6. This visual pairing emphasizes the synergistic effect of viable probiotics on functional juice properties, providing a clearer understanding of how encapsulation sustains both bacterial survival and antioxidant benefits over time.

Sensory evaluation

The sensory results reveal a decline in scores according to flavor, aroma, color, and overall acceptance over the shelf life, as displayed in Figure 3. Generally, control and T_6 received the minimum and maximum scores throughout the shelf life, respectively. The bacteria combination appeared to have a synergistic effect, helping to maintain sensory attributes and prevent spoilage, leading to higher scores compared to the individual. The synergistic

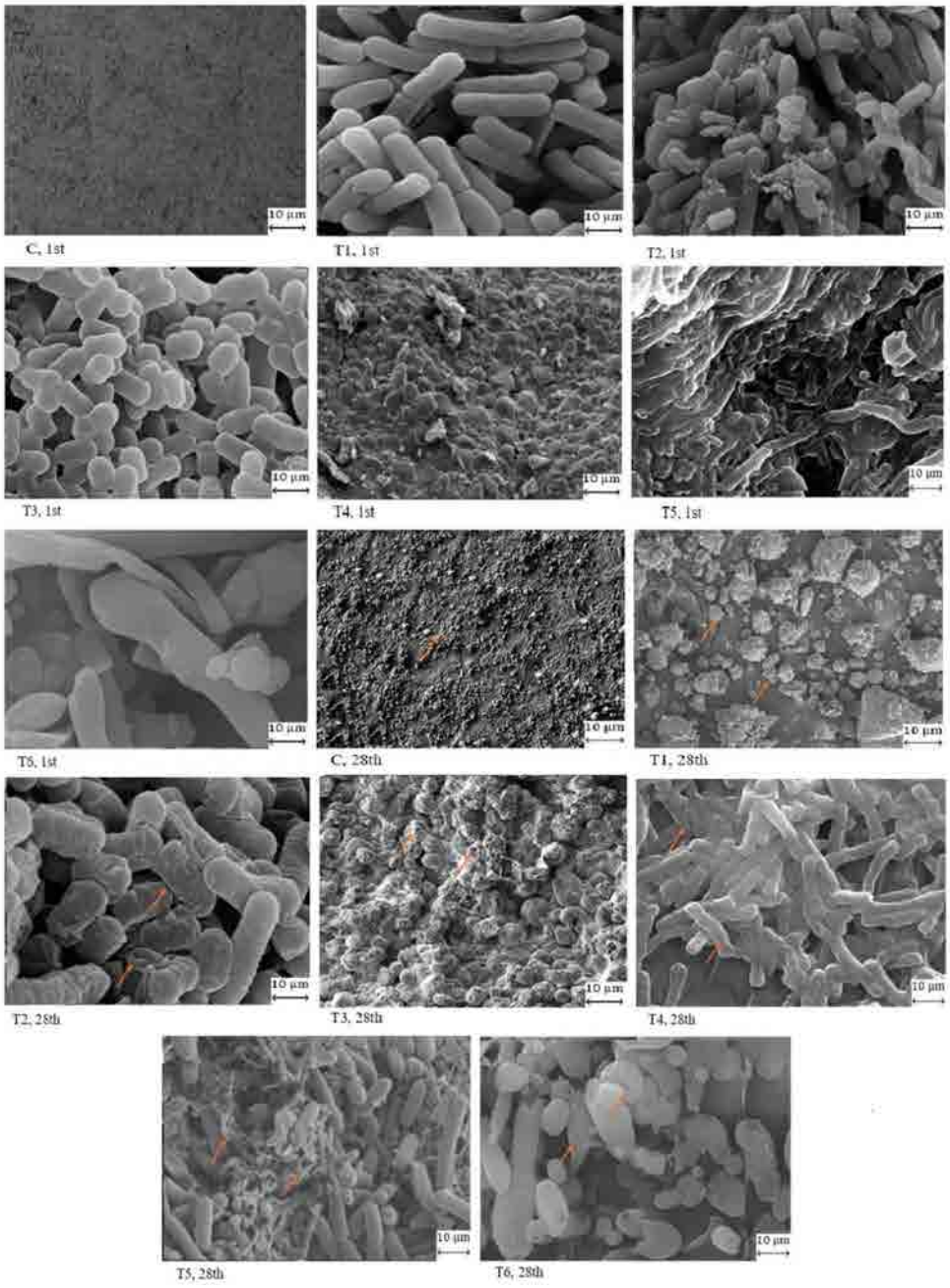

Figure 4. Scanning electron microscopy images for tomato juice treatment on the 1st and 28th days of shelf life. Control without bacteria (C), free or encapsulated *L. plantarum* (T_1 and T_2), *L. delbrueckii* (T_3 and T_4) and a mixture (T_5 and T_6).

effect in bacterial combinations for two or more agents produces a greater influence than the sum of their individual effects, which can enhance antimicrobial activity, quality, and shelf life, prevent spoilage, and also preserve sensory attributes in food products (Gan *et al.,* 2023). Supporting findings, bilayer microencapsulation with calcium alginate enhanced *L. acidophilus* survival in tomato juice during storage, resulting in better sensory characteristics (Ghobadi Dana and Rashnavadi, 2016). Similarly, samples containing *L. plantarum* exhibited the highest sensory scores for flavor and overall acceptance, making it a promising probiotic candidate to utilize unripe tomato fruits (Pereira *et al*., 2023). Moreover, probiotic fruit juices had been well-received by consumers due to their improved flavor with a notable difference in sensory scores (Naga *et al*., 2016). The present findings align with other research, such as bilayer microencapsulation with calcium alginate to improve *L. acidophilus* survival (Ghobadi Dana and Rashnavadi, 2016) and positive sensory effects of fermented tomato juice with *L. plantarum* and *L. casei* (Liu *et al*., 2018).

Morphology examination

Figure 4 illustrates microscopic structures for tomato juice samples on the 1st and 28th days of shelf life. On the 1st day, control displayed a uniform and stable texture; however, it showed structural decomposition at the 28th day. In contrast, T_1, T_3, and T_5 exhibited a homogeneous distribution of rod-shaped bacterial cells on the 1st day. Meanwhile, T_2, T_4, and T_6 revealed micrographs of encapsulated probiotic tomato that displayed irregularly shaped and larger structures attached to fibers, a result attributed to lyophilization on the 1st day. These samples exhibited a homogeneous distribution of rod-shaped bacterial cells trapped within the matrix, indicating probiotic protection (Ephrem *et al.,* 2018).

On the 28th day, bacterial structures in T_1, T_3, and T_5 had changed, appearing wrinkled, and also T_2, T_4, and T_6, with high porosity observed in certain areas, suggesting a collapse of the encapsulation wall structure. Overall, the structure of encapsulated samples (T_2, T_4, T_6), particularly T_6, remained more intact than the others on the 28th day. The observed porosity was likely a result of increased moisture in alginate-based microparticles. These findings are in line with previous research on *L. rhamnosus* encapsulated *via* water-in-oil emulsion using carboxymethyl cellulose and gelatin, which noted foam-like microstructures with a porous appearance, alveolar morphology, and bacteria present on pore surfaces (Ghafari and Ansari, 2018). Numerous studies indicated that insoluble fibers could protect probiotics and minimize bacterial loss, particularly during gastric transit and intestinal residence (Naga *et al.,* 2016; Ephrem *et al.,* 2018; Ghafari and Ansari, 2018). As depicted in Figure 4, probiotics adhere to the fiber surface with some trapped within the matrix, helping to ensure a sufficient number of viable bacteria.

Limitations and future perspectives

This study provided valuable insights into the fortification of tomato juice with encapsulated probiotics, but certain limitations warranted consideration. The observed decline in probiotic viability after day 14 suggested challenges such as nutrient depletion and accumulation of metabolic by-products, which were not fully explored mechanistically. Additionally, the study did not include in vitro gastric/intestinal resistance assays, which were critical for assessing probiotic survival during gastrointestinal passage, a key factor for functional food efficacy (Fernandes *et al*., 2024). The absence of volatile compound analysis (*e.g*., *via* headspace gas chromatography-mass spectrometry) limited understanding of flavor changes during shelf life, which could complement sensory evaluation data (Liu *et al*., 2018). Future studies should incorporate these assays to validate the gastrointestinal survival of encapsulated probiotics and profile volatile compounds to elucidate sensory impacts (Naga *et al.,* 2016; Ephrem *et al.,* 2018; Ghafari and Ansari, 2018). From a technological perspective, the use of calcium alginate encapsulation is cost-effective and scalable, offering potential for industrial application in functional beverage production (Ghobadi Dana and Rashnavadi, 2016). Economically, probiotic-fortified tomato juice could meet growing consumer demand for non-dairy, health-promoting beverages, particularly in markets targeting lactose-intolerant or vegan consumers (Dzandu *et al*., 2022). Environmentally, utilizing tomato pulp as a prebiotic reduces food waste, aligning with sustainable food production practices, and nutritionally, the enhanced antioxidant activity and probiotic viability suggest significant health benefits, including improved gut health and reduced oxidative stress (Giordano *et al*., 2022). These findings supported the potential for scale-up, with future research focusing on optimizing encapsulation materials, extending shelf life beyond 28 days, and conducting clinical trials to confirm health benefits.

Conclusions

The present research highlighted enriching tomato juice with encapsulated probiotics to enhance functional attributes, offering a viable option for health-conscious consumers. The encapsulated *L. plantarum* and *L. delbrueckii* significantly improved various functions, including viability, stability, and antioxidant activity. The encapsulated dual-bacteria treatment outperformed the free probiotic according to maintaining higher bacterial population, stability, and turbidity over the 28th day. Encapsulation not only protects probiotics during storage but also enhances the health promotion of tomato juice, making it a promising functional beverage for consumers. Future studies will focus on *in vitro* gastrointestinal resistance assays, volatile compound analysis, and extended shelf-life testing to further validate and optimize functional food for industrial applications.

References

Ahmadmoradi M, Alishahi M, Soltanian S, Shahriari A, Yektaseresht A, 2024. Effects of encapsulation of Lactobacillus plantarum on probiotic potential and reducing lead toxicity in rainbow trout (Oncorhynchus mykiss Walbaum). Aquac Int 32:337-59.

Annan NT, Borza AD, Hansen LT, 2008. Encapsulation in alginate-coated gelatin microspheres improves survival of the probiotic Bifidobacterium adolescentis 15703T during exposure to simulated gastro-intestinal conditions. Int Food Res 41:184-93.

Budiati T, Suryaningsih W, Bethiana TN, 2022. Antimicrobial of tropical fruit and vegetable waste extract for food-borne pathogenic bacteria. Ital J Food Safety 11:10510.

Castello A, Cascio GL, Ferraro C, Pantano L, Costa A, Butera G, Oliveri G, Rizzuto ML, Alduina R, Cardamone C, 2023. Food risk associated with vegetable consumption, exposure to antimicrobial-resistant strains and pesticide residues. Ital J Food Safety 12:11134.

Chung HJ, Lee H, Na G, Jung H, Kim DG, Shin SI, Jung SE, Choi ID, Lee JH, Sim JH, Choi HK, 2020. Metabolic and lipidomic

profiling of vegetable juices fermented with various probiotics. Biomolecules 10:725-35.

Da Silva SÂ, Batista LD, Diniz DS, Nascimento SS, Morais NS, de Assis CF, Passos TS, De Sousa Júnior FC, 2023. Microencapsulation of probiotics by oil-in-water emulsification technique improves cell viability under different storage conditions. Foods 12:252-64.

Dzandu B, Chotiko A, Sathivel S, 2022. Antioxidant activity and viability of Lacticaseibacillus rhamnosus, Lacticaseibacillus casei, and co-culture in fermented tomato juice during refrigerated storage. Food Biosci 50:102085.

Ephrem E, Najjar A, Charcosset C, Greige-Gerges H, 2018. Encapsulation of natural active compounds, enzymes, and probiotics for fruit juice fortification, preservation, and processing: an overview. J Funct Foods 48:65-84.

Fernandes N, Achemchem F, Gonzales-Barron U, Cadavez V, 2024. Biopreservation strategies using bacteriocins to control meat spoilage and foodborne outbreaks. Ital J Food Safety 13:12558.

Gan C, Langa E, Valenzuela A, Ballestero D, Pino-Otín MR, 2023. Synergistic activity of thymol with commercial antibiotics against critical and high WHO priority pathogenic bacteria. Plants 12:1868-78.

Ghafari S, Ansari S, 2018. Microbial viability, physico-chemical properties and sensory evaluation of pineapple juice enriched with Lactobacillus casei, Lactobacillus rhamnosus and inulin during refrigerated storage. J Food Meas Charact 12:1-9.

Ghobadi Dana M, Rashnavadi T, 2016. Investigation on the influence of two layer microencapsulation by calcium alginate on survivability of Lactobacillus acidophilus in tomato juice during shelf life. Innov Food Technol 4:151-60.

Giordano I, Abuqwider J, Altamimi M, Di Monaco R, Puleo S, Mauriello G, 2022. Application of ultrasound and microencapsulation on Limosilactobacillus reuteri DSM 17938 as a metabolic attenuation strategy for tomato juice probiotication. Heliyon 8:10969.

Goderska K, Dombhare K, Radziejewska-Kubzdela E, 2022. Evaluation of probiotics in vegetable juices: tomato (Solanum lycopersicum), carrot (Daucus carota subsp. sativus) and beetroot juice (Beta vulgaris). Arch Microbiol 204:300-15.

Isnaini AA, Trimulyono G, 2024. Aktivitas antibakteri coconut yoghurt terhadap Streptococcus mutans secara in Vitro. LenteraBio: Berkala Ilmiah Biologi 13:393-401. [Article in Indonesian].

Kaur S, Kaur HP, Grover J, 2016. Fermentation of tomato juice by probiotic lactic acid bacteria. Int J Adv Pharm Biol Chem 5:212-9.

Liu Y, Chen H, Chen W, Zhong Q, Zhang G, Chen W, 2018. Beneficial effects of tomato juice fermented by Lactobacillus plantarum and Lactobacillus casei: antioxidation, antimicrobial effect, and volatile profiles. Molecules 23:2366-79.

Ma F, Ma R, Zhao L, 2025. Effects of antimicrobial peptides on antioxidant properties, non-specific immune response and gut microbes of tsinling lenok trout (Brachymystax lenok tsinlingensis). Biochem Genet 63:85-103.

Mantzourani I, Terpou A, Alexopoulos A, Bezirtzoglou E, Bekatorou A, Plessas S, 2019. Production of a potentially synbiotic fermented Cornelian cherry (Cornus mas L.) beverage using Lactobacillus paracasei K5 immobilized on wheat bran. Biocatal Agric Biotechnol 17:347-51.

Martin MJ, Lara-Villoslada F, Ruiz MA, Morales ME, 2013. Effect of unmodified starch on viability of alginate-encapsulated Lactobacillus fermentum CECT5716. LWT 53:480-6.

Naga SS, Ramesh B, Umamahesh K, Vijaya SRO, 2016. Probiotication of tomato and carrot juices for shelf-life enhancement using micro-encapsulation. J Food Biosci Technol 6:13-22.

Nikolaou A, Mitropoulou G, Nelios G, Kourkoutas Y, 2023. Novel functional grape juices fortified with free or immobilized Lacticaseibacillus rhamnosus OLXAL-1. Microorganisms 11:646-58.

Othman NB, Roblain D, Chammen N, Thonart P, Hamdi M, 2009. Antioxidant phenolic compounds loss during the fermentation of Chétoui olives. Food Chem 116:662-9.

Özdemir N, 2022. A multi-functional survey of the properties of Lacticaseibacillus paracasei subsp. tolerans NOC-122, Levilactobacillus parabrevis NOC-111 and Latilactobacillus curvatus NOC-110. Microbiology 168:001239.

Pereira N, Farrokhi M, Vida M, Lageiro M, Ramos AC, Vieira MC, Alegria C, Gonçalves EM, Abreu M, 2023. Valorisation of wasted immature tomato to innovative fermented functional foods. Foods 12:1532-45.

Rengadu D, Gerrano AS, Mellem JJ, 2021. Microencapsulation of Lactobacillus casei and Bifidobacterium animalis enriched with resistant starch from vigna unguiculata. Starch□Stärke 73:2000247.

Wang W, Xu H, Ye Q, Tao F, Wheeldon I, Yuan A, Hu Y, Wu J, 2022. Systemic immune responses to irradiated tumours via the transport of antigens to the tumour periphery by injected flagellate bacteria. Nat Biomed Eng 6:44-53.

Yang F, Wang YP, Zhao H, 2019. Quality enhancement of fermented vegetable juice by probiotic through fermented yam juice using Saccharomyces cerevisiae. Food Sci Technol 40:26-35.