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BIOTECHNOLOGY AND INDUSTRIAL MICROBIOLOGY - RESEARCH PAPER

Encapsulation of probiotic *Lactobacillus acidophilus ATCC 4356* in alginate–galbanum (*Ferula Gummosa* Boiss) gum microspheres and evaluation of the survival in simulated gastrointestinal conditions in probiotic Tahini halva

Seyed Saeed Sekhavatizadeh[1] · Forough Afrasiabi[2] · Zahra Montaseri[3]

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Abstract

One of the famous traditional confectionery products is Tahini halva. The aim of this study was the production of probiotic halva using free *Lactobacillus acidophilus* (FLA) and microencapsulated *Lactobacillus acidophilus* (MLA) with sodium alginate and galbanum gum as the second layer. The survival rate of MLA and FLA during heat stress, storage time, and simulation gastrointestinal condition in Tahini halva was assessed. The survival rates of MLA and FLA under heat stress were 50.13% and 34.6% respectively. During storage in Tahini halva, the cell viability loss was 3.25 Log CFU g^{-1} and 6.94 Log CFU g^{-1} for MLA and FLA, separately. Around 3.58 and 4.77 Log CFU g^{-1} bacteria were reduced after 6 h of exposure in simulated gastrointestinal conditions, for MLA and FLA respectively. These results suggest that the use of alginate and galbanum gum is a promising approach to protecting *L. acidophilus* against harsh environmental conditions.

Keywords *Lactobacillus acidophilus* · Microencapsulation · Galbanum gum · Gastrointestinal condition

Introduction

It is well known that the consumption of adequate amounts of probiotics as a common ingredient in functional foods has deliberate general health benefits. These health benefits are including reduction of *Helicobacter pylori* infection, prevention of gastrointestinal cancer, reduction of lactose intolerance, reduction of symptoms of inflammatory bowel disease, and prevention of diarrhea [1]. One of the most important bacteria which successfully colonize the gastrointestinal tract of humans since birth is *Lactobacillus* spp. They are Gram-positive, rod bacilli, homofermentative, and catalase-negative microorganisms, from which, *Lactobacillus acidophilus* (LA) is classified as thermophilic strains growing well at 30–45 °C and pH 4–5 [2]. The *Lactobacillus* has many functions such as alleviating metabolic disorders, reducing fat accumulation, resistance to oxidative stress and toxicity, alleviating inflammation, protecting against pathogenic infections, and improving cognition, and neurodegenerative diseases [3]. The most common probiotic products using LA are yogurt, various kinds of cheese like white, minas fresh, cheddar, Gouda cheese, vegetable-based drinks, sweet acidophilus milk, frozen desserts, soy-based drink, and oat-based puddings. The beneficial effects of *L. acidophilus* supplements are currently emphasized by researchers, elsewhere [4]. Its survival depends on numerous factors, including storage and growth conditions, such as stabilizers, temperature, relative humidity, and oxygen content. This is a very important advantage as functional products contain which to remain within the range 10^6–10^7 CFU mL^{-1} by the expiration date of the product [2]. Moreover, these bacteria should remain to persist under the harsh condition of the gastrointestinal tract of the hosts, and thus the application

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✉ Seyed Saeed Sekhavatizadeh
s.sekhavati@areeo.ac.ir

[1] Fars Agricultural and Natural Resources Research and Education Center, AREEO, Post Box: 7155863511, Shiraz, Fars, Iran

[2] Food Science and Technology, Islamic Azad University, Sarvestan, Fars, Iran

[3] Department of Infectious Diseases, School of Medicine, Fasa University of Medical Sciences, Fasa, Iran

of encapsulation techniques is a crucial step in the survival of probiotics [5].

Microencapsulation is a technique for the protection of bioactive compounds and bacteria from moisture, oxygen, light, and several other environmental factors. Microencapsulation acts as a barrier to control release, solubility, bioavailability, ease of handling, and transport, and can also hide unpleasant tastes and aromas when microcapsules are incorporated into food. In most cases, the growth of probiotic bacteria is reduced because the bacteria do not have ideal growth conditions [6]. There are many techniques available for microencapsulation. One of the methods for bacterial encapsulation is extrusion. This technique is used to encapsulate probiotics in microspheres. Different approaches currently applied to achieve this purpose are related to size, dispersal size, production scale, and the maximum tolerance of probiotics against shearing [7]. Low cost, easy to use, and simplicity, the high survival rate of the encapsulated cells is guaranteed in this relatively mild process. Essentially, in this technique a hydrocolloid solution containing a microbial culture is extruded through a nozzle in a cross-linking solution, providing an immediate transfer from the hydrocolloid solution to a gel and ultimately forming a sphere [8]. Different kinds of materials have been used to encapsulate probiotics. Among them, alginate is the most popular coating material because it is simple, low cost, generally available, not toxic, and acceptable as a food additive [9]. Alginates are linear binary copolymers consisting of β-D-mannuronic acid and α-L-guluronic acid [10]. Providing an additional coating of the second layer to the alginate microcapsules addresses these issues. For example, the chitosan-coated alginate capsules reduce the porosity of alginate microcapsules and provide better physicochemical stability during gastric transit than capsules solely made up of alginate [11]. Galbanum (*Ferula gummosa* Boiss) gum is one of the materials that may be used in the second layer of the bead. *Ferula gummosa* (FG) is recognized as Ghasni, Balijeh, Barijeh, and Barzard in Persian. It is classified as the *Ferula* genus in the family *Apiaceae*. At least 112 genera, including aromatic plants with hollow stems and umbrella inflorescences, are presented in this family. FG shows important applications in cosmetics, pharmaceuticals, and animal breeding. Few pharmaceutical applications of FG-based products are including pain relievers mainly in the joints and stomach, mucolytics, and energizers. The resin of FG is also used as a cosmetic in skin care creams [12]. Galbanum gum is an aromatic gum that is produced from traditional Iranian species with the common Iranian names "Barijeh" (*Ferula gummosa, Ferula persica* and *Ferula tabasensis*) [13]. Galbanum occurs usually in hard or soft, irregular, more or less translucent and shining lumps, occasionally in separate tears, of a light-brown, yellowish or greenish-yellow color, and a specific gravity of 1.212. It contains about 8% of terpene, 67–69% resin which contains sulfur, 17–19% of gum, and 3–6% of volatile oils. One of the advantages of galbanum gum is the swelling index. It swelled to about 190% of the initial volume in distilled water. Thus, galbanum gum can hydrate and swells in cold water [14].

In microencapsulation, suitable polymeric materials protect their core from harmful environmental conditions (e.g., reducing acid-induced degradation of probiotics by gastric fluid in the stomach) and against encapsulated materials. To achieve higher encapsulation efficiency, it must be inactive and able to control the release of the encapsulation. However, the use of the optimal material is essential for the encapsulation of probiotics as key parameters to ensure an efficient delivery strategy, where the rough conditions and GI stimuli are principally avoided [15].

Sesame seed is currently used to produce various commodities such as sesame oil, roasted seeds, and Tahini halva in industries [16]. Tahini halva is an old homogenous solid confection with a thin fibrous structure and made up of Tahini (sesame seeds pasts >45%) sugar (45–55%) [17, 18]. This halva is rich in fats, carbohydrates and has high levels of essential amino acids such as methionine and lysine [19]. It is widely consumed in North Africa, Mediterranean countries, the Middle East, and Asia as a nutritious breakfast and dinner [20]. In addition, the consumption of this halva or Tahini (sesame seed paste), its major ingredient, has increased in European countries and the USA due to its valuable nutrition and health properties [21]. Tahini was made by adding sugar syrup, prepared from crystal sugar containing 10–15% water. Excess water is removed from the solution to obtain the desired structural wax. Next, a 0.1% soapwort extract was added to the solution for providing a sugar-bleaching solution which finally changed color to fully white. The resulting network was mixed in the ratio of 1: 1, and the flavoring agents were then added to the mixture. The final structure of the Tahini halva was molded and packed [19]. It may also be diversified by adding cocoa, flavors, and nuts [17]. In some research, the probiotic Tahini halva was produced. For example, Khaji et al. (2021) investigated the possibility of developing a novel functional sesame paste-based dessert-bearing probiotic bacteria (*Lactobacillus paracasei* and *Bifidobacterium lactis*). The use of mixed cultures in the dessert formulation could guarantee acceptable probiotic viability and provide better texture attributes. Moreover, the probiotic Tahini halva composed of sesame seeds, wheat germ, and olive oil and fermented by *B. longum* might have anti neurodegeneration properties in Alzheimer's disease [22, 23].

The novelty of this study was to investigate the effect of *Lactobacillus acidophilus* (as a free and microencapsulated form) on the textural and sensory properties of Tahini halva, in order to determine its technological suitability for the production of probiotic Tahini halva.

Due to the hot filling packaging of Tahini halva, no research has been done on the production of probiotic Tahini halva. Therefore, the present study investigated the effects of free *L. acidophilus* (FLA) and microencapsulated *L. acidophilus* (MLA) on the viability of LA, physicochemical, and textural properties of Tahini halva. These experiments were assessed during 1, 7, 14, 21, and 28 days of storage. In addition, sensory evaluation of all Tahini halva samples was run during storage.

Materials and methods

Material

Lyophilized *L. acidophilus* ATCC 4356 *was* prepared from the Persian Type Culture Collection (PTCC)s, Tehran, Iran. MRS agar (de Man, Rogosa, and Sharp, 1960), peptone water, MRS broth, and sodium citrate were purchased from Merck company (Merck, Darmstadt, Germany). Sodium alginate, clindamycin, and ciprofloxacin were prepared by Sigma Company (Sigma, Steinheim, Germany). Galbanum was purchased from a local market (Shiraz, Iran). Pepsin was obtained from Merck (Darmstadt, Germany). All other chemicals were at the analytical quality provided by Merck (Darmstadt, Germany).

Preparation of bacterial inocula

Under the aerobic condition, LA was grown in the final clindamycin, and ciprofloxacin concentration of MRS-clindamycin-ciprofloxacin (MRS-CC) was 0.1 mg L^{-1} or 10.0 mg L^{-1} respectively. The MRS-CC was used as a selective enumeration media for *L. acidophilus.* Because the addition of clindamycin and ciprofloxacin does not affect the growth of *Lactobacillus acidophilus.* But they suppressed the growth of unwanted bacteria (Cichońska et al. 2022; Izadi et al. 2014; Varga et al. 2014). This selective media was used for the cultivation of LA at 37 ° C for 72 h, under aerobic conditions, which helps to prevent the growth of food-borne pathogens [16]. The preparation was then centrifuged at 2264 g, 4 °C for 10 min. It was washed twice with sterile saline before re-suspending in peptone water (0.1%).

Microencapsulation method

Microencapsulation of LA was performed using the extrusion techniques as follows. Five milli of LA culture (9×10^{10} CFU mL^{-1}) was added to 15 ml of 2% 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 h, and the beads were washed with 0.1% peptone water and gently shaken at 1 g for 40 min in (0.2, 0.4, 0.6, 0.8, and 1% w/w) galbanum gum 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 (1).

$$EY = (Log\ N / Log\ N0) \times 100 \quad (1)$$

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) [24].

Light microscopy and TEM

The size and shape of the beads are obtained from the microscope (Olympus Optical Microscope BX51, Japan) image. For the determination of the galbanum layer dimension, the outer layer was measured by micro-measure version 1.07 software. The beads aspect ratio was determined by the length-to-width ratio of 20 beads [11]. To perform the transmission electron microscopy (TEM) and also to avoid the destructive effects of fixative solutions, on the adjacent layers of microencapsulated cells, the method of Karimi et al. (2021) was used as follows: MLA was initially dehydrated in a series of ethanol (50, 75, and 95%) for 20 min and separately immersed in pure ethanol for 20 min. It was then inserted in Spurr resin (in the ratio of 1:3 for 10 min, 1:1 for 30 min, and 3:1 for 24 h at room temperature) which was finally placed in the pure Spurr resin at 70 °C for 2 h. The silver-gold sections on an ultra-microtome were prepared using a diamond knife (om u3, C.reichert, Austria). Next, the cut section was placed on a naked copper grid. A saturated methanolic uranyl acetate solution was used to stain the end of the section and post-stained using Reynold's lead citrate for 10 min which was subsequently viewed in a Philips 410 TEM operated at 80 kV. Images were finally analyzed on a computer program [24].

Heat tolerance

The heat resistance of FLA and MLA was investigated by exposing the organism (~8.9–9.55 Log CFU mL^{-1}) to 72 °C in peptone water (pH 6.4 ± 0.2) (Oxoid Ltd.) for up to 5 min. The tube was then cooled down to 22 °C. A citrate buffer (pH 7.0) was used to prepare the serial dilution of the cooled sample which could aid in the release of encapsulated probiotic bacteria. Bacterial counts were determined for FLA and MLA at (0, 1, 2, 3, 4, and 5 min). Thermostability was determined by comparing the number of free bacteria to the number of microencapsulated bacteria. The experiment was performed 3 times [24].

Osmotic stress conditions

A medium containing high sugar concentration was employed to evaluate the resistance of FLA and MLA cells in the osmotic stress conditions. In particular, 100 g of sugar syrup (concentrated sucrose, without any nitrogen sources and other growth factors) was inoculated with 1 g of MLA and the equal number of bacterial concentration FLA (~ 9 Log CFU g^{-1}) in samples, respectively. The viable cell count was then analyzed in the samples stored for 1, 2, 3, and 4 h at 4 °C promptly. The samples were decimally serially diluted in Ringer, supplemented with 100 g/L sucrose (Sigma) and plate counted [25].

Preparation of Tahini halva

Tahini halva is generally made from two main ingredients including Tahini and sugar syrup. Tahini was prepared from sesame seeds firstly washed and cleaned, dehulled, and roasted at about 100°C, and then cooled, sieved, and ground. Sugar syrup was made by mixing water, sugar, and citric acid at around 140 °C. The Tahini and sugar syrup (6:4, %w/w) were mixed to get Tahini halva. Finally, the halva was kneaded (for 10 min), molded, cooled (at room temperature), and packed. The FLA and MLA were added to Tahini halva before its hardening as the initially estimated concentration of LA was ~ 9 Log CFU g^{-1} for FLA and MLA in Tahini halva before packaging. We chose another Tahini halva as the control (C) (Fig. 1a–c). All samples were stored at 4 °C [26].

Survivability of LA in Tahini halva

The survivability of FLA and MLA in Tahini halva was determined weekly for a total of 28 days. We used clindamycin and ciprofloxacin to prevent the growth of food-borne pathogens. Degrading of the MLA was applied with sodium citrate (0.1% m/v) at the ratio of 1:9 (%v/v) at 37 °C which was then counted using MRS-CC (clindamycin and ciprofloxacin) agar medium incubated aerobically at 37 °C for 48 h [16].

Survival of FLA and MLA under simulated gastrointestinal conditions

For this purpose, 1 g of Tahini halva containing MLA and FLA was added to 9 mL of salt solutions (0.5%) in sterile tubes separately; then, the pH of all samples was adjusted to 1.4 to 1.9 with hydrochloric acid 1 N. Then the enzymes lipase (Fluka 62305) and pepsin (porcine, stomach mucosa, Sigma) were added to the concentration of 0.9 and 300 mg L^{-1}, respectively. The samples were then placed in a shaker incubator (Lab tech, Korea) at 3 g and 37 °C for 2 h (Gastric phase). An alkaline solution (containing 150 mL of NaOH 1N and 14 g disodium hydrogen phosphate) was then used to adjust the pH of the samples to 4.3–5.2. The bile (oxgall, Sigma B838) and Pancreatin 1750 (Pancreatin pancreas, Sigma) with concentrations of 10 and 1 g L^{-1} were prepared and applied, respectively. The samples were placed in a shaker incubator at 3 g and 37 °C for 2 h (first intestinal phase). Finally, at the previous pH, with an alkaline solution, the pH of the samples was increased to 6.5–7.5. Bile and pancreatin were added to samples as the last-mentioned concentrations. Then, they were placed in an incubator at 3 g and 37 °C for 2 h (second intestinal phase). Sampling for bacterial culture and counting was done at 0, 0.5, 1, 2, 4, and 6 h after beads and free cell injection [11, 24]. The survival rate was calculated based on Eq. 2.

$$Survival\ rate\ (\%) = (Log\ \text{CFU}\ N / Log\ CFU\ N0) \times 100 \quad (2)$$

where N is the viable count after the simulation gastrointestinal stage and $N0$ is the initial viable count [27].

Sensory analysis

The sensory analysis of C, MLA, and FLA Tahini halva was performed by 45 professional panelists who were divided into two age groups of 18–24 years and 24–51 years (a total of 42% males and 58% females). The 5-point hedonic scale was applied to the Tahini halva samples. The flavor, odor, color, texture, and overall acceptability were assessed by panelists. A scale of 5 indicated "like extremely" and 1 "1dislike extremely" in comparison with the C Tahini halva.

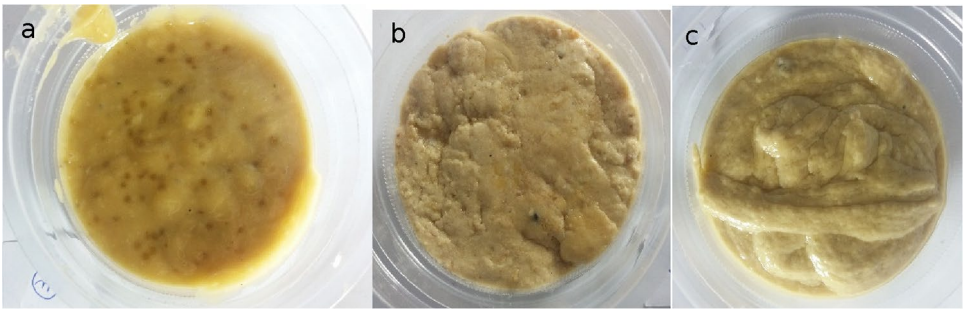

Fig. 1 Microencapsulated *Lactobacillus acidophilus* (**a**); free *Lactobacillus acidophilus* (**b**) and control Tahinin halva (**c**)

The samples were evaluated by the panelists every 7 days during the storage period at 4 °C [11].

Colors

Tahini halva 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.

Texture

Texture Analyzer CT3 (Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA) was used to record the texture profile (TPA). The TPA of the Tahini halva samples was recorded on the first and the 60th day of storage. A sample (diameter 20 ± 0.5 mm, height 10 ± 0.5 mm) was taken from the center of the Tahini halva. Next, the cylindrical Tahini halva was covered with a stretch film and brought to room temperature of 20 ± 1 °C. The following analysis conditions were as follows: TA11/1000 aluminum cylinder probe (25.4 mm in diameter), compression 20% of the initial height, test speed 1 mm s^{-1}, penetration rate 2 mm s^{-1}, pretest speed 2 mm s^{-1}, and retention time 5 s. The textural parameters including hardness (g), chewiness (mJ) adhesiveness (mJ), gumminess (g), springiness (mm), and cohesiveness were obtained from the device [28].

Statistical analysis

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

Results and discussion

Encapsulation yield, light microscopy, and TEM

In this study, microencapsulation efficiency was 93.04 ± 3.06%. A previous study by De Almeida Shoaei et al. (2022) reported that the microencapsulation efficiency of *L. plantarum* coated with Arabic gum and gelatin ranged from 90.97 ± 4.68 to 98.86 ± 2.97%. This was about the same as our result. The formation of thicker layer microcapsules finally led to higher probiotic viability. On the other hand, thicker coatings may protect the survival of probiotics during microencapsulation [29]. MLA photography and light microscope details were shown in Fig. 2. The solutions containing various proportions of galbanum gum can produce whole beads. The LA beads appeared white and were surrounded by a thin layer of membrane. The bead without a galbanum gum layer was selected as a control (Fig. 2a–f). The microbeads had a spherical shape with a smooth and uniform bead surface under a light microscope (Fig. 2g–l). The smooth surface of microbeads is important because the beads with broken surfaces can reduce the viability of encapsulated cells. All beads are spherical (aspect ratio 1.12). This finding is consistent with the results reported by Yin Lai et al. (2022) who have observed that the viscosity of sodium alginate can also affect the shape of microbeads [30]. The second layer average diameter of 10 beads containing various concentrations of galbanum gum was measured respectively. The diameter of the layer increased by galbanum gum concentration (Fig. 3). But no second layer was seen in the control sample second layer. Therefore, the encapsulated bacterial cells were formed in an intact physical barrier.

The internal appearances of coated alginate and galbanum gum bead are shown in the TEM photograph in Fig. 2m. The formation of two distinct layers with entrapped LA is demonstrated in this photograph. Moreover, FLA entrapped in 2 distinct layers, a tick light, and narrow dark lines appeared due to the formation of the alginate and galbanum layer in the beads respectively. The dimension of the alginate layer was greater than galbanum. The FLA was encapsulated properly.

Heat tolerance

Microcapsules usually need to withstand a variety of food processing conditions, including exposure to high temperatures. The heat resistance test results of FLA and MLA are given in Fig. 4A. The FLA was not able to survive in a minimal probiotic concentration (10^6 CFU mL^{-1}) in 72 °C treatment at 2 min. By comparison, microencapsulated MLA showed higher viability during 3 min of treatment at 72 °C and maintained an average count (6 Log CFU mL^{-1}). These results are consistent with the results reported by Morsy et al. (2022) who discovered that microencapsulated probiotics showed higher stability to heat treatment with double alginate microencapsulation [31]. On the contrary, our findings are in contrast to the findings of Wang et al. (2019) who reported that *Lactobacillus pentasus* was able to survive for 30 min 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. The microencapsulated wall material was effectively delaying the penetration of heat into the probiotic cells; however, this may cause a slight loss of viability [32, 33]. The complexity is another factor affecting heat tolerance [34]. Studies suggest that extreme temperatures beyond 65°C are highly damaging to probiotic cells due to thermal stress. Microencapsulation is recommended to shield the probiotics as it can reduce the heat transfer from the surrounding

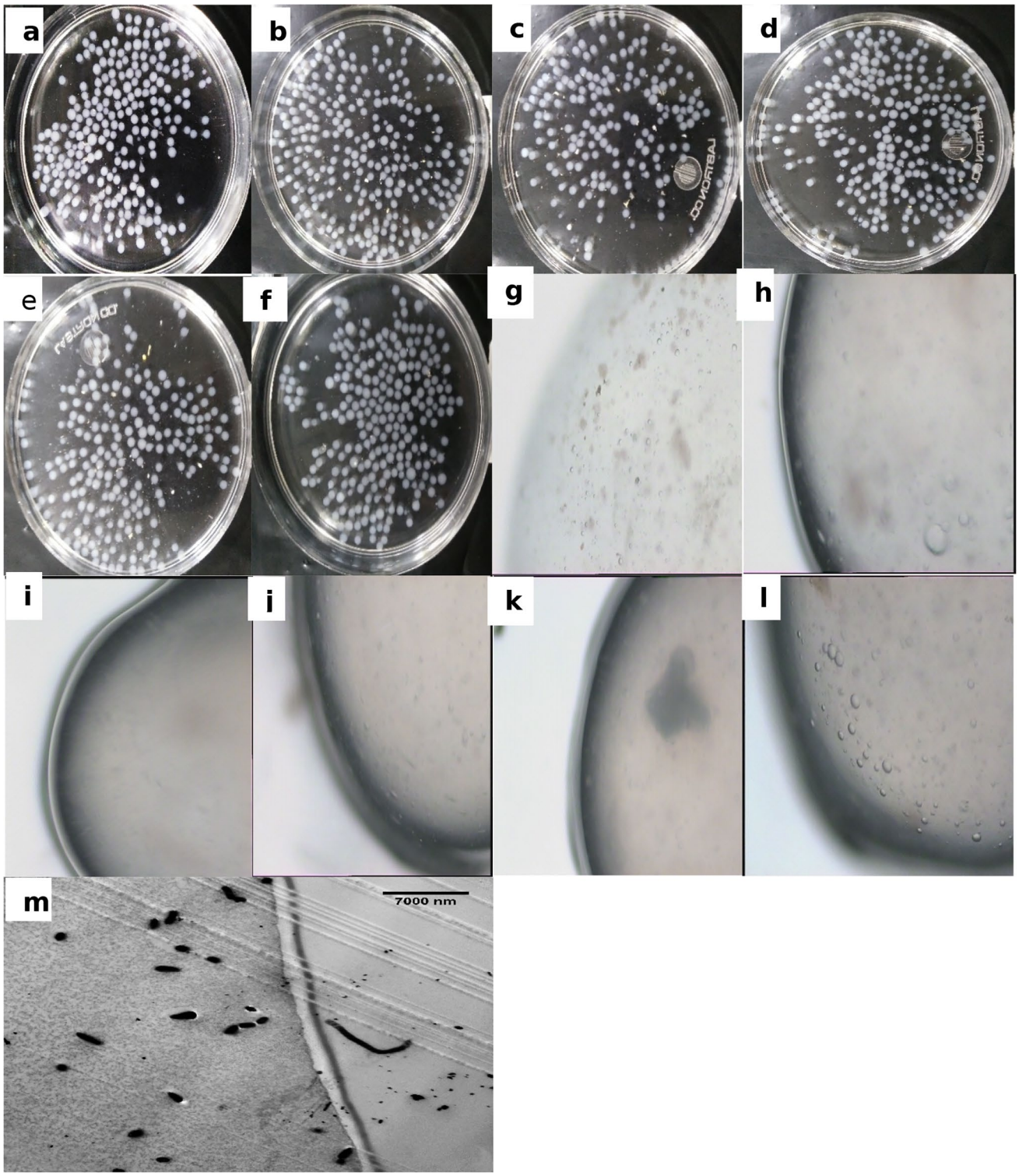

Fig. 2 Photography image (**a–f**), light microscopy (**g–l**), and transmission electron microscope (TEM) images (**m**) of microencapsulated *Lactobacillus acidophilus* (MLA) contain galbanum as a second layer in different concentrations (0.2, 0.4, 0.6, 0.8, and 1) % respectively. The MLA contains 1% galbanum gum as a second layer was used for TEM

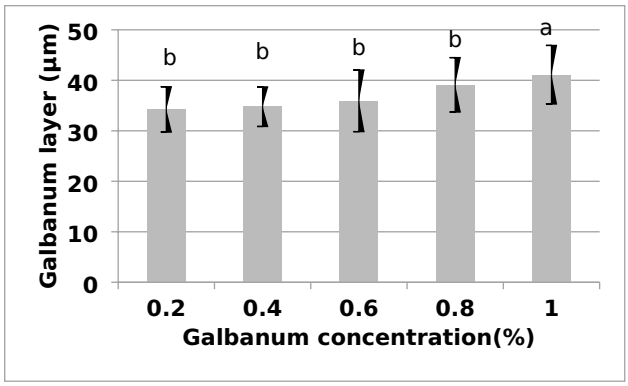

Fig. 3 Galbanum layer diameter in beads (microencapsulated bacteria) contains various concentrations of galbanum (0.2, 0.4, 0.6, 0.8, 1) %. 1. Data (mean ± standard error) are from three replications ($n = 3$). 2. Lowercase letters (a–b) show significant different ($p \leq 0.05$) between beads diameter

medium to the cell interiors [35]. So, the survivability of LA was improved by the microencapsulation technique.

Osmotic stress conditions

The results of this experiment are shown in Fig. 4B. It was observed that both FLA and MLA decreased during the stress time, but the rate of reduction was lower in MLA (25.19%) than in FLA (39.6%). A similar result was reported by De Prisco et al. (2015), who found that at the time of 3 h of exposure to osmotic stress, for free cells, a 0.7 log cycle reduction was observed, but for microcapsules, only a 0.4 log reduction was recorded [25]. Sunny-Roberts and Knorr (2008) reported the loss of *culturability* of *Lactobacillus rhamnosus* at sucrose osmotic stress. The reduction was similar to the results reported here. However, the population heterogeneities or the physiology of individual organisms might underestimate the numbers of actually viable bacteria [36]. In this case, the non-culturable live cells can still display metabolic activity under stress conditions and/or revealed sub-lethal injury. Therefore, we suggest the use

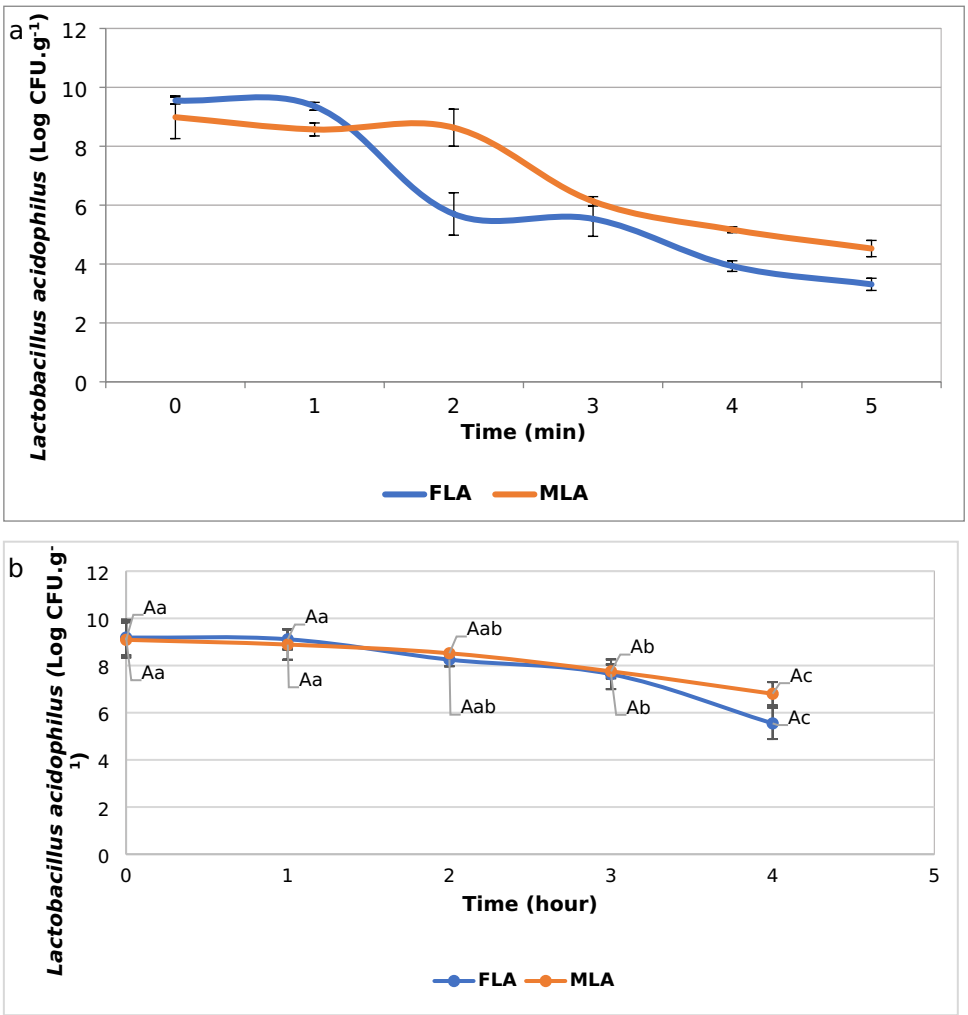

Fig. 4 The free *Lactobacillus acidophilus* (FLA) and microencapsulated *Lactobacillus acidophilus* (MLA) (1%) survive at 72 °C (**a**) and in osmotic pressure (**b**). 1. Data (mean ± standard error) are from three replications ($n = 3$). 2. Uppercase letters (A) show insignificant different ($p > 0.05$) between FLA and MLA treatment at the same time, and lowercase letters (a–c) show significant different ($p \leq 0.05$) between FLA and MLA samples during exposure time to osmotic pressure respectively

of a more accurate method such as flow cytometry to count the actual number of live bacteria [37].

Survivability of MLA and FLA in Tahini halva

Figure 5a shows the extrusion encapsulated method used to evaluate the viability of probiotics at 4 °C for up to 28 days. The reductions of LA in MLA and FLA samples were 3.16 and 6.94 Log CFU g^{-1} during storage time, respectively. The MLA Tahini halva under refrigerated conditions contained a viable count of *L. acidophilus* at an acceptable level (> 10^6 CFU g^{-1}) until 18 days of storage (based on the MLA trend line equation).

The presence of galbanum gum in the particles was very effective to keep the survival of the probiotic culture, as it may reduce the porosity of the particles and therefore decrease the susceptibility of microorganisms to harsh environmental conditions. This result indicates that a similar result was also observed by Silva et al. (2018) who reported *L. acidophilus* 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} [38].

Survival of LA under simulated gastrointestinal conditions

The mean values of the survival rate of FLA and MLA exposed to the simulated gastrointestinal conditions (SGI)

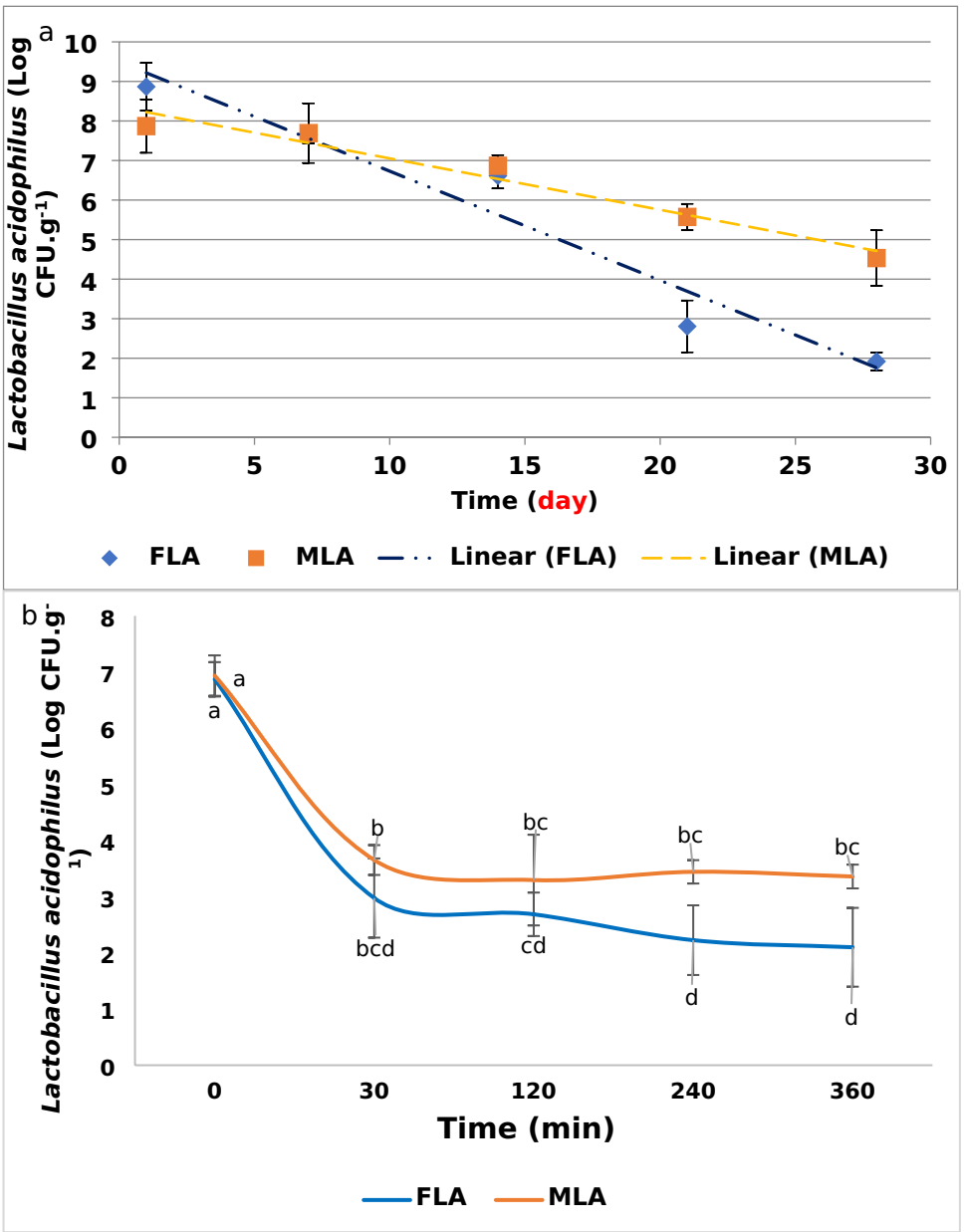

Fig. 5 The free *Lactobacillus acidophilus* (FLA) and micro-encapsulated *Lactobacillus acidophilus* (MLA) survive at 4 °C (**a**) and in simulated gastrointestinal condition (**b**) in Tahini halva. 1. Data (mean ± standard error) are from three replications ($n = 3$). 2. Lowercase letters (**a**–**d**) show significant different ($p \leq 0.05$) between FLA and MLA samples during exposure time in simulated gastrointestinal condition

are presented in Fig. 5b. This study found that survival rates for both free and microencapsulated probiotics were reduced during the SGI state. Contact with gastrointestinal fluid can reduce bacterial survival due to factors such as gastric acidity, enzyme activity, environmental competition, and small intestinal bile salts [39]. The FLA survival rate (43.33, 39.11, 32.41, 30.57%) was lower than MLA (52.67, 47.53, 49.63, 48.43%) at (0.5, 1, 2, 4, and 6 h) respectively. The results of this study showed that microencapsulation enhances probiotic protection because it is observed that there is less reduction in survival. The formation of capsules and entrapping of the bacteria was the main factor in the protection of the bacteria against the gastric low pH and enzymes. Moreover, the gum prevents the diffusion of bile salt solution into the beads. The FLA and MLA survival was significantly reduced with the gastric condition for the first 30 min ($p \leq 0.05$) [40, 41]. Wall material and its concentrations are two factors that are responsible for *Lactobacillus acidophilus* survival under simulated gastric conditions [42]. After that, survival rates gradually declined until they were exposed to SGI conditions for 4 h. Again, the protective effect of galbanum gum in the intestinal condition was observed. These results are also consistent with the findings of Saeed et al. (2022) and Afzaal et al. (2019) [43, 44].

Sensory analysis of Tahini halva

Differences in the flavor, odor, texture, color, and total acceptability of samples in each Tahini halva sample were observed during the storage (Fig. 6). The highest mean score (flavor, odor, texture, color, and total acceptability) among Tahini halva samples belong to the control (C) sample in the 28th of storage time. Flavor, odor, color, and total acceptability were constant over time, but texture decreased in all samples. The beads appeared in the structure of microencapsulated bacteria without melting. The appearance/taste of MLA was considered a defect in the halva by the panelists. Similar results were reported by Kılıç, et al. (2022) who observed yogurt containing encapsulated bacteria had lower scores for each sensory trait than yogurt containing free starter culture [45]. The grain texture was seen in MLA halva. One of the main reasons for this finding may be the existence of beads containing galbanum gum. In addition, calcium ions which may be present in some sodium alginate gels used for encapsulation are probably responsible for the MLA graininess in Tahini halva. In the latest research, replacing sodium in hydrogel alginate with calcium ions may have increased graininess [24]. As such, the increased gritty mouthfeel of the Tahini halva happened due to the diffusion of the galbanum gum from the capsule into the Tahini halva matrix. These results agree with Kailasapathy (2006) [46].

Color

The individual color of whiteness, redness-green, and yellowish-blue appearance of the samples is estimated by L *, a *, and b * values. The incorporation of MLA significantly increased ($p > 0.05$) the values of L* in the Tahini halva on the first and the 28th day (Table 1). It is probably due to water absorption by MLA during the storage time. This is because the L* value of food is related to the amount of free water on the surface of the product. This type of water is more important than the water that exists in the whole product. On the 28th day, the MLA surface adsorbed the water. So, the MLA whiteness had the highest value among the samples [24]. In FLA, the amount of bacterial preparation was too low to substantially change the L* value. The a* and b* parameters were the highest in MLA samples. The * parameter increased in MLA samples during the storage time. However, there are no significant differences ($p \leq 0.05$) in the b* parameter observed in the MLA sample during the storage time. The galbanum exists as yellow to red, translucent viscous, and tears [47]. So, MLA increased the a* and b* parameters.

Texture

Texture analyses of three types of Tahini halva are present in (Table 1). Hardness, cohesiveness, springiness, gumminess, chewiness, and adhesiveness were analyzed. Through many texture parameters, hardness [g] is one of the main important characteristics being defined as the maximum load required to compress a sample. For hardness tests performed, the MLA Tahini halva sample recorded the highest values of hardness (742.25+24.25 g and 1242.88+91.12) g, on the first and last day of storage respectively. Hardness was increased in each sample during the storage time. In a similar study, Mureșan et al. (2015) recorded the hardness of Tahini halva manufactured from low particle size Tahini was 0.89 kg, which is in agreement with the current study [48]. All texture parameters increased during storage time, except adhesiveness. A study by Guneser and Zorba (2014) revealed that storage enhances the penetration force in Tahini halva samples. The separation of oil from the Tahini halva sample and a slow increase in the water content of the sample may be the main reasons for the increase in hardness during storage [49]. Because probiotic Tahini halva has not been produced, there is no literature related to probiotic Tahini halva texture, to compare the obtained results. However, for a similar product, probiotic dessert is used for comparison. Significant changes in texture parameters were expected due to changes in the cross-linking pattern of the halva protein. The incorporated beads seem to make Tahini halva harder, which was likely associated with a decrease in the amount of absorbent in the network and an increase in the solid

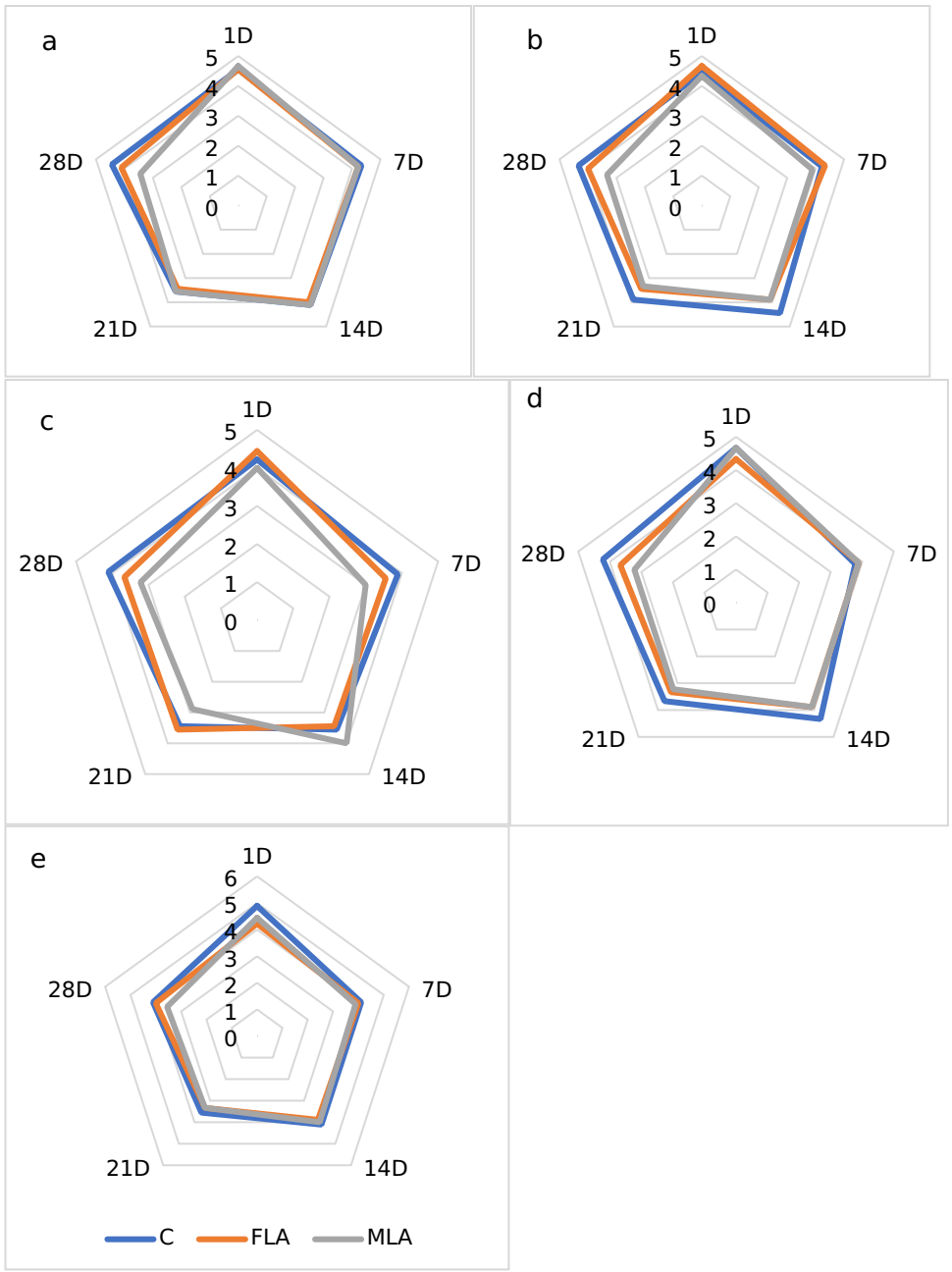

Fig. 6 Sensory characteristics (**a**: flavor; **b**: color; **c**: texture; **d**: odor; **e**: overall acceptability) of free *Lactobacillus acidophilus* (FLA), microencapsulated *Lactobacillus acidophilus* (MLA), and control (C) tahini halva during the storage time at 4 °C (n=45) (D=days)

structure (beads). Moreover, the hardness (1.95–6.80) was increased in the gelatin desserts incorporating microencapsulated *Lactobacillus fermentum* beads following storage at 4 °C for 25 days. In addition, dairy desserts containing microencapsulated *Lactobacillus rhamnosus* increased in hardness during the 21 days of storage [11, 50].

In contrast, applying FLA and MLA reduced the adhesive texture profile, indicating that the stickiness and adhesiveness of the sample were reduced. Adhesive parameters increased during storage. Aragon-Alegro et al. (2007) focus on condensed milk-based ready-to-eat desserts and show that desserts become more sticky during storage [51].

Cohesiveness was equal among the samples on the 28th day of storage. This result matched previous studies of dairy desserts with encapsulated *Lactobacillus reuteri* [24].

The greatest values of gumminess and chewiness were shown in MLA dessert during the storage time. Although hardness and cohesiveness parameters affected the gumminess. The cohesiveness value was remarkably the same in all kinds of desserts. So, the hardness parameter was the

Table 1 Color and texture analysis in supplemented Tahini halva samples during storage time

Physical parameters	Samples	Day 1	Day 28
(L*)	C	58.00± 7.53 ^{ab}	59.44± 4.59^{a}
	FLA	58.33±13.55^{ab}	60.78± 12.95^{a}
	MLA	58.00 ± 2.18^{ab}	49.89±4.96^{b}
(a*)	C	4.00 ± 1.79^{c}	1.78±1.30^{cd}
	FLA	2.33±2.06^{d}	2.11 ± 1.96^{d}
	MLA	6.33 ± 2.29^{b}	9.44± 2.60^{a}
(b*)	C	28.50± 2.01^{c}	34.33± 2.55^{b}
	FLA	29.78±3.53^{c}	33.00 ± 3.32^{b}
	MLA	40.78±3.67^{a}	39.89± 2.03^{a}
Hardness (g)	C	469.38±16.63^{d}	521.75±18.75^{cd}
	FLA	550.25±14.00^{cd}	642.75±9.00^{bc}
	MLA	742.25±24.25^{b}	1242.88±91.12^{a}
Adhesiveness (mJ)	C	58.50±1.0^{a}	43.80±0.59^{b}
	FLA	28.52±0.91^{c}	23.95±1.06^{d}
	MLA	17.66±1.55^{e}	12.46±1.32^{e}
Cohesiveness	C	0.73±0.03^{a}	0.75±0.04^{a}
	FLA	0.55±0.06^{b}	0.74±0.05^{a}
	MLA	0.69±0.07^{ba}	0.65±0.03^{ba}
Chewiness	C	38.41±3.05^{d}	43.36±1.83^{c}
	FLA	50.62±0.77^{c}	89.97±3.14^{b}
	MLA	96.85±2.47^{b}	153.48±6.21^{a}
Springiness (mm)	C	15.44±0.33^{ab}	16.44±0.37^{a}
	FLA	12.70±0.25^{c}	14.46±0.5^{b}
	MLA	11.80±0.72^{c}	12.92±0.2^{c}
Gumminess (g)	C	290.08±9.78^{d}	355.90±6.70^{b}
	FLA	383.21±13.0^{b}	550.72±28.62^{c}
	MLA	621.62±11.31^{b}	883.10±10.80^{a}

1. Data (mean ± standard error) are from three replications ($n = 3$)

2. Free *Lactobacillus acidiphilus* (FLA), microencapsulated *Lactobacillus acidiphilus* (MLA), and control (C) were the Tahini halva samples

3. Lowercase letters (a–e) show significant differences ($p \leq 0.05$) among samples in each parameter during storage time

only factor to change the gumminess value. The gumminess can influence the chewiness [52]. Therefore, the gumminess value causes a significant difference in chewiness between MLA and other samples. During 28 days of storage, the springiness was approximately increased and was the highest for the control dessert. This result was also supported by a previous study in which an increase in springiness occurred following the increased incubation time [53]. The texture integrity of the samples is indicated by the springiness value of the sample [54]. Therefore, the addition of MLA and FLA reduces the integrity of the texture and provides a reasonable rationale for interpreting the less springiness of desserts containing MLA and FLA compared to the control sample.

Conclusions

This study was the first attempt to produce probiotic Tahini halva. Probiotic Tahini halva was supplemented with FLA and MLA. The results showed the improvement of viable cell survival by MLA up to 72 °C. The resistance of the entrapped *L. acidophilus* in the galbanum was significantly improved compared to free bacterial cells in the Tahini halva. The MLA contained a viable count of *L. acidophilus* at an acceptable level ($> 10^6$ CFU g^{-1}) until 18 days of storage under refrigerated conditions.

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Author contributions SSS carried out the experiments and wrote the MS, FA carried out the experiment, and ZM edited manuscript.

Data availability The data are available from the corresponding author upon reasonable request.

Declarations

Ethics approval This study does not involve any human or animal testing.

Consent to participate This study does not involve any human or animal testing.

Conflict of interest The authors declare no competing interests.

Permission to reproduce material from other sources In this study, we didn't reproduce material from other sources.

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