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Scientific Research

Investigating of physicochemical, microbial and sensory properties of Chavil yogurt drink containing *Lactobacillus rhamnosus* nanoencapsulated with Persian gum and whey protein isolate

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ABSTRACT

Yogurt drink is one of the fermented dairy products. Probiotic yogurt drink has special health properties due to the presence of probiotics. Also, adding herbal-medicinal plants including chavil to this product can make it more attractive. However, the shelf life of probiotic bacteria in yogurt drink is not very long. This research was therefore carried out to increase the shelf life of *Lactobacillus rhamnosus* in yogurt drink by nanoencapsulation technique with Persian gum and whey protein isolate. Particle size and SEM was used for determination nanoencapsulated bacteria (bead) characteristics. In this regard, three samples of yogurt drink containing free *Lactobacillus rhamnosus*, its bead and the control were produced. Some characteristics including pH, acidity, sensory including color, taste, consistency and overall acceptability of yogurt drink were measured as well as the shelf life of probiotic bacteria under gastrointestinal conditions and storage in the refrigerator. The results showed that the beads had a size from 246.98 to 356.2 nm. The efficiency of nanoencapsulation was 87.2%, and the SEM images showed that the bacteria were covered well. At the end of the storage time, the decrease in pH (3.41) and increase in acidity (97.3 °D) of the sample containing beads was highest. The counting of free and beads also showed that the shelf life of bead was greater than free bacteria in yogurt drink. In the 21st day of storage, the product was eligible for probiotics. Moreover, the bead survival in simulation gasterointestinal condition was 3.33 log cfu/ml. The evaluation of the sensory parameters also showed that the sample containing bead had the lowest score among the panellist in all the sensory components except the texture. In conclusion, it can be stated that the yogurt drink containing the bead sample can qualify as a probiotic product during 21 days of storage time.

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1- Introduction

Today, the demand for yogurt drink has increased by consumers due to its uniqueness. It has many properties and benefits include the presence of protein, vitamin B, adequate calories, calcium, and potassium, which help to improve the immune system. It is very important [1]. Various additives are added to yogurt drink. These ingredients include vegetables and fruits such as apple, carrot, lemon, orange, pineapple, raspberry, and strawberry concentrates [2].

Adding vegetables to yogurt drink is important because they add a large number of phytochemicals containing bioactive and polyphenolic compounds that improve the antioxidant content of the product. The availability of bioactive compounds is necessary. Because they influence the metabolism of lactic acid-producing bacteria, leading to overgrowth [3]. Chavil, which is known by the scientific name *Ferulago angulata*, is a perennial plant that grows as a bush with a height of 60-150 cm, at an altitude of 1900-2300 meters. It grows in the pastures of Iran. This aromatic plant has nutritional value. It was traditionally used as a medicine plant in food for better digestion and help to suppress stomach parasites. Chavil fruit powder was used as a food flavour enhancer and leaves for oil and yogurt drink. The most important feature is preventing the growth of cancer cells and metastasis [4].

Functional foods provide energy and nutrients to the body. Moreover, they improve multiple desirable functions in the body and help improve human health. Antioxidants, essential oils, minerals, vitamins, and useful microorganisms make these foods more valuable. Probiotics are microorganisms that cause health-improving properties in humans. Most of them are bacteria belonging to the genus *Lactobacilli*, *Bifidobacterium*, *Bacillus*, and yeasts such as *Saccharomyces cerevisiae* [5]. *Lactobacillus* can survive and grows in acidic environments. One of the *Lactobacillus* species is *Lactobacillus rhamnosus*. *Lactobacillus rhamnosus* is one of the most widely used probiotics in food. This bacterium produces short-chain fatty acids. Balancing the natural intestinal flora improves the immune system and prevents digestive system disease outbreaks. The survival of *Lactobacillus rhamnosus* depends on hydrogen peroxide, oxygen and pH of food, pH of the stomach, bile salts, enzymes, and natural intestinal flora. From this point of view, the food industry is looking for new strategies to preserve the survival of probiotics during food storage and after entering the body [6].

One of the ways to improve the survival of probiotics is microencapsulation. In this method, the creation of multilayers around the bacteria can protect them from harsh environmental conditions. In microencapsulation, the use of gum is important. Persian gum is obtained from the almond plant (*Amygdalus scoparia Spach*) with light white yellow, red, or brown colour. Persian gum may be used as a medicinal plant and applied in food and industries. This gum

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has suspending, emulsifying, and adhesive properties [7].

In a research, corn starch, and sodium alginate were used as nanofibers in wall materials for *L. acidophilus, Lactobacillus rhamnosus, Bifidobacterium bifidum*, and *Bifidobacterium animalis*. The survival rate was 94.1% and 89.4% for *Lactobacilli* and *Bifidobacteria* respectively after encapsulation using electrospinning. In addition, after exposure to simulated gasteric condition (in a presence of HCl and pepsin, at 37°C), the reduction of the bacterial population was 1.58 log CFU/ml and 1.03 log CFU/ml for nanoencapsulated *Bifidobacteria* and *Lactobacilli* in two h respectively [۸]. In another research, probiotics were encapsulated with sodium alginate and pullulan using a nanoencapsulation technique. Nanoencapsulation showed a significant effect on the survival and stability of probiotic bacteria. In general, a decrease in the viability of probiotic bacteria was observed in all treatments. A decrease in the number of free probiotic bacteria at 4°C compared with encapsulated probiotic bacteria was observed. In addition, in the simulated of gastrointestinal conditions, beads had less reduction than free bacterial cells. The viable number of probiotics is maintained at the recommended level (10^6 CFU/g) in simulated of gastrointestinal conditions conditions[۹].

The purpose of this research was nanocapsulation of *Lactobacillus rhamnosus* with whey protein isolated and Persian gum. Also, the effect of microencapsulated and free bacteria on the physicochemical, microbial, and sensory characteristics of the chavil yogurt drink during the storage period was evaluated.

2. Material and Methods

2.1. Material

Lyophilized *Lactobacillus rhamnosus* PTCC 1637 was prepared from the Persian-type culture collection. Yogurt starter culture CH1 containing *Lactobacillus bulgaricus* and *Sterptococcus thermophilus* was purchased from (Pishgaman Paksh Saddiq Company, a representative of Christian Hansen Company, Denmark. *L-rhamnose*, Iron sulfate, Manganese sulfate, Trypticase peptone, Monopotassium phosphate, Triammonium, Triphenyl tetrazolium chloride, Sodium acetate, Magnesium sulfate, Yeast extract, Bactoagar, Calcium chloride, MRS Agar and MRS broth culture media were supplied from Merck (Darmstadt, Germany). Metronidazole, Sodium alginate, Tween-80, and vancomycin were obtained from Sigma. Persian gum was obtained from Ahura Daru Company, Marvdasht, Fars. Canola oil was obtained from Laren Company, Tehran, and Chavil from local sellers.

2.2 Preparation of bacteria

To activate the bacteria, MRS broth medium was used. The MRS broth tubes were kept at 37°C for 48 h. For counting of *Lactobacillus rhamnosus*, M-RTLV[2] media containing 5 ml of salt solution (magnesium sulfate 11.5 g,

۲- Modified-rhamnose-2,3,5-triphenyltetrazolium chloride-LBS-vancomycin agar (M-RTLV agar),

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ferrous sulfate 0.68 g, magnesium sulfate 2.4 g, distilled water 100 mL), 2. 3,5-triphenyltetrazolium chloride 30 mg, tri-ammonium citrate 2 g, yeast extract 5 g, vancomycin hydrochloride 10 mg, monopotassium phosphate 6 g, trypticase peptone 10 g, Tween 80 1 g, sodium acetate 3 H_2O 25 g, Metronidazole 10 mg, L-rhamnose 20 g, Bactoagar 20 g and distilled water 950 ml were used. The plates were kept in an incubator at 37°C for 48 h [10].

First, two grams of whey protein isolate were added to two grams of Persian gum. Then 1 mL of Tween 80 was added to the mixture. In the next step, the volume of the mixture was increased to 10 mL with distilled water and homogenized at 14.336×g for 5 min. Four mL of this solution was removed and one mL of centrifuged *Lactobacillus rhamnosus* in the previous step was added and homogenized for two min. The samples were dried by a freeze dryer (Christ ALPHA 1-2 LD PLUS, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany). A scanning electron microscope (Vega3, Tescan Co. Ltd. Brno, Czech Republic), with an aluminum holder was used to stabilize and cover samples with a gold layer. The sample surface and the microscope lens had a working distance (8.91-91.03 mm). Representation was obtained by dividing length by width. After the beads were dispersed in distilled water, a dynamic light scattering laser diffractometer (SZ-100, Horiba Ltd. Kyoto, Japan) was used to measure the particle size distribution of the beads. For this purpose, polystyrene latex with a refractive index of 1.3326 was used [11].

2.3. Encapsulation efficiency

First, the number of bacteria after encapsulation was counted in terms of CFU/g (N0). The number of viable cells released after drying (N) was also counted and the encapsulation efficiency was expressed as (% microencapsulation efficiency) [12].

2.4. Yogurt drink production:

First, a milk sample with 1.5% fat and 8.2% SNF was prepared. Then the milk was transferred to the homogenizer and homogenized (150 bar), and in the next step, it was pasteurized at 90°C for 5 min. The milk was cooled to 42°C and the CH_1 starter including *L. bulgaricus* and *Sterptococcus thermophilus* was added to it at the rate of 0.04%. To prepare three treatments, milk was placed in an incubator at 42°C for 4 h and then transferred to a refrigerator (at 4°C) for 24 h. The samples were prepared in 3 distinct containers and all the experiments were carried out in three replications [13]. To prepare the yogurt drink, the samples were mixed with water at a ratio of 1 to 1 and homogenized at 150 bar pressure for 5 min. Then Chavil (2%) was added to the samples and mixed. Free *Lactobacillus rhamnosus* (FLR) and microencapsulated *Lactobacillus rhamnosus* (MLR) were added to samples separately. The final number of probiotic bacteria in the product was nearly 8 log cfu/ml. A yogurt drink sample without any bacteria was selected as a control. The samples were kept at 4°C.

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2.5. pH and acidity measurement

Acidity was measured according to the standard method 2852 based on the Iranian Standard and Industrial Research Organization (Durnic degree). The pH was measured using a calibrated pH meter (Sanxin 5021Ltd Shanghai, China) with standard glass electrodes at a temperature of 25°C in the range between 4-6 [14].

2.6. Colour analysis

To evaluate the colour parameters, a chromameter (CR-400 Konica-Minolta, Osaka, Japan) was used. The L* (white to dark), a* (red to green), and b* (yellow to blue) parameters were measured for each yogurt drink sample. For each yogurt drink sample, four points were determined and photographed [11].

2.7. Lactobacillus rhamnosus and starter counting in yogurt drink

MRS agar medium (pH=4.5) was used to count *Lactobacillus bulgaricus* starter bacteria and M17 medium was used to count *Streptoccus thermophilus*. All bacteria were counted on the first day and then every 7 days until the 28 days of storage. MRLTV medium was used to cultivate *Lactobacillus rhamnosus* [10, 15].

2.8. Viscosity measurement

The viscosity of yogurt drink samples was measured at 4°C on the first and 28th using a Brookfield DVII rotary viscometer (Brookfield, USA). A spindle (CP51 spindle, LV model) was used in 40 rpm in 90 seconds at ambient temperature. The samples were evaluated with a constant shear rate and the apparent viscosity was expressed in centipoise [16].

2.9. Survivability during simulated gastrointestinal condition

For this purpose, 1 g of beads was poured into 9 ml of 0.5% NaCl solution in sterile tubes. Then the pH of all samples was adjusted to 1.4-1.9 using 1N Hydrochloric acid. In the next step, lipase enzymes (62305, Fluka, USA) and pepsin (Stomach Porcine Mucosa, Sigma Chemical Co., St. Louis, MO) were added at 1.90 and 300 mg/l respectively. The samples were placed in a rotary incubator (Lab Tech, Korea) at 3×g at 37 °C for 2 h (gastric phase). In the next step, the pH of the samples was adjusted to 2.5-3.4 using an alkaline solution (150 ml NaOH 1.0N containing 14 g/L of disodium hydrogen phosphate). Then, bile (Oxgall B838O, Sigma) and pancreatin (Pancreatin from porcine pancreas, Sigma, 1750) were added to the samples with a concentration of 10 and 1 g/l, respectively. In the next step, the samples were agitated using a shaker incubator at 3×g at 37°C for 2 h (intestinal phase). The viable counting of probiotics was done in 30 min, 2, 4 and 6 h [10].

2.10. Sensory assessment

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Sensory analyses of the product were done on 1, 7, 14, 21, and 28 of storage. For this purpose, a 5-point hedonic test was used. The samples were subjected to the hedonic test. For this test 5 scale was used. Five means 'very much like', and 1 means 'not at all'. This test was carried out by 10 trained panellists (including 4 men and 6 women with an average age of 29 years). In this regard, the samples were compared with the control sample. The sensory parameters included smell, colour, taste, texture, and overall acceptance according to panellist score were assessed [17].

2.11. Statistical analysis

The samples included a control yogurt drink, a yogurt drink containing FLR, and a yogurt drink containing MLR. Each sample was reproduced three times. For data analysis, one-way ANOVA and Duncan's test were used to compare the means ($P<0.05$). The data was analysed by SPSS (21) software. Excel (2019) software was used to draw graphs.

3. Result and Discussion

3.1. The shape, size, and encapsulation efficiency of the beads

The diameter of the beads varied from 246.98 to 356.2 nm (Figure 1). The wall material affected the particle size. For example, the use of pectin increases the diameter of the particles. The concentration of wall materials and viscosity affect the particle diameter [18]. In a research, water-soluble starch nanocapsules were produced with yellow mustard mucilage using an electrospray atomizer. SEM images showed that the nanocapsules were spherical, smooth, and non-porous with minimal wrinkling (Figure 2). However, no surface cracks or pores were observed. As a result, these capsules reduce the permeability of gas, liquids and increase the survivability of bacteria. So, the use of Persian gum and whey protein isolate are suggested for the production of nanoparticles. In the last research, the diameter of the particles ranged from 64 to 125 nm, and the average diameter of the nanoparticles distribution was 94.46 ± 0.63 nm, which is consistent with the findings of this research [19].

The efficiency of bead microencapsulation was 87.6%. In a similar study, beads were performed using fructooligosaccharide. The results showed that the efficiency of microencapsulation was 87.2% [12]. The efficiency of microencapsulation depends on various factors such as the type and concentration of wall material, the calcium chloride concentration used in the wall formulation, the type of microorganisms, the method of microencapsulation, and the size of the particles [20].

The zeta potential of particles was -42.7 mV. The results Golkar et al. (2018) showed that the size of the zeta potential of the emulsion according to the different concentrations of Persian gum (0.5 to 3%) was from -46 to -30.5 mV, which is consistent with the results of this study. An increase in gum concentration leads

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to an increase in the negative surface charge in the emulsion droplets. So, zeta potential increased. This negative charge stabilizes the beads [21].

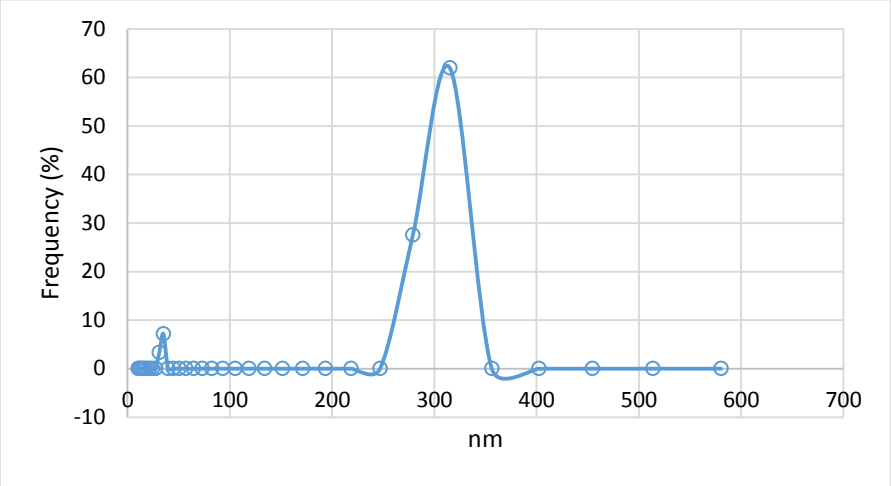

Fig 1. Initial droplet size distribution of beads encapsulated with Persian gum and whey protein isolate

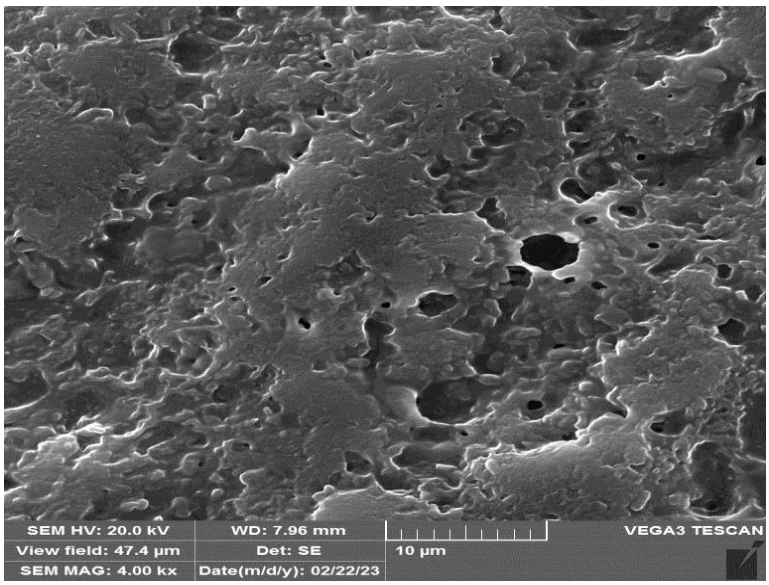

Fig 2. Scan electron microscopy of beads encapsulated with Persian gum and whey protein isolate

3.2. Measurement of acidity and pH

The pH and acidity results of control, MLR, and FLR samples are given in Figure 3. The pH of the samples was measured on 1, 7, 21, 14, and 28 days of storage. It was found that during the storage period, the pH of all samples decreased. In the MLR, the decrease was highest among the samples. The acidity was measured on 1, 7, 21, 14, and 28 days of storage. The results showed that the

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acidity increased during the storage period. Among the samples at distinct times, the acidity of MLR had the highest value. The acidity of the C sample had the lowest change during the storage time.

It is mentioned that probiotic bacteria are acid producers. For this reason, acidity increased but pH decreased in supplemented samples. Microencapsulated bacteria produce more acid at the end of storage time. This is probably due to the weakening of the microcapsule wall and better release of acid at the end of the storage period [22, 23]. The decrease in pH may be due to the production of β-galactosidase enzyme by the starters. In addition, the fermentation of the remaining carbohydrates in the product by probiotics during the storage time can lead to the production of lactic acid, formic acid, and CO2 [24]. It should be noted that the increase in acidity causes dehydration, bad taste in food, and a decrease in the number of *lactic acid bacteria* and gas. Moreover, it had a negative effect on the shelf life of probiotic foods [25].

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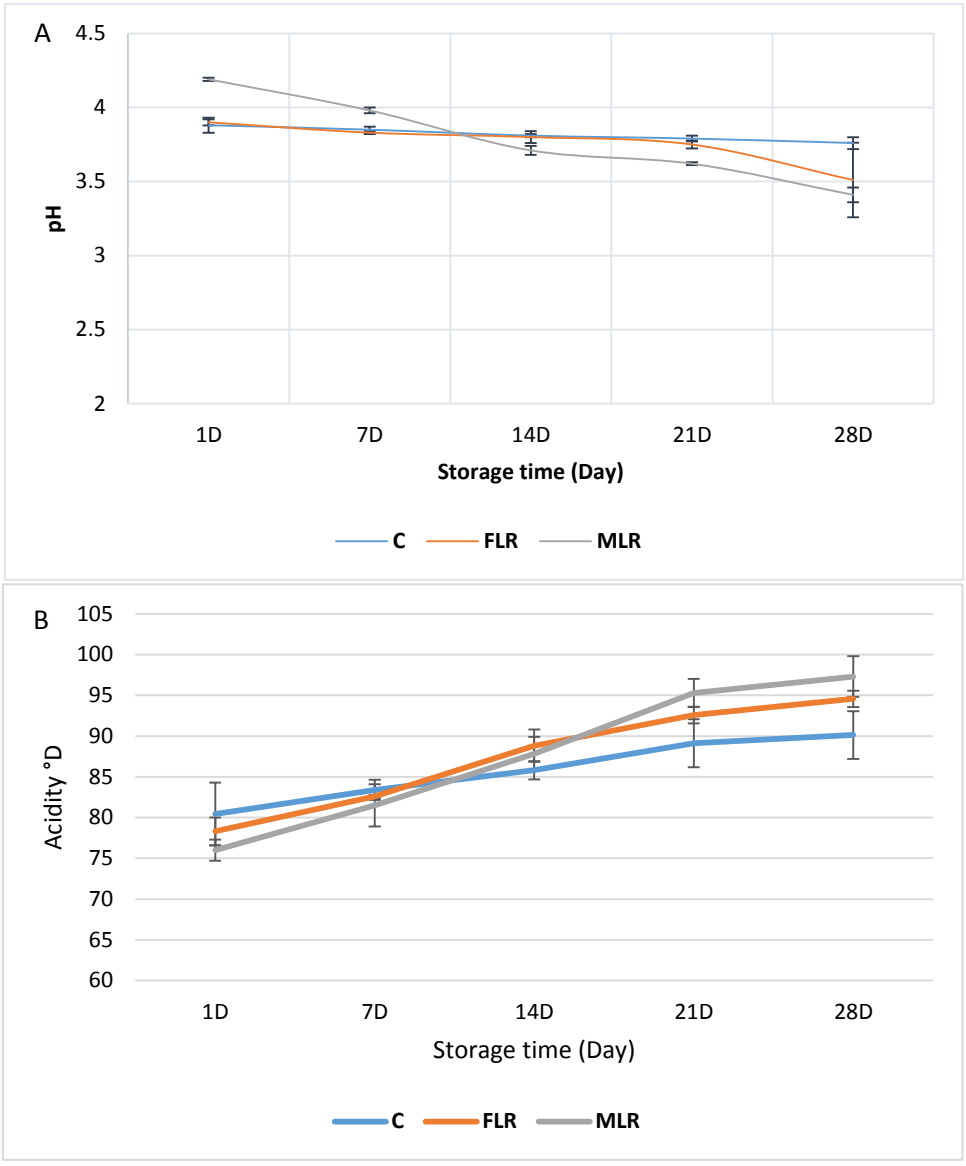

Fig 3. pH (A) and acidity(B) values of *free Lactobacillus rhamnosus* (FLR) and microencapsulated *Lactobacillus rhamnosus* (MLR) and control yogurt drink (C) during storage time.

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

3.3. Colour evaluation

Table 1 shows the evaluation of colour components in the chavil yogurt drink. The evaluation of the brightness (L*) during the storage period showed that this parameter decreased in free FLR, MLR, and control samples. Among the samples, the sample containing beads had the lowest brightness. There was no significant difference in the brightness between the sample containing FLR and the control on the 28th day of storage. The assessment of a*(green-red) during 28 days of storage showed that this parameter decreased in all samples leading to the colour of the samples tended to be red. On the first day of storage, the lowest amount of a* was related to the control yogurt drink sample, but at the end of 28 days, the highest amount of a* was related to the control yogurt drink. During the storage period, the b* (blue-yellow) increased in all the samples. Among the samples, the sample containing beads had the highest amount on the 28th day of storage. However, the control yogurt drink sample did not have a

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significant difference with FLR on the first day. But on the 28th day, the control sample had the lowest amount of b*.

Table 1. Color parameters of yogurt drink during storage time

Color parameters	Treatment	1 Day	28 Day
L*	C	51.60±3.00^{a}	43.32±5.23^{bc}
	FLR	47.63±2.69^{ab}	43.66±1.50^{bc}
	MLR	42.98±1.65^{bc}	38.22±4.42^{c}
a*	C	0.04±0.02^{f}	-0.12±0.02^{c}
	FLR	1.42±0.01^{b}	-0.66±0.01^{e}
	MLR	1.68±0.02^{a}	-0.42±0.01^{d}
b*	C	8.40±0.12^{d}	22.90±0.08^{c}
	FLR	8.57±0.12^{d}	25.42±0.19^{b}
	MLR	9.18±0.03^{d}	32.93±0.53^{a}

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

2- Means in the same row and column with different lowercase letters (a–f) among yogurt drink samples differ significantly ($P<0.05$); control yogurt drink (C); microencapsulated *Lactobacillus rhamnosus* (MLR); free *Lactobacillus rhamnosus* (FLR).

The less brightness and color tendency to the yellow of the beads may be related to the presence of proteins such as whey that have been used for encapsulation [26]. Similar results were obtained in the research of Augustin et al. (2012). In their research, the color change from white to dark ($\Delta E^* = 2.6$) was seen in the sample containing beads compared with the control. A significant difference was seen between control and MLR in whiteness [27]. One of the reasons could be the natural dark color of Persian gum. In this research, L* values of all samples increased during storage time, while a* and b* values decreased. In the current research, the redness component (a*) decreased but (b*) increased, which was not consistent with the findings of Augustin et al. (2012). One possible reason could be related to degradation of acetoxanthin, which was not used as a coating material in this research [28]. Moreover, Montero et al. (2016) reported that the color of bead powders did not change significantly during 110 days of cold storage, which is not consistent with the current research. This difference can be due to the use of different

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type and proportions of wall biopolymers in that research [29].

3.4. *Lactobacillus rhamnosus* and starter bacteria counting in yogurt drink

The survival of starter bacteria and *Lactobacillus rhamnosus* in all types of yogurt drink during 28 days of storage at 4°C is shown in Figure 4. In the control sample, the number of starter bacteria, including *Streptococcus thermophi*lus and *Lactobacillus bulgaricus*, decreased by 3.0 log cfu/ml during the storage period. In FLR, the number of *Lactobacillus rhamnosus* decreased from 8.5 to 0.9 log cfu/ml during 28 days of storage time. In MLR, the number of *Lactobacillus rhamnosus* decreased from 7.8 on the first day to 2.5 log cfu/g on the 28th day. The results showed that in the FLR and MLR samples, the viable bacterial count was 6.4 and 1.6 log cfu/g, on the 14th and 21st day of storage respectively. At these times, *Lactobacillus rhamnosus* maintains an adequate minimum limit of probiotic bacterial counting in food.

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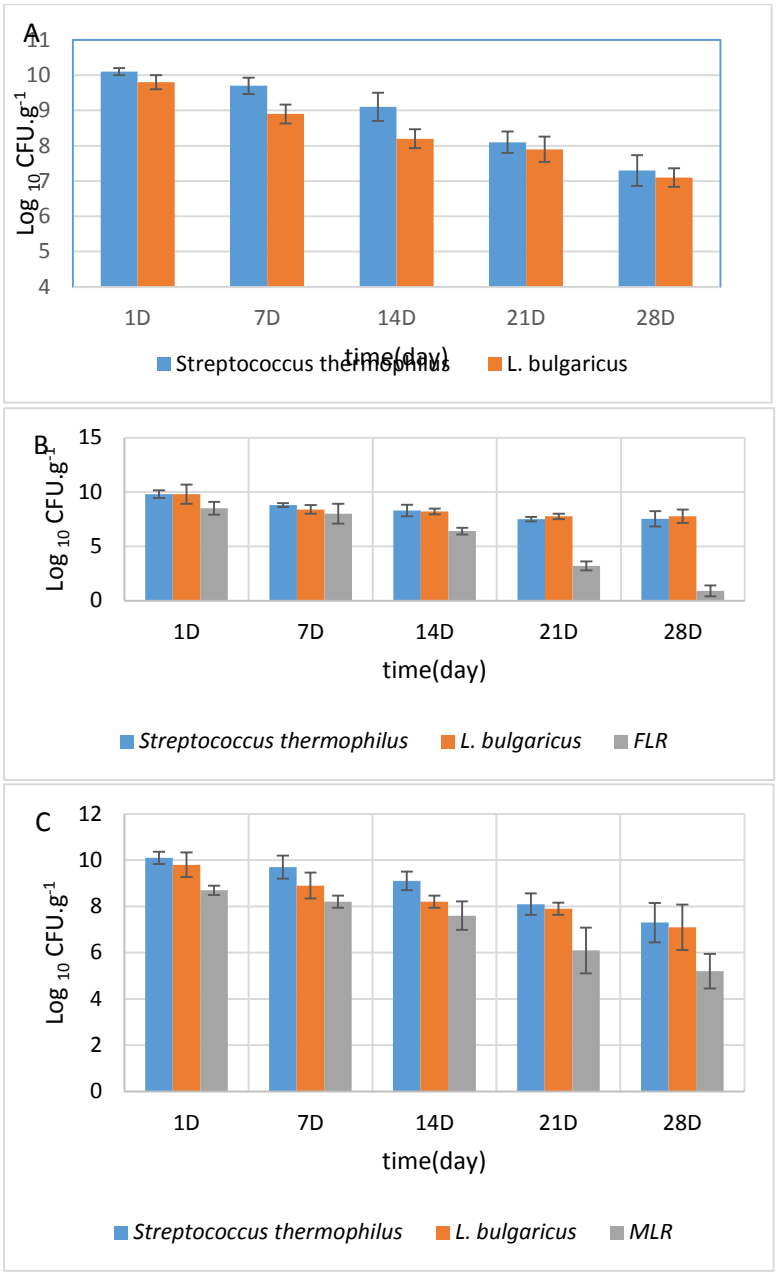

Fig 4. The culture survival rate of *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, and *Lactobacillus rhamnosus* in control yogurt drink (A); Free *Lactobacillus rhamnosus* (FLR)(B) and microencapsulated *Lactobacillus rhamnosus* (MLR)(C) during storage time.
1- Data (mean standard deviation) are from three replications.

Akbari et al. (2023) showed that the survival of bacteria without capsules decreased by 2 log cfu/g in 2 weeks of storage at 4°C. No viable count was seen after 4 weeks. The decrease in the viability of microencapsulated bacteria in the presence of bead wall material (cruciferin-alginate) after 8 weeks of storage was 1 to 1.5 log cfu/g [30]. The results of the studies showed that the protein component of the wall has no effect on the persistence of bacteria during the storage period. In the research of Würth et al. (2015), the microcapsules

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containing milk protein did not have a significant effect on the viability of the encapsulated probiotics, so only the gum Farsi has been responsible for increasing survivalability of *Lactobacillus rhamnosus* [31]. Wang et al. (2017) showed that the survival of probiotics enclosed in pea protein-alginate capsules was higher than that of free form [32].

3.5. Viscosity measurement

The apparent viscosity of control, FLR, and MLR samples on the first and 28th day of storage are presented in Figure 5. With the increase in shear rate, a decrease in apparent viscosity was observed in all samples, which indicates the non-Newtonian behaviour of the samples. Also, a rapid decrease in apparent viscosity was observed with a slight change in the shear rate. One of the reasons could be the decrease in particle size as a result of an increase in the shear rate [33]. The results showed that the viscosity increased during the storage time among all the samples, which can be related to the acidification of the yogurt drink. A decrease in the negative electric charge in the casein micelle leads to the dissolution of calcium and inorganic phosphate. It reduces colloidal stability. On the other hand, it reduces the dissolution of casein (near the isoelectric pH) (around 4.6). This phenomenon strengthens protein-protein complexes and leads to an increase in viscosity [34].

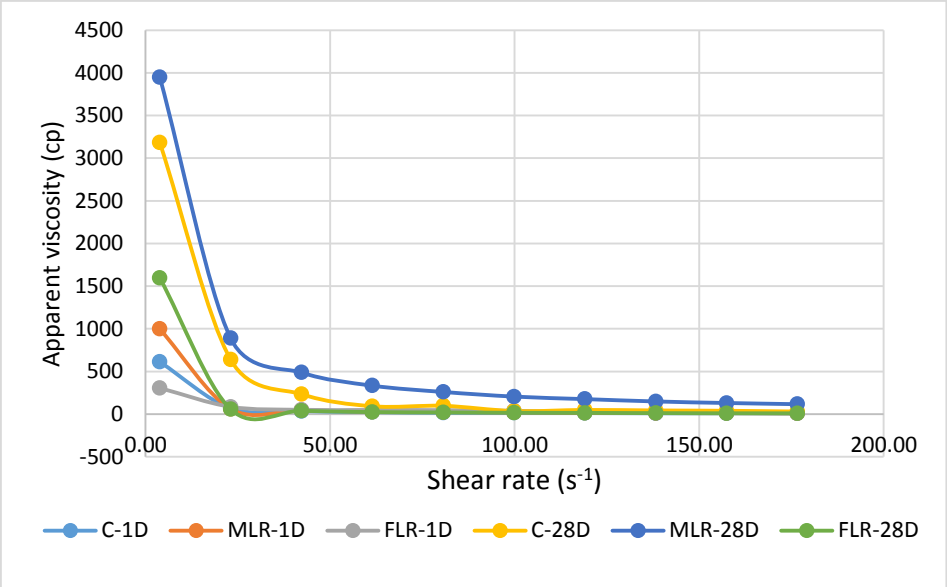

Fig 5. The apparent viscosity of yogurt drink, including the control on the 1st day of storage (C-1D), the control on the 28th day of storage (C-28D), microencapsulated *Lactobacillus rhamnosus* in the 1st of storage (MLR-1D), microencapsulated *Lactobacillus rhamnosus* in the 28th day of storage (MLR-28D), free *Lactobacillus rhamnosus* in the 1st of storage (FLR-1D), free *Lactobacillus rhamnosus* in the 28th day of storage (FLR-28D).

3.6. Survival during simulated gastrointestinal condition

The survival ability of *Lactobacillus rhamnosus* in FLR and MLR samples during

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simulated gastrointestinal conditions on the first and 14th day of storage is shown in Figure 6. On the first day, the number of *Lactobacillus rhamnosus* in was 0.8 Log cfu/ml and 3.33 Log cfu/ml in FLR and MLR respectively. During simulated gastrointestinal conditions, the reduction of *Lactobacillus rhamnosus* in FLR was much higher than MLR. The survival ability assessment on the 14th day showed that the viable number of *Lactobacillus rhamnosus* decreased in FLR and MLR, but the decrease in the FLR sample was more extensive than the MLR. *Lactobacillus rhamnosus* was not observed in the control sample.

The high survival rate of encapsulated probiotics is necessary for the production of functional foods. Probiotic present in foods with good resistance in gastric fluid increase the function of the immune system. In gastric conditions, microencapsulated probiotics with alginate and xanthan gum were more stable compared to free cells. The longer shelf life of microencapsulated probiotics with xanthan is due to the creation of a sponge gel network, which has reduced the diffusion of liquid inside the microorganisms within 2 h [35]. Jain et al. (2020) stated that the death rate of free-living cells in gastric fluid is due to the effect of pepsin and hydrochloric acid in human gastric juice. The survival of the encapsulated bacteria when exposed to gastric conditions was due to the presence of the gummy coating of the beads. They also reported that the structure of alginate beads was strengthened and densified after encapsulating with gum [36]. Chen et al. (2017) showed that the survival of beads in gastric conditions was improved due to coating with whey protein. Whey compounds have a buffering effect in an acidic environment and protect probiotic cells in this condition [37]. Bile salts and intestinal juice affect the viability of probiotics and increase the death rate of free bacteria. The viability of probiotics is increased by using coatings material that act as a protective wall during the intestinal juice diffusion [38]. Silva et al found that placing probiotic cells in the intestinal fluid causes swell and disintegration. Alginate-gelatin beads showed resistance to bile salt (intestinal fluid) by reducing the diffusion of ion exchange due to a condense bead wall structure and less porosity. These results are in agreement with the findings of this research.

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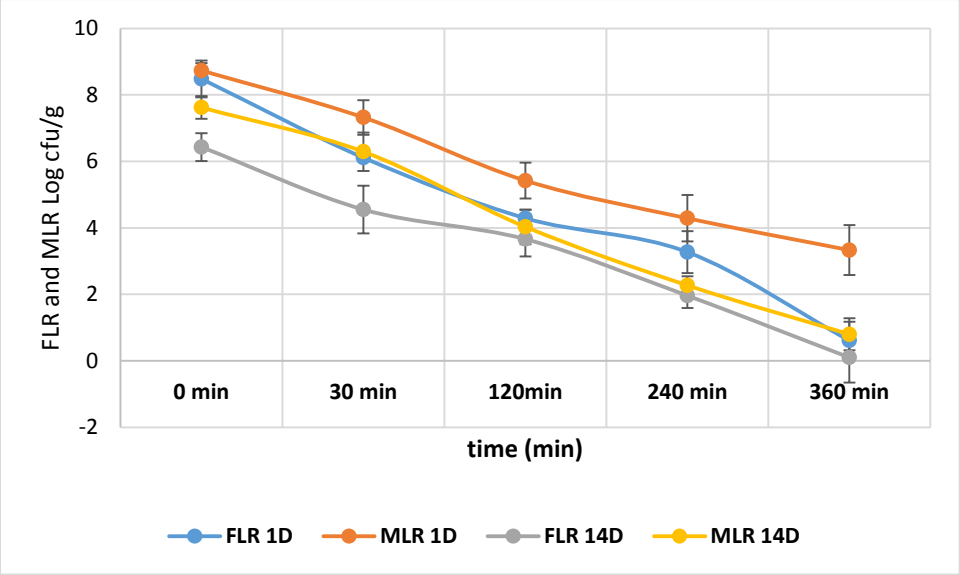

Fig 6. The survival free *Lactobacillus rhamnosus in the 1st* (*FLR 1D*) and microencapsulated *Lactobacillus rhamnosus* in the 1st (*MLR1D);* free *Lactobacillus rhamnosus in the 14th* (*FLR 14D*) and microencapsulated *Lactobacillus rhamnosus* in the 14th (*MLR 14D)* in yogurt during simulated gastrointestinal condition.
1- Data (mean±standard deviation) are from three replications.

3.7. Sensory test

The results of sensory parameters evaluation including odour, taste, colour, texture, and overall acceptance are shown in Figure 7. In the MLR sample, the odour had the lowest score compared with the others during the storage period. In terms of taste, all the treatments presented a decrease in the taste score during the storage period, but the control treatment had the highest score among the samples. The colour score of all samples decreased during the storage period, except for the MLR sample. The MLR colour score was the lowest among the samples at each time. The MLR samples had the highest textural score after the 14th day of storage. The dissolution of Persian gum and whey protein in yogurt drink increased the textural parameter. In overall acceptance, MLR had the lowest score among the samples. Kowsalya et al. (2023) presented microencapsulated *Lactobacillus plantarum* PRK7 using sodium alginate, inulin, and skim milk as microcapsule wall materials. Then the beads were added to yogurt. The sensory test of the yogurt sample showed that yogurt containing beads was better than the control sample, which is not consistent with the findings of this research. One of the reasons for this difference can be related to the positive effect of *Lactobacillus plantarum* PRK7 in organoleptic properties and flavour enhancing of yogurt samples. Hence, probiotic-supplemented yogurt obtained greater overall acceptance than control yogurt [39].

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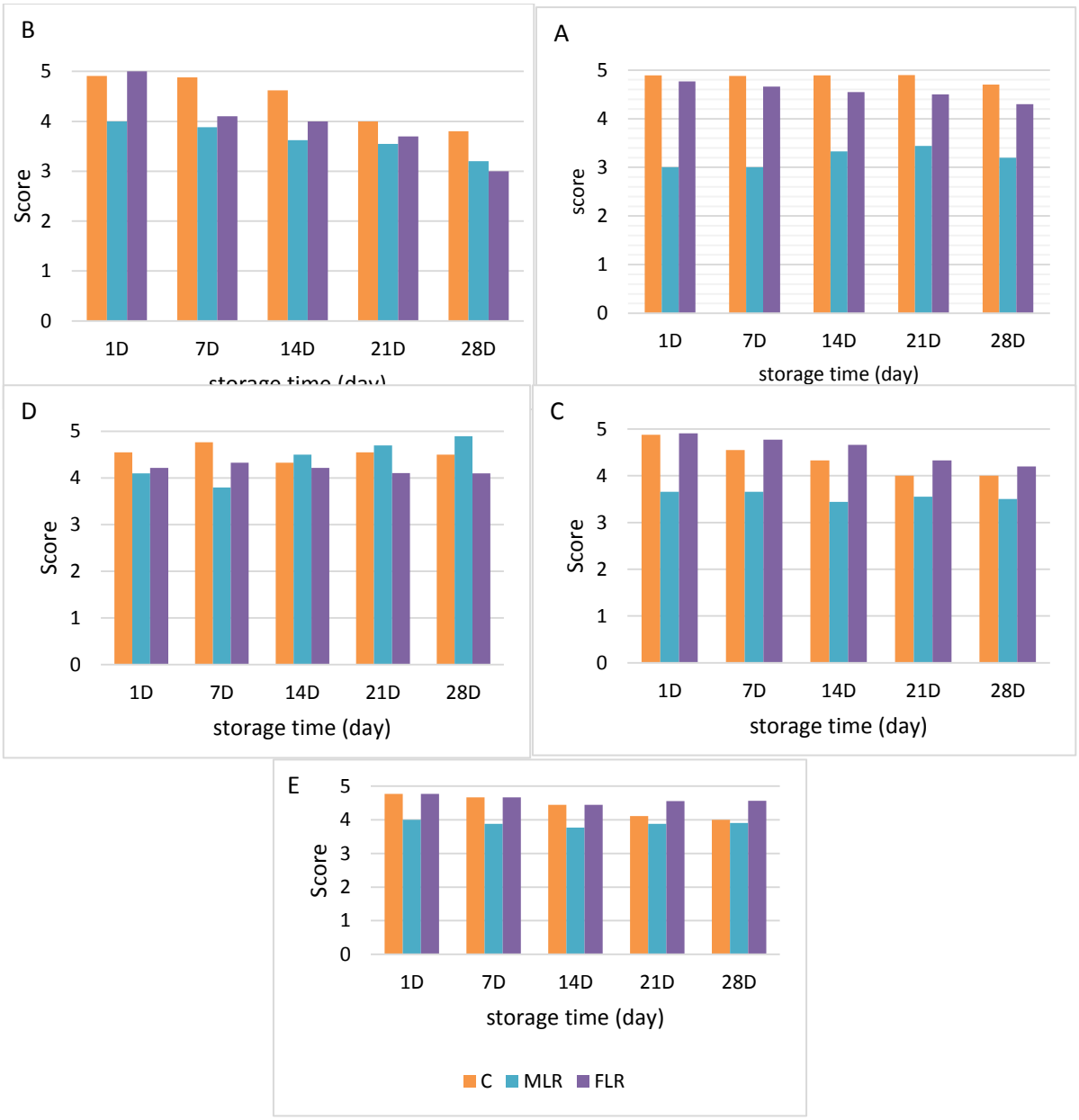

Fig 7. Sensory evaluation odor(A); taste(B); color(C); texture (D); overall acceptance(E) in control yogurt drink; Free *Lactobacillus rhamnosus* (FLR) and microencapsulated *Lactobacillus rhamnosus* (MLR) during storage time.

4. Conclusion

In this research, the *Lactobacillus rhamonosus* was encapsulated with Persian gum and whey protein. The obtained beads had suitable based diameter, and the microencapsulation efficiency was at a high level. The results showed that chavil yogurt drink containing beads had the maximum number of probiotic bacteria (10^6 cfu/g) up to 21 days of storage in the refrigerator. In addition, these beads were able to survive in simulated gastrointestinal conditions. The sensory assessment test on yogurt drink containing beads showed that, it was not accepted among the panelists. Among all sensory attributes, only the texture parameter had a good score. Low pH and high

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acidity of MLR were observed at the end of the storage time.

References

[١]. Shahein, M.R., Atwaa, E.S.H., Radwan, H.A., Elmeligy, A.A., Hafiz, A.A., Albrakati, A., and Elmahallawy, E.K. (2022). Production of a yogurt drink enriched with golden berry (*Physalis pubescens* L.) juice and its therapeutic effect on hepatitis in rats. *Fermentation*. 8,(3): 112.

[٢]. Abdeldaiem, A.M., Ali, A.H., Shah, N., Ayyash, M., and Mousa, A.H. (2023). Physicochemical analysis, rheological properties, and sensory evaluation of yogurt drink supplemented with roasted barley powder. *LWT*. 173: 114319.

[٣]. Camargo-Herrera, Á.D., Bernal-Castro, C., Gutiérrez-Cortes, C., Castro, C.N., and Díaz-Moreno, C. (2022). Bio-yogurt with the inclusion of phytochemicals from carrots (Daucus carota): a strategy in the design of functional dairy beverage with probiotics. *Journal of Food Science and Technology*. 1-12.

[٤]. Sekhavatizadeh, S.S., Karami, M., Savandroomi, A., and Sadeghisarvestani, M. (2015). Industrial Production of chvil yogurt and its chemical and sensory properties. *Food Technology and Nutrition*. 12.٧٠-٥٩ :(١),

[٥]. Šipailienė, A. and Petraitytė, S. (2018). Encapsulation of probiotics: proper selection of the probiotic strain and the influence of encapsulation technology and materials on the viability of encapsulated microorganisms. *Probiotics and Antimicrobial Proteins*. 10,(1): 1-10.

[٦]. Barajas-Alvarez, P., Gonzalez-Avila, M., and Espinosa-Andrews, H. (2022). Microencapsulation of *Lactobacillus rhamnosus* HN001 by spray drying and its evaluation under gastrointestinal and storage conditions. *LWT*.١١٢٤٨٥ :١٥٣ .

[٧]. Rahimi, S. and Abbasi, S. (2014). Characterization of some physicochemical and gelling properties of Persian gum. *Innovative Food Technologies*. 1,(4): 13-27. [In persian].

[٨]. Atraki, R. and Azizkhani, M. (2021). Survival of probiotic bacteria nanoencapsulated within biopolymers in a simulated gastrointestinal model. *Innovative Food Science & Emerging Technologies*. 72: 102750.

[٩]. Salman, H. (2022). Survival and stability of free and nanoencapsulated *Lactobacillus casei* under simulated gastrointestinal fluid and thermal conditions. *Benha Veterinary Medical Journal*. 42,(1): 68-72.

[١٠]. Sekhavatizadeh, S., Aminlari, M., and Ghaisari, H.R. (2018). The improvement of the physicochemical properties of *Lactobacillus rhamnosus* probiotic by dual layers extrusion microencapsulation with sodium alginate and zedo gum. *Journal of Food Science and Technology(Iran)*. 15,(80): 335-348. [In Persian].

[١١]. Borhanpour, F., Sekhavatizadeh, S.S., Hosseinzadeh, S., Hasanzadeh, M., Golmakani, M.-T., and Moharreri, M. (2022). Effect of microencapsulated chavil (*Ferulago angulata*) extract on physicochemical, microbiological, textural and sensorial properties of UF-feta-type cheese during storage time. *International Journal of Food Engineering*. 18,(1): 53-66.

[١٢]. Rajam, R. and Anandharamakrishnan, C. (2015). Microencapsulation of *Lactobacillus plantarum* (MTCC 5422) with fructooligosaccharide as wall material by spray drying. *LWT-Food Science and Technology*. 60,(2): 773-780.

[١٣]. Ghaderi-Ghahfarokhi, M., Yousefvand, A., Ahmadi Gavlighi, H., Zarei, M., and Farhangnia, P. (2020). Developing novel synbiotic low-fat yogurt with fucoxylogalacturonan from tragacanth gum: Investigation of quality parameters and *Lactobacillus casei* survival. *Food Science & Nutrition*. 8,(8): 4491-4504.

[١٤]. Raikos, V., Juskaite, L., Vas, F., and Hayes, H.E. (2020). Physicochemical

[DOI: 10.22034/FSCT.20.145.55]

properties, texture, and probiotic survivability of oat-based yogurt using aquafaba as a gelling agent. *Food Science & Nutrition*. 8,(12): 6426-6432.

[۱۵]. Sakai, T., Oishi, K., Asahara, T., Takada, T., Yuki, N., Matsumoto, K., Nomoto, K., and Kushiro, A. (2010). M-RTLV agar, a novel selective medium to distinguish Lactobacillus casei and Lactobacillus paracasei from *Lactobacillus rhamnosus. International Journal of Food Microbiology*. 139,(3): 154-160.

[۱۶]. Basiri, S., Haidary, N., Shekarforoush, S.S., and Niakousari, M. (2018). Flaxseed mucilage: A natural stabilizer in stirred yogurt. *Carbohydrate Polymers*. 187: 59-65.

[۱۷]. Gheybi, N. and ashrafi, r .(۲۰۲۰) .The Effect of inulin and quince seed gum powder on the physicochemical and qualitative properties of low fat yogurt. *Iranian Journal of Biosystems Engineering*. 50,(4): 963-975.

[۱۸]. Motalebi Moghanjougi, Z., Rezazadeh Bari, M., Alizadeh Khaledabad, M., Amiri, S., and Almasi, H. (2021). Microencapsulation of *Lactobacillus acidophilus LA-5* and *Bifidobacterium animalis* BB-12 in pectin and sodium alginate: A comparative study on viability, stability, and structure. *Food Science & Nutrition*. 9,(9): 51-۰۳ .۵۱۱۱

[۱۹]. Charles, A.P.R., Mu, R., Jin, T.Z., Li, D., Pan, Z., Rakshit, S., Cui, S.W., and Wu, Y. (2022). Application of yellow mustard mucilage and starch in nanoencapsulation of thymol and carvacrol by emulsion electrospray. *Carbohydrate Polymers* :۲۹۸ . .۱۲۰۱۴۸

[۲۰]. Hugues-Ayala, A.M., Sarabia-Sainz, J.A.-i., González-Rios, H., Vázquez-Moreno, L., and Montfort, G.R.-C. (2020). Airbrush encapsulation of *Lactobacillus rhamnosus* GG in dry microbeads of alginate coated with regular yogurt drink proteins .*LWT*. 117: 108639.

[۲۱]. Golkar, A., Taghavi, S.M., and Aghili Dehnavi, F. (2018). The emulsifying properties of Persian gum (*Amygdalus scoparia Spach*) as compared with gum Arabic. *International Journal of Food Properties*. 21,(1): 416-436.

[۲۲]. Leylak, C ,.Özdemir, K.S., Gurakan, G.C., and Ogel, Z.B. (2021). Optimisation of spray drying parameters for *Lactobacillus acidophilus* encapsulation in whey and gum Arabic: Its application in yoghurt. *International Dairy Journal*. 112: 104865.

[۲۳]. Zhang, C., Yang ,L., Gu, R., Ding, Z., Guan, C., Lu, M., and Gu, R. (2019). Mild heat stress limited the post-acidification caused by *Lactobacillus rhamnosus* hsryfm 1301 in fermented milk. *Biotechnology Letters*. 41: 633-639.

[۲۴]. Mahmoodi Pour, H., Marhamatizadeh, M.H ,.and Fattahi, H. (2022). Encapsulation of different types of probiotic bacteria within conventional/multilayer emulsion and its effect on the properties of probiotic yogurt. *Journal of Food Quality*. 2022: 1-12.

[۲۵]. Deshwal, G.K., Tiwari, S., Kumar, A ,. Raman, R.K., Kadyan, S.J.T.i.F.S., and Technology (2021). Review on factors affecting and control of post-acidification in yoghurt and related products. 109: 499-512.

[۲۶]. Bhagwat, A., Bhushette, P., and Annapure, U.S. (2020). Spray drying studies of probiotic Enterococcus strains encapsulated with whey protein and maltodextrin. *Beni-Suef University Journal of Basic and Applied Sciences*. 9,(1): 1-8.

[۲۷]. Augustin, M. and Hippolyte, M.T. (2012). Screening of biosurfactants properties of cell-free supernatants of cultures of Lactobacillus spp. isolated from a local fermented milk (Pendidam) of Ngaoundere (Cameroon). *International Journal of Engineering Research and Applications*. 2,(5): 974-985.

[۲۸]. Bassijeh, A., Ansari, S., and Hosseini, S.M.H .(۲۰۲۰) .Astaxanthin encapsulation in multilayer emulsions stabilized by complex coacervates of whey protein isolate and Persian gum and its use as a natural colorant in a model beverage. *Food Research International*. 137: 109689.

[DOI: 10.22034/FSCT.20.145.55]

[۲۹]. Montero, P., Calvo, M., Gómez-Guillén, M., and Gómez-Estaca, J. (2016). Microcapsules containing astaxanthin from shrimp waste as potential food coloring and functional ingredient: Characterization, stability, and bioaccessibility. *LWT*. 70: 229-236.

[۳۰]. Akbari, A., Gänzle, M.G ,.and Wu, J. (2023). Cruciferin improves stress resistance and simulated gastrointestinal survival of probiotic *Limosilactobacillus reuteri* in the model encapsulation system. *Food Hydrocolloids for Health*. 100118.

[۳۱]. Würth, R., Hörmannsperger, G., Wilke ,J., Foerst, P., Haller, D., and Kulozik, U. (2015). Protective effect of milk protein based microencapsulation on bacterial survival in simulated gastric juice versus the murine gastrointestinal system. *Journal of Functional Foods*. 15: 116-125.

[۳۲]. Wang, J., Nickerson, M., Low, N.H., and Van Kessel, A. (2016). Efficacy of pea protein isolate–alginate encapsulation on viability of a probiotic bacterium in the porcine digestive tract. *Canadian Journal of Animal Science*. 97,(2): 214-222.

[۳۳]. Sekhavatizadeh, S.S., Mirzaee, N., Golmakani, M.-T., and Hosseinzadeh, S. (2022). Physicochemical and sensory characteristics of Kashk as influenced by quinoa flour addition. *Journal of Microbiology, Biotechnology and Food Sciences*. 11,(5): e3438-e3438.

[۳٤]. Dönmez ,Ö., Mogol, B.A., and Gökmen, V. (2017). Syneresis and rheological behaviors of set yogurt containing green tea and green coffee powders. *Journal of Dairy Science*. 100,(2): 901-907.

[۳٥]. Felix da Silva, D., Junior, N.N.T., Gomes, R.G., dos Santos Pozza ,M.S., Britten, M., and Matumoto-Pintro, P.T. (2017). Physical, microbiological and rheological properties of probiotic yogurt supplemented with grape extract. *Journal of Food Science and Technology*. 54: 1608-1615.

[۳٦]. Jin, H.S., Fei, Y.S., Yan, C.K., Kuan, C.H., and Wei, S.W.Y. (2020). Effect of gums coating materials on the survival of microencapsulated probiotics under simulated gastrointestinal conditions. *Materials Today: Proceedings*. 29: 16-19.

[۳۷]. Chen, H.-Y., Li, X.-Y., Liu, B.-J., and Meng, X-.H. (2017). Microencapsulation of *Lactobacillus bulgaricus* and survival assays under simulated gastrointestinal conditions. *Journal of Functional Foods*. 29: 248-255.

[۳۸]. Ali, U., Saeed, M., Ahmad, Z., Shah, F.-u.-H., Rehman, M.A., Mehmood, T., Waseem ,M., Hafeez, H., Azam, M., and Rahman, A. (2023). Stability and survivability of alginate gum-coated L*actobacillus rhamnosus* GG in simulated gastrointestinal conditions and probiotic juice development. *Journal of Food Quality*. 2023.

[۳۹]. Kowsalya, M., Sudha, K.G., Ali, S., Velmurugan, T., and Rajeshkumar, M.P. (2023). Sustainability and controlled release behavior of microencapsulated *Lactobacillus plantarum* PRK7 and its application in probiotic yogurt production. *Food Bioscience*. 52: 102430.

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مقاله علمی_پژوهشی

بررسی خصوصیات فیزیکوشیمیایی، میکروبی و حسی دوغ چویل حاوی نانوکپسول‌های *Lactobacillus rhamnosus* ریزپوشانی شده با صمغ فارسی و پروتئین ایزوله آب‌پنیر

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چکیده

دوغ یکی از فرآورده‌های لبنی تخمیری است. دوغ پروبیوتیک به دلیل دارا بودن پروبیوتیک‌ها، خواص سلامتی-بخش ویژه‌ای دارد. اضافه‌کردن گیاهان دارویی مانند چویل به این محصول، می‌تواند آن را باارزش‌تر نماید. با توجه به کوتاه مدت بودن مدت ماندگاری باکتری‌های پروبیوتیک در دوغ، این پژوهش به‌منظور افزایش مدت ماندگاری *Lactobacillus rhamnosus* در دوغ با استفاده از تکنیک نانوریزپوشانی با صمغ فارسی و پروتئین آب‌پنیر انجام شد. آزمایش‌های اندازه‌گیری قطر ذرات و عکس‌برداری توسط میکروسکوپ الکترونی روبشی بر روی دانک‌ها صورت پذیرفت. همچنین سه تیمار دوغ شامل نمونه دارای باکتری *Lactobacillus rhamnosus* آزاد، نمونه حاوی باکتری ریزپوشانی شده و نمونه شاهد در سه تکرار مورد بررسی قرار گرفتند. ویژگی‌های حسی شامل رنگ، طعم، قوام و پذیرش کلی دوغ و همچنین pH ، اسیدیته و ماندگاری باکتری پروبیوتیکی تحت شرایط دستگاه گوارش و نگهداری در یخچال مورد سنجش قرار گرفت. نتایج نشان دادند که اندازه دانک‌ها ۲۴۶/۹۸ تا ۳۵۶/۲ نانومتر بود. میزان بازده ریزپوشانی دانک ۸۷/۶ ٪ بوده و تصاویر میکروسکوپی نشان دادند که باکتری‌ها به‌خوبی پوشانده شده‌اند. در پایان زمان نگهداری، در تیمارحاوی دانک، pH بیشترین کاهش (۳/۴۱) و اسیدیته بیشترین افزایش (۹۷/۳ ^{o}D) را داشت. شمارش باکتری‌های *Lactobacillus rhamnosus* در تیمارها نشان داد که ماندگاری پروبیوتیک ها در تیمار حاوی باکتری ریزپوشانی شده بیشتر از تیمار باکتری آزاد بود و این تیمار در روز بیست و یکم، به لحاظ تعداد باکتری دارای خواص پروبیوتیکی بود. ماندگاری دانک در شرایط مشابه دستگاه گوارش ۳/۳۳ log cfu/ml بود. ارزیابی مؤلفه‌های حسی نیز نشان داد که نمونه حاوی دانک از کمترین میزان امتیاز ارزیابان در تمام مؤلفه‌های حسی به غیر از بافت برخوردار بود؛ بنابراین به‌عنوان نتیجه‌گیری، می‌توان اظهار نمود استفاده از روش نانوریزپوشانی با صمغ فارسی و پروتئین ایزوله آب پنیر قادر به حفظ پروبیوتیک لاکتوباسیلوس رامنوسوس در محصول دوغ در طول ۲۱ روز نگهداری در شرایط یخچال شد.

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