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Application of carrot waste extract and *Lactobacillus plantarum* in *Alyssum homalocarpum* seed gum-alginate beads to create a functional synbiotic yogurt

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Abstract

One of the most recent and effective methods, which is currently receiving special attention and is being developed by numerous researchers, is production of microspheres from the probiotic cells. The largest market segment for functional foods is represented by dairy products, which have been touted as the most effective carriers of nutrients, such as probiotics, prebiotics, proteins, vitamins, and minerals. Yogurt is fermented dairy product that is popular all over the world. A new functional symbiotic yogurt fabricated by plant wastes (carrot pomace extract), *Lactobacillus plantarum*, and beads based on *Alyssum homolocarpum* seed gum (AHSG) and sodium alginate (SA) using extrusion technique was produced and characterized. Evaluation of the functional properties of yogurts indicated that the total phenolic content and DPPH radical scavenging activity were in the range of 16.13–48.30 µg GAE/ml and 7.4–14.64%, respectively. The acidity, pH, syneresis, water holding capacity, lightness, redness, and yellowness of the yogurts were in the range of 1.50–2.90, 4.07–4.38, 49.00–57.24%, 46.8–57.3%, 57.16–61.25, − 0.20–0.91, 6.40–13.06 on the 28th day storage, respectively. The panelists confirmed the sensory properties of yogurt samples. Probiotic survival rate of the functional yogurts were in the range of 6.37–8.13 log CFU/g, on the 28th day. Based on the results, bead production by AHSG and SA and the use of carrot pomace extract enhanced the survival of probiotic bacteria significantly in yogurt during storage compared to free cells.

Keywords Carrot waste, Dairy, Flavored yogurt, Microspheres, Prebiotics, Probiotics

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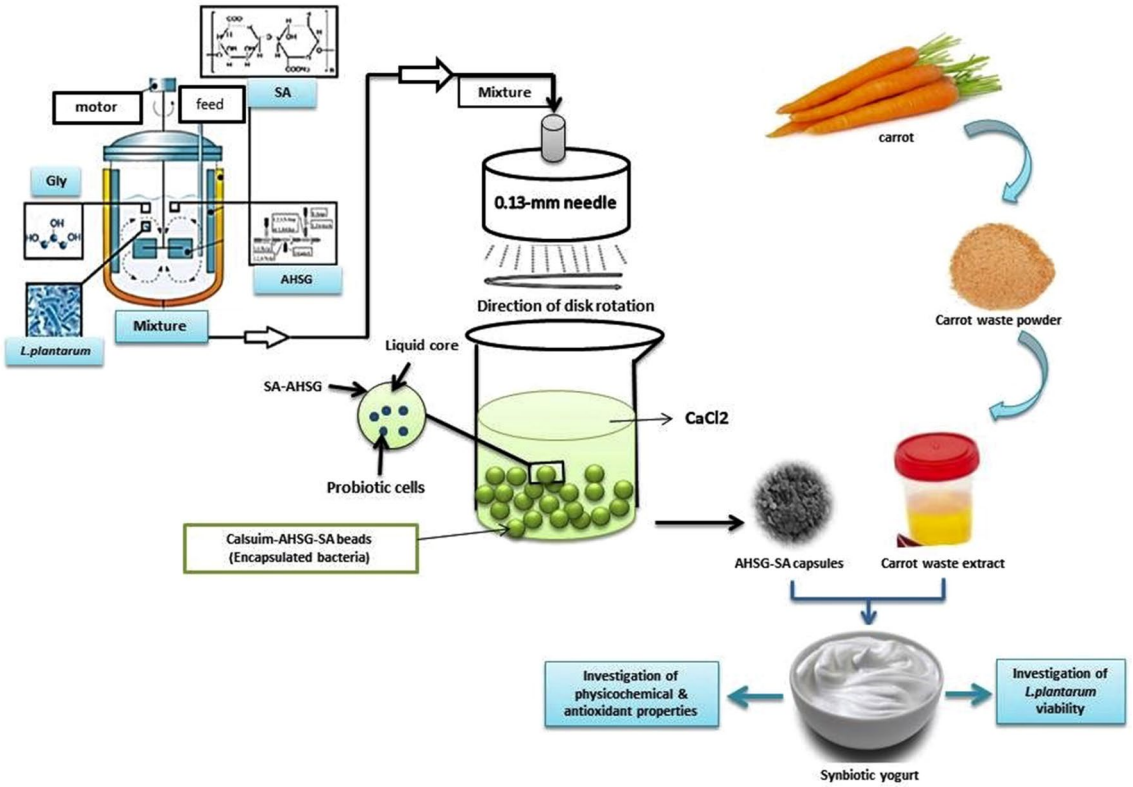
Graphical Abstract



Introduction

Due to growing public awareness of the need of good nutrition and health, as well as growing amounts of scientific data demonstrating their efficacy, interest in functional foods has grown in recent years [92]. Functional foods are characterised as food items with extra or enhanced advantages over and beyond their basic nutritional value [102]. They can help consumers by reducing lactose intolerance, controlling intestinal infections, lowering blood serum cholesterol, and boosting anticancer activity [22, 73, 88].

Probiotics are live bacteria that, when consumed in sufficient quantities, benefit the host. Many lactobacilli are generally regarded as safe (GRAS) bacteria and are found in probiotic foods. Lactobacilli also generate chemicals that inhibit pathogens. It is well-understood that gastrointestinal conditions, food processing, and storage temperature all have an impact on the viable number of probiotics. According to the International Dairy Federation (IDF), probiotics should survive at least 107 CFU/g (of the food product) at the time of ingestion [110]. *Lactobacillus plantarum* is one of the most widely used lactic acid bacterium, showing a homofermentative metabolism, moder ate acid tolerance, and is considered as a GRAS organism. Many strains of *L. plantarum* are marketed as probiotics [28].

Due to economic and environmental concerns, by-products of the fruit processing industries are regarded as waste. Some of the chemicals derived from these wastes may have functional qualities, such as water retention, gel formation, and prebiotic and antioxidant activities [44, 55, 56, 68, 113]. In recent years, there has been a lot of interest in the utilization of non-edible sections of fruits, with the majority of it focusing on the extraction of bioactive components such phenols, antioxidants, antimicrobials, fiber, and pectin. Many studies have found a negative relationship between dietary fiber consumption as a nutrient and the prevalence of chronic and cardiovascular illnesses [14, 52, 57, 70]. Furthermore, the functional features of dietary fiber, such as water—holding capacity, improve digestion, and nutrient absorption in the intestine, can reduce the risk of colon cancer [14, 47]. On the other hand, natural antioxidants reduce the risk of diabetes (type 2) by preventing peroxidation chain reactions [50].

Carrots are a Rich source of β-carotene, fiber, vitamin K1 and antioxidants. Pectin is the main form of soluble fiber can lower blood sugar levels by slowing down the digestion of sugar and starch. Natural carotenoids are more popular as food additives than synthetic colors, owing to regulatory initiatives and consumer concerns. Carotenoids are a significant natural source of provitamin A and anti-oxidant compounds [107], which are commonly associated with a variety of health benefits, such as LDL oxidation inhibition, anti-inflammatory properties, oxidative stress reduction, and immune response enhancement [108].

The initial step in the development of functional food is to identify the functional qualities of existing products, identify appropriate matrices for fortification, and design new products [65]. Yogurt and fortified yogurt production accounts for more than 70% of the overall functional food market [121]. Yogurt's beneficial characteristics have made this dairy product popular all over the world. Furthermore, this dairy fermented product plays numerous roles in human health because it contains a significant amount of natural nutrient components and improves microbiota with probiotic strains and other lactic acid bacteria (LAB) [33]. According to MollakhaliliMeybodi et al. [82], using yogurt increases resistance to dietary infections, boosts the immune system, and improves lactose and important mineral absorption. The scientific emphasis in the field of fermented dairy products has gradually shifted to the addition of ingredients with various synbiotic or bioactive activities to create yogurt with improved nutritional, sensory, physicochemical, and rheological properties when compared to traditional products [82]. Several scientific studies have described the addition of bioactive components in yogurt for the creation of fortified products, including encapsulated apple waste extract [120], tea infusion [86], hibiscus extract [27], grape seed extract [130], mushroom extracts [34], white shrimp shell extract [121], as well as carrot juice waste extract [37].

Hydrocolloids derived from numerous sources are frequently utilized in food systems as thickening and gelling agents, stabilizers, and texture modifiers. Hydrocolloids are polymers that have a significant interaction with water. Because of their low calorie value, they are particularly beneficial in the production of diet foods. Because of their potential to affect the rheological and functional qualities of food systems, hydrocolloids are also used in the food industry [40, 49, 64].

Alyssum is a flowering plant in the Cruciferae family that known by the local name Qodume shirazi in Iran. *Alyssum homolocarpum* seeds have been utilised as a traditional herbal medicine in Iran due to their high mucilaginous content [63].

Extrusion was designed to protect and preserve live probiotics in food products and during gastrointestinal transit. The most popular encapsulating agent for probiotics is sodium alginate (SA). However, the efficiency of SA is restricted because to its porosity structure, which allows other substrates to diffuse into the beads. To solve these issues, combining SA with *Alyssum homolocarpum* seed gum (AHSG) may provide improved protection for probiotics in adverse conditions due to synbiosis [87].

In this study, alginate with *Alyssum homolocarpum* seed gum was employed as the matrix for *Lactobacillus plantarum* microspheres production, which has been approved as a coating material by the Food and Drug Administration (US) and European Food Safety Authority (Europe) [76]. Therefore, the aim of this study was to produce pragmatic synbiotic yogurt using carrot waste and evaluate the effect of microspheres production processes on probiotic viability and physicochemical properties of the product during 28 days of storage.

Materials and methods

Materials

Fresh low-fat milk was provided by the Pegah Dairy Co. (Semnan, Iran). Sodium alginate (SA), was supplied from Sigma Aldrich (St. Louis, MO, USA). *A. homolocarpum* seed and Carrot were purchased from a local market in Semnan, Iran. Isolation of *L. plantarum* isolated from traditional Semnan cheese. All microbial cultures were provided by Ibersco (Karaj, Iran). All chemicals were obtained from Merck (Darmstadt, Germany).

Extraction of AHSG

AHSG was prepared based on the method suggested by Hesarinejad et al. [48]. Briefly, AHSG was extracted from *A. homolocarpum* seeds using distilled water. The swelled seeds were stirred to scrape the gum layer off the seed surface. The collected gum was filtered and dried in a freeze-dryer. The dried extracted gum was packed and stored in dry and cool conditions.

Preparation of carrot waste extract powder

Peels and pulp extract powders were prepared using a multi-stage extraction process. Following collection, the waste was dried in a hot oven at 40 °C for 48 h. The dry by-products were milled to a fine powder using a kitchen-miller (Pars-Khazar, Iran), then blended and extracted overnight with 80% ethanol in a ratio of 1:15 (w/v). The resultant solution was filtered using Whatman paper #4 and centrifuged for 10 min at 4000 rpm. Following that, the supernatant was collected and the solvent was evaporated for 6 h in a vacuum rotary evaporator at 50 °C. The concentrated extracts were finally dried using a freeze dryer (Model FDO-8606, Operon Co. Korea).

Culture preparation

The *L. plantarum* was grown in MRS broth (growth media) at 37 ± 1 °C for 18 h in an anaerobic system, and the bacterial cells were extracted using a centrifuge (for 20 min, 5000 × *g*, 4 °C). Following that, the cells were washed three times with sterile peptone solution to eliminate medium components. The final cell concentration was 3.0×10^9 cfu/ml [114].

Production of microspheres containing *L. plantarum*

The extrusion process reported by [66] with some modification was used to microspheres *L. plantarum*. AHSG and SA were hydrated with distilled water (a mix of AHSG 3%w/v and SA 3%w/v in a volume of 200 ml) at 4 °C for 12 h while gently swirling with a magnetic stirrer, and then autoclaved at 121 °C for 15 min. Sterile SA-solution (3%w/v), was created using a similar procedure. The cleaned bacterial cells (at a concentration of 3×10^9 cfu.g^{-1}) were then mixed with 20 ml of each agent and incubated at 37 °C for 4 h. After that, 5 ml of glycerol was added to the two solutions as a cryoprotectant, and the mixtures were agitated at 400 rpm for 20 min. The microbial suspensions were injected using a 0.13 mm needle into 250 ml of sterile 0.05 M $CaCl_2$ with stirring at 200 rpm and left for 45 min to gelify. Filtration via filter paper (Whatman #4) was used to collect the solidified microspheres, which were subsequently washed with 0.1%v/v sterile peptone solution and packed in sterile glass containers.

Encapsulation yield

After being added to 9 ml of 0.1 M phosphate buffer (pH 7.4), 1 g of microspheres was homogenized in a stomacher for 5 min. Following a centrifugation of the samples at 10,000 g for 10 min at 4 °C, the supernatant was used to plate the samples on MRS agar. According to a formula given by [18], the encapsulation yield—a combined measurement of the effectiveness of entrapment and the survival of viable cells during encapsulation—was measured:

$$\text{Encapsulationyield}(\%) = \frac{N_0}{N} \times 100 \quad (1)$$

where N is the number of viable entrapped cells released from the microspheres, and N_0 is the number of free cells added to the biopolymer mix during formation of the microspheres [96].

Particle size of beads

The size of the microspheres was determined using the laser diffraction particle sizer (Fritsch Particle sizer Analysette 22, Fritsch Co., Germany). The measurement of microsphere size was done immediately after sample preparation. Z-average ± standard error was reported as microspheres particle size.

Production of flavored yogurt

Yoghurts Production according to the method it was done with a few changes [59, 60]. Raw milk first was heated at 90 °C for 10 min. The sample was then cooled to 45 °C and 2% yoghurt starter culture (*Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus*) was added. Fermentation was carried out at 45 °C for 3 h until pH 4.8 was reached. After cooling to 4 °C, the mixture was divided into six equal fractions. Then, *L. plantarum* coated with SA, or with SA-AHSG, free cells, were added aseptically into the mixture. The microspheres and free cells were added to concentrations of about 3×10^9 CFU/g and 3×10^9 CFU/mL, respectively. The ratio of microspheres or free cells to yogurt was 1:10. The yoghurt samples were distributed to sterile plastic cups and packed with sterile plastic lids. The production of flavored yoghurt was done in the same way, to which 6%w/w of carrot waste extract was added.

Enumeration and viability of L. plantarum in yogurt

24 h after production and also the in second and fourth weeks, yogurt samples were tested for viability of *L. plantarum*. To evaluate the number of *L. plantarum* in the samples yogurts were prepared in sterile containers. For this, 10 ml of yogurt was uniformly suspended in 90 ml of sterile phosphate buffer (PBS; pH 7; 0.1 M; 0.85% NaCl), and stirred for 15 min on a rotatory shaker at 37 °C and 180 rpm according to [94]. Appropriate dilution of each sample was prepared in sterile peptone water and survival of *L. plantarum* was determined by plate count technique. *L. plantarum* was enumerated on MRS medium (with 20 mg.l^{-1} vancomycin), at pH 5.6 after 48 h of anaerobic incubation at 30 °C [128]. The number of probiotics bacteria was reported as a log cfu.g^{-1}.

Physical and chemical analysis of yogurt

pH measurement pH of the yogurts was determined with a pH meter (ZAG CHEMIECO) during storage at 4 °C for 4 weeks.

Water holding capacity The water holding capacity (WHC) of the yogurt was measured by centrifugation (K241R Medium Prime Centrifuge, Chichester, UK) of at 4500 rpm for 30 min at 10 °C for 5 g yogurt sample. The following formula for WHC [95] uses as inputs W = weight of separated water, and Y = yogurt weight.

$$\text{WHC} = \frac{Y - W}{Y} \times 100 \quad (2)$$

WHC was measured during the refrigerated storage for 4 weeks.

Syneresis Twenty-five grams of yogurt samples were weighed on a filter paper No. 42 placed on top of a funnel. Syneresis of whey was carried out by gravity and the quantity (grams) of whey collected in a flask of known weight was used as a syneresis value. The drainage time and temperature was 120 min and 4 °C, respectively [122].

Acidity The Dornic acidity was determined by titration of 10 ml of yogurt with 0.1 N NaOH using phenolphthalein as an indicator color. Results were expressed as degree Dornic [1].

Color The color parameters of yogurt samples were determined using WF32 colorimeter (Shanghai Jiabiao). Briefly, 50 g of each yogurt sample was poured into a container and the values of L* (lightness), a* (redness), and b* (yellowness) was measured [98]. Experiments were performed on yogurt samples in 6 replications and the effects of adding microspheres on the color characteristics of the samples compared to the control sample without microspheres were investigated. The purpose of this test was to quantitatively and accurately investigate changes in color parameters of yogurt samples to produce probiotic yogurt with color characteristics similar to the control yogurt.

Total phenolic content (TPC) and DPPH radical scavenging activity The volume of 10 ml yogurt with 10 ml ethanol: water mixture (60:40) was stirred at room temperature for 30 min. The obtained mixture was centrifuged at 5000 rpm for 15 min. The collected supernatant was stored at 2 °C and was used to evaluate TPC and antioxidant activity of the samples.

TPC was determined by [118] method using Folin–Ciocalteu reagent. Yogurt extract (0.3 ml) was mixed with 0.2 N Folin–Ciocalteu (1.5 ml). After 5 min, 1.2 ml solution of 0.7 N Na_2CO_3 was added. The mixture was incubated at room temperature for 2 h and then its adsorption was measured at 765 nm. Quantitative determination was performed based on the standard five-point calibration curve (10; 25; 50; 75, 100 $\mu g.ml^{-1}$) of gallic acid in 80% methanol. The results were expressed as µg equivalent to gallic acid (GAE) per ml yogurt.

In antioxidant measurement, similar to the previous method, 0.1 ml yogurt extract was mixed with 3.9 ml methanol DPPH solution (0.1 m $mol.l^{-1}$). The samples were kept in the dark for 30 min and then the adsorption rate was measured at 517 nm [4]. The scavenging activity of DPPH radicals (%) was calculated by the following equation:

$$\text{Radical scavenging activity}(\%) = \frac{\text{Abs DppH} - \text{Abs sample}}{\text{Abs DppH}} \times 100 \quad (3)$$

Sensory evaluation

The sensory analysis of yogurt samples was assessed accordingly as per the method described by [61]with slight modifications. In brief, yogurt samples were delivered in individual plates, each labeled with a three-digit number, to 30 untrained panelists made up of students and researchers (being 14 men and 16 women). The panelists were trained to evaluate the flavor, color, odor, and overall acceptability of yogurt samples. The panelists assessed the quality and evaluated each yogurt sample using a hedonic scale of 5 points (1 = dislike very much, 2 = dislike a little, 3 = neither like nor dislike, 4 = like a little, and 5 = like very much).

Statistical analysis

The results were evaluated by analysis of variance (ANOVA) and comparison of mean values by Duncan's multiple range test, applied at 5% level of significance ($P < 0.05$), using SPSS software version 21.0. All measurements were repeated in triplicate.

Results and discussion

Size and *L. plantarum* encapsulation efficiency

The encapsulation efficiency was 83.5% for SA microspheres and 91.4% for SA + AHSG microspheres. The results showed that the microspheres produced under the assumption of sphericity had a diameter of 5.6 ± 1.4 mm and 2.1 ± 0.9 mm for SA-based and SA + AHSG-based microspheres, respectively. The results also showed that the type of materials used in the formation of microspheres had an effect on the shape of the microspheres. Mixing SA with AHSG on one hand fills the pores in the structure of SA and on the other hand increases the survival of cells due to its prebiotic properties [81]. Several factors are effective on the diameter of microspheres. In this way, the diameter of the needle used for extrusion, the distance between the tip of the needle and the liquid surface, the concentration of $CaCl_2$ and polymers, as well as the stirring speed, have main effect on the size and shape of the microspheres, as well as the survival of the enclosed organisms [104]. In the research of Muthukumarasamy et al. [84], which was conducted on *Lactobacillus reuteri*, it was found that the type of microcoating material is effective on the diameter of the microsphere. They also found that the combination of gellan and xanthan produced the smallest microspheres diameter (2.14 mm), but when locust bean gum and κ-carrageenan were used, the largest microspheres diameter (3.72 mm)

was created [84]. In the research of Phoem et al. [93], *Bifidobacterium longum* were microcoated with SA and Eleutherine americana extract, the results of this research showed that the microspheres produced by extrusion method had a diameter of 1 to 3 μ [93]. The sphericity of the microspheres is important, because the protective layers of the microspheres are placed around the bacteria in the same ratio. These layers prevent the contact of bacteria with food, so that these bacteria cannot grow in food [38]. In addition, the large size of the microspheres produced by the extrusion method and the increase in the diameter of the protective layers of the microspheres compared to other microcoating methods increase the physical protection of the microspheres [85].

The efficiency of encapsulation with SA + AHSG (91.4%) was very sufficient in this research. One of the most important reasons can be the creation of a combination of SA with AHSG as a hydrocolloid with a low molecular weight [48], which probably reduces the porosity of the microspheres surface and prevents bacteria from leaking into the environment [105]. In a similar study conducted by Sultana et al. [119], it was also found that adding corn starch to SA can increase the efficiency of microcoating. The results of these researchers showed that the encapsulation efficiency of *Lactobacillus reuteri* with SA was 83.3%, which is close to the findings of the present study [119].

L. plantarum survival in yogurt

The results of the survival of *L. plantarum* probiotic bacteria in microspheres and free form in two types of plain and carrot yogurt during 28 days of storage at 4 °C are reported in Table 1. As can be seen, after 28 days, the highest survival rate of *L. plantarum* is related to the sample of yogurt containing carrot extract and microspheres containing SA + AHSG, and the lowest survival rate of this probiotic bacteria is related to the yogurt sample contained free bacteria, which was significantly different from other treatments ($p < 0.05$). In addition, the results showed that there was no significant difference between the treatments of carrot yogurt with SA microspheres and plain yogurt with SA + AHSG microspheres, as well as the treatments of carrot yogurt with free bacteria and plain yogurt with SA microspheres ($p > 0.05$).

In fact, it can be said that at the end of the storage, the population of probiotic bacteria has decreased in all treatments. The amount of this reduction was insignificantly higher in plain yogurt than in carrot yogurt, and yogurt containing microspheres containing bacteria and even carrot yogurt with free bacteria had an acceptable number of probiotic bacteria until the end of the storage period. Meanwhile, in the sample of plain yogurt containing free *L. plantarum*, the colony of bacteria was less than acceptable.

Table 1 Viability of *L. plantarum* (log CFU/g) in yogurt samples during storage

Samples	Day 0	Day 14	Day 28
A	$8.41 \pm 0.03^{A,a}$	$7.45 \pm 0.06^{B,c}$	$6.37 \pm 0.02^{C,d}$
B	$8.60 \pm 0.09^{A,a}$	$8.56 \pm 0.12^{A,b}$	$7.36 \pm 0.06^{B,c}$
C	$8.73 \pm 0.05^{A,a}$	$8.41 \pm 0.03^{B,b}$	$7.48 \pm 0.02^{C,bc}$
D	$8.70 \pm 0.02^{A,a}$	$8.88 \pm 0.09^{A,a}$	$7.55 \pm 0.13^{B,bc}$
E	$8.79 \pm 0.03^{A,a}$	$8.96 \pm 0.01^{\mathbf{A,a}}$	$7.73 \pm 0.04^{B,b}$
F	$8.89 \pm 0.04^{A,a}$	$9.00 \pm 0.03^{A,a}$	$8.13 \pm 0.01^{B,a}$

A: yoghurt simple containing free *L. plantarum*; B: yoghurt simple containing SA-microspheres containing probiotic; C: yoghurt simple containing SA-AHSG-microspheres containing probiotics; D: Carrot yoghurt containing free *L. plantarum*; E: Carrot yoghurt containing SA-microspheres containing probiotic; F: Carrot yoghurt containing SA-AHSG-microspheres containing probiotic

In each column and row, values with different lowercase and uppercase letters, respectively are significantly different ($P < 0.05$)

The observed results for the survival rate of *L. plantarum* indicated that the use of microcoating technique as well as the addition of carrot extract to probiotic yogurt significantly increased the survival of probiotic bacteria compared to yogurts containing free bacteria and yogurt without carrot extract ($p < 0.05$). The reason for the higher survival of probiotic bacteria in yogurts containing carrot extract can be attributed to the phenolic compounds present in the extract, which have a stimulating role and improve the growth of initiator bacteria[91] and probiotic bacteria[77]. In addition, the reason for the higher number of probiotic bacteria in this type of yogurt can be due to the antioxidant properties of these compounds and the removal of oxygen from the environment, especially by phenolic compounds. In these conditions, by removing oxygen and creating a low oxidation–reduction potential, the viability of probiotic bacteria increases [78].

The difference in the number of bacteria in the samples containing SA microspheres and the samples containing SA + AHSG microspheres probably indicated the prebiotic effect of AHSG and also the increase in the strength of the microsphere wall by this gum, which has a positive effect on the survival of bacteria [78]. In general, the production microspheres containing bacteria and the addition of carrot extract had a greater effect on maintaining the survival of cells, so there was a significant increase in the probiotic bacteria's survival when exposed to unfavorable environmental conditions compared to the free form and the sample without extract [3].

The results also revealed that storage time had a significant effect on the viability of probiotic bacteria. As can be seen in Table 1, the number of probiotic bacteria

increased in the second week of storage in some samples, especially the samples containing carrot extract, and then decreased. The reason for the increase of probiotic bacteria in the early storage period can be due to the provision of various conditions, especially the presence of nutrients necessary for the growth of probiotic bacteria [30]. The significant reduction of probiotic bacteria during the storage period is probably due to the increase in the acidity of yogurt as well as the production of hydrogen peroxide by the starter strains and the negative effect of these compounds on the viability of probiotic bacteria [26]. It should be noted that the rate of bacterial population reduction in the samples containing microspheres containing cells did not differ much, which confirms that the production of microspheres containing bacteria has a greater effect on maintaining the survival of cells than in the free form during storage. Similar results were reported by Michael et al. [80], who investigated the effect of plant extracts on improving the survival of *Lactobacillus delbrueckii* subspecies *Lactobacillus acidophilus* and *bulgaricus* in fat-free probiotic yogurt. They stated that at the end of the storage period, the number of *L. bulgaricus* and *L. acidophilus* bacteria in yogurt enriched with plant extracts was higher than that of plain yogurt, and during the storage time, the number of *L. bulgaricus* and *L. acidophilus* in plain yogurt and yogurt containing plant extracts decreased significantly in 29 days of storage [80].

On the contrary, Jaziri et al. [54] who investigated the effect of green and black tea on yogurt microflora during fermentation and storage time reached different results. They studied the acidity and microbial viability of yogurt during 42 days of storage at 4 °C and observed that the presence of green tea had no significant effect on the characteristics of yogurt microorganisms [54]. The results observed by Hadadin et al. who investigated the effect of olive leaf extract on the growth and survival of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in probiotic milk and yogurt during 21 days of storage in the refrigerator, are in agreement with the present study. They stated that the number of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in the samples containing olive leaf extract was significantly higher than the control sample. There is also a positive relationship between bacterial growth and increasing the concentration of olive leaf extract [43]. Mahdian et al. also showed the improvement of the viability of probiotic bacteria by adding beet pulp fiber to probiotics ice cream [74]. Various researchers have shown that the coating of probiotic bacteria including *Lactobacillus* and *Bifidobacterium* species in calcium alginate bed increases their viability during the storage period of ice cream, frozen yogurt, and ice milk [2, 51, 74, 111, 115] Similarly, Tavakoli et al. [125] investigated the effect of thyme and aloevera essential oil as well as storage time at 4 °C on the growth and survival of *Lactobacillus acidophilus* (La-5) in flavored probiotic yogurt drink and observed that adding aloevera and thyme essential oil increased the number of *Lactobacillus acidophilus*. The results of this research also showed that with the increase of storage time until the 11th day, the number of La-5 bacteria in the flavored probiotic yogurt drink increased, and on the 21th day, the number of this bacterium decreased compared to the 11th day, which is consistent with the present study. They also considered the reason for this decrease to be the increase in acidity during the storage period [125]. Hasani et al. [46] also obtained similar results in the study of the effect of barberry extract on the survival of *Lactobacillus acidophilus* in the flavored probiotic set and stirred yogurt during 21 days of storage. They stated that the addition of barberry extract had a significant effect on the growth process of *Lactobacillus acidophilus* during storage, and the rapid growth of this bacterium in probiotic yogurt increased with increasing the concentration of barberry extract [46].

According to these results as well as the findings of the current study, production of microspheres containing probiotics with carrot extract, while increasing the survival and stability of probiotics in yogurt samples, can be used as a strategy to improve the nutritional characteristics of food samples by incorporating bioactive compounds. Therefore, the use of SA along with AHSG strengthens the microspheres wall, and on the other hand, using prebiotic and antioxidant compounds, such as carrot extract, by reducing oxidizing compounds and inhibiting oxygen, a favorable anaerobic environment for probiotics is created, which increases stability and survival of bacteria.

pH

The trend of pH changes in yogurt samples produced during 4 weeks of storage at 4 °C is shown in Table 2. As can be seen, the pH changes during storage had a decreasing trend and the pH levels of the 1st and 2nd weeks did not have a significant difference, and the highest pH level until the end of the storage is related to the treatments of yogurt containing carrot extract containing SA-AHSG microspheres, and plain yogurt contained SA-AHSG microspheres, which had a significant difference with other treatments ($P < 0.05$). Of course, in general, the pH of carrot yogurt samples was higher than that of plain yogurts, which indicates the effect of carrot extract on increasing pH. In addition, the high pH in the samples containing SA + AHSG microspheres compared to the treatments containing SA microspheres indicates the effect of AHSG. The lowest pH level observed was related to the treatments

Table 2 pH and acidity changes in yogurt samples during storage at 4 °C

Samples	pH			Acidity		
	Day 0	Day 14	Day 28	Day 0	Day 14	Day 28
A	$4.20 \pm 0.01^{A,dc}$	$4.23 \pm 0.01^{A,c}$	$4.19 \pm 0.01^{B,cd}$	$1.02 \pm 0.05^{B,a}$	$1.01 \pm 0.03^{B,a}$	$2.48 \pm 0.44^{A,ab}$
B	$4.13 \pm 0.01^{A,d}$	$4.13 \pm 0.01^{A,dc}$	$4.07 \pm 0.01^{B,de}$	$0.96 \pm 0.08^{B,ab}$	$1.07 \pm 0.01^{B,a}$	$2.90 \pm 0.01^{A,a}$
C	$4.34 \pm 0.01^{A,b}$	$4.39 \pm 0.03^{A,a}$	$4.31 \pm 0.01^{B,b}$	$0.81 \pm 0.01^{B,b}$	$0.94 \pm 0.06^{B,a}$	$2.10 \pm 0.08^{A,ab}$
D	$4.31 \pm 0.01^{A,bc}$	$4.31 \pm 0.02^{A,b}$	$4.24 \pm 0.01^{B,cd}$	$0.94 \pm 0.06^{B,ab}$	$1.01 \pm 0.03^{B,a}$	$2.47 \pm 0.18^{A,ab}$
E	$4.20 \pm 0.01^{A,c}$	$4.21 \pm 0.01^{A,cb}$	$4.17 \pm 0.02^{B,d}$	$0.96 \pm 0.03^{B,ab}$	$1.07 \pm 0.01^{B,a}$	$2.16 \pm 0.08^{A,ab}$
F	$4.42 \pm 0.01^{A,a}$	$4.41 \pm 0.01^{A,a}$	$4.38 \pm 0.01^{B,a}$	$0.84 \pm 0.01^{B,ab}$	$0.98 \pm 0.11^{B,a}$	$1.50 \pm 0.42^{A,b}$

A: yoghurt simple containing free *L. plantarum*; B: yoghurt simple containing SA-microspheres containing probiotic; C: yoghurt simple containing SA-AHSG-microspheres containing probiotics; D: Carrot yoghurt containing free *L. plantarum*; E: Carrot yoghurt containing SA-microspheres containing probiotic; F: Carrot yoghurt containing SA-AHSG-microspheres containing probiotic

In each column and row, values with different lowercase and uppercase letters, respectively are significantly different ($P < 0.05$)

of carrot yogurt with SA microspheres and plain yogurt with SA microspheres, and this low pH content in plain yogurt containing SA microspheres was more visible than the sample of carrot yogurt with SA microspheres. According to the results, yogurts containing carrot extract had a higher pH than plain yogurts, which is probably due to the high initial pH of carrot extract, which is about 5.85. From the 2nd week onward, the gradual decrease in pH in carrot yogurts is probably due to the presence of organic acids in carrot extract and fermented sugars and acid production resulting from the activity of bacteria [101]. This gradual decrease in pH in plain yogurts is also due to the production of lactic acid by lactic acid bacteria. In addition, the reason for the high pH of the samples containing SA and AHSG microspheres compared to the samples containing SA microspheres can be related to the pH of the AHSG on one hand and to the increase in strength and uniformity of the microspheres wall with the addition of AHSG.

There are many reports about the survival of probiotic bacteria in acidic environments. The problem with fermented products such as yogurt is that the amount of acid increases during the storage period of the product, which is called excessive acidification or subsequent acidification, which is caused by active β-galactosidase enzyme remain at 0–5 °C. In these situations, the pH may even reach below 2.4 and cause the separation of yogurt serum and the survival of lactic acid producing bacteria is affected due to the increase of hydrogen ions compared to lactate ions. It has been reported that subsequent acidification occurs slower in yogurt samples containing microencapsulated probiotic bacteria than in yogurt samples containing free probiotic bacteria [59, 60]. Dakhteh et al. [25] also reached similar results regarding the effects of AHSG and Persian gum on the physicochemical properties of low-fat cream regarding changes in acidity and pH [25].

Acidity

The results of the acidity of the samples are shown in Table 2. As can be seen, the acidity of all samples has increased in the 4th week. The effect of adding carrot extract on the acidity of the treatments shows that the treatments containing carrot extract had a non-significantly lower acidity than the treatments without extract ($P > 0.05$). AHSG also caused a non-significant decrease in the acidity of samples containing SA and AHSG microspheres compared to other samples ($P > 0.05$).

According to the obtained results, the amount of acidity has also increased in all yogurt samples at the same time as the pH decreased. Due to the fact that there were phenolic antimicrobial compounds in yogurts containing carrot extract, their acidity increased to a lesser extent than plain yogurts [6]. As can be seen, the treatments containing microspheres had less acidity and this is consistent with pH changes (Table 2). Considering that the bacteria which are coated in microspheres have less acidic activity due to being placed inside the microspheres, and the pH of the samples containing the probiotic placed in microspheres was higher than the free form, as a result, the acidity of these samples was lower than other treatments. This result was observed especially in the treatments of carrot yogurt containing SA-AHSG microspheres, and plain yogurt with SA-AHSG microspheres, in which the walls of the microspheres were stronger. Because one of the factors affecting the metabolic activity of coated bacteria in products is the size of the microsphere layers, so the more layers used in the formation of microspheres, the less the acidification process [10]. In this study, adding AHSG to SA and

increasing the strength of the microsphere wall can be a reason for the lower acidity of the samples containing these microspheres.

Huma et al. [53] for yogurt containing apple pulp, Küçüköner [67] for yogurt containing date pulp and grape molasses, Cinbas and Yazici [21] for yogurt containing blueberry, Bueno et al. [17] for yogurt containing Strawberry, raspberry and Pitanga pulp, Alirezalu et al. [5] for yogurt containing blackberry and carrot extracts, Ziena and Abdelhamid [134] for yogurt containing guava leaf extract, and Joung et al. [58] for yogurt containing two types of traditional Korean plant extracts obtained similar results regarding changes in pH and acidity of yogurt during 28 days of storage. They stated that with a decrease in pH, the amount of acidity increases, and in colored yogurts containing natural extracts, acidity increases to a lesser extent due to phenolic antimicrobial compounds [5, 17, 21, 53, 58, 67, 134]. LotfizadeDehkordi et al. [72] also stated that adding *Tragopogon graminifolius* extract to yogurt decreased the rate of acidification. They stated that the extract of this plant affects the activity of lactic acid bacteria and prevents the increase of acidity and the decrease of pH of yogurt [72]. Contrary to the previously reported results, Mazloumi et al. [79] studied the effect of adding inulin on the pH and acidity of low-fat probiotic yogurt and reported that the addition of inulin did not have significant effect on the titratable acidity and pH of yogurt samples [79].

Syneresis

Syneresis is an undesirable feature during yogurt storage and its increase causes a decrease in overall acceptability [83]. Some factors such as fat content, type of starter bacteria, the amount of fat-free dry matter in yogurt, exopolysaccharide production, addition of fibers, extracts, and gums, fermentation temperature, pH of the product, and the addition of beneficial compounds are among the most critical parameters that have a significant impact on the syneresis [83].

The results of syneresis of yogurt samples are shown in Table 3. Based on these results, the amount of syneresis in the carrot yogurt sample containing SA-AHSG microsphere was significantly lower than other samples ($P<0.05$). In general, yogurts containing carrot extract showed a lower amount of syneresis than plain yogurts. The highest syneresis from the first day to the end of the storage period was related to the plain sample with free bacteria, and after that the plain yogurt sample containing SA microsphere had the highest syneresis ($p<0.05$). The results also showed that the amount of syneresis in carrot yogurt treatments was lower than plain yogurt treatments, which is due to the addition of carrot extract to these treatments, which could be due to the relatively high pH of carrot extract, which increased the pH in carrot yogurts. As mentioned, one of the important factors affecting syneresis is pH, and its high level reduces syneresis. Researches have proven that the decrease in pH changes the natural form of the protein and as a result, the water bound to the protein is released due to its denaturation and syneresis increases [11].

As can be seen, in the 2nd week, the syneresis in all treatments decreased compared to 24 h after production, and in the 4th week, the syneresis in them increased compared to the 2nd week, and these results are consistent with the trend of pH changes. In addition, the samples containing SA-AHSG microspheres had a lower syneresis than the samples containing SA microspheres, which is due to the presence of AHSG in the microspheres. The use of AHSG reduced the syneresis due to the involvement of water molecules in the gel network formed by the gum and the increase in the viscosity of the product [12]. The reason for the high syneresis in the samples containing SA microspheres, in addition to their

Table 3 Syneresis of yogurt samples during storage at 4 °C

Samples	Syneresis (%)			WHC		
	Day 0	Day 14	Day 28	Day 0	Day 14	Day 28
A	$56.56 \pm 0.13^{B,a}$	$55.52 \pm 0.22^{B,a}$	$57.24 \pm 0.06^{A,a}$	$47.10 \pm 0.14^{B,bc}$	$52.60 \pm 2.54^{A,ab}$	$50.00 \pm 0.84^{AB,ab}$
B	$54.84 \pm 0.19^{A,b}$	$52.74 \pm 0.29^{A,b}$	$54.96 \pm 0.21^{A,b}$	$43.00 \pm 1.98^{B,c}$	$47.40 \pm 0.56^{A,b}$	$46.80 \pm 3.96^{AB,b}$
C	$51.70 \pm 0.14^{B,d}$	$50.04 \pm 0.30^{B,c}$	$52.30 \pm 0.19^{A,c}$	$53.70 \pm 1.55^{A,a}$	$53.10 \pm 3.25^{AB,ab}$	$50.20 \pm 3.96^{B,ab}$
D	$52.92 \pm 0.20^{A,c}$	$52.01 \pm 0.45^{A,b}$	$52.05 \pm 0.23^{A,c}$	$53.30 \pm 1.83^{B,a}$	$55.60 \pm 2.54^{A,ab}$	$54.10 \pm 5.51^{AB,ab}$
E	$49.54 \pm 0.16^{B,e}$	$48.07 \pm 0.21^{B,d}$	$51.50 \pm 0.14^{A,d}$	$52.40 \pm 1.41^{AB,ab}$	$55.50 \pm 1.83^{A,ab}$	$51.50 \pm 1.55^{B,ab}$
F	$46.52 \pm 0.14^{B,f}$	$46.80 \pm 0.34^{B,e}$	$49.00 \pm 0.12^{A,e}$	$56.30 \pm 0.99^{B,a}$	$62.80 \pm 3.96^{A,a}$	$57.30 \pm 2.40^{AB,a}$

A: yoghurt simple containing free *L. plantarum*; B: yoghurt simple containing SA-microspheres containing probiotic; C: yoghurt simple containing SA-AHSG-microspheres containing probiotics; D: Carrot yoghurt containing free *L. plantarum*; E: Carrot yoghurt containing SA-microspheres containing probiotic; F: Carrot yoghurt containing SA-AHSG-microspheres containing probiotic

In each column and row, values with different lowercase and uppercase letters, respectively are significantly different ($P<0.05$)

low pH, is the disturbance of the structure of the yogurt gel, and the decrease in the strength of the yogurt gel. It has been found that SA microspheres can absorb part of the calcium ions in yogurt gel due to having a series of empty spaces [59, 60]. Tarakci and Kucukoner [124] and Yousef et al. [131] stated that the addition of puree or extract of fruits to yogurt does not always lead to an increase in the syneresis, as this result was also obtained in yogurt produced with banana puree. Therefore, it can be said that additive compounds, by being more coordinated with the yogurt network, cause more water to be retained in its structure, which can reduce the water content of yogurt [124, 131].

In this regard, Mahmoudi et al. [75], Razmkhah et al. [97], and AmiriAghdaei et al. [9] also stated that the addition of maltodextrin, pectin, basil, marv, and psyllium seed gum reduced the syneresis of yogurt samples [8, 9, 75, 97]. The results of current study were also consistent with the results of the research of Roy et al. [100], they showed that the syneresis of yogurt decreases in the first 10 days of storage and then increases until the end of the storage time [100].

Water holding capacity

One of the important factors in determining the quality of yogurt is the water holding capacity [83]. Many factors, including incomplete processes, high acidity, protein content, and storage temperature, affect serum release in yogurt [41]. The results of WHC were shown in Table 3. According to the results, the highest amount of WHC was related to the treatment of carrot yogurt containing SA-AHSG microspheres, and the lowest one was related to the plain yogurt containing SA microspheres. No significant difference was almost observed between all samples. Of course, the carrot yogurt treatment containing SA-AHSG microspheres among the carrot yogurt samples and the plain yogurt treatment containing SA-AHSG microspheres among the plain yogurt treatments had the highest WHC, which were not significantly different from other treatments ($P > 0.05$). In general, it was observed that the samples containing the carrot extract had a higher WHC than the samples without the carrot extract, which is consistent with the syneresis trends (previous section).

Investigating the effect of bacterial inoculation on WHC during storage showed that at the beginning of the storage period, the WHC of free samples was higher than that of samples containing microspheres, but with the increase in storage time, the WHC of free samples decreased, while it increased in samples containing microspheres. These results were consistent with the trend of syneresis in samples containing microspheres. The high WHC in carrot yogurt treatments containing SA-AHSG microspheres, and plain yogurt containing SA-AHSG microspheres, is due to the presence of AHSG in the microspheres structure of these treatments. Adding AHSG increases the percentage of water absorption. This is due to having a higher water absorption capacity than SA, which consequently increases the water absorption of yogurt. This is probably due to the presence of hydroxyl groups in the AHSG structure, which establish hydrogen bonds with water [48]. Sidhu and Bawa [117], Guarda et al. [42], Tavakolipour and Kalbasi-Ashtari [126], Shalini and Laxmi [112] also reported similar results for different gums [42, 112, 117, 126]. Therefore, it can be said that the reason for the higher WHC in yogurts containing carrot extract compared to plain yogurts was the lower acidity of these yogurts, which corresponds to the syneresis and pH.

Total phenolic content

Fruit and vegetable extracts contain different amounts of phenolic compounds[41]. Phenolic compounds have different nutritional and technological effects [69]. In addition to antioxidant, antimicrobial and anti-inflammatory properties, these compounds are used in traditional medicine as blood pressure and blood sugar reducing compounds, diuretics, anti-arteriosclerosis, as well as malaria fever and rheumatism treatment [15, 69]. Enrichment of milk and dairy products with phenolic compounds has also been investigated by Connell and Fox [23]. These researchers concluded that the presence of these compounds improves the antioxidant, sensorial, and antimicrobial properties of milk and dairy products [23].

The changes in TPC during storage are shown in Table 4. As can be seen, TPC increased in all treatments. The amount of these compounds in the samples containing carrot extract was significantly higher than the samples without extract ($P < 0.05$). This increase is quite noticeable at the end of the 4th week compared to the beginning of the storage period, which was due to the presence of phenolic compounds of carrot extract in this type of yogurt. This increase during the storage time is probably due to the release of more carrot extract from the microspheres, which causes an increase in TPC. In other words, due to changes in pH and the activity of microorganisms, the exit of the carrot extract increased and then TPC increased. This phenomena is in good agreement with Du et al. [31] who found mulberry pomace in functional yogurt increased free phenolic acids during storage. This could be due to ferulic acid esterase in these bacteria. There may be a balance between the release and degradation of phenolics in functional yogurt. Bound phenolics in carrot extract added to the yogurt were gradually liberated by LAB, while free phenolics gradually degraded due to oxidation

Table 4 TPC and radical scavenging activity of yogurt samples during storage at 4 °C

Samples	TPC (µg/ml)			DPPH		
	Day 0	Day 14	Day 28	Day 0	Day 14	Day 28
A	$14.30 \pm 2.12^{A,d}$	$15.63 \pm 2.01^{A,d}$	$16.13 \pm 1.28^{A,d}$	$5.50 \pm 1.02^{B,e}$	$12.53 \pm 1.45^{A,b}$	$7.40 \pm 1.98^{AB,c}$
B	$15.13 \pm 3.11^{A,d}$	$16.30 \pm 1.96^{A,d}$	$18.63 \pm 2.04^{A,d}$	$7.11 \pm 0.99^{B,d}$	$13.15 \pm 4.08^{A,ab}$	$8.83 \pm 2.12^{AB,bc}$
C	$20.80 \pm 1.09^{B,c}$	$24.13 \pm 2.14^{A,c}$	$25.67 \pm 1.65^{A,c}$	$9.50 \pm 0.77^{B,c}$	$14.70 \pm 1.23^{A,ab}$	$9.46 \pm 1.58^{B,abc}$
D	$28.30 \pm 3.13^{B,b}$	$31.63 \pm 3.10^{A,b}$	$31.63 \pm 3.71^{A,b}$	$10.80 \pm 0.53^{B,bc}$	$15.61 \pm 2.01^{A,ab}$	$10.22 \pm 2.08^{B,ab}$
E	$24.13 \pm 4.16^{C,b}$	$30.96 \pm 2.65^{B,b}$	$46.13 \pm 2.59^{A,a}$	$11.80 \pm 3.39^{B,b}$	$16.20 \pm 1.96^{A,ab}$	$13.38 \pm 1.16^{AB,ab}$
F	$37.63 \pm 2.14^{C,a}$	$42.50 \pm 2.47^{B,a}$	$48.30 \pm 1.46^{A,a}$	$13.60 \pm 0.64^{B,a}$	$17.50 \pm 1.45^{A,a}$	$14.64 \pm 2.45^{B,a}$

A: yoghurt simple containing free *L. plantarum*; B: yoghurt simple containing SA-microspheres containing probiotic; C: yoghurt simple containing SA-AHSG-microspheres containing probiotics; D: Carrot yoghurt containing free *L. plantarum*; E: Carrot yoghurt containing SA-microspheres containing probiotic; F: Carrot yoghurt containing SA-AHSG-microspheres containing probiotic

In each column and row, values with different lowercase and uppercase letters, respectively are significantly different ($P < 0.05$)

and utilization during the refrigerated storage. Within a certain period, the releasing amount of polyphenolics exceeded that of the degraded ones, so TPC gradually increased [31]. The carrot yogurts containing SA-AHSG microspheres had the highest TPC from the 1st week to the end of the storage period, and there was no significant difference with the carrot yogurt containing SA microspheres, but the difference was significant with other treatments. The plain yogurt samples with free bacteria was also non-significantly lower than the treatment of plain yogurt containing SA microspheres, and significantly compared to other treatments ($p < 0.05$). There was no significant difference between the treatments containing bacteria in microspheres and free forms, so it can probably be said that production of microspheres did not have a significant effect on TPC changes. In each group of yogurts, both carrot and plain yogurts, TPC in treatments containing SA-AHSG microspheres was insignificantly higher than other treatments, which could be due to the presence of phenolic compounds in AHSG [13]. The phenolic compounds present in plain yogurt samples are probably due to the presence of polyphenols in milk, which are mainly caused by animal feeding [89]. The reason for the lower amount of TPC in the treatments containing free bacteria compared to other treatments can be attributed to the metabolic activity of the bacteria in connection with the reduction or change of phenolic compounds that were able to react with Folin Ciocaltio reagent [116]. The results of this research also indicated that carrot extract yogurt containing SA-AHSG microsphere with 30.48 µg/ml had the highest amount of TPC and plain yogurt containing free bacteria had the lowest amount with 16.13 µg/ml. In this regard, various researchers investigated the enrichment of yogurt with different fruit extracts and reported that the addition of these improves the phenolic, nutritional, and sensory properties of yogurt [35, 36, 58, 133].

Antioxidant activity

The antioxidant activity of yogurt samples during 28 days of storage is shown in Table 4. According to the results, the highest level of DPPH inhibition belonged to the treatment of carrot yogurt containing SA-AHSG microspheres, which was significant with plain yogurt and insignificant with other carrot yogurts. The antioxidant activity of plain yogurt with free bacteria was insignificantly less than other plain yogurts and significantly less than carrot yogurts (Table 4). As can be seen, at the beginning of the storage period, the antioxidant activity in all samples was low, but in the 2nd week, it had an increasing trend, and from the 2nd week until the end of the period, it had a decreasing trend. The decrease in antioxidant activity during refrigerated storage may be attributed to the increase in the degradation of phenolic compounds, or it may be due to the increase in the reaction between milk proteins and polyphenols [132]. In other words, a reaction occurs between the hydroxyl groups of the extract compounds with amino acid proline and casein proteins, which are abundant in milk [32]. In the continuation of refrigerated storage, a significant increase in antioxidant activity was observed in all treatments ($P < 0.05$). The reason for this observation can be stated that microbial growth during storage may cause changes in some phenolic compounds and hence increase antioxidant activity [16]. It can also be said that the degradation of milk proteins by lactic acid bacteria helps to increase TPC. For example, the amino acid tyrosine, which is formed after the decomposition of milk proteins, has a side phenolic chain [110].

In total, the inhibitory in the samples containing carrot extract was significantly higher than the samples without extract ($P < 0.05$). According to the results, it was found that production of microspheres had no significant effect on the DPPH ($P > 0.05$). The results also indicated that by adding plant extracts to probiotic yogurt, the radical

inhibition and as a result its antioxidant activity increases significantly ($P < 0.05$), which can be attributed to phytochemical compounds (phenolic compounds) and metabolites resulting from bacterial activity were attributed [127]. β-Carotene, falcarinol and vitamin C are among the antioxidants found in carrot extract [109]. In addition, catalase and superoxidase enzymes, casein, serum proteins and uric acid present in milk and lactic acid bacteria show antioxidant activity, which causes such a feature to be observed in plain yogurt as well [19, 62, 71, 90]. Various researchers also reached similar results by investigating the effect of adding plant extracts on the antioxidant properties of yogurt [7, 24, 46, 91].

Color parameters

Color is one of the most important visual features in food products [98]. The evaluation of color parameters in yogurt treatments during 28 days of storage at 4 °C is shown in Table 5. According to the results, a significant difference was observed between the values of L*, a* and b* indices from the beginning to the 4th week. In general, the lightness index (L*) in plain yogurts was higher than that of carrot yogurts, and among the plain yogurts, the treatment containing SA-AHSG microspheres; and among the carrot yogurts of the same treatment with SA-AHSG microspheres had higher light intensity. The effect of carrot extract on the yellowness index (b*) was significant, but AHSG and SA had no significant effect on it. In addition, the decrease in redness index (a*) in carrot yogurt was more than plain yogurt ($P < 0.05$).

Color parameters are correlated with pH in such a way that decreasing pH during the storage period can decrease the lightness of yogurt [39], which is almost consistent with the results of the present study. According to the trend of pH changes observed in the previous sections, in the first week the pH was low, then it increased insignificantly, and at the end of the storage period, it started to decrease again, which is in agreement with the changes in the color index L*.

Reducing the lightness index by adding carrot extract is considered completely natural. Because the color of carrot extract plays an essential role in reducing this parameter. In normal conditions, the lightness index (L*) in yogurt treatments is extremely high, which is related to the presence of more casein micelles and increased light reflection, which in plain yogurt treatments, this index was higher than in carrot yogurts [129]. In addition, in the samples containing SA-AHSG microspheres, this index was higher than other samples, which is due to the presence of AHSG in the structure of these microspheres, because this hydrocolloid causes light reflection by absorbing moisture.

The high level of yellowness (b*) in carrot yogurts is due to the presence of carotenoid pigments in carrot extract, among which α- and β-carotene can be mentioned. In general, in the extraction process, the final liquid obtained has a yellow color, which is due to the destruction of the carrot tissue in the extraction process and finally the introduction of carotenoid-based yellow pigments into the isolated extract. Based on this, the low parameter a* can be predicted.

The results of these findings were similar to the research of Tarakci [123], who reported that adding kiwi marmalade to fruit yogurt increases the a* index compared to the control treatment [123]. Sanz et al. [103] also showed that adding asparagus fibre to yogurt will increase a* and b* and decrease L* [103]. Chouchouli et al. [20] also reported a decrease in L* of yogurt enriched with extracts of different seeds such as grape seeds compared to the control sample[20].

Table 5 Color parameters of yogurt samples during storage at 4 °C

Samples	L*			a*			b*		
	Day 0	Day 14	Day 28	Day 0	Day 14	Day 28	Day 0	Day 14	Day 28
A	$66.31 \pm 1.17^{A,a}$	$63.47 \pm 0.60^{B,bc}$	$61.08 \pm 0.71^{C,a}$	$0.41 \pm 0.55^{B,b}$	$1.05 \pm 0.12^{A,a}$	$0.85 \pm 0.19^{A,a}$	$15.28 \pm 0.75^{A,b}$	$7.14 \pm 0.13^{B,c}$	$6.46 \pm 0.35^{B,c}$
B	$63.25 \pm 1.69^{B,b}$	$66.86 \pm 1.72^{A,ab}$	$61.25 \pm 1.83^{C,a}$	$1.18 \pm 0.32^{A,ab}$	$1.08 \pm 0.41^{A,a}$	$0.91 \pm 0.16^{A,a}$	$16.55 \pm 0.16^{A,b}$	$5.79 \pm 0.45^{B,d}$	$6.95 \pm 0.46^{B,c}$
C	$61.01 \pm 0.54^{B,bc}$	$68.66 \pm 1.04^{A,a}$	$62.13 \pm 1.05^{B,a}$	$1.82 \pm 0.31^{A,a}$	$-0.28 \pm 0.04^{B,b}$	$-0.85 \pm 0.53^{B,b}$	$15.12 \pm 0.44^{A,b}$	$5.20 \pm 0.12^{B,d}$	$6.40 \pm 0.08^{B,c}$
D	$58.31 \pm 0.31^{B,cd}$	$64.75 \pm 2.36^{A,bc}$	$59.74 \pm 1.06^{B,ab}$	$-0.16 \pm 0.11^{A,d}$	$-0.18 \pm 0.26^{A,b}$	$-0.20 \pm 0.37^{A,b}$	$30.06 \pm 0.12^{A,a}$	$10.42 \pm 0.53^{B,b}$	$12.00 \pm 0.80^{B,a}$
E	$55.44 \pm 0.62^{B,d}$	$64.06 \pm 0.92^{A,bc}$	$57.16 \pm 0.64^{B,b}$	$-0.33 \pm 0.15^{A,c}$	$-0.31 \pm 0.12^{A,b}$	$-0.37 \pm 0.15^{A,b}$	$29.84 \pm 0.44^{A,a}$	$11.77 \pm 0.14^{B,a}$	$13.06 \pm 0.20^{B,a}$
F	$60.60 \pm 1.96^{A,bc}$	$61.28 \pm 0.56^{A,c}$	$60.56 \pm 1.39^{A,a}$	$-0.67 \pm 0.33^{A,c}$	$-0.61 \pm 0.13^{A,b}$	$-0.32 \pm 0.41^{A,b}$	$27.09 \pm 2.52^{A,a}$	$7.80 \pm 0.39^{B,c}$	$9.47 \pm 0.31^{B,b}$

A: yoghurt simple containing free *L. plantarum*; B: yoghurt simple containing SA-microspheres containing probiotic; C: yoghurt simple containing SA-AHSG-microspheres containing probiotics; D: Carrot yoghurt containing free *L. plantarum*; E: Carrot yoghurt containing SA-microspheres containing probiotic; F: Carrot yoghurt containing SA-AHSG-microspheres containing probiotic

In each column and row, values with different lowercase and uppercase letters, respectively are significantly different ($P < 0.05$)

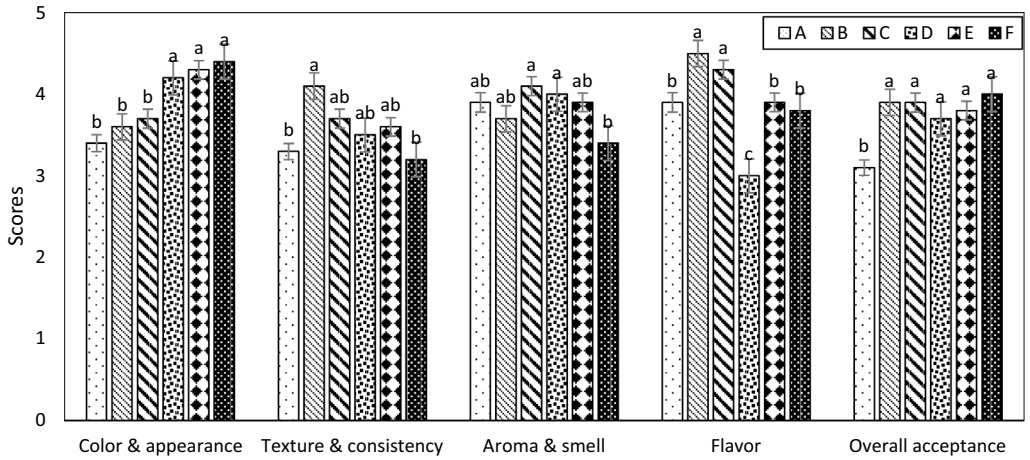


Fig. 1 Sensory evaluation of produced yogurts after 10 days of storage at 4 °C. **A** yoghurt simple containing free *L. plantarum*; **B** yoghurt simple containing SA-microspheres containing probiotic; **C** yoghurt simple containing SA-AHSG-microspheres containing probiotics; **D** Carrot yoghurt containing free *L. plantarum*; **E** Carrot yoghurt containing SA-microspheres containing probiotic; **F** Carrot yoghurt containing SA-AHSG-microspheres containing probiotic. *Values with different letters are significantly different ($P < 0.05$)

Sensory evaluations

Color and appearance

The results sensory evaluation of yogurts are presented in Fig. 1. The results showed that the color and appearance of the carrot yogurt samples were significantly better than the plain yogurt samples ($p < 0.05$). In fact, the addition of carrot extract had a significant effect on the acceptability and desirability of the color of the samples. Carrots can be used in the preparation of various products due to the presence of nutritious and suitable compounds, the presence of carotenoid pigments and also the pleasant color. There was no significant difference between the treatments containing bacteria in microspheres and free forms ($p < 0.05$). However, the color and appearance of the samples containing microspheres was slightly better than free bacteria, and among the samples containing SA beads and the samples containing SA-AHSG microspheres, the treatments containing SA-AHSG microspheres got more score ($p < 0.05$). In general, in terms of color and appearance, the highest score was given to the treatment of carrot yogurt with SA-AHSG microspheres, and the lowest score was given to the treatment of plain yogurt containing free bacteria. Kailasapathy [59, 60] also showed that probiotic yogurt containing encapsulated bacteria with alginate and resistant starch had a non-significantly better color and appearance than control yogurt due to the presence of starch [59, 60].

Texture and consistency

Texture is one of the most important features in food products that affects consumer acceptance[99]. According to the results of the texture and consistency, the highest and lowest quality belong to the order of treatments of carrot yogurt with SA-AHSG bead; and plain yogurt contained free bacteria, which were significantly different from other treatments ($p < 0.05$). Yogurts containing carrot extract had better consistency and texture than other samples, and in this regard, they showed a lower syneresis than plain yogurts, which caused them to get more scores from sensory evaluators. In addition, the samples containing beads, especially the beads containing AHSG, had a better consistency than the samples containing free bacteria due to the reduction of syneresis. Hansen et al. [45] also reported that if capsules containing probiotic bacteria are added to food, capsules larger than 1 mm cause roughening in food texture [45]. Kailasapathy [59, 60] reported that the use of SA capsules and resistant starch does not significantly affect the sensory characteristics, such as color, smell, and taste of yogurt, but it significantly changes the textural characteristics of yogurt [59, 60]. In the end, it should be noted that sensory evaluators stated that microspheres are observed that cause a sandy mouthfeel; but they liked this texture and it did not lower the texture score of the samples.

Aroma and smell

In terms of aroma and smell factor, according to sensory evaluators, the plain yogurt treatment containing SA-AHSG beads gained the highest score and the carrot yogurt treatment with SA-AHSG beads the received lowest score. Of course, there was no significant difference between all treatments in terms of aroma and smell. The reason for the slight difference in the scores of plain and carrot yogurt samples is carrot extract, which according to some evaluators, carrot yogurts did not have a

pleasant aroma. In general, the aromatic nature of yogurt is due to the breakdown of fat, lactose, proteins and citric acid of milk, producing aromatic substances that are specific to yogurt. The most important of them is acetaldehyde, which is present in the amount of 4–15 mg per kg of yogurt. Other aromatic substances include: acetone, butane, ethyl acetate, lactones and esters, in addition to the compounds of free fatty acids, diacetyl and acetone, they also play a role in creating the aroma of yogurt, and the produced carbon dioxide also creates freshness in yogurt at the same time. Free amino acids can also be used as precursors of aromatic compounds [46].

Taste

The results of the sensory evaluation showed that plain yogurts had a significantly better taste than carrot yogurts, and the plain yogurt sample containing SA beads had the best taste and the carrot yogurt sample with free bacteria had the worst taste. The superiority of plain yogurts compared to carrot is due to the fact that by adding carrot extract to the samples, the viscosity increases and this slows down the movement of the macromolecule in the complex molecular space created. This slowness of movement also occurs in the volatile and flavoring compounds of yogurt, and as a result, these compounds are less released in the mouth and affect the sensory evaluation of taste [106]. In addition, there was a significant difference in taste between the treatments containing free bacteria and the treatments containing microspheres containing bacteria ($p < 0.05$) and the samples containing free bacteria received a lower score in taste. The reason is that the probiotics are inside the bead, which has less acid activity, and as a result, the taste score of the yogurts that were used with beads containing probiotics bacteria was significantly higher than the free form. The type of wall and the use of AHSG had no significant effect on the taste [29].

Overall acceptance

In the evaluation of the acceptability of the samples, it was found that the treatment of carrot yogurt containing SA-AHSG beads had the highest quality and the treatment of plain yogurt containing free bacteria had the lowest quality in terms of sensory characteristics. Samples containing beads containing probiotic bacteria were better than samples containing free bacteria and were superior in most sensory attributes. As a result, they scored higher in terms of overall acceptance. The reason for the decrease in the overall acceptance score of the samples containing free bacteria is probably the increase in acidity and sourness of the product, which can change the overall acceptance score even in small amounts.

Conclusion

In general, it was found in this research that the microsphere production containing *Lactobacillus plantarum* probiotic bacteria can be an effective factor in increasing the viability of bacterial cells. In addition, the use of AHSG together with SA as a coating is suitable for this purpose and has a higher efficiency than alginate alone. In addition, adding carrot extract to yogurt can improve its physicochemical and antioxidant properties. In addition, carrot extract and AHSG can play the role of prebiotic for probiotic bacteria and increase their survival. From the findings of this study, it can be concluded that yogurt is a suitable substrate for the growth of *Lactobacillus plantarum* and can be considered as an environment for the transfer of probiotic microorganisms to the human body. In addition, due to increasing the viability of probiotic bacteria and having desirable quality characteristics, synbiotic yogurt containing carrot waste extract can be produced as a new dairy product on an industrial scale.

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Author contributions
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There is no conflict of interest.

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