Mesopotamia Journal of Agriculture

https://magrj.uomosul.edu.iq

SIMULATION OF THE SURVIVAL OF LACTOBACILLUS PLANTARUM IN THE GASTROINTESTINAL TRACT ENCAPSULATED WITH SODIUM ALGINATE USING THE EMULSIFICATION-SPRAY DRYING TECHNIQUE

Abdullah Anwer Nafea alani, Taha Mohammed Taki Mohammed, Sumyia Khalaf Badawi

1,2,3Department of Food Sciences, College of Agriculture and Forestry, University of Mosul, Mosul, Iraq

Article information
Article history:
Received: 20/07/2025
Accepted: 03/01/2026
Available: 31/03/2026

Keywords:
fermentation activity, probiotic viability, gastrointestinal simulation, Lactobacillus plantarum.

DOI:
10.33899/mja.2026.163080.1666

Correspondence Email:
abdullah.anwer@uomosul.edu.iq

ABSTRACT

This study aimed to simulate the gastrointestinal survival of *Lactobacillus plantarum* after microencapsulation using the emulsification–spray drying technique with sodium alginate as the wall material. The primary objective was to improve bacterial viability and preserve metabolic activity in simulated gastric and intestinal conditions. The structural and functional characterizations of the produced microcapsules were done through evaluating integrity and performance. FESEM revealed spherical, smooth, and homogeneous particles, while transmission electron microscopy disclosed the homogeneous internal distribution of *L. plantarum* cells within an alginate matrix; energy-dispersive X-ray spectroscopy indicated a key elemental component of sodium alginate, and light microscopy provided additional confirmation of particle homogeneity and surface integrity. It was shown that encapsulated cells retain acid production and fermentation capabilities, which means metabolic activity is maintained. Under simulated gastrointestinal conditions, encapsulated *L. plantarum* exhibited notably higher survival compared to free cells, hence confirming the protective role played by sodium alginate microcapsules. The findings suggest that this emulsification-spray drying with sodium alginate is a highly effective and scalable method of probiotic encapsulation with high potential for use in functional food formulations and controlled probiotic delivery systems.

INTRODUCTION

Probiotic microorganisms most importantly *Lactobacillus spp*.—are the most valuable biological reagents to preserve human health by normalizing intestinal microbiota, improving immunity, and inhibiting intestinal pathogens (Ahmed and AlJawady, 2019). *Lactobacillus plantarum* is a suitable model because it possesses comparative acid and bile salt tolerance and can generate antimicrobial metabolites (Zhang *et al*., 2021).

Despite such advantages, *L. plantarum* suffers drastic declines in numbers of viable cells on heat treatment, storage, or transit through the gastrointestinal tract due to gastric acidity, digestive enzymes, and bile salts that lyse the cell membrane and traverse probiotic components (Qasim *et al*., 2024). Recent market research suggests that no more than 30% of the initial dose of some probiotic foods survives to the small intestine (Guo *et al*., 2024). Although numerous studies have addressed probiotic encapsulation techniques, most have focused on improving survival rates without a detailed study of the composition of microparticles or the relationship between the coating type and fermentation behavior. Furthermore, only a few studies have tested bacterial survival under dynamic gastrointestinal simulation conditions using multiple imaging techniques simultaneously. Hence, the importance of this research, which aims to provide a comprehensive view encompassing the microstructure, bioactivity, and digestive resistance of encapsulated bacteria (Agriopoulou *et al*., 2024)

Consequently, microencapsulation technology has been increasingly demanded because it allows for encapsulation of cells in a barrier polymer matrix that reduces the effect of physical and chemical stress (Etchepare *et al*., 2015). Among the preferred polymers for coating probiotics, sodium alginate is due to its natural origin, bio solubility, and capability of forming stable gels with calcium present (Alkhashb *et al*., 2024). Moreover, with the co-application of emulsification with spray drying, homogenous spherical particles can be formed with prospects for bulk industrial manufacturing and extended shelf life (Sharma *et al*., 2022).

Microencapsulation is an effective process to conserve the probiotic cells from adverse environmental conditions to reach their survival as well as functional efficacy. Such a non-toxic, biodegradable, food-grade polysaccharide derived from brown seaweed is sodium alginate, which has been widely employed as a wall material due to its excellent gelling strength, acid resistance, and safety as a food-grade ingredient (Diaz-Negrete *et al*., 2025).

Spray drying emulsification is an economical scale-up process for probiotic encapsulation as stable, dry powder that can be readily consumed as food. Emulsification allows uniform distribution of the probiotic cells in the encapsulant matrix, and spray drying stabilizes the emulsion in the form of normal-morphology microcapsules (Yang *et al*., 2024).

Microencapsulation, according to the majority of researchers, is the most effective way and is extremely in vogue. It is a good technique to extend the shelf life of bacteria and deliver it in the intestine to act as a probiotic barrier (D’Amico *et al*., 2025).

The success of such a strategy is greatly attributed to the optimal selection of microencapsulation material, probiotic strain, probiotic release process, and encapsulation process (Setiarto *et al*., 2025).

MATERIALS AND METHODS

HIMEDIA (India) provided sodium alginate, OLIMP (Poland) supplied preeutin whey protein, Selcuk University (Turkey) supplied *L. plantarum*, and HIMEDIA (India) supplied MRS Broth.

Preparation of Bacterial Culture

Bacterial culture was rejuvenated in 10 ml of MRS broth, and incubation was maintained at 37 °C anaerobically for 48 h anaerobically. Logarithmic phase cells were centrifuged at 6000 × g for 10 min to provide a final concentration of approximately 107 CFU/ml (Wang *et al*., 2022).

Production of encapsulated particles:

An emulsion-based system was prepared according to (Etchepare *et al*., 2015) with some modifications and according to the following protocol:

1- **Preparation of aqueous phase:**

4% solution of sodium alginate was prepared and mixed with 2 ml of *L. plantarum* biomass. The two were mixed on a magnetic stirrer until uniform.

The pH of the solution was set to approximately 5.5 using 1 N HCl or NaOH, according to requirements for better interaction between the wall materials and emulsion stability. The methods for preparing gastric and intestinal juices followed the standard models suggested by (Mahmoud *et al*., 2020), with a few small changes made to adjust the pH to reflect actual human digestive conditions. The stability of the components was confirmed through careful calibration, and tests were performed in three replicates to ensure reliability.

2- **Surfactant (Tween 20) addition:**

To make the emulsion stable and lower the tension at the interface, a set amount of Tween 20 (0.2 mL) was added to the water phase (Jiao *et al*., 2022).

3- **Emulsion formation:**

Sunflower oil (2 mL) was added to the water phase progressively with stirring in a continuous manner to form a preliminary emulsion. The obtained mixture was homogenized with the help of a high-speed homogenizer to produce a stable water-in-oil (W/O) emulsion (Micanquer-Carlosama *et al*., 2022).

4- **Stabilization**:

The second dose of Tween 20 (0.2 mL) was administered for additional stabilization of the emulsion from phase separation.

5- **Spray drying**:

Spray drying is a common technique for microencapsulating probiotics to enhance their stability. Exposure to high temperatures during drying can reduce bacterial viability; however, this effect can be minimized by controlling the inlet temperature, using protective agents, and employing rapid drying. In this study, the final emulsion was spray dried at an inlet temperature of 120°C, and the microcapsules were stored in sealed vials at 4°C (Tatasciore *et al*., 2024). This method helps protect the cells from harsh environmental conditions, such as low gastric pH and intestinal bile salts.

Bacterial Activation: *L. plantarum* was reactivated by incubating liquid MRS medium of concentration 52.6 g/L. The medium was inoculated with the bacteria and incubated at 37°C for 48 hours under sterile conditions. This was repeated three times to activate bacterial activity (Tabaar *et al*., 2021).

Production of Encapsulated Particles: Encapsulated bacteria were formulated based on the extrusion method as per Etchepare *et al*. (2020) with slight modification. Double 2% and 4% sodium alginate solutions and 2.5% whey protein were prepared. Bacteria were incubated for 48 hours and centrifuged from YINGTAI at 700 rpm for 3 minutes. The initial 2% sodium alginate solution was extruded into 0.1 M $CaCl_2$ solution using a nozzle (a small bore nozzle). The solution was homogenized using a homogenizer to avoid the clumping of the mixture upon extrusion and mixing difficulty with a magnetic stirrer. It was mixed into the original 2.5% whey protein solution, filtered, and washed using sterile water. It was then mixed with the 4% sodium alginate solution. This was done again by extruding into a $CaCl_2$ solution, washing with distilled water, and mixing with the second and final protein solution of concentration 0.1 M, 2.5%, spray-dried of Chinese origin at 120 °C. In addition, the protective alginate–whey protein matrix acted as a thermal shield, minimizing direct heat exposure of the probiotic cells and thereby preserving their viability during the drying process (Luo *et al*., 2022).

Characterization of the encapsulated particles: Encapsulated *L. plantarum* structural integrity and morphology were evaluated by Scanning Electron Microscopy (FESEM) and Transmission Electron Microscopy (TEM). Elemental analysis of the encapsulation matrix and identification of probiotic existence were substantiated through Energy-dispersive X-ray spectroscopy (EDX). Light microscopic evaluation using a digital camera was carried out to determine the initial particle shape and aggregation. The biological activity of free and encapsulated probiotic cells was determined.

Moreover, in vitro gastrointestinal simulation experiments were conducted to find out the survival of bacteria under hostile gastric and intestinal conditions.

Bacterial activity: Prepare a medium of liquid MRS with 2% lactose by dissolving 52.6 g of MRS powder per 1 liter of distilled water, Sterilize using an autoclave at 121°C for 15-20 minutes. After cooling, distribute the medium into test tubes. Recover the encapsulated *L. plantarum* bacteria (1 g/9 ml) with distilled water, inoculate 1 ml of the medium, and incubate at 37°C for 12-24 hours. Monitor changes in acidity until the pH decreases due to lactic acid production (Parhi *et al*., 2021).

Results and Discussion

Field Emission Scanning Electron Microscopy (FESEM):

Spray-dried microcapsules were mounted on aluminum stubs using conductive carbon adhesive. To minimize surface charging and enhance image resolution, a thin layer of gold by (sputter coating) (approximately 10 nm) was sputter-coated on the samples. Samples were imaged using a field emission scanning electron microscope (FESEM).

FESEM micrographs indicated that the microcapsules possessed a regularly spherical and typical shape with smooth, unbroken surfaces and dense wall structures (Figure 1a). Particle diameters were between 8.2 ± 0.7 µm and 12.4 ± 1.1 µm, and had a narrow size range and homogeneous morphology across the sample set (Figure 1b).
The smoothness and tightness of the microcapsules reflect a very ordered sodium alginate matrix with effective cross-linking. Such dense surfaces reduce porosity to a minimum and seal the capsules against penetration by external stress factors such as gastric acid and bile salts. It also has a significant impact on the structural integrity of capsules and the survival of probiotic cells that have been encapsulated. Moreover, uniformity in capsule size and shape can also contribute to better mechanical stability through processing and handling, and uniform distribution in food matrices of function (Abdulsattar *et al*. 2024).
The results follow whose assumption was that compact and spherical surface morphology alginate microcapsules enhanced probiotic survival in acidic conditions, also established higher encapsulation efficiency and acid resistance within the same compact morphological structures (Krasaekoopt *et al*. 2015).
FESEM analysis verified that the emulsification–spray drying process produced microcapsules that were intact structurally, with irregular surface texture, low porosity, and uniform size. Such physical characteristics are applicable to the maintenance of bacterial viability by imparting high resistance against extreme gastrointestinal environments.

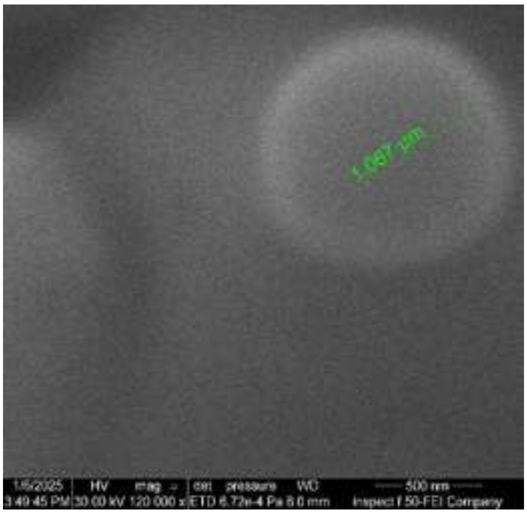


Figure (1 a) shows the size of the encapsulated granule using the scanning electron microscope.

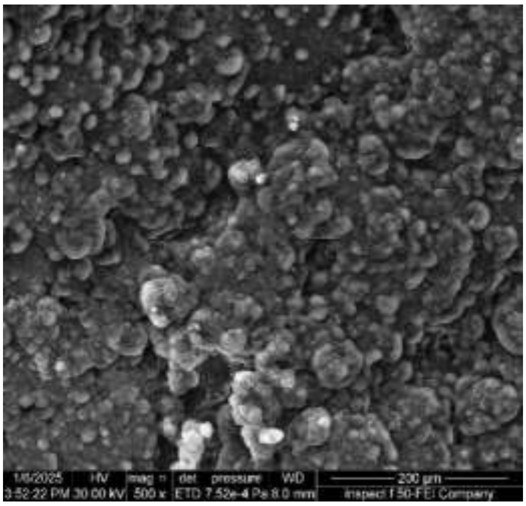


Figure (1 b) Surface structure of *L.plantarum* bacteria coated by emulsification technique

This regular, spherical surface structure indicates the success of the emulsification technique in forming cohesive microparticles capable of providing effective external protection for bacteria. The absence of cracks or overlaps also reflects the quality of the spray-drying process and enhances the suitability of these particles for food applications (Gullifa *et al.,* 2023).

Transmission Electron Microscopy (TEM):

Transmission electron microscopy (TEM) is a well-resolved technique used to image the internal structure of particle-containing capsules at the nanometer scale. The microcapsules that were employed in this experiment were chemically fixed, dehydrated through graded ethanol, and embedded in epoxy resin for the purpose of structural support. Ultrathin sections approximately 70 nm thick were prepared using an ultramicrotome and further deposited on copper grids. Imaging was conducted using a TEM at an accelerating voltage of 120 kV (Bonaccorso *et al.,* 2021).

The TEM images revealed clear internal morphology of emulsified microcapsules, e.g., spatial distribution of *L. plantarum* cells in encapsulating matrix (Figure 2a). The cells were well protected and encapsulated within the alginate-based matrix, confirming the efficiency of the encapsulation process. The size of the encapsulated granules was also similar in size in the micrometer range, as seen from the FESEM data, which ranged in the same micrometer range (Figure 2b).

TEM analysis provided information regarding the capsule wall continuity and thickness and the ultrastructural porosity of the matrix. Core–shell characteristic of the capsules was present, confirmatory to the interpretation that encapsulation matrix creates an integrated barrier to the probiotic cells. The structure is very important in preventing degradation upon passage through the gastrointestinal tract (Łętocha *et al.,* 2023).

Overall, the TEM findings verify the structural integrity and homogeneity of the encapsulated microcapsules, again verifying the effectiveness of the emulsification–spray drying methodology in producing stable and safe delivery systems for probiotic application (Gullifa *et al.,* 2023).

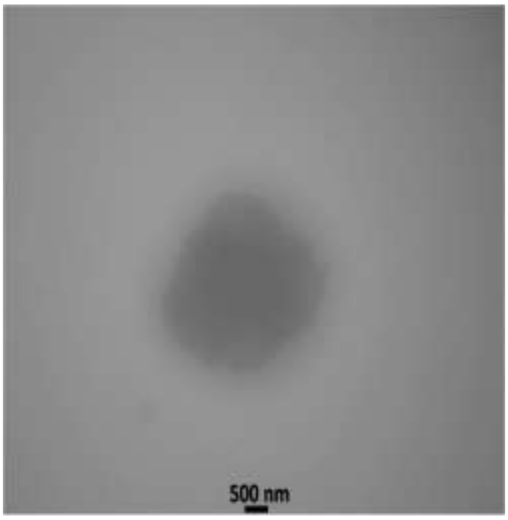


Figure (2 a) shows the size of the encapsulated granule using the Transmission Electron Microscopy

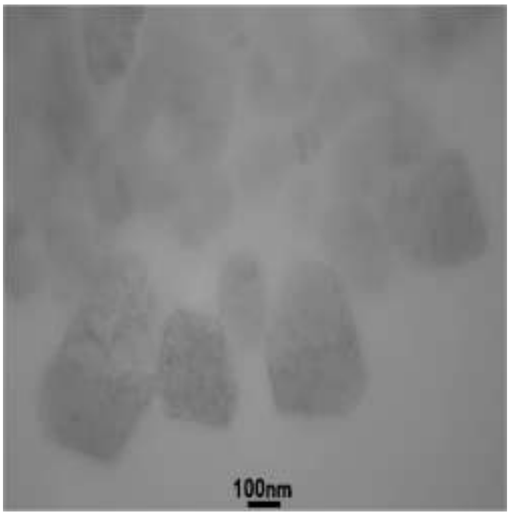


Figure (2 b) shows the internal structure of the particles encapsulated by the emulsion technique.

The homogeneous inner structure ensures the proper integration of bacteria within the alginate matrix, reducing the risk of leakage of the outer medium and enhancing the chances of survival in the gastrointestinal tract (Oberoi *et al.,* 2021).

Energy Dispersive X-ray Spectroscopy (EDX):

Energy-dispersive X-ray spectroscopy (EDX) was also conducted along with FESEM to identify the elemental composition of the encapsulated microcapsules. EDX spectra depicted the occurrence of four major elements, i.e., carbon (C), oxygen (O), sodium (Na), and calcium (Ca), as the characteristic elements of the encapsulating matrix (Zaineb *et al.,* 2022).

The most pronounced peaks were those of carbon and oxygen, which were expected from the organic polysaccharide backbone of sodium alginate. The confirmation by sodium emphasized the qualification of sodium alginate as the prime coating material because sodium ions are part of its molecular composition. Also, the trace of calcium assured effective ionic cross-linking of alginate chains upon gelation, playing an important role in giving microcapsules their mechanical stability and strength (Khosravi Zanjani *et al.,* 2014).

The overall elemental profile was as would be expected for a sodium alginate encapsulation system and indicated that the matrix had been produced with intact structure and in the absence of contamination. These results verify the ability of the emulsification–spray drying process to produce clean, chemically uniform probiotic microcapsules (Bustamante *et al.,* 2025).

Our results conform to (Premjit *et al*. 2024), which also showed good elemental consistency and established ionic cross-linking in probiotic encapsulation calcium–alginate matrices.

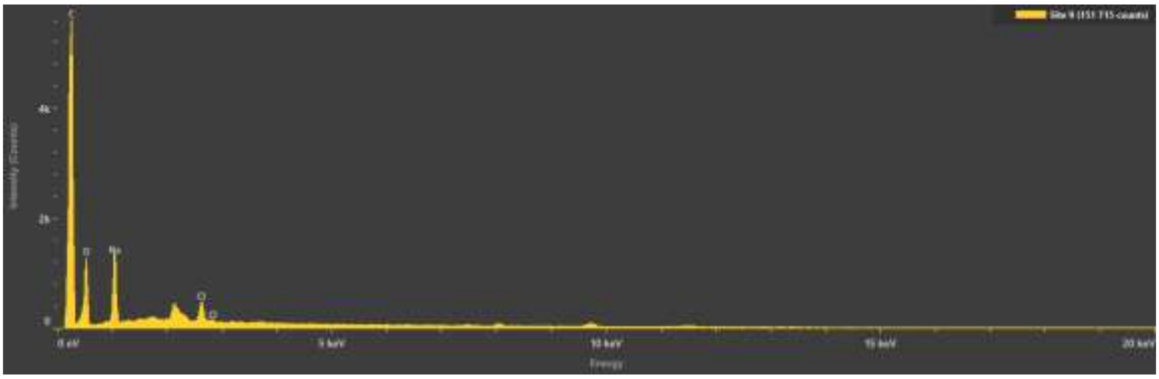


Figure (3): Energy dispersive X-ray spectroscopy and its detection of elements in *L. plantarum* bacteria encapsulated by emulsion technique.

This spectroscopic analysis supplemented the visual observation because the elements present provided hints on the purity of the material used in the coating, determining the presence of the ionic bonds to stabilize the gelatin network.

Light Microscopy and Digital Imaging

Light microscopy It is used to observe the general shape of the capsule, whether it is spherical, oval, or irregular, and to determine its size. It is also used to detect cracks or bubbles that occur between particles. to study the morphology and surface topography of the probiotic particles encapsulated. The observations were conducted using an Optika digital bright-field microscope equipped with an integrated high-resolution digital camera and LED illumination, and real-time imaging and accurate documentation of microstructure details.

A few microliters drop of very small volume microcapsule suspension was placed on a glass slide, and covered with cover slip, and viewed under various magnifications. Spherical in shape, slightly oval were the majorities of the particles, and that indicates that droplet formation during emulsification was fine. There was no variation in particle size at all, likely as a result of homogenization and drying conditions during spray drying. The average particle size of the visual was well in the range seen in spray-dried emulsified microspheres (Khosravi ZanjanI *et al.*, 2018).

The microscopic results are consistent with other structural characterization techniques such as FESEM and EDX, and cumulatively validate the effectiveness and reproducibility of the emulsification–spray drying encapsulation process.

The results are in agreement with the results by Giordano *et al*., (2023), in which they also showed alginate microspheres formulated through emulsification maintained both structural integrity and morphology homogeneity under bright-field microscopy.

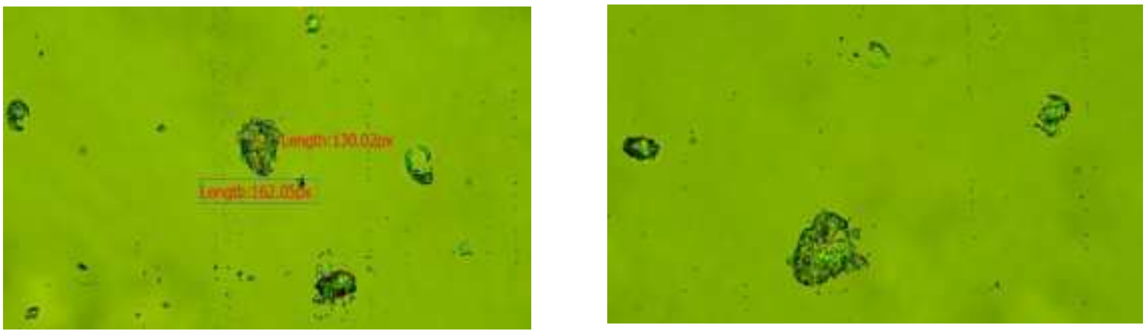


Figure 4: Light microscopy and digital imaging of *Lactobacillus plantarum* microcapsules prepared via the emulsification–spray drying technique.

Representative bright-field microscopy images of *L. plantarum* microcapsules prepared via the emulsification–spray drying technique. In the first image, microcapsules appear well-formed, displaying generally spherical to slightly irregular shapes, with clear surface definition and minimal aggregation. The second image includes particle size measurements, showing a consistent size distribution within the microscale range (130.02 px and 162.05 px), supporting the homogeneity of the encapsulation process. These measurements further confirm the effectiveness of the emulsification and spray drying conditions in producing uniform probiotic microcapsules with stable morphology (Gullifa *et al.,* 2023).

Bacterial activity:

The bacterial culture's pH profile was followed for 48 hours under incubation at 37 °C to quantify lactic acid production as a surrogate to the bacterial metabolic activity. Table (1) represents free and encapsulated *L.plantarum* cells' relative pH values. At time zero, the encapsulated samples had a slightly higher pH (pH 6.0) compared to free-cell samples (pH 5.5), which indicates the encapsulation material's buffering capacity. For the first 6 hours, a profound acidification was suffered by the free-cell culture (pH fell to 5.0), while encapsulated culture pH did not change (pH 6.0), suggesting that the encapsulation matrix decelerated lactose fermentation by inhibiting substrate diffusion (Kirmizigul *et al*. 2024).

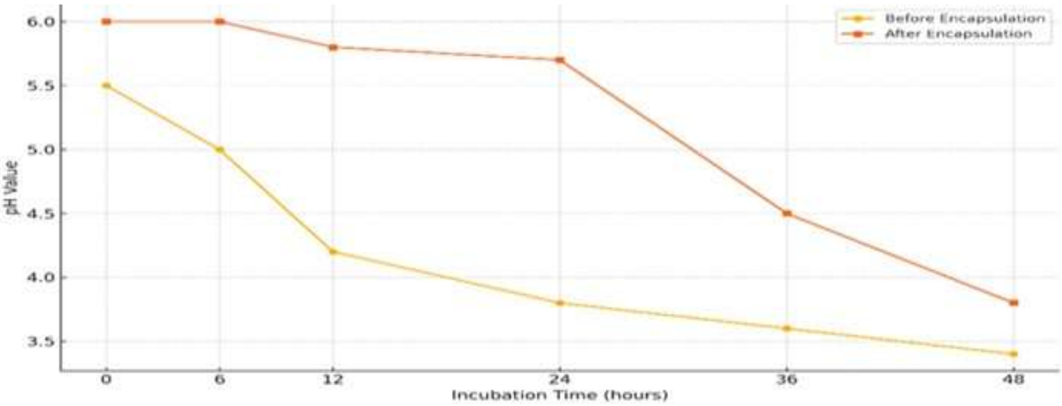
Bacterial cultures' pH was monitored over 48 hours at 37 °C to assess lactic acid production as an indicator of metabolic activity. Table 1 presents the relative pH values of free and encapsulated *Lactobacillus plantarum* cells. At the start of incubation, encapsulated samples exhibited a slightly higher pH (6.0) compared to free-cell samples (5.5), reflecting the inherent buffering capacity of the alginate-based encapsulation matrix. During the first six hours, free-cell cultures experienced a marked acidification, with pH dropping to 5.0, whereas the pH of encapsulated cultures remained stable at 6.0. This indicates that the encapsulation matrix effectively slowed lactose fermentation by limiting substrate diffusion (Kirmizigul *et al*., 2024). These results underscore the critical role of microencapsulation in protecting probiotics under acidic conditions; the matrix maintains a more balanced microenvironment, thereby enhancing bacterial survival and sustaining metabolic activity throughout the incubation period.

With continued incubation, the liberated bacteria promptly fermented lactose, resulting in a sharp pH drop to 3.4 within 48 hours. The encapsulated bacteria, on the other hand, revealed a more sustained but consistent acidifying trend, with the pH remaining at 3.8 upon termination of incubation. These results indicate that encapsulation slightly delays the onset of fermentation while maintaining bacterial metabolic activity throughout the incubation period. Controlled acidification of encapsulated samples demonstrates that microencapsulation not only preserves probiotic cells from environmental stressors but also enables continued metabolic activity, which is advantageous for extended shelf life and controlled release. Moreover, the encapsulating matrix serves as a protective barrier against harsh conditions, including elevated temperatures during spray-drying, low pH in the stomach, and bile salts in the intestinal tract. This combination of physical protection and sustained metabolic functionality ensures that the probiotics remain viable and active, supporting their efficacy in functional food and nutraceutical applications. These observations are consistent with previous studies reporting moderated acidification and enhanced survival of *Lactobacillus plantarum* within alginate microcapsules during fermentation and gastrointestinal simulation (Xin *et al*., 2025).

Evaluation of Fermentation Capability:

Fermentative activity of *Lactobacillus plantarum* immobilized with sodium alginate and whey protein via emulsification-spray drying technique was examined in 10% (w/v) reconstituted skim milk at 37°C for 24 hours. The results indicated that the immobilized cells gradually reduced pH from 6.6 to approximately 4.5 within 21 hours, while titratable acidity rose to approximately 85°D. On the other hand, free cells presented a faster acidification rate, with pH 4.3 after 18 hours, but acid production plateaued earlier, with reduced fermentation sustainability. The acidification profile of the encapsulated bacteria was more stable and extended, indicating that the coating matrix was effective in cell protection and enhancement of their metabolic activity during fermentation. These findings are consistent with recent studies by (Premjit *et al.,* 2024), who noted improved fermentation kinetics and yogurt quality when *L. plantarum* was encapsulated in a sodium alginate system.

pH changes During Fermentation Before and after Encapsulation



Figer 5: The graph above clearly shows the rapid in pH for un en capsulated bacteria compared to the gradual decrease for encapsulated baxteria. This supports the table′s results and confirms that encapsulation provides effective protection that delays but dose not halt the fermentation on process, enhancing the stability of the bactreia′s bioactive properties over the incubation period.

Fermentation Kinetics of Encapsulated vs. Free *L. plantarum*

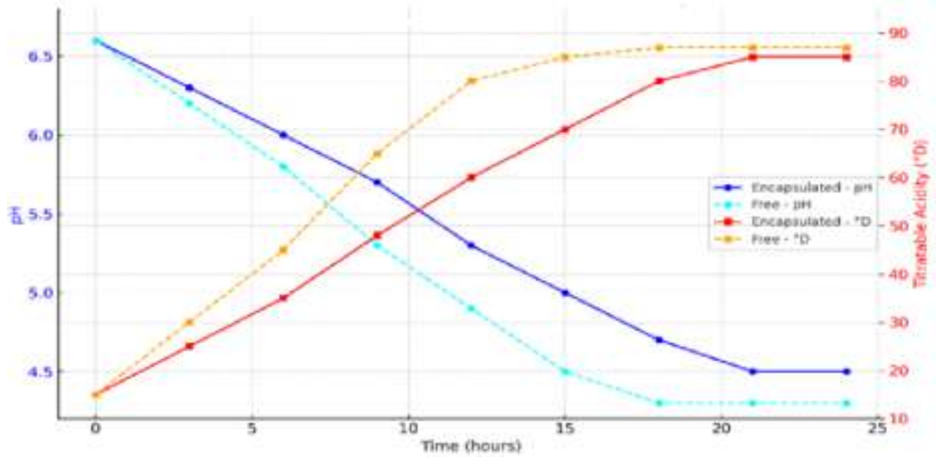


Figure 6: Graph showing comparison of fermentation dynamics between encapsulated and free *L. plantarum*. The blue lines represent pH changes, and the red lines represent titratable acidity. Encapsulated bacteria exhibit more stable fermentation behavior, while free bacteria decline more rapidly but stabilize early.

In Vitro Gastrointestinal Simulation:

In the present study, artificial gastric juice and intestinal was prepared as per an artificial model of digestion with certain adjustment of available protocols (Mahmoud *et al*., 2020).

Simulated gastric juice (SGJ) was also prepared by combining 0.2 g sodium chloride (NaCl) and 0.3 g pepsin in 100 mL distilled water and filled up to 1 N hydrochloric acid (HCl) to pH 2.0 to mimic the acidic environment of the human stomach (Mahmoud *et al*., 2020).

Simulated intestinal juice was prepared by dissolving 0.68 g KH_2PO_4 in 19 mL NaOH and making up to 100 mL with distilled water. NaOH or HCl was added for the addition of 2.0 g of bile salts and pH adjustment to 7.5 to simulate the small intestine condition. These in vitro model digestive juices were then used to examine stability and probiotic product viability under simulated conditions of the gastrointestinal tract.

Results show that free bacteria experienced a sharp decline in numbers during the gastric juice phase, reconfirming their low resistance to the acidic environment. On the other hand, samples that were encapsulated in a double layer (alginate + whey protein) experienced a high percentage of survival, an indicator that the outer layer helped in reducing cell exposure to acid components and digestive enzymes. These results confirm the idea of using multilayer encapsulation as a site-specific release system in the small intestine.

Survival of *L. plantarum* in Simulated Gastrointestinal Conditions

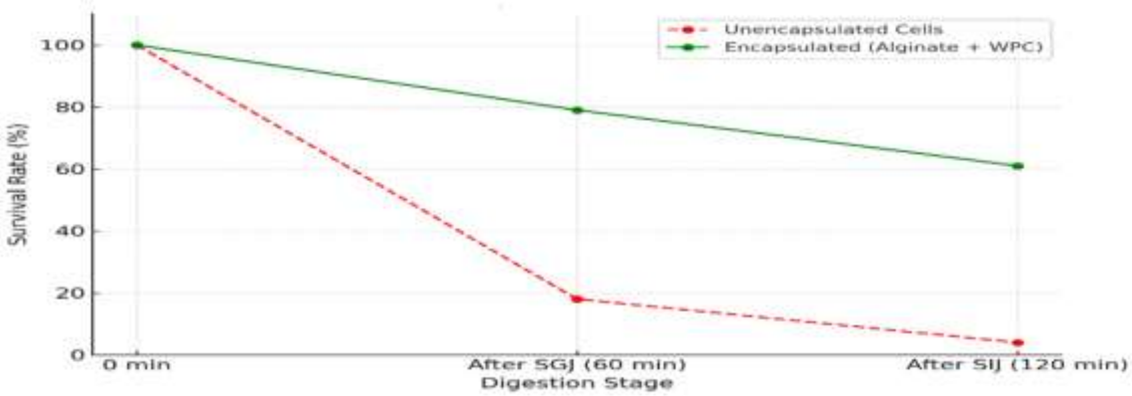


Figure 7: Graph showing the survival rate of *Lactobacillus plantarum* during the artificial digestion stages: The green line represents cells coated with two layers (alginate + whey protein) and shows positive results with a slight decrease. The dashed red line represents uncoated cells and shows a sharp decrease in survival.

Upon comparison of results from this study and results of studies carried out previously (e.g, Nuaman and Afzaal, 2024), it is clear that sodium alginate and food protein application increase encapsulation efficiency along with functional probiotic shelf life. This is due to the fact that the physicochemical characteristics of the encapsulated substance, e.g., crystallinity, viscosity, and ionic binding, influence it.

This study also involved the use of different imaging and physical analysis techniques to corroborate the activity and survival results, which have not been addressed in as much depth by most previous studies.

CONCLUSIONS

This study successfully used the integrated extrusion-spray drying process to microencapsulate *Lactobacillus plantarum* in sodium alginate and whey protein. Under harsh environmental conditions, the probiotic cells were demonstrated to be highly protected and stabilized by the nano- and microcapsules that were created.
The newly developed encapsulation process is of great practical significance because it makes production of highly viable probiotic products with a high shelf life easy. Application of encapsulated probiotics in fermented dairy foods or functional beverages has great commercial potential, particularly in the context of growing demand for digestive and immune system health-promoting products.
Additional work must be done on the in-use behavior of these encapsulated probiotics in actual food matrices, how their interactions with the other food constituents would affect them, and how well they would survive and release under simulated in vitro GI conditions, and also in vivo evaluation. Further work on other alternative or adjunct encapsulation agents, such as carrageenan or plant proteins, can facilitate further optimization of controlled release and site-specific delivery to the intestine.
Overall, the findings support emulsification–spray drying using sodium alginate as a promising and scalable method for probiotic encapsulation, with potential applications in functional food development and future in vivo studies

ACKNOWLEDGMENT

The researchers would like to thank the Deanship of the College of Agriculture and Forestry and the Department of Food Sciences for their continuous support for this work.

CONFLICT OF INTEREST

The authors stated that there are no conflicts of interest with the publication of this work.

محاكاة بقاء بكتيريا *Lactobacillus plantarum* في الجهاز الهضمي المغلف بألجينات الصوديوم باستخدام تقنية التجفيف بالرذاذ والاستحلاب

عبدالله أنور نافع العاني [1]   طه محمدتقي محمد [2]   سمية خلف بدوي [3]

قسم علوم الاغذية، كلية الزراعة والغابات، جامعة الموصل، الموصل، العراق[1,2,3]

الخلاصة

هدفت هذه الدراسة إلى محاكاة بقاء بكتيريا *Lactobacillus plantarum* في الجهاز الهضمي بعد التغليف الدقيق باستخدام تقنية الاستحلاب والتجفيف بالرذاذ مع استخدام ألجينات الصوديوم كمادة تغليف، كان الهدف

الرئيسي هو تحسين حيوية البكتيريا والحفاظ على النشاط الأيضي في ظروف محاكاة للمعدة والأمعاء. أُجريت التوصيفات الهيكلية والوظيفية للكبسولات الدقيقة المنتجة من خلال تقييم سلامتها وأدائها، كشف المجهر الإلكتروني النافذ عن جسيمات كروية وناعمة ومتجانسة، بينما كشف المجهر الإلكتروني النافذ عن التوزيع الداخلي المتجانس لخلايا *L. plantarum* داخل مصفوفة ألجينات. وأشار مطياف الأشعة السينية المشتت للطاقة إلى وجود مكون عنصري رئيسي لألجينات الصوديوم، كما قدم المجهر الضوئي تأكيداً إضافياً على تجانس الجسيمات وسلامة سطحها، وقد تبين أن الخلايا المغلفة تحتفظ بقدرتها على إنتاج الأحماض والتخمير، مما يعني الحفاظ على النشاط الأيضي، في ظل ظروف محاكاة الجهاز الهضمي، أظهرت بكتيريا *L. plantarum* المُغلّفة نسبة بقاء أعلى بشكل ملحوظ مقارنةً بالخلايا الحرة، مما يؤكد الدور الوقائي الذي تلعبه كبسولات ألجينات الصوديوم الدقيقة، تشير النتائج إلى أن هذه الطريقة الاستحلاب و التجفيف بالرذاذ باستخدام ألجينات الصوديوم تُعدّ طريقة فعّالة للغاية وقابلة للتطوير لتغليف المعزز الحيوي، مع إمكانية كبيرة لاستخدامها في تركيبات الأغذية الوظيفية وأنظمة توصيل المعزز الحيوي المُتحكّم بها.

الكلمات المفتاحية: نشاط التخمير، قابلية البروبيوتيك للبقاء، محاكاة الجهاز الهضمي، *Lactobacillus plantarum*.

REFERENCES

Abdulsattar, A. E., Bahaaldden, J. N., & Khalaf, B. S. (2024). Biosynthesis of selenium and copper oxide nanoparticles using probiotic bacteria against. *Food Spoilage Microorganisms, 19*(11); 42-48. https://doi.org/10.25303/1911rjbt042048.

Agriopoulou, S., Smaoui, S., Chaari, M., Varzakas, T., Can Karaca, A., & Jafari, S. M. (2024). Encapsulation of probiotics within double/multiple layer beads/carriers: a concise review. *Molecules*, *29*(11), 2431. https://doi.org/10.3390/molecules29112431.

Ahmed, S., & Al-Jawady, M. A. (2019). Effect of using dual-purpose oven on aflatoxins destruction and chemical composition of yellow corn. *Mesopotamia Journal of Agriculture*, *45*(1), 175–186. https://doi.org/10.33899/magrj.2019.161286.

Alkhashb, A., Alhaji, T., & Thalij, K. (2024). Effectiveness of Chitosan and Ag-Nanoparticle Films on the Quality of Chicken Meat. *Mesopotamia Journal of Agriculture*, *52*(2), 14-26. doi: 10.33899/mja.2024.145729.011337

Bonaccorso, A., Russo, N., & Romeo, A. (2021). Coating *Lacticaseibacillus rhamnosus* GG in alginate systems: An emerging strategy towards improved viability in orange juice. *AAPS Pharm. Sci. Tech., 22*(1), 123. https://doi.org/10.1208/s12249-021-01996-x.

Bustamante, M., Oomah, B. D., Burgos-Díaz, C., Vergara, D., Flores, L., & Shene, C. (2025). Viability of microencapsulated probiotics in cross-linked alginate matrices and chia seed or flaxseed mucilage during spray-drying and storage. *Microorganisms, 13*(7), 1457. https://doi.org/10.3390/microorganisms13071457.

D'Amico, V., Cavaliere, M., Ivone, M., Lacassia, C., Celano, G., Vacca, M., la Forgia, F. M., Fontana, S., De Angelis, M., Denora, N., & Lopedota, A. A. (2025). Microencapsulation of Probiotics for Enhanced Stability and Health Benefits in Dairy Functional Foods: A Focus on Pasta Filata Cheese. *Pharmaceutics*, *17*(2), 185. https://doi.org/10.3390/pharmaceutics17020185.

Diaz-Negrete, N.I., Ramos Clamont Montfort, G., Cueto-Wong, C., López-Fernández, S.G. & Balandrán-Quintana, R.R. (2025).Encapsulation of *Lactiplantibacillus plantarum* using lactoferrin and alginate: Layer-by-layer coating and dual coating air brush approaches for enhanced stability. *Dairy, 6*, 27-36. https://doi.org/10.3390/dairy6030027.

Etchepare, M. de A., Nunes, G. L., Nicoloso, B. R., Barin, J. S., Moraes Flores, E. M., de Oliveira Mello, R., & Ragagnin de Menezes, C. (2020). Improvement of the viability of encapsulated probiotics using whey proteins. *LWT - Food Science and Technology, 117*, 108601. https://doi.org/10.1016/j.lwt.2019.108601.

Etchepare, M. D. A., Barin, J. S., Cichoski, A. J., Jacob-Lopes, E., Wagner, R., Fries, L. L. M., & Menezes, C. R. D. (2015). Microencapsulation of probiotics using sodium alginate. *Ciência Rural, 45*, 1319-1326. https://doi.org/10.1590/0103-8478cr20140938.

Giordano, I., Maresca, D., & Mauriello, G. (2023). Microencapsulation and sonication: A multiple physical approach to attenuate the probiotic *Lacticaseibacillus casei* ATCC 393. *Heliyon, 9*(12), e23144. https://doi.org/10.1016/j.heliyon.2023.e23144.

Gullifa, G., Risoluti, R., Mazzoni, C., Barone, L., Papa, E., Battistini, A., Martin Fraguas, R., & Materazzi, S. (2023). Microencapsulation by a Spray Drying Approach to Produce Innovative Probiotics-Based Products Extending the Shelf-Life in Non-Refrigerated Conditions. *Molecules*, *28*(2), 860. https://doi.org/10.3390/molecules28020860.

Guo, H., Zhou, Y., Xie, Q., Chen, H., Zhang, Y., Hong, Z., Chen, S. and Zhang, M., 2024.Microencapsulation of *Lactobacillus plantarum* with improved survivability using pufferfish skin gelatin-based wall materials. *Marine Drugs*, 22, 124-132. https://doi.org/10.3390/md22030124.

Jiao Y, Zhao Y, Chang Y, et al. (2022). Enhancing the Formation and Stability of Oil-In-Water Emulsions Prepared by Microchannels Using Mixed Protein Emulsifiers. *Front Nutr.* 9:822053. . https://doi.org/10.3389/fnut.2022.822053.

Khosravi Zanjani, M. A., Ehsani, M. R., Ghiassi Tarzi, B., & Sharifan, A. (2018). Promoting probiotics survival by microencapsulation with Hylon starch and genipin cross-linked coatings in simulated gastro-intestinal condition and heat treatment. *Iranian Journal of Pharmaceutical Research, 17*(2), e124827. https://doi.org/10.22037/ijpr.2018.2199.

Khosravi Zanjani, M. A., Ghiassi Tarzi, B., Sharifan, A., & Mohammadi, N. (2014). Microencapsulation of Probiotics by Calcium Alginate-gelatinized Starch with Chitosan Coating and Evaluation of Survival in Simulated Human Gastro-intestinal Condition. Iranian journal of pharmaceutical research: IJPR, 13(3), 843–852. https://pmc.ncbi.nlm.nih.gov/articles/PMC4177644.

Kirmizigul P., A., Guney, D., & Sengun, I. (2024). The use of both free and microencapsulated *Lactiplantibacillus plantarum* and *Pediococcus parvulus* in cucumber pickles. *Food and Bioprocess Technology, 17*, 4617–4631. https://doi.org/10.1007/s11947-024-03400-5.

Krasaekoopt W, Bhandari B, & Deeth H. (2015). The influence of coating materials on some properties of alginate beads and survivability of microencapsulated probiotic bacteria. *Ciência Rural.* 45(1):1–7. https://doi.org/10.1590/0103-8478cr20140938.

Łętocha, A., Michalczyk, A., Miastkowska, M., & Sikora, E. (2023). Design of alginate microsphere formulation as a probiotics carrier. *Chemical and Process Engineering: New Frontiers, 44*(3), Article e20. https://doi.org/10.24425/cpe.2023.146722.

Mahmoud, M., Abdallah, N. A., El-Shafei, K., Tawfik, N. F., & El-Sayed, H. S. (2020). Survivability of alginate-microencapsulated *Lactobacillus plantarum* during storage, simulated food processing and gastrointestinal conditions. *Heliyon, 6*(3), e03541. https://doi.org/10.1016/j.heliyon.2020.e03541.

Micanquer-Carlosama, A., Cortés-Rodríguez, M. & Serna-Cock, L., (2022). Optimization and storage of water-in-oil emulsion using a supplemented aqueous phase and sunflower oil. *Journal of Engineering Science and Technology*, **17**(5), pp.3071–3087. https://jestec.taylors.edu.my/Vol%2017%20Issue%205%20October%202022/17_5_5.pdf

Nuaman, M., & Afzaal, M. (2024). Survivability of probiotic under simulated gastrointestinal conditions as affected by synbiotic alginate–arabinoxylan encapsulation. *International Journal of Food Properties*. Advance online publication. https://doi.org/10.1080/10942912.2024.2380376.

Oberoi, K., Tolun, A., Altintas, Z., & Sharma, S. (2021). Effect of Alginate-Microencapsulated Hydrogels on the Survival of *Lactobacillus rhamnosus* under Simulated Gastrointestinal Conditions. *Foods (Basel, Switzerland)*, *10*(9), 1999. https://doi.org/10.3390/foods10091999.

Parhi, P., Song, K. P., & Choo, W. S. (2021). Viability, Storage Stability and In Vitro Gastrointestinal Tolerance of *Lactiplantibacillus plantarum* Grown in Model Sugar Systems with Inulin and Fructooligosaccharide Supplementation. *Fermentation*, *7*(4), 259. https://doi.org/10.3390/fermentation7040259.

Premjit, Y., Pandey, S., & Mitra, J. (2024). Encapsulation of probiotics in freeze-dried calcium alginate and κ-carrageenan beads using definitive screening design: A comprehensive characterisation and *in vitro* digestion study. *International Journal of Biological Macromolecules, 258*(Part 2), 129279. https://doi.org/10.1016/j.ijbiomac.2024.129279.

Qasim, S., Mohammed, T., & Badawi, S. (2024). Estimation of the percentage of lactoferrin and immune proteins IgG in buffalo colostrum within five days of birth. Mesopotamia Journal of Agriculture, 52(4), 16-28. https://doi.org/10.33899/mja.2024.149536.1430.

Setiarto RHB, Anshory L,& Wardana AA. (2025) . Microencapsulation Technology for Probiotic Bacteria: A Review. *IOP Conf Ser Earth Environ Sci.* ;1449(1):012153. https://doi.org/10.1088/1755-1315/1449/1/012153.

Sharma, R., Rashidinejad, A. & Jafari, S.M. (2022). Application of Spray Dried Encapsulated Probiotics in Functional Food Formulations. *Food Bioprocess Technol* .15, 2135–2154. https://doi.org/10.1007/s11947-022-02803-6.

Tabaar, N., Bahramian, S., & Mir Ahmadi, F. (2021). Investigation of the survival of probiotic bacteria *Lactobacillus plantarum* in free and microencapsulated forms in barley malt extract. *Journal of Kermanshah University of Medical Sciences, 25*(3), e114693. https://doi.org/10.5812/jkums.114693.

Tatasciore, S., Santarelli, V., Neri, L., Di Mattia, C. D., Di Michele, A., Mastrocola, D., & Pittia, P. (2024). Microencapsulation of hop bioactive compounds by spray drying: Role of inlet temperature and wall material. *Current research in food science*, *8*, 100769. https://doi.org/10.1016/j.crfs.2024.100769.

Wang, X., Li, Y., Zhang, J., & Chen, H. (2022). Microencapsulation of Limosilactobacillus reuteri by spray drying using different encapsulation wall materials. *Journal of Food Processing and Preservation*, 46(7), e16880. https://doi.org/10.1111/jfpp.16880.

Xin, W.-G., Jiang, Y.-H., Zhao, Y.-T., Liu, S.-J., & Suo, H.-Y. (2025). Encapsulation of *Lactiplantibacillus plantarum* in a sodium alginate/pectin/tea polyphenol complex: Enhancing stability and bioactivity for fermented milk products. *Food Chemistry*. Advance online publication. https://doi.org/10.1016/j.foodchem.2025.144851.

Yang C, Xiong Q, Mao Y, & Rong Q. (2024). Encapsulation techniques, action mechanisms, and evaluation models of probiotics: recent advances and future prospects. *Food Front.* 5(2):374–388. https://doi.org/10.1002/fft2.374.

Zaineb, T., Uzair, B., Rizg, W. Y., Alharbi, W. S., Alkhalidi, H. M., Hosny, K. M., Khan, B. A., Bano, A., Alissa, M., & Jamil, N. (2022). Synthesis and characterization of calcium alginate-based microspheres entrapped with TiO_2 nanoparticles and cinnamon essential oil targeting clinical Staphylococcus aureus. *Pharmaceutics, 14*(12), 2764. https://doi.org/10.3390/pharmaceutics14122764.

Zhang, Y., Liu, W., Wei, Z., Yin, B., Man, C. & Jiang, Y., (2021). The incorporation of *Lactobacillus plantarum* in W/O/W double emulsion and its stability in simulated gastrointestinal conditions. *LWT - Food Science and Technology*, 135, 110590. https://doi.org/10.1016/j.lwt.2020.110590.

Luo, X., Fan, S., He, Z., Ni, F., Liu, C., Huang, M., Cai, L., Ren, G., Zhu, X., Lei, Q., Fang, W., & Xie, H. (2022). Preparation of alginate–whey protein isolate and alginate–pectin–whey protein isolate composites for protection and delivery of *Lactobacillus plantarum*. *Food Research International, 161*, 111794. https://doi.org/10.1016/j.foodres.2022.111794.