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Improving the survival of lactic acid bacteria in Tarhana soup as a non-dairy matrix: Improving the survival of probiotics

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Abstract

The objective of this study was to improve the survival of lactic acid bacteria (LAB) in Tarhana soup as a non-dairy matrix. *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophiles* were encapsulated in electrospun nanofiber mats fabricated from corn starch (CS) and sodium alginate (SA) and the protective effect of the nanofibers were investigated on the cells during the preparation of Tarhana and in the gastrointestinal tract. The moisture content of the control and nanofiber- loaded dried Tarhana samples was 8.75 and 8.71%, respectively; therefore, using nanofiber mats in the formulation had no significant effect on the moisture content of the samples. A negative zeta potential value of -15.1 mV was found for LAB- loaded nanofibers. The nanofibers mats prepared from SA and CS mix showed a bead- free and clean structure with uniformity in size. The diameter size of most of the fibers ranged from 175- 338 with an average of 265 nm. Loading nanofiber mats with *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* cells led to a uniform distributed beaded structure and the average diameter enhanced to approximately 763 nm. The viability of *L. delbrueckii* and *S. thermophilus* at the end of the electrospinning process was 92.82% and 95.83%, respectively, which indicating a slight loss in their population. Survival of nanoencapsulated *S. thermophilus* and *L. delbrueckii* was 93.50% and 89.16% respectively, while for free cells it was 85.3 and 76.4% that showed considerable protective effect of CS/SA fibers on the cells against dehydration of Tarhana medium. Nanofiber mats improved the stability of the cells against ordinary heat treatment used in preparing Tarhana soup. The survival rate of *S. thermophilus* was higher than *L. delbrueckii* subsp. *bulgaricus* and a significant difference was observed between the viability of free and nanoencapsulated bacteria. The survival of CS/SA nanoencapsulated *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* was 83.25% and 80.21%, respectively, which is indicative of the significant protective effect of fibers on the cells against the heating process. The nanofibers also provided good stability for the cells in the gastrointestinal tract as 10^6 to 10^7 $CFUg^{-1}$ of the cells were survived which is within the recommended level of potential probiotic dose to be effective. There was no significant difference in the color of all samples. Nanoencapsulation in CS/ SA nanofiber mats improved the protection of both LAB strains in simulated fluids of the stomach and intestine (Table 4). After continuous exposure to simulated gastrointestinal fluid, a significant loss of viable free LAB cells (higher than 4 log CFU/ml) was found while the population of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* encapsulated in CS/ SA nanofibers decreased only 0.45 and 0.37 log CFU after 120 min ($p> 0.01$), 0.93 and 0.80 log CFU after 180 min ($p< 0.01$), respectively. Tarhana soup prepared with probiotic– loaded nanofibers gained higher scores in terms of consistency, mouth feel, odor, taste, flavor, and overall acceptability attributes. Tarhana soup with nanofibers possessed much sour taste and flavor than samples prepared with free cells of probiotics. The results of the present study indicated that the protection obtained from CS/ SA capsules secured

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around10^6 to 10^7 CFU/g of the probiotic cells which are within the recommended level of probiotic dose to be functional in consumers' body. Therefore, this product can be used by the consumers like vegetarians and lactose or milk peptide intolerants who do not consume dairy products but need potential fermented probiotic food.

Keywords: Biopolymer, Electrospinning, Encapsulation, Lactic acid bacteria, Tarhana.

Introduction

In recent years, consumers' awareness and interest in the important effect of diet composition and quality on health and well-being has significantly enhanced and resulted in increasing the demands for health- promoting and functional probiotic foods to provide nutrients and modulate one or more targeted physiological function of the body (Nyanzi, Jooste, & Buys, 2021). These types of foods are termed "functional foods" and are considered whole, enriched, or fortified foods that provide health benefits besides providing essential nutrients (like minerals and vitamins) when consumed at efficacious amounts as part of a regular diet (Sridharan & Das, 2019). Among the different types of functional foods, maximum attention has been paid to probiotic foods both as therapeutic supplements and health- promoting foods. Probiotic microorganisms ferment sugars and produce lactic and other organic acids. Furthermore, the enzymatic activity of probiotics changes the nature of food components in a way that exerts beneficial effects on the gastrointestinal tract.

Probiotics are "live microorganisms confer health benefits on the host when adequate level is digested" (Hotel & Cordoba, 2001); therefore, these microorganisms should reach the gastrointestinal tract alive and also in sufficiently high number to provide the health benefit. Prebiotic compounds are a category of complex carbohydrates that have a synergistic effect on probiotics and enhance their growth when administered together. Most of the prebiotics are originated from plant sources, like wheat, onion, garlic, etc. that metabolized by specific members of gut microbiota. So, prebiotics in plant foods can be used as suitable substrates for probiotic microorganisms in producing healthy functional foods (Sridharan & Das, 2019). Zendeboodi et al. (2020) proposed three main groups of probiotic as 'true probiotic' referring to active viable probiotic cells, 'pseudo- probiotic' referring to inactive viable cells, and 'ghost probiotic' referring to nonviable/ dead cells, in the forms of ruptured or intact. Each of these groups are divided into two subgroups according to their site of action/impact: external *(in vitro*) or internal (*in vivo*) (Zendeboodi, Khorshidian, Mortazavian, & da Cruz, 2020). The consumption of probiotic products is associated with multiple health benefits. Grom et al. (2020) evaluated the effect of some probiotic-enriched dairy matrices containing *L. casei* on *in vitro* and *in vivo* anti-hyperglycemic potential. They demonstrated that the type of food matrix has a considerable effect on health-promoting activity (Grom et al., 2020). Probiotic food products containing plant components as substrates for probiotics are good vehicles to deliver these organisms to the gut system. In addition, fermentation of plant ingredients by probiotic bacteria provides easy digestion of food, creates desired taste, degrades anti-nutritional compounds and flatulence- causing oligosaccharides, enhances protein digestibility in tannin- rich cereals, and improves the bio-availability of minerals (Karovičová & Kohajdova, 2007).

Most of the probiotic foods available in the market are fermented milk- originated products but some consumers prefer fermented non-dairy products due to trace cholesterol content or preferring plant- originated foods. Therefore, researchers and food industry are exploring probiotic plant foods to develop industrial-scale production of these types of products.

Tarhana, a traditional fermented cereal-based food popular in the West of Iran (province of Lorestan), is prepared with a

mixting of several types of cereal flours, vegetables, herbs, spices, and yogurt. Originally, it was produced by the Turkish in Middle Asia and then it extended to different parts of the world. The cereals flour and yogurt are mixed with the ratio of 2: 1, kneaded with dried vegetables and several spices, fermented with yogurt starter culture and baker's yeast (mainly *Saccharomyces cerevisiae*) (Demirci, Palabiyik, Ozalp, & Tirpanci Sivri, 2019). The Tarhana soup is prepared from dry or wet Tarhana and has a sour taste. Tarhana has high nutritional value due to being a good source of amino acids (of cereals and yogurt), minerals, B- group vitamins, and fibers. It is also considered functional food as it contains prebiotic and probiotic microorganisms. Tarhana dough which is prepared from yogurt, wheat flour, and barley whole meal found to be a rich source of lactic acid bacteria (LAB) as probiotics and β- glucan as prebiotic (Demirci et al., 2019; Ozdemir, Gocmen, & Yildirim Kumral, 2007). Some studies proposed that yogurt microorganisms (*S. thermophiles* and *L. delbrueckii)* could now be considered probiotics as they confer health benefits to the host (Akbar et al., 2018; Guarner et al., 2005; Mater et al., 2005). During preparation, storage, and digestion of Tarhana, probiotic microorganisms may lose their viability and functionality as the result of exposure to heat and high acidity. As some consumers (*e.g.* vegetarians and lactose or milk peptide intolerants) do not use dairy products but demand probiotic food, Tarhana containing a starter culture of yogurt could be a valuable alternative for them. There are several techniques to protect probiotics against harsh conditions of food processing and digestive tract like microencapsulation and nanoencapsulation (Akbar et al., 2018; López-Rubio, Sanchez, Wilkanowicz, Sanz, & Lagaron, 2012; Zupančič, Škrlec, Kocbek, Kristl, & Berlec, 2019). For example, a spraying method was used to microencapsulate and improve the stability and survival rate of a newly isolated probiotic *Lactococcus lactis* KUMS- T18 strain (originated from traditional Tarhana) during storage at 4°C and 25°C for four months. In their study, Tarhana as a microencapsulation matrix improved the quality and sensory properties of probiotic *L. lactis* enriched potato chips (Kiani et al., 2021). Also, in a recent work by Atraki and Azizkhani (2021), the electrospinning technique was used to nanoencapsulate some strains of lactic acid bacteria and bifidobacteria and improves their survival and viability in simulated condition of gastrointestinal tract (Atraki & Azizkhani, 2021). There is no data about applying electrospinning as a nanoencapsulation method to enhance the stability of yogurt culture bacteria (as potential probiotics) in a non- dairy food matrix (like Tarhana) and also in gastrointestinal tract. Therefore, in the present study, the effect of Nano encapsulation on the survival and viability of *S. thermophiles* and *L. delbrueckii* as Tarhana's potential probiotics during production, storage, and digestion was investigated.

Materials and methods

All culture media used in this work were obtained from Merck (Germany). The chemicals and reagents were purchased from Sigma- Aldrich and Merck (Germany). Wheat flour, onions, tomato, spices (turmeric, red pepper), vegetables (tarragon, mint, and oregano), salt, and baker's yeast used in Tarhana preparation were obtained from retail shops in Sari, Iran. Corn starch and sodium alginate were obtained from Anmol Chemicals Co. (India). Pepsin, trypsin, and bovine bile salt were purchased from Sigma- Aldrich (St. Louis, USA).

Bacterial strains

Commercial starter culture of lactic acid bacteria (LAB) for yogurt preparation, containing *L. delbrueckii* subsp. *bulgaricus* (DSM 24734) and *S. thermophiles* (DSM 24731), was purchased from Danisco/ DuPont (Denmark). The bacteria were cultured in de Man Rogosa and Sharpe (MRS) broth and incubated at 37± 1°C for 18 h under anaerobic conditions (10% carbon dioxide, 10%

hydrogen, and 80% nitrogen). The pellets were then precipitated by centrifugation (6000× g, 10 minutes), rinsed with sterile deionized water, diluted to one- hundredth (in their own broth), and stored at 30± 1°C till used for nanoencapsulation.

Nano encapsulation of the strains

Nano encapsulation of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* was performed by applying the electrospinning technique. At the first step, to prepare a coating solution, the optimum concentrations of corn starch (CS) and sodium alginate (SA) were obtained through evaluating the electrospinning capacity and mechanical properties to achieve desired nanofiber mats. The best spin ability rate was obtained at a concentration of 10% (w v^{-1}) CS (in pure deionized water at 100°C for 120 min) and 5% (w v^{-1}) SA (in pure deionized water). The spinning- dope solutions of CS/ SA to fabricate control (without LAB) nanofiber mats were produced via mixing CS and SA in the ratio of 5:1 (v v^{-1}), respectively. To prepare CS/SA/LAB spinning-dope solutions CSP and fabrication of nanofiber mats loaded with LAB, CS, SA, and CSP (10 log CFU ml^{-1}) were mixed in the ratio of 5:1:2 (v: v: v), respectively, and mixed further for 30 min to obtain homogeneous solutions. Then, each solution was transferred into the injectors (10 ml) attached to needles (Zupančič et al., 2019).

A laboratory- scale electrospinner (Vira System, Tehran, Iran) was used to fabricate nanofibers and the process was optimized to obtain desired nano- scale products: different voltage values (10 to 32 kV), flow rates (0.2 to 2.0 ml h^{-1}), distances between Taylor cone and the flat collector (5 to 15 cm), and also the combinations of these parameters were tested. The optimization results showed that the best condition would be as follows: the voltage of 24 kV, the flow rate of electrospinning dope solutions at 1.5 ml h^{-1}, the current on the needle to collect the fabricated nanofibers on the aluminum plate at 10 μA, and the distance between the needle and the collector was adjusted at 12 cm. The electrospinning process was conducted at 25± 1°C and the solutions were completely volatilized during the electrospinning. The electrospun nanofiber mats were freeze- dried after collecting from the collector to remove the remained water (A. Yilmaz et al., 2016).

Preparing Tarhana samples

Tarhana samples were prepared in two groups. Samples contained free LAB cells and samples which were produced with nanoencapsulated LABs. Tarhana samples were produced according to the traditional method in Lorestan province (Iran) with a slight modification as follows: wheat flour (1000 g), barely meal (300 g), tomato (150 g), onion (150 g), paprika powder (150 g), vegetables (thyme, mint, parsley, dill, native Tarhana herb, totally 200 g), salt (40 g), baker's yeast (10 g), and 0.25 g 10 L^{-1} of the free cells of the commercial starter (Elizaquível et al., 2011) or nanofiber mats loaded with the commercial starter. To prepare Tarhana dough, onion, tomato, vegetables, and paprika powder were completely smashed, mixed, and sieved (pore diameter: 1.5 mm). The mixture was pasteurized at 65± 1°C for 30 min and cooled to the ambient temperature. Then, free or nanencapsulated starter culture, yeast, wheat flour, and barley meal were added and this mixture was kneaded to obtain a homogenous dough. The dough was subsequently fermented at 30± 1°C for 7 days. After the fermentation step, the obtained Tarhana was dried at room temperature (Demirci et al., 2019).

Zeta potential of nanofiber mats loaded with LAB

The stability of a colloidal dispersion and electrophoretic mobility of the particles are determined by the zeta potential value. A Zetasizer Nano ZS (ZEN 3600, Malvern Instruments, Worcestershire, UK) was applied to determine the zeta potential of the nanofiber mats. The samples were prepared by dispersion of 1 mg of nanofiber mats in 5 ml of PBS and run 20 times at 25± 1°C.

Scanning electron microscopy (SEM)

One layer of two-sided tape was fixed to the sample stub of the scanning electron microscope and the freshly prepared nanofiber mat samples were sprayed onto one side of the tape following by gold spraying. The samples were observed on a high-resolution and low-vacuum scanning electron microscope (MIRA3 FEG-SEM, Tescan Co., Czech).

Survival of LAB during the electrospinning process

Since the viability and survival of LAB may be affected by the electrospinning process, the population of these microorganisms was determined at the end of nanoencapsulation in CS/SA nanofiber mats. The plate counting method was used for this purpose as follows: briefly, the LAB loaded-nanofiber mats were mixed with phosphate buffer saline (PBS) at the ratio of 1:1 and incubated for 1 h at 25± 1°C. Then, 10-fold serial dilutions were prepared and cultured on MRS agar. Free LAB strains were also cultured as described above. The plates were incubated at 37± 1°C in anaerobic condition (20% CO_2 in the atmosphere) for 48 h; the population of the free and nanoencapsulated strains was showed as log CFU/ml (López-Rubio et al., 2012).

Moisture content

The moisture content was determined for each sample as the percentage ratio of the weight loss to the initial weight of the sample as in Eq. 1. (AOAC, 2006). Samples were dried at 105ºC for 5 h.

$$MC = \frac{(W_i - W_f)}{(W_f)} \times 100 \quad (1)$$

W_i = initial weight; W_f = final weight, and MC = the moisture content.

Survival of LAB during the preparation of dried Tarhana

Tarhana samples were ground to powder and 25 g of each sample was diluted in 225 ml sterile PBS, homogenized in a stomacher (Stomacher® 400 Circulator, Seward Co., UK), decimal dilutions were prepared, and cultured on MRS agar. The plates were incubated at 37± 1°C for 48 h in anaerobic condition as mentioned above. The population of the LAB cells was reported as log CFU/ml (López-Rubio et al., 2012).

Survival of LAB during the preparation of Tarhana soup

Tarhana pieces were first soaked in cold water (1:5), allowed to dissolve and rehydrate for about 3 hours, and boiled for 20 minutes with occasional stirring. Then, the soup samples were cooled to room temperature, 10-fold serial dilutions were prepared, cultured on MRS agar. Plates were then incubated at 37± 1°C in anaerobic condition for 48 h; the population of the free and nanoencapsulated strains was showed as log CFU ml^{-1}.

Survival of LAB in the simulated gastrointestinal environment

The stability of LAB in Tarhana soup in the continuous model of gastrointestinal fluid was investigated. The simulated gastric fluid was prepared briefly as follows: the pH of the PBS solution was adjusted to 2.5 using 1 M HCl and then 3 $g.l^{-1}$ pepsin was added. To prepare simulated intestinal fluid, 13.6 g of dipotassium hydrogen phosphate, 77 ml of 0.2 M solution of NaOH, 10 g of trypsin, and 1 g of bovine bile salt were mixed with 250 ml deionized distilled water. The pH of the solution was adjusted to 6.80 using 0.2 M NaOH, and the final volume was adjusted to 500 ml using deionized distilled water. The simulated gastric and intestinal fluids were sterilized applying an MF-Millipore™ membrane filter (47 mm diameter, 0.22 µm pore size). Ten ml of Tarhana soup samples were, separately, added to 90 ml of the simulated gastric and incubated at 37ºC on a shaker (100 rpm) (Incu-Shaker, Benchmark Scientific, Canada) for 2 h. Then 25 ml of the gastric digested solution was transferred into 225 ml of simulated intestinal fluid. After 0, 30, 60, 120, and 180 min incubation at 37ºC, 100 µl from each sample was taken for viable cell counting. To count the viable cells, decimal

serial dilutions from each sample was prepared using PBS solution, cultured on MRS agar, and incubated under anaerobic conditions at 37°C for 48 h (Ji et al., 2019a; Yasmin, Saeed, Pasha, & Zia, 2019).

Sensory analysis

Sensory evaluation was performed on prepared Tarhana soups by twelve trained panelists (six male and six female, 20- 52 years old). The training of the panelist group was carried out for 6 h (6 sessions, each session 1h) in Amol University of Special Modern Technologies. The ingredients of the samples were explained to the panelists. Both groups of the soups were served to panelists in porcelain bowls at 50°C and the sensory evaluation was carried out in a room with daylight condition. Scoring of Tarhana soup samples was performed regarding color, odor, mouth feel, consistency, flavor, sourness, and overall acceptability applying the five-point hedonic scale (as 1= extremely disliked, 2= dislike a little, 3= neither like nor dislike, 4= like a little, and 5= extremely liked) (Demirci et al., 2019).

Statistical analyses

All the experiments were performed in triplicate. Data were analyzed by Independent Samples t-test at 95% confidence level ($p<0.05$) using SPSS (version 22.0) and presented as the mean± standard deviation. Mann-Whitney test was used as a non-parametric test to compare the sensory scores of two groups of Tarhana samples.

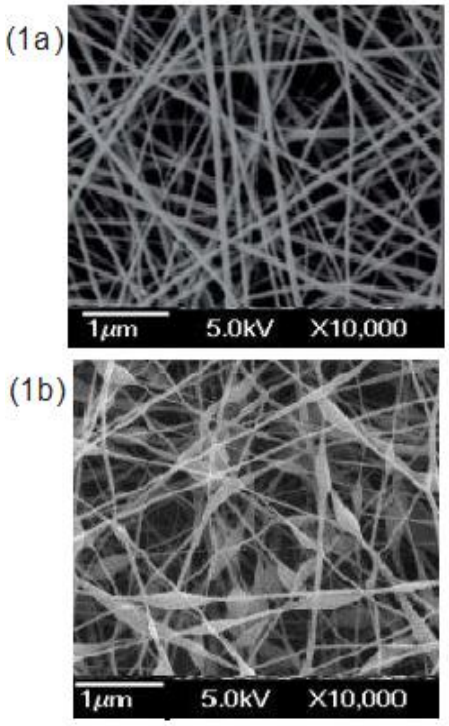

Fig. 1. SEM images of the LAB free (control) and probiotic-loaded nanofiber mats

Results and discussion

Zeta potential of nanofiber mats loaded with LAB

Zeta potential shows the particle surface charge and is used as a determining parameter in characterizing nano-scaled particles. Zeta potential presents the electrostatic potential value and it is claimed that zeta potential values of ±30 mV are representative of well-stabilized particles (Vogel et al., 2017). In this work, a negative zeta potential value of -13.84± 0.50 and -15.1± 0.77 mV was obtained for the control (nanofiber mats without LAB) and LAB-loaded nanofiber mats, respectively ($p<0.05$).

SEM images

Figure 1 shows the SEM images of the LAB-free (control) and LAB-loaded nanofiber mats. As seen, the nanofibers mats prepared from SA and CS mix showed a bead-free and clean structure with uniformity in size. The diameter size of most of the fibers was ranged from 175-338 with an average of 265 nm (Fig. 1a). Loading nanofiber mats with *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* cells led to a uniform distributed beaded structure as seen in Fig. 1b and the average diameter enhanced to approximately 763 nm.

Survival of LAB during the electrospinning process

A viability test was conducted to investigate the effect of encapsulation in electrospun fibers on LAB. According to the data presented in Table 1, the viability of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* cells at the end of the electrospinning were 92.82% and 95.83%, respectively, which indicated a slight loss in their population.

Table 1- Viability of the *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* during nanoencapsulation by electrospinning

	***L. delbrueckii* subsp. *bulgaricus* (log CFU ml^{-1})**	***S. thermophilus* (log CFU ml^{-1})**
Initial free cells	10.18± 0.75^{a*}	10.33± 1.15 ^{b}
Electrospun nanoencapsulated cells	9.45± 1.02 ^{a}	9.90± 0.83 ^{b}

*Different lowercase superscripts in a column express significant difference between means

Survival of LAB during the preparation of dried Tarhana

The moisture content of the control and nanofiber-loaded dried Tarhana samples was 8.75 and 8.71%, respectively; therefore, using nanofiber mats in the formulation had no significant effect on the moisture content of the samples ($p>0.05$). Survival of LAB during the drying step was measured to evaluate the effect of encapsulation within nanofibers on the stability of the bacteria in the dehydration process and low moisture content. As presented in Table 1, nanofiber mats had a protective effect on the cells, and *S. thermophilus* showed a higher viability rate in comparison to *L. delbrueckii* subsp. *bulgaricus* cells during the drying step ($p<0.05$). This might be due to lower water activity (~ 0.95) (Zhou et al., 2008) *S. thermophilus* needs to survive and proliferate in comparison to *L. delbrueckii* (~0.97) (Kamel, Gomma, Osman, & Hassan, 2018). At the end of the drying Tarhana samples, there was a significant difference between the survival rate of free and encapsulated bacteria ($p< 0.05$). The viability of nanoencapsulated *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* was 93.50% and 89.16%, respectively, while for free cells was 85.3 and 76.4%, respectively, that indicated the considerable protective effect of CS/SA fibers on the cells against dehydration of Tarhana medium.

Survival of LAB in Tarhana soup

To investigate the effect of nanoencapsulation within CS/SA fiber mats on the thermal stability of the bacteria in the heating process, the viability of LAB during preparing Tarhana soup was evaluated. As presented in Table 3, nanofiber mats improved the stability of the cells against ordinary heat treatment used in preparing Tarhana soup. The survival rate of *S. thermophilus* was higher than *L. delbrueckii* subsp. *bulgaricus* ($p< 0.05$) and a significant difference was observed between the viability of free and nanoencapsulated bacteria ($p< 0.05$). The survival of CS/SA nanoencapsulated *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* was 83.25% and 80.21%, respectively, which is indicative of the significant protective effect of fibers on the cells against the heating process.

Table 2- Viability of the *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* during drying process of Tarhana samples

	L. delbrueckii subsp. *bulgaricus* (log CFU ml^{-1})	*S. thermophilus* (log CFU ml^{-1})
Free cells in wet Tarhana	9.35± 0.75^{a*}	9.26± 1.15 ^{a}
Free cells in dried Tarhana	7.15± 1.02 ^{b}	7.90± 0.83 ^{b}
Nanoencapsulated cells in wet Tarhana	9.42± 0.75^{a}	9.89± 1.15 ^{a}
Nanoencapsulated cells in dried Tarhana	8.39± 1.02 ^{c}	9.24± 0.83 ^{a}

*Different lowercase superscripts in a column express significant difference between means

Table 3- Survival of the *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* during cooking process of Tarhana soup

	L. delbrueckii subsp. *bulgaricus* (log CFU ml^{-1})	*S. thermophilus* (CFU ml^{-1})
Free cells in dried Tarhana (before cooking)	7.15± 1.02^{a*}	7.90± 0.83 ^{a}
Free cells in Tarhana soup	3.85± 0.27 ^{b}	4.49± 0.61 ^{b}
Survival rate of free cells (%)	53.8	56.8
Nanoencapsulated cells in dried Tarhana (before cooking)	8.39± 1.02^{c}	9.24± 0.83 ^{c}
Nanoencapsulated cells in Tarhana soup	6.73± 0.80 ^{d}	7.69± 0.55 ^{a}
Survival rate of nanoencapsulated cells (%)	80.21	83.25

*Different lowercase superscripts in a column express significant difference between means

Survival of LAB in simulated gastrointestinal model

The free and CS/SA nanoencapsulated LAB were exposed to simulated gastrointestinal environment and the viability of the cells was studied. Nanoencapsulation in CS/SA nanofiber mats improved the protection of both LAB in simulated fluids of the stomach and intestine (Table 4). After continuous exposure to simulated gastrointestinal fluid, a significant loss of viable free LAB cells (higher than 4 log CFU/ml) was found while the population of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* encapsulated in CS/SA nanofibers decreased only 0.45 and 0.37 log CFU at 120 min ($p > 0.01$), 0.93 and 0.80 log CFU at 180 min ($p < 0.01$), respectively.

Table 4- Survival of free and nanoencapsulated *S. thermophilus* (*S*) and *L. delbrueckii* subsp. *bulgaricus* (*L*) upon exposure to continuous simulated gastrointestinal fluid.
Data are presented as mean± standard deviation log CFU.ml^{-1} from triplicate experiments (n =3)

	Exposure Time (min)				
	0	**30**	**60**	**120**	**180**
Free *S*	4.49± 0.61^{b*}	ND†	ND	ND	ND
Nanoencapsulated *S*	7.69± 0.55 ^{a}	7.50± 0.18^{b}	7.41± 0.35^{c}	7.32± 0.25^{c}	6.89± 0.47^{d}
Free *L*	3.85± 0.27 ^{b}	ND	ND	ND	ND
Nanoencapsulated *L*	6.73± 0.80 ^{d}	6.56± 0.71^{b}	6.39± 0.50^{c}	6.28± 0.10^{c}	5.80± 0.33^{c}

†ND: No cell detectable.
*Different lowercase superscripts in a row express significant difference between means during the incubation time ($p<0.01$).

Sensory properties

Table 5 presents the sensorial properties of the Tarhana samples. There was no significant difference in color of the samples ($p > 0.05$). Tarhana soup prepared with probiotic–loaded nanofibers gained higher scores in terms of

consistency, mouth feel, odor, taste, flavor, and overall acceptability attributes. Tarhana soup with nanofibers showed much sour taste and flavor than samples prepared with free cells of probiotics ($p< 0.05$).

Table 5- sensory analysis of Tarhana soup samples

Sensory properties	Tarhana soup samples	
	with free LAB	with nanoencapsulated LAB
Color	3.25± 0.28 [a*]	3.50± 0.31 [a]
Flavor	4.36± 050 [a]	3.95± 0.12 [b]
Taste	4.18± 0.35 [a]	3.77± 0.25 [b]
Odor	3.85± 0.10 [a]	3.80± 0.42 [a]
Sourness	4.55± 0.21 [a]	3.70± 0.18 [b]
Consistency	3.44± 0.16 [a]	2.91± 0.13 [b]
Mouth feel	3.57± 0.29 [a]	2.82± 0.34 [b]
Overall acceptability	3.64± 0.20 [a]	3.05± 0.45 [b]

*Different lowercase superscripts in a row express significant difference between Means during the incubation time ($p< 0.01$).

According to the results, the control and probiotic-loaded nanofiber mats had a negative zeta potential value of -13.84± 0.50 and -15.1± 0.77 mV, respectively. The negative charge and the resulted negative zeta potential of the nanofibers may be due to the detachment of protons from the acid groups of sodium alginate (Borumand, 2013) and also the presence of free carboxylic acid groups at the surface of alginate molecules (Borumand, 2013). Also, the corn starch molecule has a negative charge at the surface due to the de-protonation of some hydroxyl (OH) groups and dissociation of carboxylic (COOH) groups during the mixing-heating process (Robinson, Coustel, Abdelmoula, & Mallet, 2020). In a study by Wang et al., the nanoparticles prepared from starch and sodium alginate showed a zeta potential of -10.5 mV (Wang et al., 2017). Our findings demonstrated that loading nanofibers with probiotics caused lower zeta potential compared to unloaded nanofibers. It can be stated that loading nanofiber mats with probiotics increased the negativity of the zeta potential value. The higher negative zeta potential values of probiotic-loaded nanofiber mats can be explained by the net negative charge and negative zeta potential value of *Lactobacilli* cells (Dean, Leary, Sullivan, Oh, & Walper, 2019; Ji et al., 2019b; Murga, de Valdez, & Disalvo, 2000; Pérez, Minnaard, Disalvo, & De Antoni, 1998) that shows efficient nanoencapsulation of probiotics within nanofibers in our work.

The average diameter of the fibers was 265 nm and loading nanofiber mats with *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* cells led to a uniform distributed beaded structure with the average diameter of 763 nm, demonstrating the successful nanoencapsulation of the bacteria in the CS/ SA nanofibers. In research by Yilmaz et al. (2020), loading *L. paracasei* into the nanofiber mats (initial diameter size of 305 nm) resulted in a beaded structure of fibers with an increase of diameter to 842 nm (M. T. Yilmaz, Taylan, Karakas, & Dertli, 2020) which is similar to our findings Skrlec et al. (2019) prepared the poly (ethylene oxide) nanofibers loaded with *L. plantarum* cells with a diameter of 492 nm which had a lower size compared to the loaded nanofibers of our study (Škrlec et al., 2019) due to the difference between the coating material used to produce the nanofibers and the effect of the bacterial characteristics on the fiber diameter size.

The viability rates of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* cells during the electrospinning process was found 92.82% and 95.83%, respectively, which indicated a slight

loss in their population. Factors such as rapid evaporation of water content and high rate changes of osmotic pressure affect the survival of the cells during the electrospinning process (López-Rubio et al., 2012). In a previous study, a high viability rate was observed for *Bb. animalis* subsp. *Lactis Bb*12 and combination of *Streptococcus thermophilus*, *L. paracasei* and *Bb*-12 following encapsulation using the electrospun poly (vinyl alcohol) fibers (Akbar et al., 2018). Similar results were claimed by other researchers that prepared electrospun nanofibers from alginate (M. T. Yilmaz et al., 2020) and starch (Lancuški et al., 2017) to nanoencapsulate *L. paracasei* which provided good cell viability (higher than 85%). It is indicated that calcium alginate improved the viability of *L. rhamnosus, L. casei, L. acidophilus,* and *Bifidobacterium.* spp. in fermented dairy products and the survival rate was increased by combining calcium alginate and starch as the coating material (Chen, Wang, Liu, & Gong, 2017). The survival rate of the LAB cells in the present work was considerably higher than other studies that show good compatibility of the starch and sodium alginate to provide protection for probiotics.

In this work, there was a significant difference between the survival rate of free and encapsulated bacteria at the end of the drying process (93.50% and 89.16% for *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus,* respectively, verses 85.3 and 76.4%). The protecting activity of alginate capsules was also investigated by Albadran et al. (2015). They evaluated the stability of fluid bed and freeze-dried chitosan-coated alginate microcapsules loaded with LAB. The viability loss was about 0.8 and1.3 log for fluid bed and freeze-dried samples. In both samples, the moisture content and aw were lower than 10% w/w and 0.25, respectively, which is necessary for high stability during long storage (Albadran, Chatzifragkou, Khutoryanskiy, & Charalampopoulos, 2015). It is demonstrated by several studies that decreasing the moisture content and aw increases the survival of the dried encapsulated cells (Bora, Li, Zhu, & Du, 2019; Dianawati, Mishra, & Shah, 2016; Liu et al., 2019). Donthidi et al. evaluated the effect of starch on the viability of alginate encapsulated probiotics at different temperatures. The incorporation of starch improved the entrapment efficiency and the viability of encapsulated bacteria that is similar to our results (Donthidi, Tester, & Aidoo, 2010). The use of starch for the encapsulation of LAB can provide technological benefits like stability against high temperature and low aw. Furthermore, combining starch with alginate promotes a synergistic effect on the gelation of starch and provides further protection to LAB cells (de Araújo Etchepare et al., 2016; Donthidi et al., 2010; Mirzaei, Pourjafar, & Homayouni Rad, 2011).

The survival rate of CS/SA nanoencapsulated *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* (83.25% and 80.21%, respectively) showed the significant protective effect of fibers on the cells against the thermal processing. Mahmoud et al. (2020) studied the viability of alginate-microencapsulated *L. plantarum* during food processing. They reported that survival of the microencapsulated *L. plantarum* cells improved over the initial count, which was about 8 log CFU g^{-1} sample, upon exposure to 40 and 45˚C for 24 h and 30 min, respectively. In contrast, exposure to 65˚C for 30 min decreased the viability of the encapsulated bacteria (Mahmoud, Abdallah, El-Shafei, Tawfik, & El-Sayed, 2020). Also, Ouled-Haddar et al. (2016) found 100% and 90% survival of SA-encapsulated *L. plantarum* upon heat treatment at 40 and 50˚C for 20 min, respectively. SA-Skimmed milk encapsulating material maintained 10^6 CFU g^{-1} of *L. plantarum* after exposure to 65˚C for 30 min, which is the dietary recommended dose of probiotics in the food to exert functional effects (Bilenler, Karabulut, & Candogan, 2017; Teoh, Mirhosseini, Mustafa, Hussin, & Abdul Manap, 2011).

Nanoencapsulation in CS/SA nanofiber mats increased the viability of both LAB in simulated fluids of the stomach and intestine.

Our findings demonstrated that CS/SA nanofiber mats protected the LAB from adverse effects of gastric acid condition and bile salt. The technique used in our study to fabricate nanocapsules resulted in a higher level of protection in comparison to other methods (like microemulsification, nanoemulsification, extrusion, etc.) applied in previous studies (Coghetto, Brinques, & Ayub, 2016; Ji et al., 2019a; Liu et al., 2018; Yeung, Üçok, Tiani, McClements, & Sela, 2016). Mahmoud et al. (2020) reported the viability loss of 1.24, 1.71, and 2.47 log CFU for *L. plantarum* cells entrapped within SA/chitosan, SA/skimmed milk, and SA/dextran, respectively, upon 120 min exposure to gastrointestinal fluid. Also, combining our data with other studies 'data showed that the incorporation of sodium alginate and corn starch in nanofibers provides a higher survival rate in gastrointestinal fluids in comparison with alginate combined with other compounds (Ji et al., 2019a; Yeung et al., 2016; M. T. Yilmaz et al., 2020). In order to perform their functional activity in human body, probiotics must reach the small intestine and then colonize there in enough number, that is 10^6 -10^7 CFU g^{-1} and encapsulation seemed to be a promising method for increasing the viability of LAB cells in gastrointestinal tract conditions (Shori, 2017). Several studies have proved that combining SA with other polymers increases alginate's protective effect on probiotics. For example, it is reported that chitosan coating of alginate microcapsules resulted in a high level survival of *L. plantarum* (Fareez, Lim, Mishra, & Ramasamy, 2015). Ramirez et al. (2015) reported that alginate and starch granules exert a protective effect on each other (Ramírez et al., 2015) that can prevent or reduce the action of digestive enzymes on the encapsulated object. This finding is related to the electrostatic interactions between alginate and other molecules that forms a strong membrane and decreases the likelihood of leakage of the capsulated cells. The results also indicated that the protection provided by CS/SA maintained around10^6 to 10^7 CFU g^{-1} of the cells which is within the recommended level of probiotic dose to be functional and effective.

Tarhana soup prepared with probiotic–loaded nanofibers gained higher scores in terms of consistency, mouth feel, odor, taste, flavor, and overall acceptability attributes. This might be due to higher survival of nanoencapsulated bacteria which resulted in higher rate of fermentation and acid and flavoring metabolites production. For the overall acceptability, the panelists recorded lower scores for Tarhana samples prepared with free cells of *S. thermophilus* (*S*) and *L. delbrueckii* subsp. *bulgaricus*. The slight difference between consistency and mouth feel of Tarhana samples with nanofibers and the control is related to the presence of SA and CS (as bodying agents) that improved the consistency and texture of the samples. The overall sensorial data showed that encapsulating *S. thermophilus* (*S*) and *L. delbrueckii* subsp. *bulgaricus* in CS/SA nanofiber mats resulted in acceptable Tarhana soup properties according to the overall preference of the panelists.

Encapsulating *S. thermophilus* (*S*) and *L. delbrueckii* subsp. *bulgaricus* as the fermenting bacteria in preparation process of Tarhana improved their survival and viability during drying step and heat treatment during preparation of Tarhana soup and also provided a considerable protective effect on probiotics in gastrointestinal tract. The Tarhana soup prepared with encapsulated cells of *S. thermophilus* (*S*) and *L. delbrueckii* subsp. *bulgaricus* in corn starch and sodium alginate nanofiber mats was highly preferred in terms of the sensory properties in comparison to the samples containing free cells.

Conclusion

The results of the present study indicated that the protection obtained from CS/SA capsules maintained around10^6 to 10^7 CFU/g of the probiotic cells which are within the recommended level of probiotic dose to be functional in consumers' body. Therefore, this product can be used by the consumers like vegetarians and lactose or milk peptide

intolerants who do not consume dairy products but need potential fermented probiotic food.

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Conflict of interest statement

No conflict of interest declared.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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افزایش بقای باکتری‌های اسید لاکتیک در سوپ ترخینه به‌عنوان ماتریکس غیرلبنی

مریم عزیزخانی*[۱]- رفعت کاربخش راوری

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چکیده

ترخینه، غذای تخمیری سنتی ایران بر پایه غلات، از مخلوطی از آرد غلات، سبزیجات، سبزی، ادویه‌جات و ماست تهیه می‌شود. هدف از این مطالعه بهبود بقای باکتری‌های اسید لاکتیک (LAB) در سوپ ترخینه به‌عنوان یک ماتریکس غیرلبنی بود. *لاکتوباسیلوس دلبروکئی* زیرگونه *بولگاریکوس* و *استرپتوکوکوس ترموفیلوس* در نانوالیاف الکتروریسی شده تهیه شده از نشاسته ذرت (CS) و سدیم آلژینات (SA) ریزپوشانی شدند و اثر محافظتی نانوالیاف بر سلول‌ها در طول تهیه ترخینه و نیز در دستگاه گوارش مورد بررسی قرار گرفت. نتایج نشان داد که مقدار پتانسیل زتا ۱۵/۱- میلی ولت برای نانوالیاف بارگذاری شده با LAB مشاهده شد. نانوالیاف حاوی LAB دارای ساختار مهره‌ای با توزیع یکنواخت و قطر متوسط ۷۶۳ نانومتر بودند. بقای *لاکتوباسیلوس دلبروکئی* و *استرپتوکوکوس ترموفیلوس* در پایان الکتروریسی ۹۲/۸۲ و ۹۵/۸۳ درصد بود که نشاندهنده کاهش جزئی در جمعیت آنها است. بقای *استرپتوکوکوس ترموفیلوس* و *لاکتوباسیلوس دلبروکئی* نانوکپسوله شده ۹۳/۵۰و ۸۹/۱۶ درصد بود، در حالی که برای سلول‌های آزاد ۸۵/۳ و ۷۶/۴ درصد بود که اثر محافظتی قابل توجه الیاف CS/SA را بر سلول‌ها در برابر دهیدراتاسیون محیط ترخینه نشان داد. نانوالیاف پایداری قابل توجهی برای سلول‌ها در برابر فرآیند حرارتی تهیه سوپ و همچنین در دستگاه گوارش فراهم آوردند، زیرا بقای $۱۰^{۶}$ تا $۱۰^{۷}$ سلول تامین شد که در محدوده دوز توصیه‌شده پروبیوتیک جهت تاثیرگذاری بر سلامت مصرف‌کننده قرار دارد. مصرف‌کنندگانی مانند گیاه‌خواران و افراد مبتلا به عدم تحمل لاکتوز/ پپتید شیر که لبنیات مصرف نمی‌کنند، لیکن به مصرف مواد غذایی پروبیوتیکی نیاز دارند، می‌توانند از این محصول استفاده نمایند.

واژه‌های کلیدی: الکتروریسی، باکتری های اسید لاکتیک، پلیمر زیستی، ترخینه، ریزپوشانی.

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