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Vacuum oven drying technology used in the microencapsulation of *Limoslactobacillus reuteri*

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ABSTRACT

Microencapsulation processes are used to preserve the viability of probiotic bacteria. This study prepared the encapsulating mixture using whey proteins and gum Arabic to capsulate *Limosilactobacillus reuteri* bacteria. The vacuum oven was used for the encapsulation process and followed the proportions recommended by the Design of Experiments program. Moisture content, powder yield, changes in bacterial viable cell numbers, and the efficiency of the encapsulated bacteria were estimated. Subsequently, the best conditions for producing encapsulated bacteria were determined and the encapsulating material surrounding the bacteria was examined using a Scanning Electron Microscope (SEM). The results of the Design of Experiments showed that the optimal volume of *Limosilactobacillus reuteri* was 3 mL containing 11.74 log CFU/mL, mixed with a mixture of an encapsulating solution consisting of 10 g of whey protein and 3.75 g of gum Arabic. The optimal conditions for the encapsulation process were found to be a temperature of 50°C and a pressure of 0.6 bar for 180 minutes. The logarithmic value of the bacterial enumeration after the encapsulation procedure was recorded at 9.12 CFU/g, whereas the encapsulation effectiveness was 77.68%, accompanied by a moisture content of 4.26%. The yield of powder exhibited a mass percentage of 83.58%. The morphological analysis conducted through Scanning Electron Microscopy illustrated the envelope enveloping *Limosilactobacillus reuteri* bacteria. The diameter of the shell encasing the bacteria attained a measurement of 68.29 nm. The storage period did not significantly influence bacterial counts or encapsulation efficiency at 4°C and 25°C for 6 months. The possibility of using a mixture of whey proteins and gum Arabic in microencapsulation of bacteria by vacuum oven and maintaining the bacterial viable number under storage conditions.

استعمال تقنية التجفيف تحت التفريغ **Vacuum oven** في التغليف الدقيق لبكتريا *Limosilactobacillus reuteri*

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الخلاصة

تستعمل عمليات التغليف الدقيق للحفاظ على حيوية البكتريا العلاجية. وقد أعدت هذه الدراسة خليط التغليف باستخدام بروتينات شرش الحليب والصمغ العربي لتغليف بكتريا *Limosilactobacillus reuteri*. باستعمال فرن التفريغ لعملية التغليف واتبع النسب الموصى بها في برنامج تصميم التجارب. وتم تقدير محتوى الرطوبة، وحاصل المنتج، والتغيرات في أعداد الخلايا البكتيرية القابلة للحياة، وكفاءة عملية التغليف. وبعد ذلك، تم تحديد أفضل الظروف لإنتاج البكتريا المغلفة وفحص مادة التغليف المحيطة بالبكتريا باستعمال المجهر الإلكتروني الماسح (SEM). وأظهرت نتائج تصميم التجارب أن الحجم الأمثل لبكتريا *Limosilactobacillus reuteri* كان 3 مل يحتوي على 11.74 لوغاريتم وحدة تكوين مستعمرة \ مل، مخلوطًا بمزيج من محلول تغليف يتكون من 10 غرام من بروتينات الشرش و3.75 غرام من الصمغ العربي. وقد وجد أن أفضل الظروف لعملية التغليف هي درجة حرارة 50 م وضغط 0.6 بار لمدة 180 دقيقة. وكان لوغاريتم العد البكتيري بعد إجراء التغليف بلغ 9.12 وحدة تكوين مستعمرة\ غم، في حين بلغت كفاءة عملية التغليف 77.68% مصحوبة بمحتوى رطوبة 4.26%. وأظهر الحاصل النهائي من المسحوق 83.58%. بين التحليل المظهري الذي أجري من خلال المجهر الإلكتروني الماسح الغلاف الذي يغلف بكتيريا *Limosilactobacillus reuteri*. ان قطر الغلاف الذي يغلف البكتريا كان 68.29 نانومتر. ولم تؤثر فترة التخزين بشكل كبير على أعداد البكتيريا أو كفاءة عملية التغليف عند 4 م° و25 م° لمدة 6 أشهر. إمكانية استعمال خليط من بروتينات شرش الحليب والصمغ العربي في تغليف البكتريا بوساطة فرن التفريغ والحفاظ على العدد الحيوي للبكتريا تحت ظروف التخزين.

الكلمات الافتتاحية: بكتريا *Limosilactobacillius reuteri*، صمغ عربي، شرش حليب الجاموس.

INTRODUCTION

Probiotic bacteria have a beneficial effect on human and animal health. This necessitates a heightened focus on preservation techniques to create products containing these bacteria. Various factors can impact the quality and stability of these bacteria in food products, including thermal, osmotic, oxidative, and acidic stresses. Additionally, manufacturing and storage processes, along with environmental stresses, may result in a decline in the viability of probiotic bacteria. Consequently, several methods have been employed to enhance preservation factors for live bacterial cells and shield them from external influences (Jakub and Radoslaw, 2022).

Previous studies have shown that food products containing probiotic bacteria must have a sufficient survival rate of viable microorganisms before consumers ingest them. This is necessary for the organisms to reach the small intestine and exert a beneficial effect. The

bacterial count should be higher than 10^6–10^7 CFU/g or mL. However, most commercial products do not contain this number of bacteria (Al-Sahlany and Niamah, 2022). The main issue with food products containing these microorganisms is that their numbers decrease during the storage and transportation period (Wang and Zhong, 2024). *Limosilactobacillus reuteri* is a type of lactic acid bacteria widely used in human life. It produces many important metabolic products and is utilized in various functional foods. One of its significant products is the antimicrobial compound ß-3-hydroxypropionaldehyde, known as Reuterin. Reuterin is a low molecular weight compound that is soluble in water under anaerobic conditions. It is a non-protein compound that is resistant to many proteolytic and lipolytic enzymes. Reuterin is effective in a wide range of pH levels and exhibits activity against Gram-positive and Gram-negative bacteria, such as *E. coli*, *Salmonella*, *Shigella*, *Proteus*, *Pseudomonas*, *Clostridium*, *Staphylococcus*, and *E. coli* O157:H7, as well as some types of yeasts and molds (Mohammed *et al*., 2020; Niamah *et al*., 2023).

Many methodologies were utilized to control the concentrations of probiotic microorganisms within the requisite parameters. These methods included adding chemicals to adjust the acidity caused by bacterial metabolic activities, controlling oxidation potential, reducing food product content, adjusting storage temperature, and shortening storage periods. Encapsulating bacteria or yeast with natural materials can enhance their ability to survive and maintain their population numbers (Raise *et al*., 2020; Niamah *et al*., 2018). Xie *et al*. (2023) mentioned the possibility of using proteins, including denatured proteins, in the microencapsulation of active compounds and microorganisms, and these molecules are inactive. The encapsulation process involves drying processes, resulting in the production of a low-moisture mixture ranging between 4-7%. The dry mixture is stable in storage. Adding carbohydrates to the mixture lowers production costs, enhances the desired qualities, and is a naturally occurring, low-toxic, and biodegradable substance. Vacuum drying is similar to freeze drying, with the main difference being that the samples are dried through evaporation rather than sublimation. In freeze-drying, the samples should first be frozen before the water is removed, whereas in vacuum drying, the samples remain in a liquid state. Vacuum dryers typically operate at higher temperatures and pressures compared to freeze dryers but at lower temperatures than spray drying. Typical pressure values for vacuum dryers are typically more than 10 mbar, whereas for freeze dryers, they are less. The vacuum drying process is less effective in terms of the heat impact on the viability of probiotic bacteria. Low pressure reduces the temperatures required to evaporate the solvent, thereby minimizing heat-related damage (Fathi *et al*., 2022). The current study aimed to utilize vacuum drying technology to encapsulate *Limosilactobacillus reuteri* bacteria. A mixture of whey proteins and gum Arabic was used, and its components were determined through the Design of Experiments program.

MATERIAL AND METHODS

Obtained *Lim. reuteri* bacteria from the Department of Food Sciences at the College of Agriculture, University of Basrah, were activated on MRS-broth media after being incubated for 18 hours at 37°C. Utilize whey derived from buffalo milk processed at the Agricultural Research

Station/College of Agriculture. Gum Arabic was supplied by Sigma-Aldrich, Germany. Peptone water of 0.1% is prepared by dissolving 1g of peptone in a liter of distilled water. The solution is then distributed into test tubes (9 ml) and sterilized at 121°C for 15 minutes, following the method described by Harrigan and McCane (1966).

Prepare the microencapsulation solution:

Encapsulation *Lin**euteri* solution was prepared by mixing 1-5 mL of activated bacteria containing 11.74 log CFU/g, 1-5 gm of gum Arabic, and 1-15 gm of whey protein. Made use of the experiment design software and mixed the ingredients for 20 minutes with a magnetic stirrer, then placed them in a vacuum oven until the samples were dry. The encapsulation efficiency was calculated by dissolving one gram of encapsulated bacteria in 10 ml of phosphate buffer (PBS 0.1 M, pH = 7.2), and stirring for 20 minutes to release the bacterial cells. A serial dilution was performed and the diluted bacteria on MRS agar (Hi-media, India). The samples were incubated anaerobically at 37°C for 48 hours (Eckert *et al*., 2017). The percentage efficiency of the microencapsulation process was calculated using the following equation:

Microencapsulation efficiency (%) = (N/N0) × 100

Where: N = number of viable encapsulated bacteria after drying. N0 = number of viable bacteria in the encapsulation solution before drying.

The optimal conditions for the encapsulation process: The optimum conditions for encapsulated bacteria were studied using the Design of Experiments program. Temperatures ranging from 40 to 60 °C and pressure from 0.3 to 0.9 bar were tested, along with different periods ranging from 1 to 5 hours. Then calculate the efficiency of microencapsulation (%) of bacteria according to the previous equation.

Encapsulated Bacteria Tests: Moisture estimation: The moisture percentage of the encapsulated bacteria was determined by using an air oven. Five grams of encapsulated bacteria were weighed and dried at 105°C until a stable weight was achieved. Moisture was estimated according to the following equation (AOAC, 1975).

Moisture %= [(weight of sample before drying-weight of sample after drying)/ weight of sample] × 100

Estimating the total number of coliform bacteria: MacConkey Agar (MA) media was used to enumerate total coliform bacteria in the product containing encapsulated bacteria. The plates were incubated at 37°C for 48 hours.

Powder yield: The dry powder mass of encapsulated bacteria was measured, and the encapsulation yield was done as explained by Arepally and Goswami (2019):

Powder yield (%) = $(M/M_1) \times 100$

Where: M is the mass of dry powder, and M_1 is the mass of the encapsulated material used.

Scanning Electron Microscope (SEM): The internal appearance of the encapsulated bacteria was studied using a scanning electron microscope (SEM). The encapsulated bacterial cells were placed on a conductive carbon tape (metal support), and covered with gold nanoparticles using a Hitachi E-1045 Ion Sputter Encapsulator. Subsequently, their shapes were analyzed with a Hitachi SU8010 scanning electron microscope at an accelerating voltage of 15 kV and various magnifications ranging from 2 nanometers to 25 micrometers, including 200 and 500 nanometers. Particle size was measured using Size Meter software (version 1.1), with a minimum of 50 particles per experiment. Statistica® 5.0 software was used to calculate the particle size distribution (Narathip *et al*., 2021).

The change of viable numbers of encapsulated *Lim. reuteri* bacteria during storage stages: The viability of *Lim. reuteri* bacteria and the microencapsulation efficiency was estimated during the storage period at 1, 2, 3, 4, 5, and 6 months of storage at 4 °C and 25°C. The viable counts of the encapsulated bacteria were determined by following the procedure outlined in APHA (1978). One milliliter of the last serial dilution was transferred to Petri dishes, and MRS agar media was poured over them. The plates were then incubated at 37°C for 48 hours, after which the number of growing bacteria was calculated (Jaafar *et al*., 2024).

RESULTS AND DISCUSSION

Table 1 presents the proportions of ingredients used to achieve optimal encapsulation and their application in the microencapsulation of *Lim. reuteri* bacteria. Calculate the efficiency of the encapsulation process based on the specified ratios using the Design of Experiments program. The results indicated that the optimal mixture for encapsulating bacteria consisted of 3.25 grams of gum Arabic and 12 grams of dried buffalo whey, with the addition of 2.2 milliliters of active *Lim. reuteri* bacterial culture. The logarithm of the number of encapsulated bacteria reached 8.95 CFU/g, and the conversion efficiency percentage was 76.23%. Whey proteins work with Gum Arabic to form bonds that trap bacteria, keeping them from drying out while remaining permeable. This allows the bacteria to benefit from the nutrient media, grow, and release beneficial metabolic products to the host. Despite the protective cover from harsh conditions, these findings align with Phùng *et al.* (2021), who utilized whey proteins and lignin in encapsulating *Lim. reuteri* KUB-AC5 bacteria through the spray drying technique. Similarly, Kajal *et al.* (2021) demonstrated that gum Arabic is among the best carbohydrates for encapsulating *Lacticaseibacillus rhamnosus* bacteria. Furthermore, Sharifi *et al.* (2021) isolated whey proteins and gum Arabic for encapsulating *Lactiplantibacillus plantarum*, using complex coacervation technology, which exhibited the highest.

Table 1: The logarithm of the number of *Lim. reuteri* and the efficiency percentage of the encapsulation process using various component percentages in the encapsulation mixture

Bacterial inoculum (ml)	Gum Arabic (g)	Whey (g)	Log. Bacterial numbers (CFU/g)	Percent (%)
5.00	5.00	5.00	7.46	63.54
5.00	3.51	11.00	7.46	63.54
1.00	4.13	15.00	8.63	73.50
2.62	5.00	10.95	8.60	73.25
3.58	2.50	15.00	8.06	68.65
3.00	4.75	5.95	8.41	71.63
5.00	5.00	15.00	7.39	62.94
1.00	5.00	5.00	7.46	63.54
2.62	3.50	5.00	7.60	64.73
2.62	3.50	5.00	7.50	63.88
3.28	2.50	8.30	7.50	63.88
5.00	3.51	11.00	8.16	69.50
1.00	4.18	9.45	8.16	69.50
1.00	2.50	8.55	8.16	69.50
3.58	2.50	15.00	8.16	69.50
2.20	3.25	12.00	8.95	76.23
2.62	5.00	[illegible]	8.39	71.46
5.00	2.50	5.00	7.46	63.54
3.36	4.11	15.00	7.06	60.13
5.00	4.51	[illegible]	7.76	66.09

Figure 1 shows the results obtained from the Design of Experiments program, which aimed to determine the optimal proportions of the components in the encapsulating mixture. The mixture consisted of 10 g of dried whey proteins, 3.75 g of gum Arabic, and 3 ml of activated bacteria. The logarithm of the bacterial count after the encapsulation process reached 9.12 CFU/g, while the efficiency of the encapsulation process was 77.68%. These values are lower than the proposed theoretical value of the logarithm of 10.20 CFU/g.

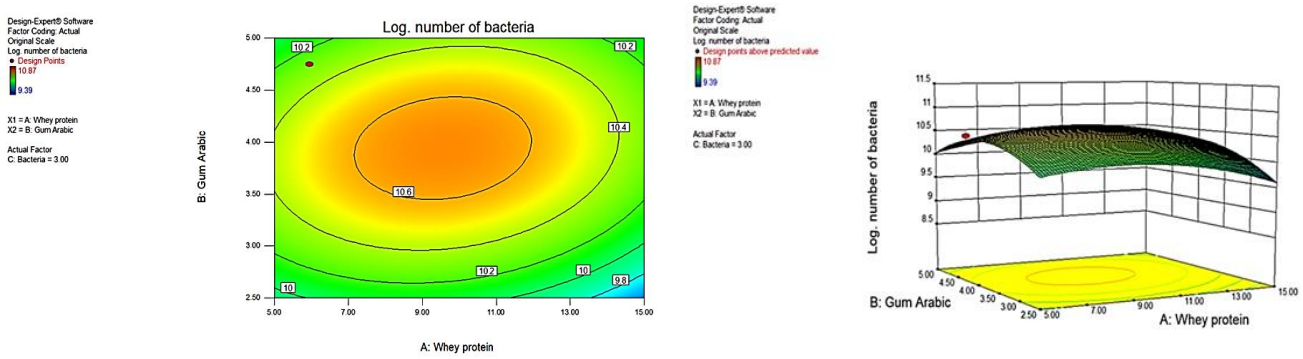

Figure 1. The proposed proportions of the components of the encapsulating mixture for bacteria using the Design of Experiments program

Figure 2 shows the results of the optimal conditions for the vacuum oven drying device, using the Design of Experiments program. The proposed theoretical value was 9.35 log CFU/g when drying is carried out at a temperature of 50°C and a pressure of 0.6 bar for 180 minutes. The practical result slightly exceeded the theoretical value, reaching 9.8 log CFU/g. The efficiency of the encapsulation process was 84.15%. This high efficiency can be attributed to the short drying time, low temperature, and optimal pressure, which promote higher growth rates for the encapsulated *Lim. reuteri*. Additionally, the presence of suitable encapsulating materials helps protect the viable cells. These findings align with those of Foerst *et al.* (2012) in their study on encapsulating *L. paracasei* F19 bacteria using a vacuum oven. Hongpattarakere and Uraipan (2015) found that the survival rates of *L. plantarum* SIF17AN2 bacteria after drying were higher when a shorter drying time was used (12 hours, 37°C, 0.4 bar) compared to using room temperature and pressure of 0.4 bar for 5 days. It can be concluded that shorter drying times and lower temperatures are preferable for the viability of the encapsulated microorganisms. It should be noted that water activity also plays a crucial role in viability, not only after drying but also during storage**.**

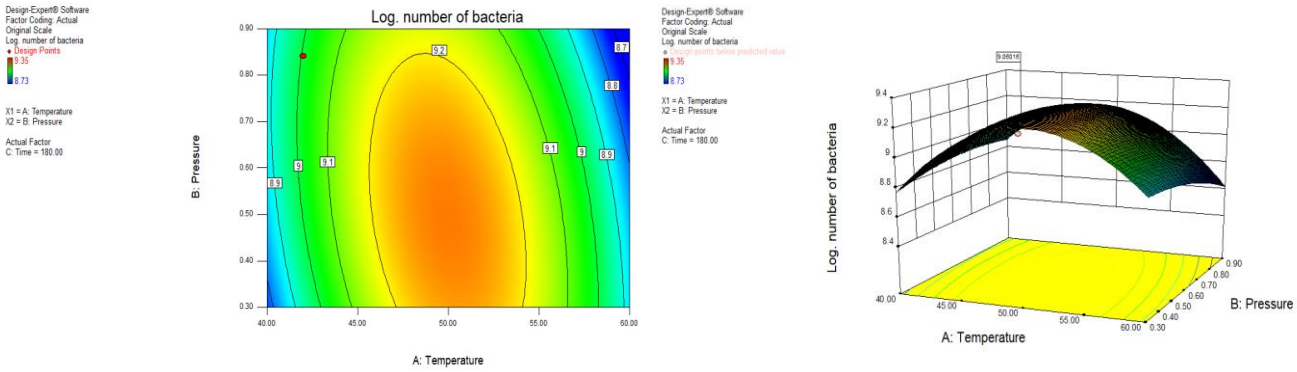

Figure 2. Optimal conditions for encapsulating *Lim. reuteri* using a vacuum oven and the Design of Experiments program.

Table 2 presents some microbial and chemical characteristics of the dry powder of encapsulated bacteria. There was no growth of Coliform group bacteria colonies, indicating that the samples dried in the vacuum oven device remained uncontaminated after drying. The drying was carried out under sterile conditions, and the resulting powder weighed 14 g out of 16.75 g of the mixture powder. The powder yield percentage was 83.58% after drying at a temperature of 50°C and a pressure of 0.6 bar for 180 minutes. The moisture content was 4.26%, and the brick percentage was within the permissible range for dried products, which should not exceed 5%. The lack of moisture in the resulting powder increases the bond strength between whey proteins and Arabic gum via glycosidic and peptide bonds. This, in turn, enhances the resistance of the encapsulated bacterial colonies to temperature and pressure. These results closely align with the findings of previous studies.

Sharifi *et al.* (2021) found that the moisture content and water activity of both microcapsules produced by spray-drying and freeze-drying methods were less than 4.0% when encapsulating *L. plantarum* bacteria using gum Arabic and whey proteins. Gardiner *et al.* (2000) stated that the shelf life of powder-containing therapeutic bacteria *L. salivarius* UCC 118 and *L. paracasei* NFBC 338 improved when the moisture content was reduced by 4% or less.

Table 2. Chemical and microbial characteristics of dry powder of encapsulated *Lim. reuteri.*

Traits	Results
Powder quantity (gm)	14.00±0.09
Productivity percentage (%)	83.58±3.82
Moisture (%)	4.26±0.03
Total coliform bacteria number (CFU/g)	Nil

Figure 3 shows the morphological analysis of the envelope surrounding *Lim. reuteri* bacteria, using a Scanning Electron Microscope (SEM). The diameter of the bacteria reached 68.29 nanometers with an applied electronic voltage of 15 kV and a magnification power of 200 nanometers, as shown in the cross-section in the picture, the bacteria are attached to the wall of the shell, which is composed of gum Arabic and whey, with a network of protein and sugar bonds, which protects bacteria from external conditions. The presence of a small vacuum allows bacteria to produce their metabolic products in the intestines and the media, taking the food it needs to grow and reproduce its colonies, it works by different mechanisms to maintain the balance of intestinal flora. The results were consistent with those of Mina *et al*. (2021), Phùng *et al*. (2021), and Wang & Mutukumira (2022).

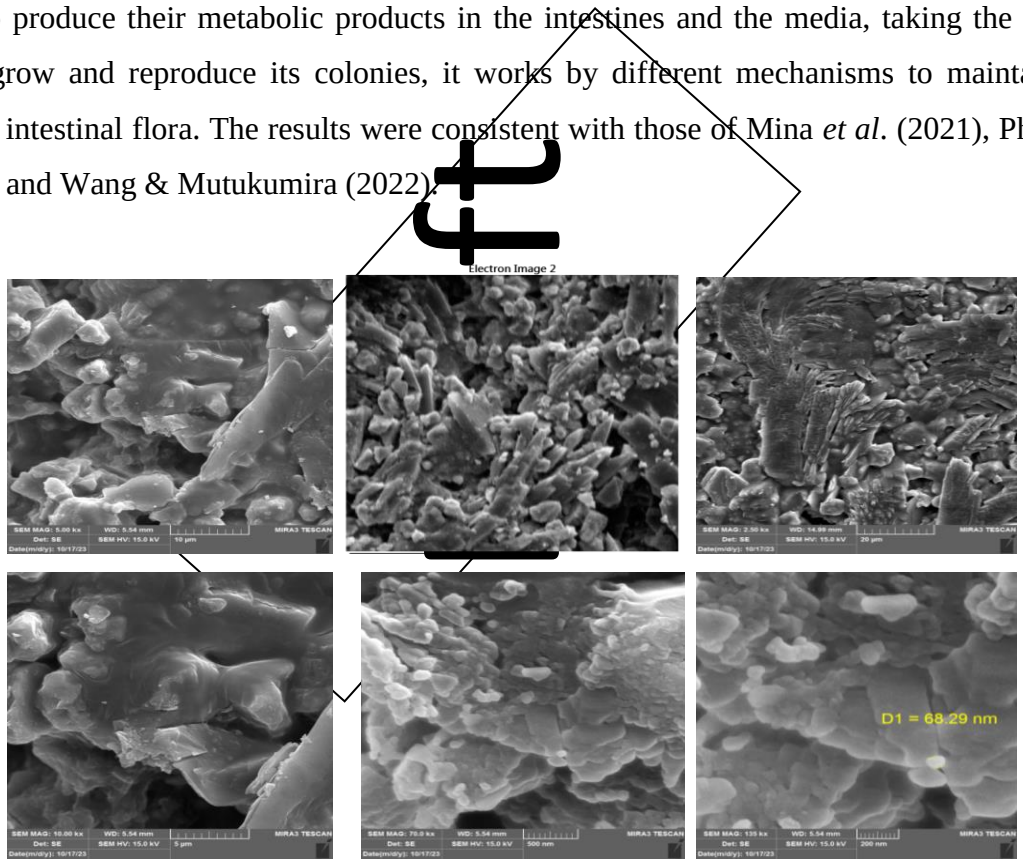

Figure 3. Morphological analysis of encapsulated bacteria using scanning electron microscopy (SEM).

Table (3) presents the logarithm of the number of live bacteria and the percentage of efficiency of the microencapsulation process during the storage period at 4 and 25°C. There was no significant effect on the logarithm of the live numbers of encapsulated bacteria during the storage period at 4 and 25 °C; it reached 9.57 and 9.42 CFU/g, respectively. The percentage of efficiency of the microencapsulation process in the first month was 99.19% and 98.98%, respectively. However, this percentage decreased as the storage period progressed, reaching 96.86% and 95.34% at the end of the storage period (the sixth month) for temperatures of 4 and 25°C, respectively. The process of carefully encapsulating probiotic bacteria, such as *Lim. reuteri* helps preserve the living cells of the bacteria from external influences, such as temperature. This process increases the lifespan of the bacteria and maintains the living cells at the required level.

Table 3. The logarithm of the number of viable bacteria and the efficiency percentage of the microencapsulation process for *Lim. reuteri* bacteria when stored at temperatures of 4°C and 25°C for 6 months.

Storage periods (months	**The logarithm of bacterial counts (CFU/g)**		**Microencapsulation Process Efficiency Percentage (%)**	
	4 °C	25 °C	4 °C	25 °C
1	9.80±0.23	9.78±0.30	99.19±2.55	98.98±3.13
2	9.76±0.11	9.70±0.15	98.78±2.87	98.17±2.30
3	9.70±0.21	9.66±0.09	98.17±2.48	97.77±2.18
4	9.65±0.16	9.54±0.13	97.67±1.99	96.55±1.78
5	9.62±0.19	9.50±0.19	97.36±2.09	96.15±1.95
6	9.57±0.09	9.42±0.25	96.86±1.87	95.34±1.90

CONCLUSION

The process of carefully encapsulating bacteria and active compounds increases their shelf life and protects them from external influences. Carbohydrates and proteins are utilized as packaging materials for therapeutic bacteria, such as *Lim. reuteri* bacteria provide a robust network of synergistic bonds to support the optimal growth potential of bacterial cells after drying. The utilization of a vacuum oven in the microencapsulation process helps to maintain the viability of bacteria cells. This is achieved by employing moderate temperatures under reduced pressure, which reduces drying time. Consequently, encapsulated bacteria have an extended shelf

life. Therefore, vacuum oven drying technology can be applied on an industrial scale to extend the storage duration of therapeutic probiotic cells and active compounds that are susceptible to external conditions.

CONFLICT OF INTEREST

The authors declare no conflicts of interest associated with this manuscript.

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REFERENCES

A.O.A.C. (1975). Official Methods of Analysis (Horwitz) 12th ed, *Association of Official Analytical Chemists Washington D.C., U.S.A.*

Al-Sahlany, S. T., and Niamah, A. K. (2022). Bacterial viability, antioxidant stability, antimutagenicity, and sensory properties of onion types fermentation by using probiotic starter during storage. *Nutrition & Food Science*, 52(6), 901-916.

APHA, (1978). Standard methods for examination of dairy products, 14th ed. Marth, E.H. (ed.) *American Public Health Association. Washington*, D.C.

Arepally, D., and Goswami, T. K. (2019). Effect of inlet air temperature and gum Arabic concentration on encapsulation of probiotics by spray drying. *Lwt*, 99, 583-593.

Eckert C., Serpa V.G., Santos A.C., Costa S.M., Ilalpubel V., Lehn D.N. and Souza C.F.V. (2017). Microencapsulation of *Lactobacillus plantarum* ATCC 8014 through spray drying and using dairy whey as wall materials. *LWT-Food Sci. Technol*., 82: 176-183.

Fathi, F., N. Ebrahimi, S., Matos, L. C., PP Oliveira, M. B., & Alves, R. C. (2022). Emerging drying techniques for food safety and quality: A review. *Comprehensive Reviews in Food Science and Food Safety*, *21*(2), 1125-1160.

Foerst P., Kulozik U., Schmitt M., Bauer S. and Santivarangkna C. (2012). Storage stability of vacuum-dried probiotic bacterium *Lactobacillus paracasei* F19. *food and bioproducts processing*, 90:295-300.

Gardiner, G.E., O'Sullivan E., Kelly J., Auty M.A., Fitzgerald G.F., Collins J.K., Ross R.P. and Stanton C. (2000). Comparative survival rates of human-derived probiotic *Lactobacillus*

paracasei and *L. salivarius* strains during heat treatment and spray drying. *Applied and Environmental Microbiology*, 66(6): 2605-2612.

Harrigan W.F and McCance M.E. (1966). Laboratory Methods in Microbiology. Academic press. London. U.K.

Hongpattarakere T. and Uraipan S. (2015). Bifidogenic characteristic and protective effect of Saba starch on survival of *Lactobacillus plantarum* CIF17AN2 during vacuum-drying and storage. *Carbohydr. Polym*, 117: 255–261.

Jaafar, F. E., Rubaiy, H. H. M. A., & Niamah, A. K. (2024). Effect of Different Air Oven Temperatures on Chemical, Physical, and Microbial Properties of Dried Bio-Yoghurt Product. *Dairy*, *5*(1).

Jakub K. and Radosław D. (2022). Current Trends in the Production of Probiotic Formulations. *Foods*, 11(15):2330. https://doi.org/10.3390/foods11152330

Kajal F., Rajab S., Tabandeh F., Shahraky M.K., Maghsoudi A. and Ashengroph M. (2021). Efficient Spray-Drying of *Lactobacillus rhamnoses* PTCC 1637 Using Total CFU yield as the decision factor. *Food Bioscience*, 40: 100816.

Mina P., Stojanović M., Veličković Z., Kovačević A., Milkovich R., Mirković N. and Marinković A. (2021). Characterization of potential probiotic strain, *Lim. reuteri* B2, and its microencapsulation using alginate-based biopolymers. *International Journal of Biological Macromolecules*, 183: 423-434.

Mohammed, A.A., N.A. Hussain and A.K. Niamah (2020). Antibacterial spectrum of produced Reuterin from new isolates of *Lactobacillus reuteri*. *Journal of Microbiology, Biotechnology and Food Sciences*, 10(1):134-39.

Narathip P., S. Thangrongthong, K. Kasemwong, P. Kerdsup and M. Taweechotipatr(2021). Spray-drying microencapsulation using whey protein isolate and nano-crystalline starch for enhancing the survivability and stability of *Lactobacillus reuteri* TF-7. *Food Sci. Biotechnol*., 30(2):245-256.

Niamah, A. K., Al-Manhel, A. J., & Al-Sahlany, S. T. G. (2018). Effect microencapsulation of *Saccharomyces boulardii* on the viability of yeast in vitro and ice cream. *Carpathian Journal of Food Science & Technology*, 10(3): 100-107.

Niamah, A.K., A.A. Mohammed and N.A. Alhelf (2023). Antibacterial activity and identification of produced Reuterin from local *Lactobacillus reuteri* LBIQ1 isolate. Journal Microbiol Biotech Food Sciences, 12 (5) e4701.

Phùng, D., H. Vu, A. Rodklongtan and P. Chitprasert (2021). Whey protein isolate-lignin complexes as encapsulating agents for enhanced survival during spray drying, storage, and in vitro gastrointestinal passage of *Lactobacillus reuteri* KUB-AC5.*LWT-Food Science and Technology*. 148: 111725.

Raise, A., S. Dupont, C. Iaconelli, C. Caliri, A. Charriau, P. Gervais, O. Chambin and L. Beney (2020). Comparison of two encapsulation processes to protect the commensal gut probiotic bacterium *Faecalibacterium prausnitzii* from the digestive tract. *Journal of Drug Delivery Science and Technology*, 56: 101608.

Sharifi, S., M.R. Bari, M. Alizadeh, H. Almasi and S. Amir (2021). Use of whey protein isolate and Arabic gum for the co-encapsulation of probiotic *Lactobacillus plantarum* and phytosterols by complex coacervation: Enhanced viability of probiotic in Iranian white cheese. *Food Hydrocolloids*. 113: 106496.

Wang, A., & Zhong, Q. (2024). Drying of probiotics to enhance the viability during preparation, storage, food application, and digestion: A review. *Comprehensive Reviews in Food Science and Food Safety*, 23(1), e13287.

Wang, F. And A.N. Mutukumira (2022). Microencapsulation of *Limosilactobacillus reuteri* DPC16 by spray drying using different encapsulation wall materials. *Journal of Food Processing and Preservation*, 46(10): e16880.

Xie, A., Zhao, S., Liu, Z., Yue, X., Shao, J., Li, M., & Li, Z. (2023). Polysaccharides, proteins, and their complex as microencapsulation carriers for delivery of probiotics: A review on carrier types and encapsulation techniques. *International Journal of Biological Macromolecules*, *242*, 124784.