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Research article

# Enhancing *Lactobacillus plantarum* viability using novel chitosan-alginate-pectin microcapsules: Effects on gastrointestinal survival, weight management, and metabolic health ☆

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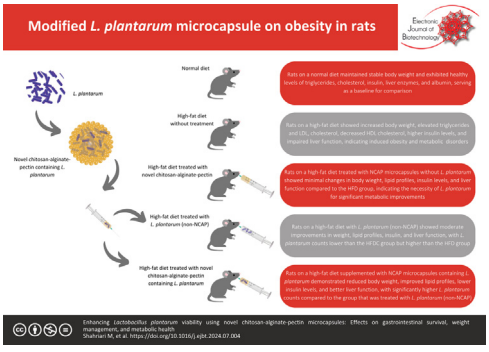
## GRAPHICAL ABSTRACT




Modified *L. plantarum* microcapsule on obesity in rats

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## ABSTRACT

*Background:* Probiotics, like *Lactobacillus plantarum*, show promise in managing obesity, but delivery challenges hinder their effectiveness. This study explores the effects of NCAP microcapsules with *L. plantarum* on weight, lipids, liver function, and insulin in rats over eight weeks, enhancing the bacteria's survivability through microencapsulation.
*Results:* NCAP microcapsules (10–15 μm, >79% survival) effectively protected L. plantarum. In rats, a high-fat diet with NCAP microcapsules (HFDC) significantly reduced body weight (176 g vs 179 g, $p < 0.001$), low-density lipoprotein (33 mg/dL vs 44 mg/dL, $p < 0.001$), and improved liver markers (aspartate transaminase 56 U/L vs 69 U/L, $p = 0.008$; alanine transaminase 36 U/L vs 38 U/L, $p < 0.001$). *L. plantarum* counts were notably higher in HFDC (870,963,590 CFU/g vs 14,454 CFU/g, $p < 0.001$).
*Conclusions:* NCAP microcapsules enhance *L. plantarum* survivability and improve weight, lipids, liver function, and insulin. This study addresses the need for effective probiotic delivery systems, offering insights into metabolic health promotion.

☆ Audio abstract available in Supplementary material.

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## 1. Introduction

*Lactobacillus plantarum* is a probiotic bacterium that has gained significant attention owing to its potential health benefits [1,2,3,4]. However, the viability of *L. plantarum* can compromised by various challenges, such as exposure to bile acids and high salt concentrations. To overcome these challenges and enhance the viability of *L. plantarum*, researchers have explored using microcapsules as protective shields [5,6,7,8].

Microencapsulation involves encapsulating active ingredients within a protective matrix that provides a physical barrier against harsh environmental conditions. Chitosan, alginate, and Pectin are natural polymers widely used for microencapsulation owing to their biocompatibility, biodegradability, and gel-forming properties [9,10,11].

Several studies have investigated using chitosan-alginate-based microcapsules to encapsulate *L. plantarum* and other probiotic bacteria. They demonstrated that chitosan-coated alginate-xanthan gum beads provided superior protection to *L. plantarum* against low pH and high temperature [10,12,13]. Similarly, *L. plantarum* encapsulated in alginate/chitosan/alginate microcapsules showed enhanced immunomodulatory effects [14,15]. These studies highlight the potential of chitosan-alginate-based microcapsules for improving the viability and functionality of *L. plantarum*. In addition to chitosan and alginate, Pectin has been incorporated into microcapsules to enhance their protective properties further. Alginate-pectin-based microcapsules have been shown to provide a more rigid and regular structure compared to alginate-only microcapsules [16]. Combining alginate and Pectin can improve the stability and encapsulation efficiency [17].

The protective mechanism of CAP microcapsules involves the formation of a polyelectrolyte complex between chitosan and alginate, which tightens and stabilizes the surface of microcapsules [11]. This complex is highly hydrophilic, leading to increased swelling of the microcapsules and improved release of the active agents [11]. The electrostatic interaction between chitosan and alginate also limits the diffusion of competing ions, such as $H^+$ and $Na^+$, into the inner core of the microcapsules [11].

*L. plantarum* was selected for encapsulation within chitosan-alginate-based microcapsules based on its documented resilience and efficacy [14,15]. Previous studies have demonstrated that chitosan-coated alginate-xanthan gum beads provide superior protection to *L. plantarum* against adverse environmental conditions such as low pH and high temperature. Additionally, *L. plantarum* encapsulated in alginate-chitosan-alginate microcapsules has shown enhanced immunomodulatory effects.

Furthermore, incorporating Pectin into microcapsules enhances the mechanical stability and porosity of the gel matrix [9]. The presence of Pectin can also lead to the forming of a calcium pectinate network, which further influences the size and properties of microcapsules [9].

CAP microcapsules have been explored in various applications, including encapsulating other probiotic bacteria such as *Bifidobacterium longum* and *Lactobacillus salivarus* [9]. These studies demonstrate the potential of CAP microcapsules as a delivery system for probiotics with enhanced viability and immunomodulatory effects.

This study aims to investigate the viability of modified microcapsules containing *L. plantarum* under acidic and bile salt conditions and assess their potential effects on obesity in rats. Leveraging the resilience and beneficial properties of *L. plantarum* within CAP microcapsules, this research contributes to developing probiotic-based interventions for various health conditions.

## 2. Materials and methods

### 2.1. Preparation of bacterial cultures

The initial bacterial culture of *L. plantarum* ATCC 14917 was obtained from the Iran University of Medical Sciences. Stock solutions were meticulously maintained by preservation at −80°C in an MRS medium supplemented with 0.05% L-cysteine in a 25% glycerol solution. The bacteria were cultured in MRS medium under anaerobic conditions at 37°C for 48 h. After incubation, the bacterial cells were harvested by centrifugation at 4000 rpm for 10 min, followed by washing with 0.85% NaCl (physiological saline) solution (25 mL). The cellular suspension was resuspended in 0.85% NaCl (2 mL) [18]. Salinized cellular suspensions were used to assess free cell survival.

### 2.2. Production of modified three-layer L. plantarum microcapsules

The *L. plantarum* strain used in this study was obtained from the Iran University of Medical Science. A meticulous cryopreservation procedure was employed at −80°C to maintain the bacterial stock solutions, utilizing MRS medium supplemented with 0.05% L-cysteine and 25% glycerol as cryoprotectants. Following storage, the bacterial cultures were cultivated in MRS broth at 37°C for 48 h, ensuring that strict anaerobic conditions were maintained. For cell harvesting, centrifugation was executed at 4000 rpm for 10 min. The resultant cell pellets were washed twice with 0.85% NaCl (physiological saline) solution, with 25 mL of each wash. After washing, the bacterial cells were resuspended in 2 mL of 0.85% NaCl solution [19].

The viability assessment of free cells involved the utilization of the cell mentioned above suspensions. Additionally, these cell suspensions served as the foundation for evaluating the survivability of the encapsulated cells in the subsequent phases of the study.

### 2.3. Fourier Transform Infrared Spectroscopy of CS-P-SA scaffolds

Fourier Transform Infrared Spectroscopy (FTIR) explored the bonding characteristics within the microcapsule layers. FTIR involves measuring the absorption or transmission of infrared light to discern the chemical composition of a material and shed light on its functional groups, chemical bonds, and molecular structures [20]. The assessment was conducted using an Equinox 55 FTIR instrument (Bruker, Germany) housed at the Tarbiat Modares University. This study aimed to unveil the intricate molecular interactions inherent in CS-P-SA scaffolds.

### 2.4. Acid and bile resistance of chitosan-alginate-pectin-modified microcapsules

The NCAP Microcapsules were incubated in sterile simulated gastric juice without pepsin for 120 min, transferred to sterile

simulated intestinal fluid without bile, and incubated at room temperature for another 120 min. The surviving bacteria were counted using the pour plate method.

The microcapsules were incubated in a tube containing 10 ml of sterilized 0.6% bile salt solution (pH 8.25) for 120 min at 37°C for bile tolerance assays. Surviving bacteria were counted using the pour plate method after disintegrating the microcapsules and were incubated aerobically at room temperature on MRS agar for 48 h [21].

### 2.5. Assessment of the impact of NCAP microcapsules containing L. plantarum on biochemical markers in blood and body weight of rats

Twenty-five male Wistar rats aged 50–60 d, with an average weight of 160 ± 10 g, were obtained from Tarbiat Modares University (Tehran, Iran). The rats were housed under a 12 h light/12 h dark cycle in a controlled room with a temperature of 23 ± 3°C and humidity of 50% ± 10% [22]. Ethical approval was obtained from the Research Ethics Committee of Tarbiat Modares University (IR.MODARES.AEC.1401.009), and all procedures were performed following the International Guidelines for Laboratory Animals.

The rats were randomly assigned to one of the five groups: the control group, which received a regular diet and no treatment (ND); high-fat diet without treatment (HFDB), high-fat diet with treatment with *L. plantarum* (HFDM); and high-fat diet with NCAP microcapsules (HFDC), high-fat diet with NCAP microcapsules containing *L. plantarum*. The experimental interventions were orally administered via gavage twice daily for eight weeks, with an approximate dosage of $1 \times 10^8$ CFU per animal. At weekly intervals throughout the eight weeks, the rats were anesthetized using ketamine/xylazine, and blood samples were obtained from their tails. Serum was subsequently extracted from the blood samples through centrifugation at 1400 rpm. The levels of triglycerides, cholesterol, HDL, and LDL were quantified using a test kit sourced from Asanpharm in Seoul, Korea.

### 2.6. Investigation of L. plantarum population in stool samples

The *L. plantarum* population in stool samples was investigated multiple times to assess its dynamics comprehensively. Before the intervention, baseline stool samples were collected to establish the initial population. Subsequent sampling was conducted in the first, fourth, and eighth weeks following the commencement of the intervention. These time points were strategically chosen to monitor the changes in the *L. plantarum* population throughout the study. Colony-forming units (CFUs) in each stool sample were counted using standard microbiological techniques to quantify the presence and abundance of *L. plantarum* and provide valuable insights into its persistence and impact throughout the study period [23].

### 2.7. Statistical analysis

Genomic DNA was extracted from intestinal contents and fecal samples using a GENDIA kit (Iran). Real-time PCR (qPCR) was used to amplify DNA using the Qiagen Rotor-Gene Real-Time PCR System. qPCR was performed using TaqMan probes. Amplification was programmed to begin at 95°C for 30 s, followed by 40 cycles.

## 3. Results

### 3.1. Confirmation of L. plantarum isolates

The bacterial culture was confirmed to be gram-positive, rod-shaped with round ends, and catalase-negative due to Gram staining, and it was confirmed to be *L. plantarum* by polymerase chain reaction (PCR).

### 3.2. Chitosan-alginate-pectin microcapsule quality control

NCAP microcapsules with a mean diameter of 10–15 μm were successfully produced. Most microcapsules (72%) were ~ 10 μm in diameter, 7% were less than five μm, and 21% were > 15 μm. The efficiency yield (EY) results showed that the microcapsules effectively protected the *L. plantarum* strain from harsh gastrointestinal tract and bile conditions. The EY was 79.80%, indicating that more than 79% of the probiotic bacteria survived in microcapsule form. This significantly improved the probiotic population before encapsulation (13.67 log CFU/ml), suggesting that the microcapsules could provide a protective barrier against harsh environmental conditions (Table 1).

Additionally, NCAP microcapsules were prepared and evaluated for their ability to enhance the viability of *L. plantarum* in simulated bile and gastrointestinal (GI) tract conditions. The NCAP microcapsules showed an acceptable survival rate of > 68% in the bile and 54% in the GI tract, significantly higher than that of the control group ($P < 0.05$). These results suggest that the NCAP microcapsules can effectively protect *L. plantarum* from the harsh environment of the gastrointestinal tract (Table 2).

This study showed that the microencapsulation of *L. plantarum* significantly increased its resistance to acid and bile salts. Under acidic conditions, the survival rate of the microencapsulated bacteria was 54%, which was considerably higher than that of the non-microencapsulated bacteria (20%). Similarly, under bile salt conditions, the survival rate of microencapsulated bacteria was 68%, significantly higher than that of non-microencapsulated bacteria (18%). These results suggested that microencapsulation can protect bacteria from harsh gastrointestinal conditions.

### 3.3. Fourier Transform Infrared Spectroscopy characterization

FTIR analysis revealed distinct molecular signatures for the individual components of the microcapsules as well as interactions within the chitosan-pectin-alginate scaffold.

The characteristic peak of alginate was evident at 1620 $cm^{-1}$, indicative of a carbonyl bond (Fig. 1A). Chitosan exhibited peaks at 3422 $cm^{-1}$ corresponding to O—H stretching, 1653 $cm^{-1}$ for the amide II band, 1592 $cm^{-1}$ for the C—O stretch of the acetyl group, and 1173 $cm^{-1}$ and 1071 $cm^{-1}$ representing the amide II band and N—H stretching, respectively, along with skeletal vibrations involving bridge C—O stretching (Fig. 1B). In the case of pectin, the undissociated carboxyl acid (COOH) was characterized by a band at 1733 $cm^{-1}$ due to the —CO of the methyl ester group ($COOCH_3$), whereas the band at 1610 $cm^{-1}$ was assigned to the asymmetric stretching vibration of the carbonyl group of the carboxylate ion (COO—) (Fig. 1C).

Upon analysis of the chitosan-pectin mixture scaffold, two relatively broad bands emerged within the 1800–1600 $cm^{-1}$ region, suggesting the presence of overlapping bands from the amino

**Table 1**
**Quality control of chitosan-alginate-pectin microcapsules.** This table presents the efficiency yield, indicating the percentage of viable probiotic bacteria that survived under gastrointestinal tract and bile conditions. The data show that the microencapsulated *L. plantarum* strain's survival rate exceeded 79%.

| | |
|---|---|
| Probiotic population before encapsulation (log CFU/ml) | 13.67 ± 0.16 |
| Encapsulated probiotic population (log CFU/g) | 10.91 ± 0.34 |
| Efficiency yield (EY%) | 79.80 ± 3.18 |

**Table 2**
The study found that microencapsulation improved the survival rate of *L. plantarum* under acidic conditions from 20% to 54% and in sharp bile salts from 18% to 68%. These results highlight the significant effect of microencapsulation on bacterial survival under challenging conditions.

| | Without microcapsule | | | With microcapsule | | |
|---|---|---|---|---|---|---|
| | Time 0 (log CFU/ml) | Time 4 h (log CFU/ml) | SR (%) | Time 0 (log CFU/ml) | Time 4 h (log CFU/ml) | SR (%) |
| Acid resistance | 9.48 ± 0.06 | 1.9 ± 0.18 | 20.04% | 9.82 ± 0.33 | 5.39 ± 0.09 | 54.55% |
| Bile resistance | 9.61 ± 0.01 | 1.73 ± 0.6 | 18.00% | 8.74 ± 0.17 | 5.98 ± 0.02 | 68.42% |

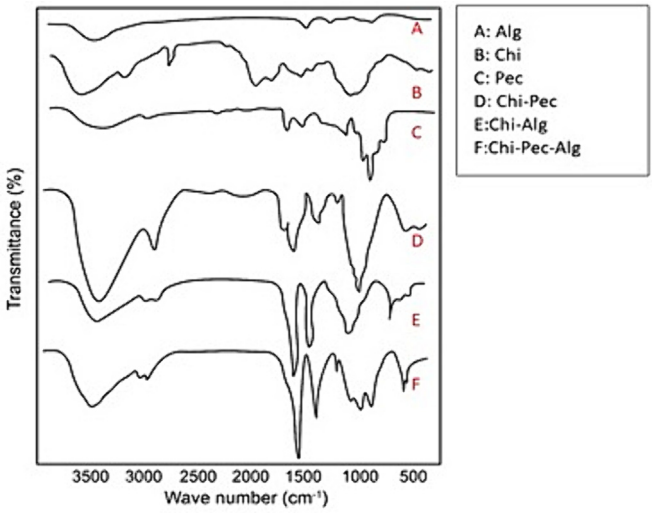


**Fig. 1.** Distinct molecular signatures and interactions within chitosan-alginate-pectin microcapsules revealed by FTIR analysis showed unique molecular characteristics of the microcapsule components and their interactions within the chitosan-alginate pectin scaffold. Alginate's carbonyl bond (1620 $cm^{-1}$), chitosan's functional groups (3422 $cm^{-1}$, 1653 $cm^{-1}$, 1592 $cm^{-1}$, 1173 $cm^{-1}$, 1071 $cm^{-1}$), and Pectin's carboxyl acid and carbonyl groups (1733 $cm^{-1}$, 1610 $cm^{-1}$) were identified. The Chitosan-Pectin mixture displayed overlapping bands (1800–1600 $cm^{-1}$) indicating molecular interactions. The amide I peak shifted (1643 to 1654 $cm^{-1}$) in the Chitosan-Alginate spectrum. The Chitosan-Alginate-Pectin ternary scaffold exhibited an additional band (1578 $cm^{-1}$), reflecting interactions between amino and carboxylic groups. These patterns signify a chitosan-alginate-pectin copolymer complex formed via ionic interactions between the negatively charged carbonyl groups of alginate and pectin and the positively charged amino groups of chitosan.

and carboxylic groups, indicative of molecular interactions (Fig. 1D). Within the chitosan-alginate spectrum, the amide I peak shifts from 1643 to 1654 $cm^{-1}$ (Fig. 1E). Notably, the chitosan-pectin-alginate ternary scaffold exhibited an additional band at 1578 $cm^{-1}$ (Fig. 1F), indicating interactions between amino and carboxylic groups within the interacting molecules. Collectively, these spectral shifts and patterns show the formation of an NCAP copolymer complex, which is attributed to the ionic interactions between the negatively charged carbonyl groups (—COOH) of alginate and pectin and the positively charged amino groups (—NH2) of chitosan.

### *3.4. Effect of chitosan-alginate-pectin microcapsule on rat's body weight*

The regular diet (ND) group observed a slight increase in body weight. In contrast, the high-fat diet (HFD) group displayed a significant and more rapid increase in body weight than the ND group. Additionally, both the high-fat diet with *L. plantarum* (HFDB) and the high-fat diet with microcapsules (HFDMs) groups showed a significant increase in body weight. Intriguingly, in the high-fat diet with NCAP microcapsules containing *L. plantarum* (HFDC) group, there was a notable and substantial decrease in body weight, particularly after the fourth and eighth weeks of the study, as illustrated in Fig. 2.

### *3.5. Effect of modified microcapsules on triglycerides*

Although TG levels at the fourth and eighth weeks did not show significant differences, in addition to a substantial increase in TG levels in the HFD, HFDB, and HFDM groups, there was a notable and significant decrease in TG levels in the high-fat diet with chitosan-alginate-pectin-modified microcapsules containing *L. plantarum* (HFDC) group, particularly evident after the fourth and eighth weeks of the study, as illustrated in Fig. 2.

### *3.6. Effect of modified microcapsules on total cholesterol*

Although TC level comparison at weeks four and eight did not differ significantly, in addition to a significant increase in TC levels in the HFD, HFDB, and HFDM groups, the level of TC did not change in the HFDB group. Intriguingly, in the HFDC group, there was a notable decrease in TC levels after the eighth week of the study, as illustrated in Fig. 2.

### *3.7. Effect of modified microcapsules on LDL*

However, LDL levels at weeks four and eight did not significantly differ, in addition to a significant increase in LDL levels in the HFD, HFDB, and HFDM groups. Intriguingly, in the HFDC group, there was a notable decrease in LDL levels after the eighth week of the study, as illustrated in Fig. 2.

### *3.8. Effect of modified microcapsules on HDL*

Although HDL level compression at week four did not differ significantly, at week eight, the HDL level was significantly higher than that in the HFDB group. Besides a significant decrease in HDL levels in the HFD, HFDB, and HFDM groups. Intriguingly, in the HFDC group, after a notable reduction in the first week of HDL level, there was a noteworthy increase in the fourth and eighth weeks of the study, as illustrated in Fig. 2.

### *3.9. Effect of modified microcapsules on insulin*

Insulin level compression at week four revealed a significant reduction in insulin levels in the HFDC group compared with the HFD group. Besides the substantial increase in insulin in HFD in the fourth week versus the first week, intriguingly, in the HFDC group, there was a notable reduction of insulin in the fourth week versus the first week of the study, as illustrated in Fig. 2.

### *3.10. Effect of modified microcapsules on ALT and AST*

The ALT and AST levels at weeks four and eight showed significant differences between the groups. In addition to the substantial increase in ALT and AST levels in the HFD and HFDB groups, the changes in the HFDM group were not significant. Intriguingly, after a notable increase in AST and ALT levels in the first week, a

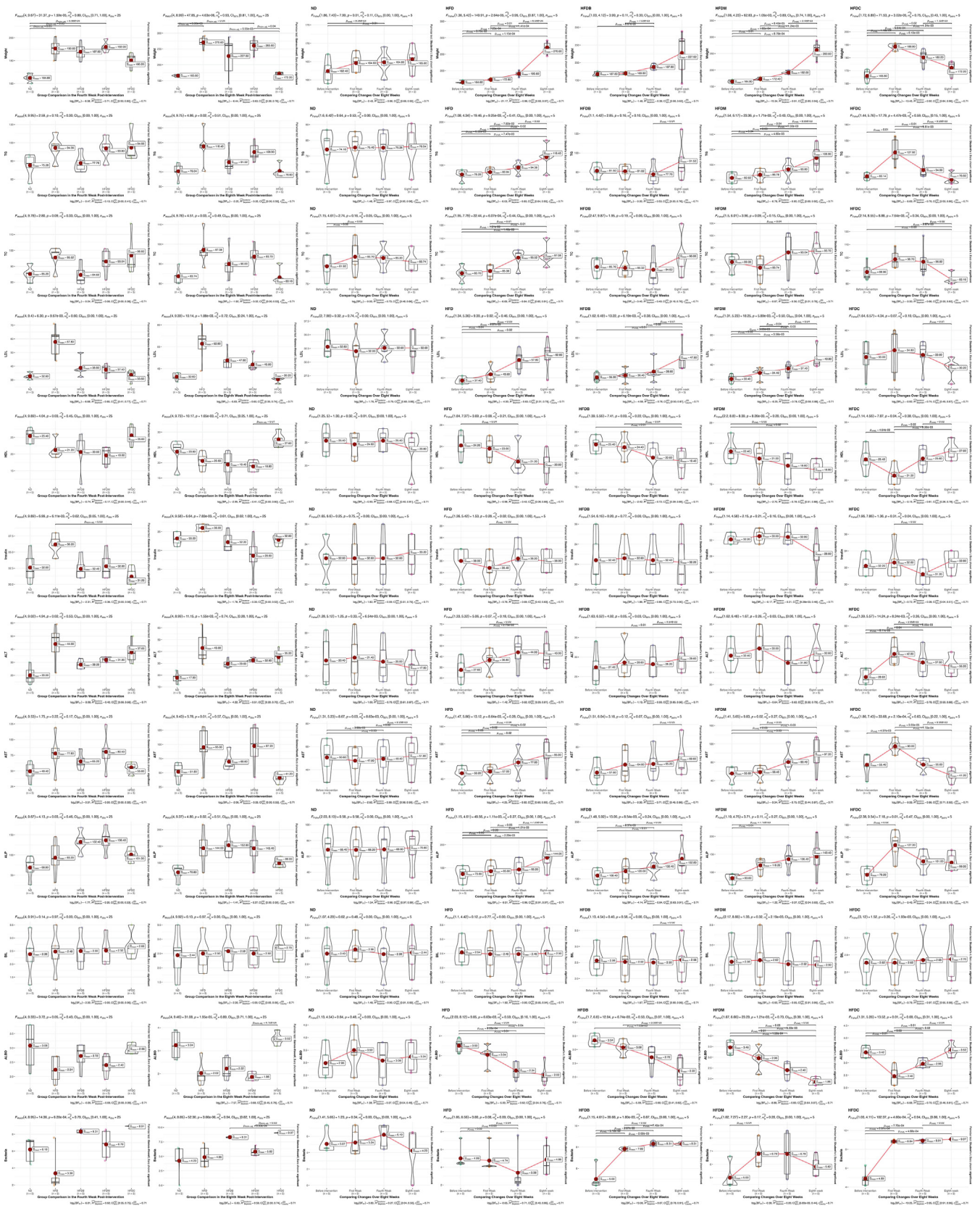

**Fig. 2.** Comparative analysis of blood biochemical parameters and body weight in rats across different groups. The figure includes two rows of data: Row 1 displays body weight, and Row 2 includes triglycerides (TGs), total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), insulin, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), bilirubin (BIL), albumin (ALBO), and bacteria levels. The groups analyzed are ND (normal diet), HFD (high-fat diet), HFDB (high-fat diet treated with *L. plantarum*), HFDM (high-fat diet treated with microcapsules), and HFDC (high-fat diet treated with NCAP microcapsules containing *L. plantarum*). Measurements were taken at Weeks 4 and 8, with an additional assessment of intra-group variability over time.

noteworthy reduction was observed in the fourth and eighth weeks (Fig. 2).

### 3.11. Effect of modified microcapsules on BIL

The BIL level compression did not show any significant differences between the groups. However, there was a considerable increase in the HFDM group at week four of the study (Fig. 2).

### 3.12. Effect of modified microcapsules on albumin

The albumin level compression at week four did not show any significant difference between the groups; however, at week eight, a considerable difference was found between the HFDM and HFDC groups. In addition to the substantial reduction in albumin levels in the HFD, HFDB, and HFDM groups, in the HFDC group, after a notable decrease in albumin levels in the first week, there was a significant increase in the fourth and eighth weeks of the study (Fig. 2).

### 3.13. Effect of modified microcapsules on L. plantarum

The count of *L. plantarum* in week four did not show any significant difference between groups, but in week eight, the count of *L. plantarum* in the HFDC group was significantly higher than that in the HFDB and HFDM groups. The number of *L. plantarum* cells in the HFD group was notably reduced in the first and fourth weeks. In the HFDB group, the count of *L. plantarum* significantly increased until the fourth week, and in the HFDM group, a significant increase was observed in the first week. Intriguingly, in the HFDC group, the count of *L. plantarum* over eight weeks is illustrated in Fig. 2.

## 4. Discussion

*L. plantarum*, a probiotic bacterium revered for its remarkable health benefits, is frequently encountered in fermented foods like sauerkraut, kimchi, and pickles [24]. This beneficial microorganism is celebrated for its positive impact on digestive health, immune support, and anti-inflammatory properties [25]. However, environmental stressors such as temperature, pH, and oxygen exposure during storage or transit can adversely affect its viability. Microcapsule technology has been developed to address these challenges as an effective strategy to preserve and protect *L. plantarum* [26].

Microcapsule technology entails encapsulating particles or droplets within a protective barrier, safeguarding them from environmental threats [27]. This technique serves as a protective mechanism for *L. plantarum*, ensuring its stability and viability during storage and transit [27]. Extensive research has explored various encapsulating materials, among which the combination of NCAP has emerged as an up-and-coming option [28,29]. These microcapsules are crafted from chitosan derived from crustacean shells, alginate extracted from seaweed, and Pectin obtained from plant-based sources [30].

Recent studies have explored various methods to improve the viability and functionality of *L. plantarum* for its potential applications in food and health industries [31,32]. Encapsulation techniques using biopolymers like chitosan, alginate, and Pectin have shown promising results in enhancing the survival of these probiotics under harsh gastrointestinal conditions [33].

Wang et al. [34] investigated the use of NCAP microcapsules for encapsulating *L. plantarum*, demonstrating significant improvements in gastrointestinal survival, weight management, and metabolic health in treated subjects. The study found that microencapsulation protected the probiotics from the stomach's acidic environment and improved their release and activity in the intestines.

Brinques and Ayub [35] and Trabelsi et al. [36] similarly reported that encapsulating *L. plantarum* in alginate and chitosan significantly enhanced its viability in simulated gastrointestinal conditions and during storage, suggesting that the protective coating provided by these biopolymers is crucial for maintaining the probiotic's functionality.

Furthermore, Jiang et al. [37] explored the immunomodulatory effects of *L. plantarum* encapsulated in alginate-chitosan microcapsules, highlighting the potential health benefits beyond just enhanced viability. The study demonstrated that these microcapsules could induce the secretion of beneficial cytokines, supporting encapsulated probiotics in therapeutic applications.

Karakas et al. [38] and Mahmoud et al. [39] provided additional evidence that alginate-based microencapsulation can improve the thermal stability and survivability of *L. plantarum* during food processing and storage. This is particularly relevant for developing functional foods that require processing at higher temperatures.

The findings from these studies collectively underline the effectiveness of biopolymer-based encapsulation techniques in improving the viability and functionality of probiotics like *L. plantarum*. This aligns with the results of Brinques and Ayub [35], who demonstrated that NCAP microcapsules enhance gastrointestinal survival and contribute to improved weight management and metabolic health.

Our investigation focused on a novel approach to microencapsulating *L. plantarum*, assessing its impact on survival over an extended period. The data from our study revealed that NCAP microcapsules substantially enhanced the viability of *L. plantarum* compared to its non-encapsulated counterparts, ensuring higher survival rates and maintaining consistent cell numbers. This improved efficacy can be attributed to the protective shell of the microcapsules, which acts as an impenetrable barrier against environmental stressors such as oxygen, moisture, and temperature fluctuations.

Microencapsulation technology has garnered significant attention for its pivotal role in shielding probiotics from environmental stressors and augmenting their viability. Our study vividly demonstrated the effectiveness of NCAP microcapsules in upholding the viability of *L. plantarum* while concurrently improving various health parameters. These findings align with the research conducted by Elsherief et al. [40], who delved into nanoemulsions and polymer coatings to enhance the quality and shelf life of food products. Their study underscored the antimicrobial potential of essential oils and nanoemulsions in preserving food integrity, mirroring our observations regarding the protective prowess of microencapsulation in sustaining probiotic viability.

Additionally, the study by Afzaal et al. [41] further enriches the discussion on probiotic encapsulation strategies. They investigated the potential of cellulose-chitosan (Cl-Ch)-based hybrid encapsulation in preserving the viability and stability of probiotics, specifically *L. plantarum*, under simulated gastric transit and in kefir. Their findings highlighted the promising potential of Cl-Ch hybrid-based encapsulation for targeted probiotic delivery and maintenance of viability in gastrointestinal conditions and food matrices.

The development of innovative systems for storing and delivering therapeutic agents is crucial for ensuring their long-lasting functionality and diverse delivery modes. One such advancement is the Chitosan–Alginate–Pectin-coated Suspended-Liquid-Encapsulating (CAPSuLE) marbles, introduced by Kim et al. [42]. This quasi-spherical triple-layered capsule contains suspended liquid droplets and allows multi-modal delivery of therapeutic agents in the aqueous phase. The CAPSuLE marble utilizes pectin as a liquid–air interfacial barrier to maintain the integrity of the liquid droplets within the core zone [43]. Anionic alginate and cationic chitosan layers are sequentially formed around the pectin-coated

droplet, resulting in a system with sufficient mechanical strength to resist external harsh environments. The CAPSULE system offers eco-friendly sustainability, responsiveness to external stimuli, coacervate-driven coalescence for linking adjacent marbles, and a self-repairing ability, making it a promising candidate for biomedical applications in biology and applied engineering.

In a related study by Afzaal et al. [44], the focus was on assessing the survival and stability of probiotic bacteria under simulated gastrointestinal digestion and thermal conditions. Using an encapsulate, probiotics were encapsulated with hydrogel matrices, specifically sodium alginate and carrageenan. The developed microbeads were characterized to elucidate the interaction between hydrogel matrices and probiotics. Encapsulation significantly enhanced the survival and stability of probiotics, with encapsulated cells exhibiting higher viability under thermal and gastrointestinal conditions than free cells. These findings underscore the potential of encapsulation in preserving the viability of probiotic bacteria, highlighting its importance in ensuring the efficacy of probiotic formulations under challenging environmental conditions.

Storage conditions, particularly temperature, were found to significantly influence the viability of microencapsulated *L. plantarum*, with colder storage temperatures (4°C) favoring higher survival rates than room temperature storage, highlighting the importance of refrigeration.

The study also observed a marked weight reduction in rodents fed a high-fat diet supplemented with NCAP microencapsulated *L. plantarum*, indicating its potential for weight management. This observation is supported by similar research showing the effectiveness of microcapsules in weight regulation. Studies by Mooranian et al. [45] and Tuch et al. [46] have explored the role of microencapsulation in insulin production and the viability of encapsulated human islets for diabetes treatment, respectively.

Regarding lipid metabolism, the group fed the high-fat diet with NCAP microcapsules demonstrated a significant drop in triglyceride levels, suggesting an advantage in lipid management. This is consistent with other research, such as Song et al. [47], who studied the impact of microcapsules on lipid metabolism.

Moreover, reductions in total cholesterol and LDL cholesterol levels were noted in the same group, indicating effective cholesterol management. This finding aligns with Yao et al. [48], who discussed the potential of microcapsules in cholesterol regulation.

Liver health was notably improved in the group treated with NCAP microcapsules, as indicated by the reduced levels of liver enzymes, suggesting a hepatoprotective effect. This observation aligns with our study results, where we found significant enhancements in liver function markers such as aspartate transaminase and alanine transaminase levels. These improvements underscore the potential of NCAP microcapsules in maintaining *L. plantarum* viability and promoting liver health. Additionally, our findings are consistent with the study by German et al. [14], which demonstrated the capability of microcapsules in liver-targeted drug delivery, further supporting the relevance of our research in this context.

Our study highlights the multifaceted efficacy of NCAP microcapsules, demonstrating their significant benefits in weight management, lipid optimization, cholesterol regulation, and liver function support. These findings showcase the versatility of microencapsulation technology and highlight its usefulness in various biomedical applications. Future research should focus on refining these formulations for potential clinical use.

Microencapsulation shows excellent potential in enhancing the viability of probiotics, bringing essential implications for nutrition and pharmacology. This innovative technique protects probiotics from harsh gastrointestinal conditions, extends their shelf life, and allows for controlled release, thus increasing their therapeutic potential. Additionally, microencapsulation allows researchers to customize probiotics for specific health needs, enabling the incorporation of other bioactive ingredients to enhance their health benefits and achieve synergistic effects.

## 5. Conclusions

The study showed that microencapsulated *L. plantarum* effectively improves its viability and resistance to harsh environmental conditions, especially in the gastrointestinal tract. These findings suggest that microencapsulation technology can potentially preserve probiotic bacteria and promote better health outcomes. Moreover, the study emphasized the advantages of NCAP microcapsules in weight management, lipid metabolism, and liver health, indicating their potential use in functional foods and therapeutics. Further research is needed to investigate the broader implications of microencapsulation in enhancing the effectiveness of probiotics and their health benefits.

## 6. Limitations

- Limited Sample Size: The study was limited in sample size. The relatively small sample size, especially in the experimental groups, may have affected the statistical power of the results and the ability to detect more minor but potentially significant effects. This limitation highlights the need for large-scale human trials to validate and generalize our findings.
- Differences Between Rats and Humans: It is essential to acknowledge that while the study's findings in rats are promising, translating these results directly to humans can be challenging because of the inherent physiological differences between the two species. Rats and humans have variations in anatomy, metabolism, and immune responses, which can affect how interventions such as microencapsulation impact health outcomes in each species.
- Duration of the Study: The study duration of eight weeks might not fully capture the long-term effects or extended viability of microencapsulated *L. plantarum*. Long-term studies are essential to assess the sustainability of the observed outcomes and account for potential variations over time.
- Study Population and Generalizability: The study primarily focused on a specific population, which might limit the generalizability of the findings to a broader demographic. Expanding the participant pool to include more diverse individuals with varying health conditions would enhance their ability to apply these findings to a broader range of people and health scenarios.

## CRediT authorship contribution statement

**Mahla Shahriari:** Writing – original draft, Validation, Software, Methodology, Investigation, Formal analysis, Data curation. **Ashraf Mohabati Mobarez:** Writing – review & editing, Validation, Supervision, Project administration, Conceptualization. **Amin Talebi Bazminabadi:** Writing – review & editing, Visualization, Supervision. **Masoumeh Tavakoli Yaraki:** Writing – review & editing, Visualization, Project administration, Methodology.

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## Conflict of interest

None declared.

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## Supplementary material

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## Data availability

The authors do not have permission to share data.

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