Survival of *Lactobacillus acidophilus* La-5 Encapsulated Along with *Spirulina platensis* and the Application of this Capsule in Sour Cherry Juice as a Probiotic Drink

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ABSTRACT: *This study investigated the effects of the microencapsulation of Lactobacillus acidophilus La-5 along with Spirulina platensis on bacterial survival in sour cherry juice containing synbiotic capsules. S. platensis powder was used as a prebiotic and the microencapsulation of bacteria and S. platensis was conducted using the spray dryer method. S. platensis and bacteria were encapsulated in maltodextrin and cross-linked alginate. Bacterial survival, thermal tolerance, morphology, efficiency, and resistance under simulated gastric and intestinal conditions in sour cherry juice were examined. The results showed a decrease in probiotic bacterial death in sour cherry juice containing bacteria and algae encapsulated at 4°C on the 28th day of storage (7.9 ± 0.10) (Log CFU/mL) as well as an increase in temperature resistance in fruit juice containing bacteria capsules and S. platensis. The results of Scanning Electron Microscopy (SEM) analysis revealed that the capsules contained L. acidophilus La-5 and S. platensis with round shapes and had an average diameter of 12.80 ± 1.43 µm. The examination of bacterial encapsulation efficiency indicated that the highest and lowest values for bacteria encapsulated with Spirulina and bacteria capsules without Spirulina were 81.9% and 79.86%, respectively. In addition, organoleptic analysis of sour cherry juice at 4°C and at the end of storage duration in the refrigerator demonstrated the highest general acceptance (4.60 ± 0.10) for the juice, containing bacteria and Spirulina compared to other bacterial groups (p<0/05). L. acidophilus La-5 capsules containing Spirulina showed the largest viability during the 0-10 min period and 60-80°C temperature (p<0/05). Moreover, bacteria encapsulated with Spirulina exhibited the highest survival rate under simulated gastric and intestinal conditions over a 0-120 min incubation time. Overall, using S. platensis as a prebiotic can significantly stimulate the growth of L. acidophilus La-5 as a beneficial probiotic, which results in the production of healthier and more nutritious sour cherry juice.*

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INTRODUCTION

Nowadays, the majority of consumers are interested in eating healthy foods because of their nutritional values and safety [1-3]. These properties are found in functional foods containing a diverse range of probiotics [4]. Probiotics are microorganisms that can provide many health benefits to the human body [5-7]. Due to the advantages of probiotic foods, including their effects on the treatment of alcohol-related liver diseases and colorectal inflammation, treatment of irritable bowel syndrome, gastrointestinal effects [8, 9], immune response stimulation [10], improving the absorption of Ca, Cu, and P and the digestibility of different ingredients, e.g., fat and proteins, they should be continuously consumed (>100 g/day or 10-20 billion CFU per day) [11, 12].

The microencapsulation of herbal essential oils and extracts has received much attention because of the unique beneficial attributes of these oils to human health [13, 14]. This novel technique can improve the stability of bioactive compounds by protecting them against chemical, enzymatic, environmental, ionic, and thermal changes. In addition, microencapsulation can provide buffering properties and protect against undesirable odors and tastes when herbal supplements are administered [15]. Moreover, microencapsulation is useful for unmixable materials and can be beneficial for controlling the release of fine-grained materials [16].

Microencapsulation is carried out using different methods in the industry, the most important of which are spray drying, freeze drying, spray cooling, and cooling, coacervation, liposome systems, and fluid bed systems. Among the various micro-coating methods in the food industry, the spray drying method is more commonly used because of its low cost, high speed of operation, high reliability, and high flexibility [17-22].

Polysaccharides (e.g. alginate) extracted from different algal species are utilized to microencapsulate probiotic bacteria [23, 24]. Such therapeutic bioactive compounds are widely used for the microencapsulation of probiotic bacteria in order to enhance their viability and efficiency in the digestive system of organisms [25]. The survival rate of probiotics is directly related to alginate concentrations as a substance for microencapsulation [26].

Spirulina platensis is one of the most well-known cyanobacterial species owing to its unique nutritional benefits. Due to its abundance of vitamins, omega-3 and omega-6 fatty acids, minerals, amino acids, and essential fatty acids, *S. platensis* is known as a functional food and dietary supplements in the food industry [27]. The dried biomass of *S. platensis* is composed of 1-1.5% chlorophyll-a, 8-10% fiber, 6-8% lipids, 7-10% ash, 55-60% protein, 3-7% moisture, and 12-20% carbohydrates [28]. *S. platensis* contains a substantial amount of proteins [29] as well as high levels of vitamin E, vitamin B12, cryptoxanthin, and β-carotene [30,31].

The Food and Drug Administration (FDA) has categorized *Spirulina platensis* as a "Generally Recognized as Safe" (GRAS) for human consumption and the Dietary Supplements Information Expert Committee (DSI-EC) concluded that there is no serious risk to health with the consumption of Spirulina [32]. Spirulina has been proposed by the European Space Agency as a dietary supplement during long-duration space missions [33].

Various post-harvest technologies have been developed for the processing of fruits to make value-a ed products such as jams, jelly, and fruit juices. Fruit juices are much preferred for their nutritional benefits [34].

Cerasus vulgaris L. (sour cherry), which contains a considerable amount of anthocyanin, particularly cyanidin 3-glucoside, is one of the most well-liked fruits in the juice industry [35]. Anthocyanin is a polyphenolic compound and an important group of water-soluble pigments in plants. This flavonoid possesses antioxidant effects that protect cells against Reactive Oxygen Species (ROS) and prevent many disorders such as cancer and cardiovascular diseases [36,37]. In addition, this therapeutic compound has antihyperglycemic effects, especially in patients with chronic diabetes [38].

Lactobacillus acidophilus La-5, as a functional microorganism, plays a leading role in the development of a symbiotic relationship with the human host. In this study, sour cherry juice was used, which is a non-fermenting type of functional food that contains ingested live microorganisms. Microalga can play an essential role

in providing vitamins, free amino acids, and other nutritive compounds to better support probiotics and increase the efficiency and probiotic effects of *L. acidophilus* La-5 in juicy foods [39,40,1]. Therefore, in the present study, the effects of the microencapsulation of *L. acidophilus* La-5 along with *S. platensis* on bacterial survival, temperature tolerance, and viability under simulated gastric and intestinal conditions in sour cherry juice, as a functional beverage, were investigated.

EXPERIMENTAL SECTION

S. platensis powder was obtained from Arian Gostar Company (Tehran, Iran), and lyophilized *L. acidophilus* La-5 was purchased from Chr Hansen (Denmark). All the chemicals including sodium chloride (≥ 99.0%), hydrochloric acid (37%), sodium hydroxide (0.1 N), De Man, Rogosa, and Sharpe (MRS) agar, peptone water, MRS broth, low viscosity sodium alginate, maltodextrin (DE = 18), ammonium hydroxide (25%), succinic acid (≥ 99%), dicalcium phosphate dehydrates (98.0-105.0%), sodium citrate (≥ 99%), pepsin (Sigma Aldrich P7000), and pancreatin (P1500) were purchased from Sigma-Aldrich (Merck, Germany). Bile salt was provided by the Oxoid Company (Basingstoke, UK). Fresh sour cherries were purchased from a fruit market in Tehran (Iran) and carefully washed before the removal of kernels, impurities, and seeds. After that, the juice was filtered to remove pulps and then pasteurized for 5 min at 80°C in a hot water bath [41]. Finally, the samples were rapidly cooled and the pasteurization process was completed.

Prepration of L. acidophilus La-5

To prepare the *L. acidophilus* La-5 stock, 10 g of probiotic bacteria was rehydrated in 100 mL of MRS broth at room temperature for 10 min and incubated anaerobically at 37°C for 24 h using an Anaerocult C system (Merck, Germany). Then, 1 mL of saturated culture was mixed with 0.25 mL of sterile 25% (v/v) glycerol in sterile cryogenic vials and stored at -80°C until starting the experiments. On the day of the experiment, each stock culture was thawed at ambient temperature for 30 min and then added to 100 mL of MRS broth.

Following the procedure, 10 mL of the culture was inoculated in 1 L of MRS broth and incubated under anaerobic conditions for 20 h to reach the stationary stage. The obtained biomass was isolated by centrifugation (Hettich Universal, Germany) at room temperature and 2500 *g* (or 16000 rpm) for 30 min and washed twice with sterile peptone water. Afterward, the cell pellets were resuspended in sterile peptone water to obtain a bacterial concentration of 10^{11} CFU/mL [42]. Bacterial counting was conducted by both serial dilutions and colony counting as well as OD measurement and comparison with McFarland's table.

Bacterial microencapsulation

About 5 mL of the bacterial solution prepared in the previous step was collected and after centrifugation at room temperature and 2500 *g* for 30 min, the cell pellet was added to the solution of capsule wall material. Maltodextrin and cross-linked alginate microcapsules were made using a spray dryer (Buchi B290). This process was performed under the conditions reported by *Strobel et al*. [43]: Inlet air temperature was set to 130°C, the aspirator airflow rate was set to maximum (35 m^3/h), the peristaltic pump was set to 45% of maximum, and nozzle airflow was set to 50 mm on the Q-flow indicator. Briefly, a suspension containing 6.6 g of maltodextrin, 1 g of sodium alginate, 0.4 g of $CaHPO_4$, and 2 g of succinic acid (pH 5.6) were mixed with water and utilized for microcapsules containing bacteria without Spirulina, while in another suspension which contains 2.4 g of Spirulina powder, 4.5 g of maltodextrin, and 0.7 g of sodium alginate were added to 0.4 g of $CaHPO_4$ and 2 g of succinic acid (pH 5.6) and mixed with water for the capsules containing Spirulina and bacteria.

Finally, 200 mL of each of these wall material solutions were loaded into the spray dryer device to produce dry microencapsulated probiotic bacteria.

Inoculation of bacteria

For bacteria inoculation, 1 g of dry microencapsulated probiotic bacteria obtained from the microencapsulation stage was added to 240 mL of pasteurized sour cherry juice. Two separate amounts of 0.4 mL and 0.42 mL of the bacterial solution, which was obtained in the last stage of bacterial preparation, were individually centrifuged. The cell pellet from this process was mixed with the fruit juice samples to prepare samples containing free bacteria and samples containing free bacteria along with *Spirulina platensis*.

In general, there were four types of treatment in this article: Sour cherry juice containing free bacteria,

Sour cherry juice containing free bacteria and *Spirulina platensis*, Sour cherry juice containing encapsulated bacteria, and sour cherry juice containing encapsulated bacteria along with *Spirulina platensis*. After adding bacteria to the juice samples, the samples were kept at 4°C for 28 days and their microbial count was performed at 7-day intervals in three replicates.

Sour cherry juice characterization

Sour cherry juice characterization was conducted using a five-point hedonic scale ranging from a score of 1 (very unpleasant) to 5 (very pleasant), and 25 human subjects were asked to rate the general acceptance, texture, color, odor, and taste at the end of day 28 [44]. The color was characterized by the Gretag Macbeth CE7000A colorimeter, using spherical geometry and transfer method at the end of day 28. The color attributes of the samples were assessed with a 10-degree standard observer under the D65 optical standard. Samples were transferred into the spectrometer and tested for the type of color. The basis of colorimetry in this system is the measurement of L* (darkness-brightness), a* (green-red), and b* (blue-yellow) indices [45].

Particulate capsule morphology

The Scanning Electron Microscopy (SEM) technique (TESCAN brand and MIRA LMU model) was used to determine the morphology and appearance of the capsules. The capsules were fixed on the coater (SC7620, England) with double-sided adhesive and covered with gold and palladium for 2 min. Finally, capsules were observed by electron microscopy and analyzed using electron radiation of 10 kV [46].

Count of probiotic Bacteria

About 1 g of the bioencapsulated samples was added to 9 mL of 2% w/v sterile sodium citrate (pH 6). The prepared sample was then homogenized by a stomacher (Seward Laboratory, London, UK) at 260 rpm for 5 min at room temperature to fully open the capsules. Then, the extracted bacteria were added to an MRS agar medium and incubated for 72 h at 37°C under anaerobic conditions. The number of bacteria was then counted in three replications [47].

Encapsulation Efficiency (EE)

EE was calculated using the following equation:

$$EE\ (\%) = (X_t / X_i) \times 100$$

where X_t is the total amount of probiotics loaded in the formulated capsules and X_i is the initial amount of probiotics added in the preparation process [48].

Bacterial survival under simulated gastric and intestinal conditions

To investigate the survival rate of microencapsulated bacteria, gastric and intestinal conditions were simulated. For gastric conditions, pepsin was mixed with a 0.5% solution of NaCl to reach a concentration of 3 g/L, and sterile HCl was used to reach a pH of 1.5 [49].

Then, 1 mL of juice samples containing encapsulated bacteria along with Spirulina and 1 mL of juice samples containing free bacteria were poured separately into 9 mL of simulated gastric juice and incubated in a shaker incubator at 37°C for 2 h. Bacterial survival was evaluated at intervals of 30 min (0, 30, 60, 90, and 120 min) during the incubation period in gastric juice [44,50].

To simulate intestinal conditions, pancreatin was mixed with 0.5% NaCl until reaching a final concentration of 1 g/L with a 4.5% bile salt solution [49]. Then, the pH was adjusted to 8 by adding 0.1 M sterile NaOH. Both solutions were filtered using 0.22-µm filters. To evaluate the viability of the bacteria in the intestine, the prepared samples were kept in the simulated gastric juice vial for the requisite time before being transferred to the vial containing the simulated intestinal extract for an incubation period of 2 h at 37°C. The survival rate was determined at 30-min intervals in a duration of 0, 30, 60, 90, and 120 min in intestinal juice [44,50].

Evaluation of bacterial viability in response to heat

The survival rate of both encapsulated and free bacteria in response to thermal variations was investigated using a water bath at 60°C, 70°C, and 80°C for 0, 1, 2.5, 5, and 10 min. In addition, D-value was calculated by plotting the number of remaining bacteria at different heating times [44].

Statistical analysis

Statistical analysis of the obtained data was performed using SPSS software. A one-way analysis of variance was used to compare the means. Duncan's test was also applied to compare the means in cases where the overall effect of the treatments was found to be significant. It should be

Table 1: Changes in the number of L. acidophilus La-5 (Log CFU/mL) in sour cherry juice during storage at 4°C.

Treatment				
Days	Encapsulated bacteria	Free bacteria	Encapsulated bacteria + Spirulina	Spirulina + free bacteria
1	8.24 ± 0.08^{Aa}	8.22 ± 0.16^{Aa}	8.22 ± 0.12^{Aa}	8.24 ± 0.06^{Aa}
7	8.12 ± 0.00^{Ba}	8.18 ± 0.10^{Ba}	8.33 ± 0.06^{Aa}	8.16 ± 0.10^{Ba}
14	8.07 ± 0.24^{Ba}	7.16 ± 0.10^{Cb}	8.25 ± 0.05^{Aa}	7.22 ± 0.06^{Cb}
21	7.88 ± 0.17^{Bb}	6.11 ± 0.09^{Dc}	8.12 ± 0.04^{Aa}	6.42 ± 0.09^{Cc}
28	7.59 ± 0.10^{Bb}	5.89 ± 0.13^{Dd}	7.90 ± 0.10^{Ab}	6.22 ± 0.03^{Cc}

The numbers in the table are the average of three replicates ± standard deviation. Different uppercase letters in each row and different lowercase letters in each column indicate a significant difference in the probability of P < 0.05.

noted that all analysis stages were conducted at a significance level of 0.05. The General Line Model (GLM) test was employed to compare the mean responses on different days to evaluate changes in chemical properties and viability of probiotics.

RESULTS AND DISCUSSION

A change in the number of *L. Acidophilus* La-5 was observed in sour cherry juice during storage at 4°C. It was determined that the number of *Lactobacillus acidophilus* was 8.22 ± 0.16 (Log CFU/mL) on the first day of incubation in the group of free bacteria and reduced to 5.89 ± 0.13 (Log CFU/mL) on day 28. Compared to the other treatments, the highest reduction in the number of bacteria was shown in this sample. In addition, in this group, a statistically significant difference was observed between the number of bacteria on different days from day 1 to 28 ($P < 0.05$). Spirulina-encapsulated *L. acidophilus* showed the least change in a 28-day period and no significant changes were observed between the studied days ($P > 0.05$). Moreover, in the group of encapsulated bacteria, no significant difference was observed on the first day of incubation with days 7 and 14, but days 1, 7, and 14 were significantly different from days 21 and 28 ($P < 0.05$). These findings were in line with the results reported by *Akalin et al*. [51], *Guldas* and *Irkin* [52], and *Beheshtipour et al*. [53]. Vitamins, free amino acids, and other nutritive compounds in *S. platensis* minimized the reduction in the number of bacteria [54]. Moreover, these microalgae exert positive effects on bacterial growth due to their anti-pathogenic characteristics [55] and prebiotic capabilities [56]. Such results were in line with our findings, which confirmed that the highest number of bacteria was observed in the group containing bacteria encapsulated with *S. platensis.* Changes in the number of *L. Acidophilus* La-5 (Log CFU/mL) in sour cherry juice during storage at 4°C are reported in Table 1.

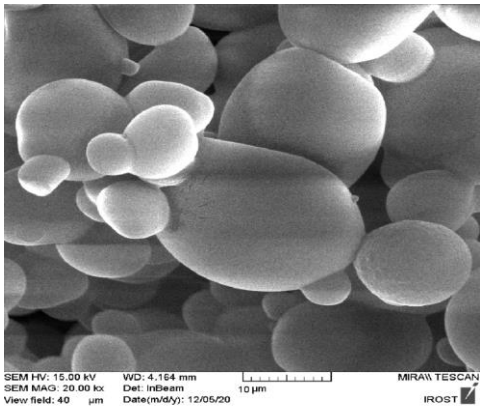

Fig. 1: The morphology (SEM image) of capsules containing Lactobacillus acidophilus La-5 and S. platensis.

The SEM analysis of encapsulated bacteria and *S. platensis* is presented in Fig. 1. The spherical microcapsules shown by SEM cause a softer texture in food products with a mean diameter of 12.80 ± 1.43 µm in comparison to capsules with a diameter in mm [57]. The results are in line with the findings of *Hansen et al*. [58] who reported that capsules with a diameter higher than 1 mm caused the rough texture of food products.

The EE results are presented in Table 2. The results showed efficiency percentages of 81.9% and 79.86% for bacteria encapsulated with and without *S. platensis*, respectively.

General acceptance, texture, color, odor, and taste of sour cherry juice in different studied groups are displayed in Table 3. The results of organoleptic analysis have shown higher values for general acceptance of juice containing

Table 2: Encapsulation efficiency of Lactobacillus acidophilus La-5 in the spray drying process.

Encapsulation	Input bacteria	Output bacteria	Efficiency (%)
Bacteria	$(2.335 \pm 0.15) \times 10^9$ CFU/mL × 200 mL	$(4.20 \pm 0.29) \times 10^{10}$ CFU/g × 8.88 g	79.86±5.62
(Bacteria + Spirulina)	$(2.23 \pm 0.14) \times 10^9$ CFU/mL × 200 mL	$(4.05 \pm 0.46) \times 10^{10}$ CFU/g × 9.02 g	81.9±7.40

Table 3: Organoleptic evaluation of sour cherry juice containing Lactobacillus acidophilus La-5.

Treatments	Organoleptic attributes				
	General acceptance	Texture	Color	Odor	Taste
Encapsulated bacteria + *S. platensis*	4.60 ± 0.10^a	4.45 ± 0.14^a	5.00 ± 0.00^a	5.00 ± 0.00^a	4.50 ± 0.12^a
Encapsulated bacteria	4.50 ± 0.11^a	4.40 ± 0.15^a	5.00 ± 0.00^a	5.00 ± 0.00^a	4.55 ± 0.14^a
Free bacteria + *S.platensis*	3.40 ± 0.17^b	4.50 ± 0.11^a	2.90 ± 0.12^c	2.90 ± 0.14^c	3.10 ± 0.13^c
Free bacteria	3.60 ± 0.15^b	4.50 ± 0.12^a	3.45 ± 0.11^b	3.40 ± 0.11^b	3.55 ± 0.12^b
Control	4.80 ± 0.20^a	4.70 ± 0.20^a	5.00 ± 0.00^a	5.00 ± 0.00^a	4.70 ± 0.25^a

Different lowercase letters in each column indicate a significant difference in the probability of $P < 0.05$.

Table 4: Comparison of the colorimetric attributes of sour cherry juice containing Lactobacillus acidophilus La-5 and S. platensis capsules with control+ and control at the end of day 28.

Treatments	b*	a*	L*
Control+	26.02 ± 0.08^a	39.13 ± 0.42^c	12.03 ± 0.00^c
Optimal	21.24 ± 0.17^b	40.20 ± 0.24^b	13.17 ± 0.19^b
Control	20.63 ± 0.35^b	41.81 ± 0.14^a	16.24 ± 0.10^a

Different lowercase letters in each column indicate a significant difference in the probability of $P < 0.05$.
Control $^+$: Sour cherry juice containing S. platensis + bacteria without capsule
Control: Sour cherry juice without additives
Optimal: Sour cherry juice containing encapsulated bacteria along with S. platensis

encapsulated bacteria and *S. platensis* than other treated groups, including free bacteria with *S. platensis* and free bacteria without any additives (Table 3). It was proven that the addition of free bacteria with or without *S. platensis* could deteriorate the taste, odor, and color of the final product.

Colorimetric characteristics of enriched sour cherry juice containing encapsulated bacteria and *S. platensis* are presented in Table 4. Comparison of the brightness components of the samples revealed that the positive control sample, which contained free bacteria with *S. platensis*, had the lowest brightness compared to the improved and control sample, while the control group exhibited the highest brightness.

The results of changes in the color components a* and b* showed the lowest amount of yellow in the control and the highest amount of yellow in the positive control sample in which bacteria and Spirulina were not encapsulated. In addition, the positive control sample portrayed less redness than the control sample and the optimal sample. In the positive control sample, the color of the sour cherry juice changed more than in the optimal sample because of the existence of free microalgae and bacteria. In general, the results obtained from the evaluation of the color of the product in the organoleptic test confirmed the results of the colorimetric test.

The effect of temperature variations on the viability and tolerance of bacteria was investigated in capsules containing bacteria and *S. platensis* and free bacteria (Table 5). The results showed that capsules containing bacteria and *S. platensis* had the highest survival rate compared to free bacteria. Thus, the temperature tolerance of bacteria at 80°C for 5 min was higher in the encapsulated bacteria and *S. platensis* compared to free bacteria (Table 5). The use of bacteria encapsulated

Table 5: The effect of heat on the survival of Lactobacillus acidophilus La-5 (Log CFU/mL).

Form	Temp (°C)	Time (min)					D-value
		0	1	2.5	5	10	
Encapsulated bacteria and Spirulina	60	8.22 ± 0.00^{Aa}	8.00 ± 0.07^{Ba}	7.65 ± 0.08^{Ca}	7.00 ± 0.06^{Da}	5.82 ± 0.09^{Ea}	4.1
	70	8.22 ± 0.00^{Aa}	7.91 ± 0.05^{Ba}	7.50 ± 0.06^{Ca}	6.78 ± 0.04^{Db}	5.34 ± 0.05^{Eb}	3.5
	80	8.22 ± 0.00^{Aa}	7.24 ± 0.09^{Bb}	5.75 ± 0.11^{Cb}	3.20 ± 0.10^{Dc}	-	1
Free bacteria	60	8.22 ± 0.00^{Aa}	7.90 ± 0.07^{Ba}	7.45 ± 0.07^{Ca}	6.70 ± 0.11^{Da}	5.00 ± 0.06^{Ea}	3.1
	70	8.22 ± 0.00^{Aa}	7.30 ± 0.05^{Bb}	5.95 ± 0.07^{Cb}	3.65 ± 0.07^{Db}	-	1.1
	80	8.22 ± 0.00^{Aa}	4.85 ± 0.13^{Bc}	-	-	-	0.3

The numbers in the table are the average of three replicates ± standard deviation. Different uppercase letters in each row and different lowercase letters in each column indicate a significant difference in the probability of $P < 0.05$.

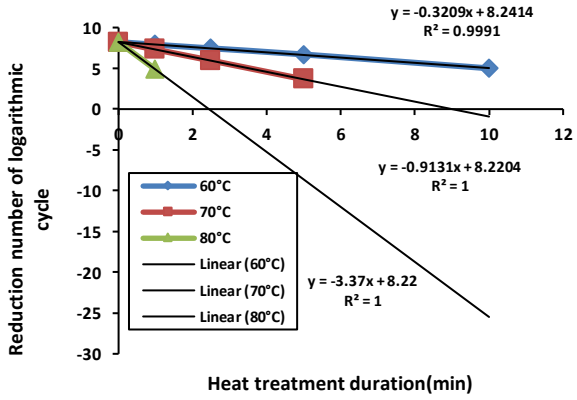

Fig. 2: The effect of the heating process at three temperatures of 60°C, 70°C, and 80°C on the survival of Lactobacillus acidophilus La-5 in free forms.

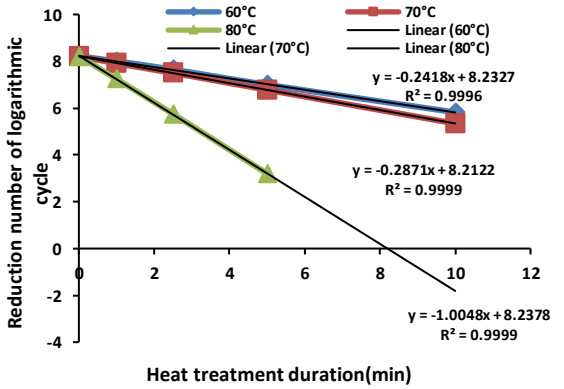

Fig. 3. The effect of the heating process at three temperatures of 60°C, 70°C, and 80°C on the survival of Lactobacillus acidophilus La-5 in the capsule form.

with *S. platensis* increased thermal resistance, due to the formation of a protective coating layer caused by encapsulation and the provision of nutrient-rich media high in antioxidants and vitamins by *S. platensis*. Accordingly, in 2019, *Praepanitchai et al.* [59] reported the effect of temperature changes on the stability of *L. casei* at different temperatures of 55-60°C and 65°C for 20 min in the form of free bacteria and bacteria encapsulated with alginate at different concentrations. They outlined that encapsulation significantly increased the probiotic resistance of *L. casei* to temperature changes.

Figs. 2 and 3 show the effect of the heating process at three temperatures of 60°C, 70°C, and 80°C on the survival of *Lactobacillus acidophilus* La-5 in free and capsule forms. Using Figs. 2 and 3, the slope in each graph was converted to a negative number, which was then inverted to obtain the D-value. The D-value for the encapsulated bacteria was more than the free-form bacteria, which demonstrated that in the encapsulated samples, the time it took for the number of microorganisms to decrease by one logarithmic cycle was longer than the free-form, which was due to the effect of the capsule in protecting bacteria.

The survival of *Lactobacillus acidophilus* La-5 (Log CFU/mL) under the simulated stomach and intestinal conditions is shown in Table 6. The capsules containing Spirulina and bacteria exhibited a high survival rate under gastrointestinal conditions. Overall, prebiotic ingredients provide nitrogen and carbon sources for probiotics [60]. In this research, a decrease was observed in the number of bacteria during the experiment, which can be due to the acidic and alkaline conditions of the stomach and intestine,

Table 6: Survival of Lactobacillus acidophilus La-5 in different treatments under simulated gastric and intestinal conditions during 2 h (Log CFU/mL).

Bacterial form	Time (min) in gastric juice				
	0	30	60	90	120
Encapsulated bacteria + *S. platensis* after 28 days	7. 90 ± 0.07^{Ab}	7.63 ± 0.14^{Aa}	7.36 ± 0.05^{Ab}	6.98 ± 0.09^{Bb}	6.60 ± 0.05^{Cb}
Encapsulated bacteria + *S. platensis* on the first day	8.22 ± 0.08^{Aa}	7.94 ± 0.06^{Aa}	7.76 ± 0.09^{Aa}	7.48 ± 0.08^{Aa}	7.04 ± 0.05^{Ba}
Free on the first day	8.22 ± 0.04^{Aa}	7.75 ± 0.09^{Ba}	6.33 ± 0.07^{Cc}	5.95 ± 0.07^{Dc}	5.02 ± 0.13^{Ec}
Bacterial form	Time (min) in the intestinal juice				
	0	30	60	90	120
Encapsulated bacteria + S. *platensis* after 28 days	6.60 ± 0.05^{Ab}	6.22 ± 0.08^{Bb}	6.10 ± 0.10^{Bb}	5.80 ± 0.11^{Cb}	5.50 ± 0.05^{Db}
Encapsulated bacteria + *S. platensis* on the first day	7.04 ± 0.05^{Aa}	6.79 ± 0.06^{Aa}	6.62 ± 0.05^{Aa}	6.36 ± 0.08^{Ba}	6.14 ± 0.12^{Ba}
Free on the first day	5.02 ± 0.13^{Ac}	4.70 ± 0.11^{Bc}	3.85 ± 0.7^{Cc}	3.49 ± 0.5^{Dc}	3.22 ± 0.7^{Ec}

The numbers in the table are the average of three replicates ± standard deviation. Different uppercase letters in each row and different lowercase letters in each column indicate a significant difference in the probability of P<0.05.

respectively. Moreover, a significant increase in the viability of encapsulated bacteria and *S. platensis* was observed under simulated gastric and intestinal conditions, which was in line with the results of previous studies by *Fritzen-Freire et al.* [61], *Chávarri et al.* [62], *Darjani et al.* [63], and *Foroutan et al.* [64]. According to the results, fresh capsules containing bacteria and Spirulina show the highest resistance to gastrointestinal conditions, while bacteria in free form have the lowest survival.

CONCLUSIONS

This study investigated the effects of *S. platensis* on the viability and survival of capsulated *L. acidophilus* La-5 in cross-linked maltodextrin and alginate used in sour cherry juice. Using live microorganisms as probiotics plays a key role in producing healthy foods and nutritional beverages. Beneficial compounds in aquatic microalgae can provide a wide range of essential nutritive ingredients with therapeutic benefits. Using such substances in the encapsulation of probiotic microorganisms during the production of functional beverages can affect the efficiency of coexisting bacteria in the digestive system. The results of this study showed that the encapsulation of probiotics and the presence of *S. platensis* as a prebiotic have a significant effect on the survival of probiotics in sour cherry juice during 28 days of storage at 4°C. Capsules containing bacteria and Spirulina increased the thermal resistance of probiotics to different temperatures. In addition, the coexistence of encapsulated bacteria and *S. platensis* increased the resistance of probiotics to the simulated intestinal and gastric juice. In conclusion, the use of *S. platensis* as a prebiotic can increase the growth and survival of probiotic bacteria *L. acidophilus* La-5, which is significantly nutritious and beneficial in the production of a functional beverage.

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