DOI: http://dx.doi.org/ 10.22104/IFT.2023.5978.2127

Innovative Food Technologies, 11(1), 11-24, Autumn 2023

Research Article

Evaluation of Physicochemical Properties of Lactobacillus acidophilus Cells Encapsulated with Sodium Alginate and Balangu Seed Mucilage

Seyed Saeed Sekhavatizadeh[1*] , Tina Yaghoobpour[2]

1. Fars Agricultural and Natural Resources Research and Education Center, AREEO, Shiraz, Fars, Iran.

2. Department of Clinical Sciences, School of Veterinary Medicine, Shiraz University, Shiraz, Iran.

(Received 27 November 2022, Received in revised form 28 January 2023, Accepted 7 February 2023)

Abstract

This study aimed to improve the survival rate of *Lactobacillus acidophilus (LA)* under harsh conditions. Therefore, the extrusion encapsulation technique was used to apply the Balangu (*Lallemantia royleana*) Seed Mucilage (BSM) as a second coating material at concentrations of 0.2, 0.4, 0.6, and 0.8%. We evaluated the physicochemical properties and feasibility between the free and microencapsulated forms during simulation gastrointestinal conditions. The results showed that the beads produced were spherical. Increasing the concentration of BSM significantly increased the bead diameter, while the L* parameter remained constant. It was obvious that the free and microencapsulated forms decreased at 72° C whereas a longer shelf life was observed in the beads compared to the free bacteria. In addition, the non-capsulated bacteria and the microencapsulated forms decreased through storage and under simulated gastrointestinal conditions. *LA* counts revealed the decreased levels of 6.47 and 4.65 log units, respectively, after exposure to simulated gastrointestinal conditions. Microencapsulated LA (MLA) had a 77.56% survival ability in the MRS broth after 28 days in cold storage. The results of this study showed that the use of BSM could extend the survival ability of MLA (43.22, 22.58, 24.76 and 1.46%) in comparison to FLA, during the heat stress, salt and acid condition, refrigerated storage, and simulation gastrointestinal condition, respectively. In comparison to quince seed mucilage, the BSM bead revealed a greater survival rate during the simulated gastrointestinal condition but lower survival at 72° C.

Keywords: **Lactobacillus acidophilus, Microencapsulation, Lallemantia royleana, Gastrointestinal condition, Extrusion**

How to cite this article:

Sekhavatizadeh S.S & Yaghoobpour T (2023). Evaluation of Physicochemical Properties of Lactobacillus acidophilus Cells Encapsulated with Sodium Alginate and Balangu Seed Mucilage. *Innov. Food Technol., 11(1)*, 11-24.

* Corresponding author: s.sekhavati@areeo.ac.ir

1. Introduction

It is repeatedly confirmed that adequate amounts of probiotics revealed beneficial influences such as regulating the microbial flora of the gastrointestinal tract, inhibiting the evolution of pathogenic microorganisms, and regulating the overall immune responses [1].

The most important probiotic microorganisms in food are Lactic acid bacteria (including *lactococci* and *lactobacilli*) in products [2]. Numerous fermented foods are produced by *Lactobacillus* species as starter cultures. Several *lactobacillus* strains have potential therapeutic properties. *Lactobacillus acidophilus* (*LA*) is naturally present in the gastrointestinal tract of humans and animals, and some strains may have probiotic properties [3]. The *LA* is a catalase-negative, homofermentative, gram-positive, microorganism with rod morphology [4].

Studies report the use of probiotics in dairy products such as butter, ice cream, cheese, and yogurt. To market a food product claiming health benefits from the addition of probiotics, a large number of viable cells at least 10^6-10^7 CFU.g^{-1} must reach the colon where their beneficial effect must occur. They must be able to withstand stomach acid and bile salts. However, approximately 2 log CFU.g^{-1} is lost during digestion, so an amount of 10^8-10^9 CFU.g^{-1} present in the product before consumption is recommended [5].

In food, the concentration and viability of probiotics before consumption and at the time of expiration are important parameters. The type of starter culture, probiotic strain, storage conditions, and lactate and oxygen concentrations are the main parameters affecting MO viability in probiotic yogurts. Therefore, maintaining a constant level of probiotic concentration and viability has become a key challenge in the development of probiotic products[6].

Survival of probiotic bacteria is compromised by a variety of harsh conditions. Therefore, microencapsulation of probiotics as an effective technique has been introduced to prolong cell viability [7].

Microencapsulation is an advanced food processing technique that allows the encapsulation of the arbitrary compounds in specific materials, resulting in small spheres ranging from 1 μm to 100 μm in diameter. Microencapsulation is done to protect sensitive compounds and ensure safe delivery. Microencapsulation extrusion technology can be used to produce high-density microcapsules. The immiscible wall material is necessary. Here, the wall materials surround the core which finally passes through concentric nozzles to form droplets including the surrounding core. Solidification then occurs by cooling or by using a suitable gelling bath, the droplets fall off and solidify due to the formation of complexes [8]. The wall material is an important component of microencapsulation, and the properties of the wall material, including rheological properties, wall material film properties, molecular structure, and emulsion stability, determine the yield and efficiency of microencapsulation. The basic features of wall material include sensory properties, physicochemical stability, bioactive retention, loading, and release [9]. Due to the availability, biocompatibility, and low-cost values of alginate, it is currently used as a safe polymer to encapsulate probiotics. Alginate is a linear heteropolysaccharide natural derivative of brown algae which is composed of β-D-mannuronic (M) acid and α-L-guluronic (G) acid. However, developing a susceptible microcapsules spongy structure is resulted from using alginate susceptible to breakdown in the occurrence of extra monovalent ions and as Ca^{2+} chelating agents. Mixing alginate with other coating materials can be viewed as an optimal approach to strengthen the microcapsule structure [10]. To solve this problem, you can use mucilage as a second layer (coating material).

One of the folk medicinal plants is Benth (*Lallemantia royleana)* belonging to the *Labiatae* family. This is one of the major typical families of flowering plants, having 220 genera and almost 4000 species, worldwide. The Balangu or Balangu Shirazi is the native names of *Lallemantia royleana's* seed in Iran [11]. Balangu seeds are an excellent source of protein (25.60%), crude fiber (30.67%), oil (18.27%), ash (3.63%), and carbohydrates (45.25%). Moreover, it has health-enhancing properties because of several medicinal and nutritional components [12]. Elemental analysis of the gum indicated that *Lallemantia royleana* had a carbon, hydrogen, oxygen and nitrogen content of 33.39%, 4.05%, 35.45% and 0.31%, respectively. Monosaccharide composition consists of arabinose, rhamnose, mannose, fructose, galactose, α-and β-D-glucose, glucuronic acid, galacturonic acid, and glucosamine although it is mainly composed of rhamnose, galactose, arabinose and galacturonic acid [13]. Traditionally consumed as a stimulant, reconstituting agent, diuretic, and expectorant, balangu seeds are used in many products manufactured in traditional or industrial applications such as beverages. Due to its high mucus content, this seed quickly absorbs water when soaked, producing a cloudy, sticky, and tasteless liquid. It can be used in food formulations, as a new food hydrocolloid [14].

The purpose of this study was to evaluate the ability of alginate and BSM microspheres to encapsulate *LA* through an extrusion process. Encapsulation efficiency was measured to assess the viability of encapsulated *LA*. The encapsulated and free forms of *LA* were exposed to heat (72° C) to assess the protective effect of the microspheres. Moreover, salt and acid conditions (NaCl 15% at pH=1.5) were assessed for selecting the bead's resistance. Furthermore, the survival ability of beads during storage time was determined.

2. Materials and Methods

2.1. Material

Lyophilized *LA* ATCC 4356 was prepared from the Persian Type Culture Collection, Iranian Research Organization for Science and Technology (IROSt), Tehran, Iran. The BSM was purchased from a local market (Shiraz, Iran). Sodium-alginate was prepared by Sigma Company (Sigma, Steinheim, Germany). Peptone water, MRS broth, sorbitol agar, and sodium citrate, MRS agar, de Man, Rogosa, and Sharp, 1960, were purchased from (MERCK Company, MERCK, Darmstadt, Germany). Pepsin (derived from porcine stomach mucosa), Bile (bovine bile), pancreatin (from porcine pancreas) and lipase from *Rhizopus oryzae* provided by Sigma Aldrich, Dorset, UK.

2.2 Preparation of bacterial inoculum

Under aerobic condition, *LA* was grown in MRS sorbitol agar. This selective media was used for the cultivation of *LA* at 37° C for 72 hours. The preparation was then centrifuged at 4500 rpm and 4° C for 10 minutes. It was washed twice with sterile saline before re-suspending in peptone water (0.1%) [15].

2.3 Microencapsulation method

Microencapsulation of *LA* was performed using the extrusion techniques as follows. 5 ml of *LA* culture (10^{10} CFU.ml^{-1}) was added to 15 ml of 1.5% sodium alginate solution. Then, the suspension was injected into the sterile 0.1 Mol.L^{-1} $CaCl_2$ through a 0.11 mm needle. The suspension was refrigerated for 12 hours, and the beads were washed with 0.1% peptone water and gently shaken at 100 rpm for 40 minutes in (0.2, 0.4, 0.6, and 0.8 % w/w) BSM solution separately (Orbital shaker, two-step method). The beads were ultimately washed with sterile peptone water (0.1%) several times. The encapsulation yield (EY) was calculated using the following formula.

$$EY=(\text{Log } N / \text{Log } NO)\times 100 \quad \text{(Equ 1)}$$

Where N stands for the intact viable cells once the microcapsule is produced (CFU.g^{-1} beads) and N_0 stands for the number of viable cells before making the beads (CFU.g^{-1} mixed alginate).

For the determination of *LA* on the surface of beads (un-capsulated *LA*), equation 2 was used

$$\text{un- capsulated LA}=100-EY \quad \text{(Equ 2)}$$

2.4 Light microscopy, SEM, and color analysis of beads

The morphology and aspect ratio of 20 gel beads prepared using different concentrations (0.2, 0.4, 0.6, and 0.8 w/v %) of BSM were evaluated with a light microscope (Olympus BX51, Japan). Gel beads were examined under ×40 magnification. The bead layer size was analyzed with micromeasure ver1.07 software. The aspect ratio was measured using equation (3).

$$\text{Aspect Ratio= Major axis(mm)/ Minor axis(mm)} \quad \text{(Equ 3)}$$

[16]

To prepare the SEM image, the lyophilized sample was fixed in an aluminum holder and scanned by electron microscope (SEM, VEGA3, TESCAN, Czech Republic). Next, the sample was observed at an acceleration voltage of 10.0 kV. The sample surface of 7.03-8.91 mm ranged between the microscope objective and was then applied. Beads colors were measured using a Chroma-meter CR-400 (Konica-Minolta, Osaka, Japan). The L* value is an indicator of lightness (black to white lightness). The a* values indicate green and red, and b* indicates blue and yellow [17].

2.5 Texture of beads

In order to evaluate the texture profile, hardening of bead were applied after 24 h of its production. A texture analyzer (Brookfield CT3 4500, USA) was used to measure the gel strength of the beads. The 35mm diameter cylindrical aluminum probe was at a speed of 0.1mms^{-1} in a compression mode and the rupture distance of 1.0mm was used. The peak force was measured in grams. Ten beads were tested each time and 3 replications were applied for each treatment. The textural parameters including hardness (g), adhesiveness (mJ), gumminess (g), springiness (mm), and cohesiveness were obtained from the device.

2.6 Heat tolerance

To assess the thermotolerance of MLA cells, bacterial survival was tested against time and temperature combinations. Samples were prepared as follows.

1 ml of free *LA* (FLA) cells or microcapsules was suspended in 9 ml of MRS broth. Samples were placed in water bath at a constant temperature and processed under the conditions indicated previously. The glass tube was then rapidly cooled under running water. Aliquots were removed and heat treated (0, 1, 2, 3, 4, and 5 min), and serially diluted 1 mL aliquots of the test material were plated on MRS agar and incubated at 37° C under aerobic conditions, counted by incubating for 48 hours. Results were expressed as survival rates. Equation 4 was used to determine the survival rate.

Survival rate= $\frac{LogN}{LogNO}\times 100$ Equ (4)

where N is the number of bacteria in a determined time, N_0 is the number of bacteria at the beginning of the experiment [18].

The half time of FLA and MLA were evaluated based on regression curve equation. When Y was sand for 50% of survival rate.

2.7 Tolerance to NaCl and acid

The effect of NaCl (15)% concentration and acidic condition (pH=1.5) were evaluated according to Ilha et al. (2015): 0.1 g of the microencapsulated culture and 1 mL of a free cells suspension (10^8 CFU.mL^{-1}) were added to 10 mL MRS broth containing (NaCl 15%, pH 1.5) and incubated at 37° C for 30 min, 1, and 1.5 h. The enumeration of free and beads was performed after 48 hour [19].

2.8 Survival in cold storage

The storage stability of FLA and MLA cells was tested in MRS medium at 4° C for 4 weeks. The samples were taken for viability measurements at predetermined time points of 1, 7, 14, 21, and 28 days. The MRS agar was used for culturing bacteria [20].

2.9 Survival in Simulated Gastrointestinal Conditions

To perform the simulated gastrointestinal conditions, one gram of the FLA and MLA were independently added to normal saline, on the 1st day of production. The prepared 1 mol.L^{-1} HCl was employed to adjust the pH to 1.4–1.9. The samples were then supplemented with 3 g L^{-1} pepsin and 0.9 mg.L^{-1} lipase to reach the final desired concentrations. To mimic the gastric phase, samples were subsequently incubated at 37◦C (in a shaker incubator), with the approximate agitation at 110 rpm, for 2 hours. The 150 mL of 1 mol.L^{-1}, NaOH, 14 g of $NaH_2PO_4.2H_20$, and distilled water at the final volume of 1 L. The 10 g L^{-1} of Bile was added to raise the pH to 4.3–5.2, and 1 g.L^{-1}of pancreatin were added to the samples to reach the final concentrations. The enteric phase 1 was also prepared following incubation at 37 °C for 2 hours under agitation. The enteric phase 2 was adjusted by adding 10 g.L^{-1} and 1 g.L^{-1}, raised pH to 6.7–7.5, and re-incubated at 37◦C for 2 hours under agitation, following 6 hours of the experiment. The values of *LA* were recorded after 30 min, 2, 4, and 6 hours of incubation. The assay of the correspondence volumes (varying from 0.01 to 1 mL) were completed in triplicates. Aliquots of 0.01 mL and 0.1 mL of each preparation was finally pour plated in the MRS agar before being further incubated at 37 °C for 48 h under anaerobic condition [17].

2.10 Statistical analysis

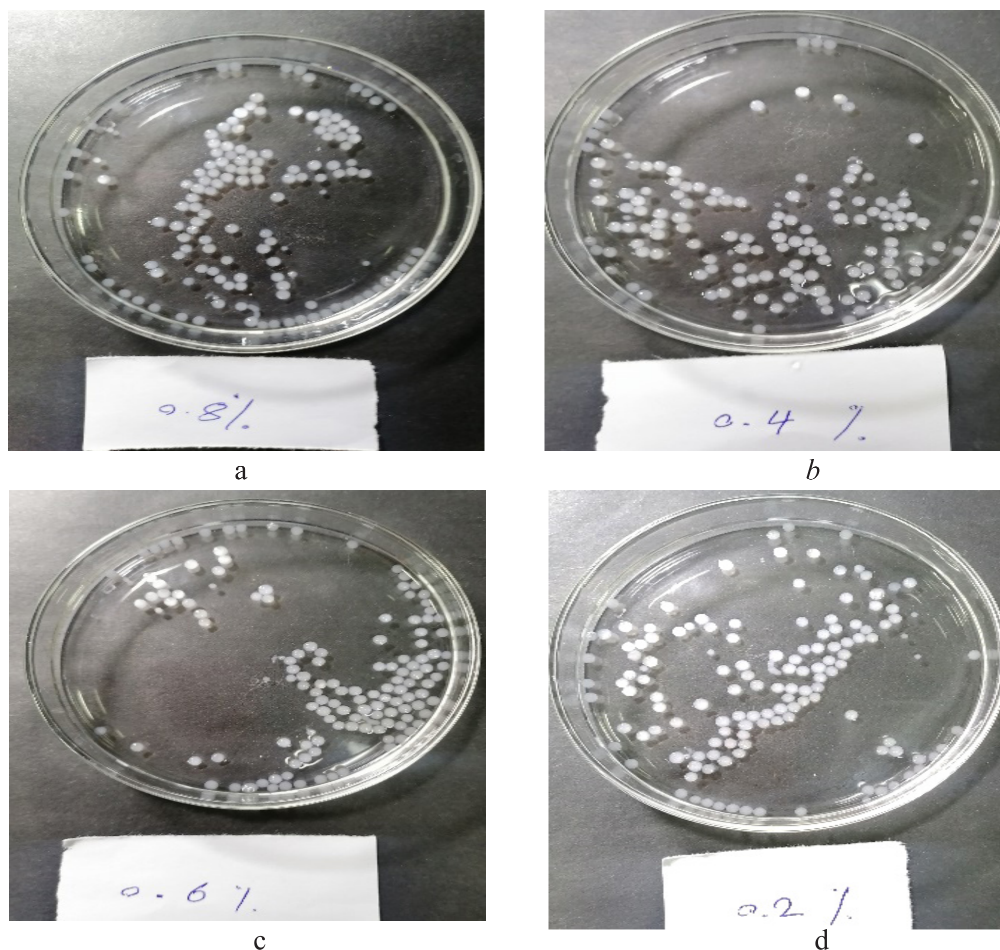
Data were presented as mean ± SEM. The data were statistically analyzed using SPSS (Ver. 21). One-way ANOVA followed by Duncan's post-test was used to compare the mean values between groups, and the significance level was set at $p \leq 0.05$.

3. Results and Discussion

3.1 Encapsulation yield and Light microscopy

In this study, microencapsulation efficiency was 91.54±3.38, 88.74±2.16, 87.60±3.19, 85.94±4.42% and un-capsulated *LA* 8.46+3.38, 11.26+2.16, 12.40+3.19 and 14.05+4.43% in MLA contains 0.2, 0.4, 0.6, and 0.8% BSM, respectively. The authors found that using higher concentrations of BSM improved encapsulation efficiency. This discovery was due to the presence of protein and fiber. The presence of protein in BSM formed a protective layer on probiotic bacteria, and the presence of dietary fiber may have partially replaced water molecules within cells, avoiding cell membrane damage [21,13]. A previous study by Frakolaki *et al*. (2022) reported that the efficacy of microencapsulation (76.55–85.75%) was obtained for alginate, carrageenan, inulin, and Glycerol [22]. This was different from this study's result. The MLA photography and light microscope details were shown in Figures 1 and 2. The MLA appeared dark and were surrounded by a thin layer of membrane. The average size of beads containing different concentrations of BSM was (3280.40±14.04 to 3300.50±44.50 µm). In the same study, probiotic-loaded with the alginate macro beads produced by the extrusion process revealed an average size of 1.9 mm (1.8–1.9 mm range) [23].

All beads were spherical aspect ratio 1.13. The results of this study are in agreement with Abbaszadeh et al. (2013) who observed that the aspect ratio of the chitosan-coated alginate beads was 1.03±0.01 to 1.11±0.05. The previous similar studies, using the same extrusion technique confirmed that the wall materials process had an impact on the sphere morphology of the beads. The bead size varied from 1.8 to 2.5 mm depending on the distance between the syringe and CaCl2 solution and, in particular, on the diameter of the needle orifice [18]. The average diameter of the BSM layer increased by its concentration (Figure 2 and Table 1). Therefore, the encapsulated bacterial cells were formed in an intact physical barrier. The SEM images of beads (Figure 3) revealed that with increased BSM concentration, the cavity and porous texture of the bead decreased. In the same study, the microcapsules obtained by the extrusion method were droplet-like particles with a mean diameter of approximately 1.5 mm [24].



a b c d

Fig 1. The images of the microencapsulated bacteria from left to right, the upper row: 0.2, 0.4 (a and b) and lower rows of 0.6, 0.8% (c and d) of the Balangu Seed Mucilage (BSM) respectively that used in the microencapsulation

Table 1. Physical properties of beads encapsulated with BSM

Parameters		BSM			
		0.2%	0.4%	0.6%	0.8%
Layers dimension (µm)	Alginate	3280.40±14.04a	3301.40±35.13a	3324.20±33.27a	3300.50±44.50a
	BSM	9.99±1.12c	13.52±0.71b	15.82±0.86b	19.92±1.48a
Color	L*	78.25±1.31a	75.25±0.75ab	75.75±1.63ab	73.50±0.29b
	a*	1.25±0.25a	1.25±0.25a	0.75±0.25a	0.75±0.25a
	b*	-1.25±0.96a	-1.50±0.58a	-1.50±0.58a	-1.75±0.96a
Texture	Hardness/g	18.75±1.83a	20.25±1.61a	19.75±0.52a	21.25±0.60a
	adhesiveness	0.02±0.003c	0.03±0.005bc	0.05±0.005b	0.07±0.01a
	Cohesiveness	0.67±0.08a	0.51±0.04b	0.39±0.02bc	0.28±0.05c
	Gumminess/g	12.48±0.55b	13.20±0.86b	16.88±0.25a	16.43±0.17a
	Springiness/mm	0.76±0.17a	0.76±0.06a	0.74±0.10a	0.68±0.15a

Data (mean ± standard error) are from three replications.

The results with different upper letters in each row are statistically significant ($p \leq 0.05$).

Balangu Seed Mucilage (BSM)

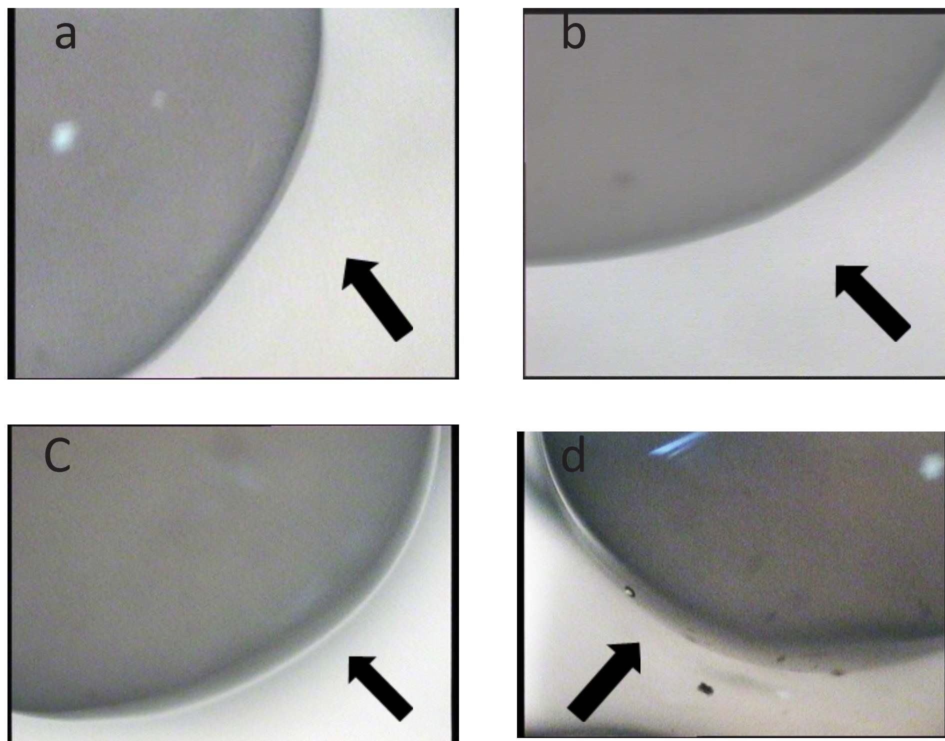


Fig 2. Light microscopy image from microencapsulated bacteria, from left to right, the upper row: 0.2, 0.4 (a and b) and lower rows of 0.6, 0.8% (c and d) of Balangu Seed Mucilage (BSM) respectively that used in microencapsulation(40X); Black arrows show second layer (BSM) of beads.

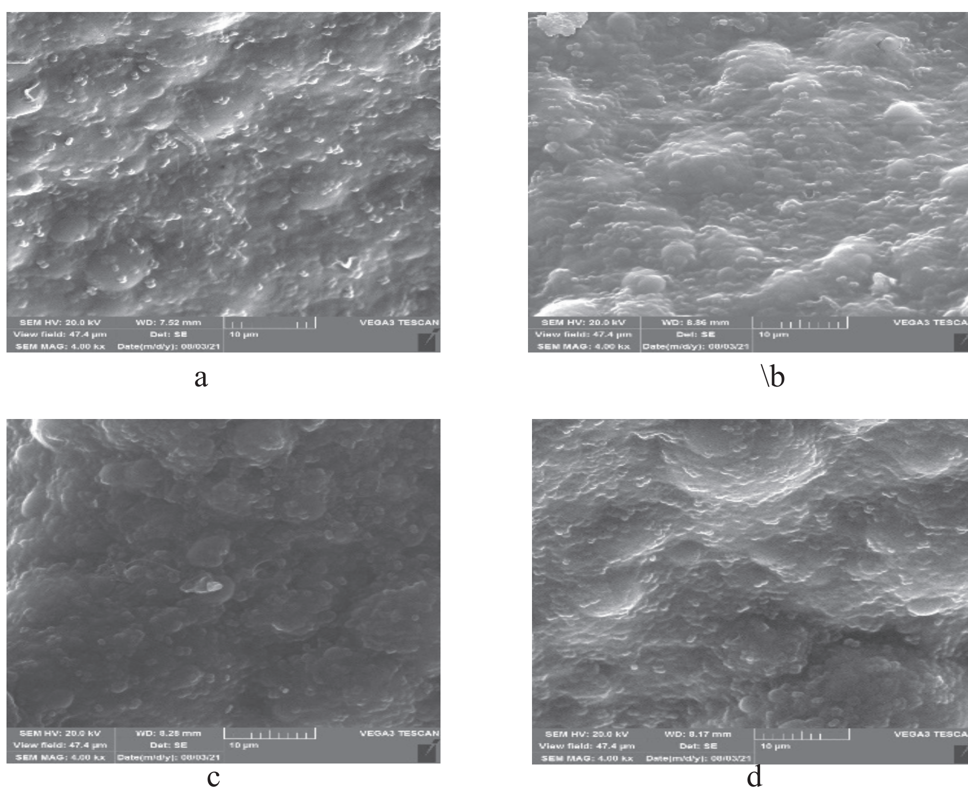


Fig 3. SEM images of microencapsulated *Lactobacillus acidophilus* (MLA); from left to right, the upper row: 0.2, 0.4 (a and b) and lower rows of 0.6, 0.8% (c and d) of Balangu Seed Mucilage (BSM) respectively.

3.2 Color and texture of beads

The color of beads was an important quality attribute. Color could be influenced by the structure of the beads, especially the second layer of beads. Because BSM was opaque, as BSM concentration stand up, the L* parameter dropped (Table 1). We showed that both a* and b* parameters were constant with increasing BSM in the bead. The BSM had no color. This may be one reason for the color parameters (a* and b*) stability among MLA.

We hypothesis, the degree of cross-linking reaction between calcium alginate and BSM may affect the mechanical response to bead deformation. Moreover, BSM polymer showed the presence of carboxyl groups, which may serve as binding sites for ions like Ca^{+2} that exists in monolayer bead structure. BSM has a high molecular weight and intrinsic viscosity, rather flexible chain, low stiffness parameter and hydrogel content [25]. No significant difference was observed in hardness and springiness among MLA samples (Table 1). Adhesiveness, springiness, and gumminess increased by rising BSM concentration but cohesiveness decreased. BSM has a high molecular weight and intrinsic viscosity, rather flexible chain, low stiffness parameter and hydrogel content. BSM has a lot of un-substituted mannose in its structure. It may be bonded with calcium. Therefore, the mentioned parameters were increased [25, 26]. Hardness and chewiness are two terms used to describe the force needed to compress a sample which represent the resistance and energy required to chew a sample, respectively [17]. It was inversely related to the length of the reaction period and was directly proportional to the thickness of the biopolymer-gelled fraction in the bead. The speed at which a sample assumes its original shape is known as springiness [27].

From a sensory perspective, it is important to consider the hardness and gumminess properties of the beads when incorporating microcapsules into foods. The beads can be hard or soft depending on the desired texture. Another textural characteristic measured by TPA is cohesiveness, which correlates with the tensile strength of the internal linkages that make up the product's body. The analysis of all alginate/BSM formulations (Table 1) of the beads revealed that there was no discernible difference in the outcomes ($p > 0.05$). This implied that the internal structure may create between the polymer matrix like sugar and the active ingredient of the beads (strength of bonds) formed by BSM. In a similar research, combining alginate with other biopolymers such as flaxseed mucilage in the structure of microcapsules' walls could improve the strength of chemical bonds between these two biopolymers (glucomannan and alginate) creating a less penetrable wall on the surface of the capsules [28]. A similar cohesiveness for each type of gelation appeared to be independent of calcium and BSM concentration utilized in the study's range [29].

3.3 Heat tolerance

The effect of temperature treatment showed that heat tolerance improved by BSM concentration in beads. The resistance ability of beads containing various concentrations of BSM against high temperatures (72° C) was shown in Figure 4. Microcapsules usually need to withstand a variety of food processing conditions, including exposure to high temperatures. In this r study, the survival rate of FLA (8.4%) was lesser than MLA containing 0.8% BSM (51.62%). The half-life for *LA* in MLA (0.2, 0.4, 0.6, 0.8%) and FLA were 5.30, 5.02, 5.61, 5.81 and 3.47 min, respectively. Therefore, the survival of MLA can improve compared to the FLA during heat stress. According to the authors, the surface hydrophobicity of the alginate capsules may improve by coating them with hydrophobic substances (BSM containing oil), which interfere to decrease the transfer of moist heat. Moreover, the coating protected the cells on the capsules surface and also blocked the surface pinholes on the alginate capsules [30]. These results were encouraging and consistent with those of Ji *et al*. (2019), discovering that the heat resistance of encapsulated *B. longum* was significantly improved compared to the non-encapsulated *B. longum* [31]. On the contrary, our findings are in contrast to the findings of Wang *et al*. (2019), reporting that *Lactobacillus pentosus* was able to survive for 30 minutes at 65° C encapsulated with a double coating of chitosan and sodium phytate. One of the reasons may be related to the species of bacteria and the kind of coating wall material [32].

The microencapsulated wall material was effectively delaying the penetration of heat into the probiotic cells. Moreover, different temperatures and wall materials showed different effects on the viability of the probiotics [33].

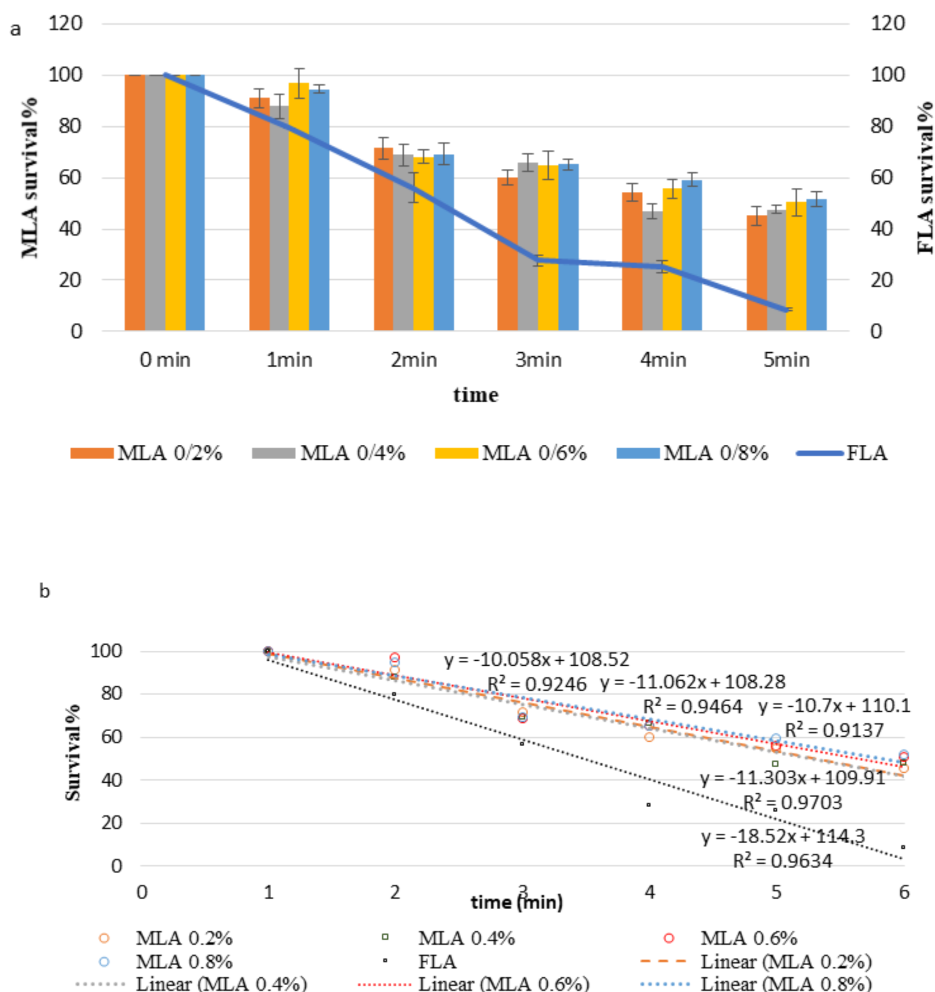


Fig 4. The free *Lactobacillus acidophilus (FLA)* and microencapsulated *Lactobacillus acidophilus* (MLA) survival rate against the 72° C; survival cylindrical curve (a); survival regression curve(b)

1- Data (mean ± standard deviation) are from three replications.

3.4 Survivability of MLA and FLA in acid and salt

The main obstacle to the survival of probiotic microorganisms is an acidic environment. Figure 5 shows the survival rate of FLA and MLA in salt (NaCl 15%) and acid (pH=2) condition. The BSM was the most appropriate encapsulating agent to enhance the tolerance of LA to NaCl and acidic condition. The survival rate of LA in MLA and FLA samples were 72.5 and 52.7% during storage time, respectively. In addition, according to Ilha et al. (2015), the viability of encapsulated cells can improve, which resulted in average loss of 1 log CFU compared to the average loss of 4.25 log CFU in the free cells, following exposure to pH 2.0 and 3.0, respectively [19]. Salt and acidic conditions can destroy microorganisms. Therefore, microencapsulation can be used as an effective technique dealing with this destructive effect. In this study, beads size and BSM are two factors that affected the salt and acid tolerance. In a research, Hansen et al. (2002) found that a positive relationship exists between bead size and salt tolerance. Therefore, the salt tolerance increased by bead size diameter [34].

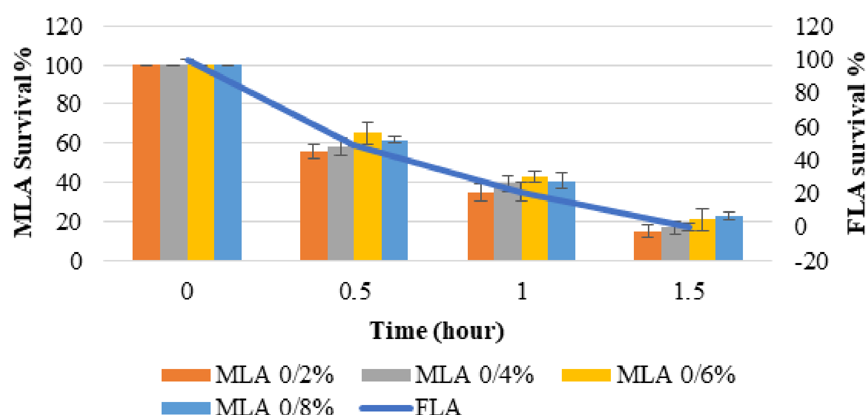


Fig 5. The free *Lactobacillus acidophilus (FLA) and* microencapsulated *Lactobacillus acidophilus* (MLA) survival rate against the salt (15%) and acid (pH=2)

1- Data (mean ± standard deviation) are from three replications.

3.5 Survival in cold storage

Damaged cell membranes are another main factor contributing to the survival of probiotics in food products during storage, while the storage conditions such as temperature are critical factors that affect cell viability crucial issues [35]. Figure 6 shows that the survival ability of free and all the encapsulated LA increased throughout the storage period. The survival rate of MLA count increased with the increased BSM concentrations. Therefore, it can be concluded that BSM as an encapsulating agent maintains the viability of encapsulated cells under refrigerated conditions. The presence of BSM in the particles was very effective to keep the survival of the probiotic culture, as it may reduce the porosity of the particles (Fig 3), and therefore decrease the susceptibility of microorganisms to harsh environmental conditions. In this research, the survival rate of MLA (77.56%) was greater than FLA (52.85%). These results were in agreement with those obtained by El-Shafei et al. (2018), which also showed that the encapsulated probiotic count in labneh was significantly greater than the control in each week of the storage period [36].

A similar result was also observed by Silva et al. (2018) who reported LA encapsulated with the extrusion technique had less than 6 Log10 $CFU.ml^{-1}$ after 30 days of storage. At the end of storage, the population of probiotics in the beads produced by extrusion was reduced to approximately 4 Log $CFU.g^{-1}$ [37].

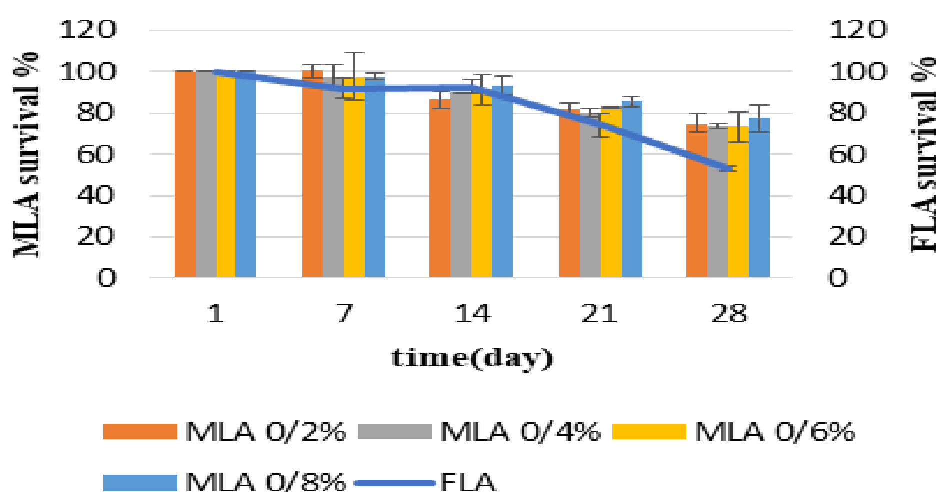


Fig 6. The free *Lactobacillus acidophilus (FLA) and microencapsulated Lactobacillus acidophilus* (MLA) survival rate during refrigerated storage at 4° C

1- Data (mean ± standard deviation) are from three replications.

3.6 Survival in SGI

Regarding FLA survival, it decreased with all treatments during exposure to simulated gastrointestinal conditions (SGI), as shown in Figure 7. However, following exposure to the simulated gastric and intestinal juices, the free cells respectively exhibited reduced levels of 6.13 and 6.47 log units while the encapsulated probiotic bacteria showed reduced levels of 3.6, 4.41 log cycles for microencapsulation conferring LA protection during exposure to simulated gastric juice and simulated intestinal juice, respectively. Therefore, the BSM coating of microparticles confers additional protection to LA, once it prevented the diffusion of acidic groups and enzymes into the MLA. The sugar and fat content of BSM may protect the beads from destruction.

Favorable and unfavorable results, reported in the literature regarding the efficiency of particle coatings aimed at improving the survival of probiotics in the intestinal tract. The results presented in this study compared simulated gastric juice (pH 1.8 in the presence of pepsin) and simulated intestinal fluid (pH 6.5 in the presence of pancreatin, trypsin, and bile salts) to uncoated particles [38].

The study's findings involved to maintain viability of probiotic bacteria due to several factors such as the microorganism studied, different encapsulation conditions, and method of evaluating encapsulation efficiency (pH, presence or absence of enzyme, and comparison between coating conditions). Limited efforts have been made to find the best coating technology for gastrointestinal tract [39].

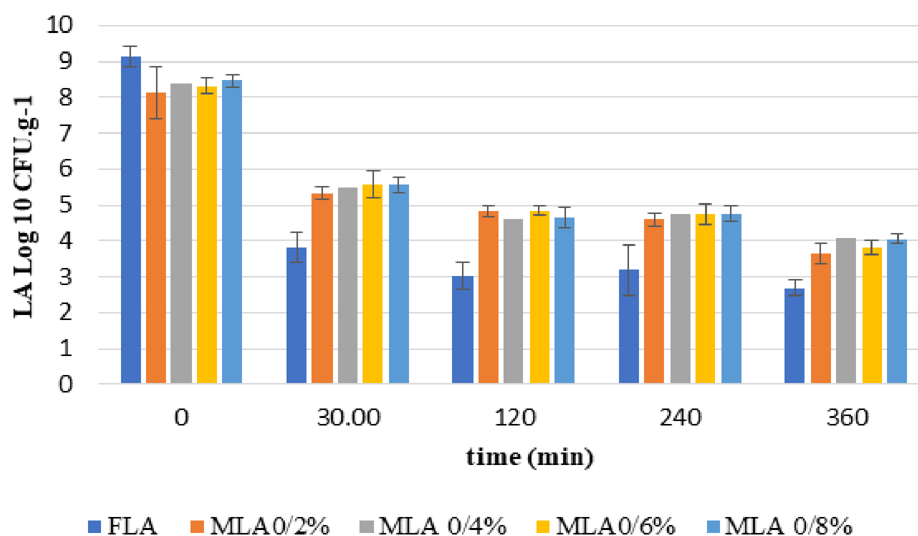


Fig 7. The free *Lactobacillus acidophilus (FLA) and microencapsulated Lactobacillus acidophilus* (MLA) survival rate during simulated gastrointestinal condition

1- Data (mean ± standard deviation) are from three replications.

4. Conclusion

This study was the first attempt to produce MLA using BSM. The microcapsules exhibited smooth spherical morphologies and high encapsulation efficiency. The results showed that better viable cell survival was observed by MLA up to 72° C. The resistance of MLA using BSM significantly improved compared to FLA. There are considerable differences between the adhesiveness and gumminess of MLA and other samples. However, the a* and b* parameters are equal in MLA samples. The survival rate of MLA was greater than FLA during storage and salt and acid conditions. One of the main characteristics of beads is survivability during passage through gastrointestinal tract. The use of BSM in second layer of beads may improve resistance of LA to acid and enzyme in SGI.

Disclosure statement

The authors report no conflict of interest. The authors are responsible for the content and writing of the article.

Funding

This study received no specific grant from funding agencies in the public, commercial, or non-profit sectors.

Ethical approval

This article does not contain studies with human participants or animals.

References

[1] Ghasemi L, Nouri L, Mohammadi Nafchi A, Al-Hassan AA. (2022). The effects of encapsulated probiotic bacteria on the physicochemical properties, staling, and viability of probiotic bacteria in gluten-free bread. *J Food Process Preserv*. 46(3), e16359. https://doi.org/10.1111/jfpp.16359

[2] Moghaddam MFT, Jalali H, Nafchi AM, Nouri L. (2020). Evaluating the effects of lactic acid bacteria and olive leaf extract on the quality of gluten-free bread. *Gene Reports*. 21, 100771. https://doi.org/10.1016/j.genrep.2020.100771

[3] Tantratian S, Wattanaprasert S, Suknaisilp S. (2018). Effect of partial substitution of milk-non-fat with xanthan gum on encapsulation of a probiotic Lactobacillus. *J Food Process Preserv*. 42(7), e13673. https://doi.org/10.1111/jfpp.13673

[4] Altamirano-Ríos AV, Guadarrama-Lezama AY, Arroyo-Maya IJ, Hernández-Álvarez AJ, Orozco-Villafuerte J. (2022). Effect of encapsulation methods and materials on the survival and viability of Lactobacillus acidophilus: A review. *Int J Food Sci Tech*. 57(7), 4027-40.

https://doi.org/10.1111/ijfs.15779

[5] da Silva MN, Tagliapietra BL, Pivetta FP, dos Santos Richards NSP. (2022). Nutritional, functional and sensory profile of added butter from Lactobacillus acidophilus encapsulated and hyposodium salt. *LWT*. 161, 113385. https://doi.org/10.1016/j.lwt.2022.113385

[6] Mahmoodi Pour H, Marhamatizadeh MH, Fattahi H. (2022). Encapsulation of different types of probiotic bacteria within conventional/multilayer emulsion and its effect on the properties of probiotic yogurt. *J Food Qual*. 2022, 7923899. https://doi.org/10.1155/2022/7923899

[7] Kazemi M, Shahidi F, Varidi MJ, Roshanak S. (2022). Encapsulation of Lactobacillus acidophilus in solid lipid microparticles via cryomilling. *Food Chem*. 395, 133564. https://doi.org/10.1016/j.foodchem.2022.133564

[8] Choudhury N, Meghwal M, Das K. (2021). Microencapsulation: An overview on concepts, methods, properties and applications in foods. *Food Frontiers*. 2(4), 426-42. https://doi.org/10.1002/fft2.94

[9] Lu W, Yang X, Shen J, Li Z, Tan S, Liu W, et al. (2021). Choosing the appropriate wall materials for spray-drying microencapsulation of natural bioactive ingredients: Taking phenolic compounds as examples. *Powder Technol*. 394, 562-74. https://doi.org/10.1016/j.powtec.2021.08.082

[10] Fathi F, Saberi-Riseh R, Khodaygan P. (2021). Survivability and controlled release of alginate-microencapsulated Pseudomonas fluorescens VUPF506 and their effects on biocontrol of Rhizoctonia solani on potato. *Int J Biol Macromol*. 183, 627-34. https://doi.org/10.1016/j.ijbiomac.2021.04.159

[11] Naghibi F, Mosaddegh M, Motamed SM, Ghorbani A. (2022). Labiatae family in folk medicine in Iran: from ethnobotany to pharmacology. *Iran J Pharm Res*. 4(2), 63-79.

[12] Razavi SM, Karazhiyan H. (2009). Flow properties and thixotropy of selected hydrocolloids: Experimental and modeling studies. *Food hydrocoll*. 23(3), 908-12. https://doi.org/10.1016/j.foodhyd.2008.05.010

[13] Farhadi, N. (2017). Structural elucidation of a water-soluble polysaccharide isolated from Balangu shirazi (*Lallemantia royleana*) seeds. Food Hydrocoll. 72, 263-270.https://doi.org/10.1016/j.foodhyd.2017.05.028

[14] Razavi SMA, Cui SW, Ding H. (2016). Structural and physicochemical characteristics of a novel water-soluble gum from Lallemantia royleana seed. *Int J Biol Macromol*. 83, 142-51. https://doi.org/10.1016/j.ijbiomac.2015.11.076

[15] Al-Sahlany St, Niamah A. (2022). Bacterial viability, antioxidant stability, antimutagenicity and sensory properties of onion types fermentation by using probiotic starter during storage. *Nutr Food Sci*. 52, 901-16. https://doi.org/10.1108/NFS-07-2021-0204

[16] Dokoohaki ZN, Sekhavatizadeh SS, Hosseinzadeh S. (2019). Dairy dessert containing microencapsulated Lactobacillus rhamnosus (ATCC 53103) with quince seed mucilage as a coating material. *LWT*. 115, 108429. https://doi.org/10.1016/j.lwt.2019.108429

[17] Karimi M, Sekhavatizadeh SS, Hosseinzadeh S. (2021). Milk dessert containing Lactobacillus reuteri (ATCC 23272) encapsulated with sodium alginate, Ferula assa-foetida and Zedo (Amygdalus scoparia) gum as three layers of wall materials. *Food Bioprod Process*. 127, 244-54. https://doi.org/10.1016/j.fbp.2021.03.003

[18] Abbaszadeh S, Gandomi H, Misaghi A, Bokaei S, Noori N. (2014). The effect of alginate and chitosan concentrations on some properties of chitosan-coated alginate beads and survivability of encapsulated Lactobacillus rhamnosus in simulated gastrointestinal conditions and during heat processing. *J Sci Food Agric*. 94(11), 2210-6. https://doi.org/10.1002/jsfa.6541

[19] Ilha EC, Da Silva T, Lorenz JG, de Oliveira Rocha G, Sant'Anna ES. (2015). Lactobacillus paracasei isolated from grape sourdough: acid, bile, salt, and heat tolerance after spray drying with skim milk and cheese whey. *Eur Food Res Technol*. 240(5), 977-84. https://doi.org/10.1007/s00217-014-2402-x

[20] Kim JU, Kim B, Shahbaz HM, Lee SH, Park D, Park J. (2017). Encapsulation of probiotic Lactobacillus acidophilus by ionic gelation with electrostatic extrusion for enhancement of survival under simulated gastric conditions and during refrigerated storage. *Int J Food Sci Tech*. 52(2), 519-30. https://doi.org/10.1111/ijfs.13308

[21] Arepally D, Reddy RS, Goswami TK. (2020). Encapsulation of Lactobacillus acidophilus NCDC 016 cells by spray drying: characterization, survival after in vitro digestion, and storage stability. *Food Funct.* 11(10), 8694-706. https://doi.org/10.1039/D0FO01394C

[22] Frakolaki G, Kekes T, Lympaki F, Giannou V, Tzia C. (2022). Use of encapsulated Bifidobacterium animalis subsp. lactis through extrusion or emulsification for the production of probiotic yogurt. *J Food Process Eng*. 45(7), e13792. https://doi.org/10.1111/jfpe.13792

[23] Sohail A, Turner MS, Coombes A, Bostrom T, Bhandari B. (2011). Survivability of probiotics encapsulated in alginate gel microbeads using a novel impinging aerosols method. *Int J Food Microbiol*. 145(1), 162-8. https://doi.org/10.1016/j.ijfoodmicro.2010.12.007

[24] Pupa P, Apiwatsiri P, Sirichokchatchawan W, Pirarat N, Muangsin N, Shah AA, et al. (2021). The efficacy of three double-microencapsulation methods for preservation of probiotic bacteria. *Sci Rep*. 11(1), 1-9. https://doi.org/10.1038/s41598-021-93263-z

[25] Razavi, S. M. A., Cui, S. W., & Ding, H. (2016). Structural and physicochemical characteristics of a novel water-soluble gum from *Lallemantia royleana* seed. *Int J Biol Macromol*. 83, 142-151. https://doi.org/10.1016/j.ijbiomac.2015.11.076

[26] Zameni, A., Kashaninejad, M., Aalami, M., Salehi, F., & Shirvani, G. (2015). Rheological Properties, Texture and Color of Balangu (*Lallemantia royleana*) Seed Gum Affected by Different Temperatures. *Iran J Biosyst. Eng*. 46(2), 185-192. https://doi.org/10.22059/ijbse.2015.55678

[27] Rodríguez-Huezo M, Lobato-Calleros C, Reyes-Ocampo JG, Sandoval-Castilla O, Perez-Alonso C, Pimentel-Gonzalez D. (2011). Survivability of entrapped Lactobacillus rhamnosus in liquid-and gel-core alginate beads during storage and simulated gastrointestinal conditions. *Revista mexicana de ingeniería química*. 10(3), 353-61. https://doi.org/10.1007/s11694-021-01022-5

[28] Nasiri H, Golestan L, Shahidi SA, Darjani P. (2021). Encapsulation of *Lactobacillus casei* in sodium alginate microcapsules: improvement of the bacterial viability under simulated gastrointestinal conditions using wild sage seed mucilage. *J Food Measure Character*. 2021 15(5):4726-34. https://doi.org/10.1007/s11694-021-01022-5

[29] Lupo B, Maestro A, Gutiérrez JM, González C. (2015). Characterization of alginate beads with encapsulated cocoa extract to prepare functional food: Comparison of two gelation mechanisms. *Food Hydrocolloids*. 49, 25-34. https://doi.org/10.1016/j.foodhyd.2015.02.023

[30] Mizielińska M, Łopusiewicz Ł. (2018). Encapsulation and evaluation of probiotic bacteria survival in simulated gastrointestinal conditions. *Rom Biotechnol Lett*. 23(3), 13690-96.

[31] Ji R, Wu J, Zhang J, Wang T, Zhang X, Shao L, et al. (2019). Extending viability of Bifidobacterium longum in chitosan-coated alginate microcapsules using emulsification and internal gelation encapsulation technology. *Front Microbiol*. 10, 1389. https://doi.org/10.3389/fmicb.2019.01389

[32]. Wang M, Yang J, Li M, Wang Y, Wu H, Xiong L, et al. (2019). Enhanced viability of layer-by-layer encapsulated Lactobacillus pentosus using chitosan and sodium phytate. *Food Chem*. 285, 260-5. https://doi.org/10.1016/j.foodchem.2019.01.162

[33] Soltani Lak A, Marhamatizadeh MH, Fattahi H. (2021). Stability of Encapsulated Lactobacillus reuteri during Harsh Conditions, Storage Period, and Simulated In Vitro Conditions. *J Food Qual*. 2021. https://doi.org/10.1155/2021/3872190

[34] Hansen LT, Allan-Wojtas P, Jin Y-L, Paulson A. (2002). Survival of Ca-alginate microencapsulated Bifidobacterium spp. in milk and simulated gastrointestinal conditions. *Food Microbiol*. 19(1), 35-45. https://doi.org/10.1006/fmic.2001.0452

[35] Li K, Wang B, Wang W, Liu G, Ge W, Zhang M, et al. (2019). Microencapsulation of Lactobacillus casei BNCC 134415 under lyophilization enhances cell viability during cold storage and pasteurization, and in simulated gastrointestinal fluids. *Lwt*. 116, 108521. https://doi.org/10.1016/j.lwt.2019.108521

[36] El-Shafei K, Elshaghabee FMF, El-Sayed HS, Kassem JM. (2018). Assessment the viability properties of Lactobacillus casei strain using labneh as a carrier. *Acta Scientiarum Polonorum Technologia Alimentaria*. 17(3), 267-76. https://doi.org/10.17306/J.AFS.2018.0583

[37] Silva MP, Tulini FL, Martins E, Penning M, Favaro-Trindade CS, Poncelet D. (2018). Comparison of extrusion and co-extrusion encapsulation techniques to protect Lactobacillus acidophilus LA3 in simulated gastrointestinal fluids. *LWT*. 89, 392-9. https://doi.org/10.1016/j.lwt.2017.11.008

[38] Gbassi GK, Vandamme T, Ennahar S, Marchioni E. (2009). Microencapsulation of Lactobacillus plantarum spp in an alginate matrix coated with whey proteins. *Int J Food Microbiol*. 129(1), 103-5. https://doi.org/10.1016/j.ijfoodmicro.2008.11.012

[39] Gebara C, Chaves KS, Ribeiro MCE, Souza FN, Grosso CR, Gigante ML. (2013). Viability of Lactobacillus acidophilus La5 in pectin-whey protein microparticles during exposure to simulated gastrointestinal conditions. *Food Res Int*. 51(2), 872-8. https://doi.org/10.1016/j.foodres.2013.02.008

DOI: http://dx.doi.org/ 10.22104/IFT.2023.5978.2127

فصلنامه فناوری های جدید در صنعت غذا، دوره ۱۱، شماره ۱، صفحه ۲۴-۱۱ پاییز ۱۴۰۲

مقاله پژوهشی

ارزیابی خواص فیزیکوشیمیایی باکتری لاکتوباسیلوس اسیدوفیلوس ریزدرون پوشانی شده با آلژینات سدیم و موسیلاژ دانه بالانگو

سید سعید سخاوتی زاده*[1]، تینا یعقوب پور[2]

۱. مرکز تحقیقات و آموزش کشاورزی و منابع طبیعی فارس، سازمان تحقیقات، آموزش و ترویج کشاورزی، AREEO، شیراز، فارس، ایران

۲. گروه علوم بالینی، دانشکده دامپزشکی، دانشگاه شیراز، فارس، ایران

(تاریخ ارسال:۱۴۰۱/۰۹/۰۶، تاریخ آخرین بازنگری: ۱۴۰۱/۱۱/۰۸، تاریخ پذیرش: ۱۴۰۱/۱۱/۱۸)

چکیده

این مطالعه با هدف بهبود میزان ماندگاری باکتری لاکتوباسیلوس اسیدوفیلوس در شرایط سخت انجام شد؛ بنابراین، از روش ریزدرون پوشانی اکستروژن به همراه موسیلاژ دانه بالنگو (*Lalemantia royleana*) به عنوان لایه دوم ریزدرونپوشانی در غلظت‌های ۰/۲، ۰/۴، ۰/۶ و ۰/۸ درصد استفاده شد. خواص فیزیکوشیمیایی و بقای باکتری در فرم‌های آزاد و ریزدرونپوشانی شده در شرایط شبیه‌سازی معده‌ای رودهای ارزیابی شد. نتایج نشان داد که دانه‌های تولیدشده کروی بودند. افزایش غلظت موسیلاژ دانه بالنگو، قطر دانک را به طور معنیداری افزایش داد، اما پارامتر L^* ثابت بود. فرم‌های آزاد و ریزدرونپوشانی شده باکتری در دمای ۷۲ درجه سانتی‌گراد کاهش یافتند. علاوه بر این، باکتری‌های آزاد و باکتریهای ریزدرونپوشانی شده در مدت نگهداری و تحت شرایط شبیه‌سازی شده گوارشی، کاهش تعداد داشتند. شمارش لاکتوباسیلوس اسیدوفیلوس پس از قرار گرفتن در شرایط شبیه‌سازی‌شده گوارشی، به ترتیب سطوح ۶/۴۷ و ۴/۶۵ واحد لگاریتمی کاهش داشتند. لاکتوباسیلوس اسیدوفیلوس ریزدرونپوشانی شده توانایی بقای ۷۷/۵۶٪ را در محیط مایع MRS طی ۲۸ روز نگهداری در سردخانه داشت. نتایج این مطالعه نشان داد که استفاده از موسیلاژ دانه بالنگو می‌تواند توانایی بقای لاکتوباسیلوس اسیدوفیلوس ریزدرون پوشانی شده را در شرایط تنش گرمایی (۴۳/۲۲٪)، شرایط نمک و اسید (۲۲/۵۸٪)، نگهداری در یخچال (۲۴/۷۶٪) و شبیه‌سازی شرایط گوارشی(۱/۴۶٪) نسبت به باکتری آزاد افزایش دهد. دانک حاوی موسیلاژ بالنگو در شرایط شبیه‌سازی شده گوارشی میزان بقای بیشتری را نسبت به دانک حاوی موسیلاژ بهدانه داشت؛ اما در دمای ۷۲ درجه سانتی‌گراد از نرخ بقای باکتری کمتری برخوردار بود.

واژگان کلیدی: **لاکتوباسیلوس اسیدوفیلوس؛ ریزدرونپوشانی؛ *royleana Lallemantia*؛ شرایط مشابه معده‌ای روده‌ای؛ اکستروژن.**

* نویسنده مسئول: s.sekhavati@areeo.ac.ir