Original Article
Food Science and Technology

ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.1590/fst.40322

Improvement of the quality parameters of a novel synbiotic yogurt sauce using microencapsulated *Lactobacillus paracasei* and natural prebiotics

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Abstract

This study aimed to examine the production of a synbiotic yogurt sauce and to evaluate its physicochemical, microbiological, sensory, and rheological properties during storage. *Lactobacillus paracasei* was microencapsulated with resistant starch and sodium alginate by the emulsion technique. Two forms of probiotic bacteria (microencapsulated and free), together with prebiotic compounds (flaxseeds and chia seeds each one at two concentrations of 2% and 4%), were added to the yogurt sauce. Samples were kept for 30 days at 4 °C and observed for physicochemical, microbiological, sensory, and rheological characteristics within specific intervals. The results revealed that the produced synbiotic yogurt sauce was characterized as a pseudoplastic fluid. However, its viscosity increased due to the presence of flaxseeds and chia seeds. The addition of flaxseeds and chia seeds improved the survival of bacteria. The post-acidification values were lower in samples containing the microencapsulated bacteria compared to samples containing the free bacteria. Among the produced samples, the highest acceptable score for the sensory characteristic was obtained for the sample containing microencapsulated *L. paracasei* and 2% flaxseeds. Therefore, it is possible to produce a synbiotic yogurt sauce with desirable properties.

Keywords: chia seeds; flaxseeds; *Lactobacillus paracasei*; yogurt sauce.

Practical Application: In this study, *Lactobacillus paracasei* (free and microencapsulated) together with natural prebiotics (flaxseeds and chia seeds) were added to the yogurt sauce. It was shown that functional yogurt sauce with desirable quality could be produced using microencapsulated *L. paracasei* and 2% flaxseeds. Therefore, it is recommended to use of them in yogurt sauce production and it can be extended to industrial scale.

1 Introduction

Synbiotic foodstuffs have been defined as food products, containing both probiotics and prebiotics that can improve human health (Kearney & Gibbons, 2018). The positive effect of synbiotic food products on the host has been attributed to the activated metabolism of a number of health-promoting bacteria, and/or stimulation of the growth of useful bacteria, and/or increased survival of nutritious microbial communities in the gastrointestinal tract (Shaghaghi et al., 2013).

Probiotics are living microorganisms, which confer health benefits to consumers when used at suitable quantities (Afzaal et al., 2019; Zendeboodi et al., 2020). Many reports have described the potential therapeutic effects of probiotics, such as enhancement of the innate immune function, reduction of cholesterol levels, and treatment of gastrointestinal diseases (Bron et al., 2017; Han et al., 2020; Miremadi et al., 2014; Gibson et al., 2017). Prebiotic compounds are non-digestible and non-viable food ingredients, which are selectively processed by probiotics as energy sources to enhance their activity or growth (Fahimdanesh et al., 2013; Xavier-Santos et al., 2022). The synergy between probiotics and prebiotics in synbiotic foodstuffs has been confirmed in many studies (Homayouni et al., 2008; Markowiak & Śliżewska, 2017; Mohanty et al., 2018).

Probiotics refer to a variety of microorganisms, including bacteria (Lee et al., 2020). The most investigated probiotics include lactic acid bacteria, particularly *Lactobacillus* (Verdenelli et al., 2009). *Lactobacillus paracasei* is considered a probiotic agent with therapeutic properties (Shaghaghi et al., 2013). This bacterium is commonly used in dairy products, such as fermented milk, yogurt, and sauce (Afzaal et al., 2019; Guerra et al., 2018). Natural prebiotics from sources, such as grains and vegetables, are consumed in everyday meals and are considered as the most important prebiotic compounds. Chia seeds and flaxseeds are known as excellent sources of numerous nutrients with health benefits, which can reduce the risk of some diseases, such as cardiovascular disease (Shaghaghi et al., 2013). Chia seeds are ideal sources of antioxidants, proteins, minerals, and polyunsaturated fatty acids (PUFAs) (Rojas et al., 2019). Besides, flaxseeds have a high content of dietary fibre, mineral substances, linoleic acids, and also proteins (Alexeev et al., 2015).

Received 25 Mar., 2022
Accepted 20 May, 2022
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The efficiency of probiotic bacteria depends on several factors, such as the type of food used for the delivery of these bacteria to the human body, viability of bacteria during product storage, and tolerance of probiotics under gastrointestinal conditions (Afzaal et al., 2019; Pena et al., 2021; Qi et al., 2019). Consumption of probiotic bacteria via dairy food products, including yogurt sauce, is an ideal method for the delivery of these bacteria (Homayouni et al., 2008). However, for survival, they need to tolerate toxic metabolites, produced during digestion or under gastric acidic conditions and resist bile salts in the lower intestine. The survival of probiotic bacteria in these difficult conditions is essential to induce their health benefits (Afzaal et al., 2019).

So far, several protection methods have been utilized to enhance the survival of probiotics in food products. Among these methods, microencapsulation has currently gained more attention, as it can increase the viability of probiotic bacteria during processing and in the gastrointestinal tract (Afzaal et al., 2019; Liu et al., 2020). Microencapsulation is a technique, in which a substance is confined by a wall material (Muzzafar & Sharma, 2018). This substance is a probiotic bacterium that can be coated by some materials, such as chitosan, resistant starch, and sodium alginate, to create a microencapsulating matrix (Krasaekoopt et al., 2003; Krasaekoopt et al., 2006). Among techniques that have been used to produce microcapsules, the emulsification technique has been introduced as the best procedure due to simple reactive conditions, processing, and equipment, together with the smaller size of the produced alginate (Qi et al., 2019).

Yogurt is the most common dairy food, with health benefits for consumers due to the presence of lactic acid bacteria (Afzaal et al., 2019). Novelty in the production of yogurt products leads to the increased consumption of yogurt, which can result in improved health outcomes (Eor et al., 2020; Hadjimbei et al., 2020; Lucatto et al., 2020). Yogurt sauce is one of the derivatives of yogurt, which is prepared by adding some dressings, such as lemon juice or vinegar, garlic, pepper, salt, thyme, parsley, tarragon, and olive oil, to plain yogurt. Today, there is a great interest in the use of yogurt sauce as a flavoured dressing for various salads and foods. Apart from common bacteria that can be found in yogurt products, the addition of other suitable bacteria, as well as prebiotic compounds, to yogurt products can confer more health benefits to consumers. Therefore, this study aimed to produce a synbiotic yogurt sauce and evaluate its physicochemical, microbiological, sensory, and rheological characteristics during 30 days of storage.

2 Materials and methods

2.1 Preparation of probiotic bacterial culture

A pure culture of probiotic bacterium, namely, *L. paracasei* ATCC 18036, was obtained from Iranian Research Organization for Science and Technology. The freeze-dried culture was transferred to a De Man, Rogosa and Sharpe (MRS) broth and incubated at 37 ˚C for 24 h under anaerobic conditions (Golestani & Pourahmad, 2017). Next, probiotic cells were centrifuged at 4000 rpm for 15 min at 4 ˚C and washed in a sterile 9% saline solution using a similar centrifugation procedure. Later, probiotic cells were used in the microencapsulation process. A cell count exceeding 10^9 CFU/mL was considered for the cell concentration (Afzaal et al., 2019).

2.2 Microencapsulation of L. paracasei

All solutions and glassware used were sterilized at 121 ˚C for 15 min. The microencapsulation method was applied using the emulsion procedure. First, 2 g of resistant starch (HI-MAIZE® 260 starch, UK) and 2 g of sodium alginate (product number 71238, Sigma-Aldrich, USA) were slowly added to 100 mL of distilled water until entirely dissolved. Next, 0.1% probiotic cultures were transferred into the solution and stirred for five minutes. To create an emulsion, the final mixture was suspended in 200 mL of corn oil, containing 0.2% Tween 80 (Merck, Germany) as an emulsifier and then mixed using a stirrer at 350 rpm for 20 min to obtain a homogenous emulsion.

Afterward, to prepare the alginate capsules, 200 mL of calcium chloride (0.1 M) was added to the final emulsion, resulting in the emulsion phase separation. Next, the mixture was allowed to rest for 30 min to obtain alginate capsules at the bottom of the container. Finally, the alginate capsules were separated from the mixture by centrifugation at 350 rpm for 15 min. The capsules were washed with 1% peptone water and stored at 4 ˚C (Sultana et al., 2000).

2.3 Survival of microencapsulated and free bacteria in the Simulated Gastric Fluid (SGF)

The SGF was prepared from chloride sodium (0.2% w/v, pH = 2), and pepsin was added to the solution at a final concentration of 3.2 g/L. Next, it was filtered through a 0.22-µm sterile membrane. The microencapsulated (0.5 g) and free (0.5 mL) bacteria were added to the SGF (9.5 mL) and incubated at 37 ˚C for 0, 30, 60, 90, and 120 min in a shaking incubator at 150 rpm. At the end of incubation, the SGF was neutralized with phosphate buffer (0.2 m/L, pH = 7) at 4 ˚C. The survival of microencapsulated and free bacteria was measured at the specified intervals. All experiments were repeated in triplicate (Chen et al., 2017).

2.4 Survival of microencapsulated and free bacteria in the Simulated Intestine Fluid (SIF)

The SIF was prepared by dissolving potassium hydrogen phosphate (K_2HPO_4) in distilled water at a final concentration of 6.8 g/L (pH = 7.4). Next, trypsin was added to the solution to reach a final concentration of 10 g/L. The mixture was then filtered through a 0.22-µm membrane filter for sterilization. Afterward, microencapsulated (0.5 g) and free (0.5 mL) bacteria were added to the SIF (4.5 mL) and incubated at 37 ˚C for four hours in a shaking incubator at 150 rpm. At the determined time intervals, the total viable count of probiotic bacteria was measured using the pour-plate technique with a nutrient yeast extract salt medium (NYSM) agar (Chen et al., 2017).

2.5 Characterization of microencapsulated bacteria

Scanning electron microscopy (SEM, LEO 440 I, Leo Electron Microscopy Ltd., Cambridge, UK) was applied to observe the surface and morphology of microencapsulated bacteria. To prepare the microcapsules, they were located on a specimen stub and coated with gold using a sputter coater. After ten minutes, the surface of the prepared samples was examined by SEM at an accelerating voltage of 15 kV and a power of 20 W (Mokarram et al., 2009).

2.6 Production of synbiotic yogurt sauce

Fresh pasteurized yogurt (2.5% fat, Choopan Dairy Company) was purchased from the local market and used for the preparation of yogurt sauce. To produce the yogurt sauce samples, ingredients, such as sugar (5%), salt (1.2%), mustard (0.3%), and citric acid (0.03%), were added to water (12%) and mixed completely. Next, a proper amount of yogurt (35%) was added to the prepared mixture. Carrageenan powder was then gradually added to the final mixture, followed by eggs (9%), and mixed thoroughly until obtaining a uniform substance. The last additive, that is, oil droplets (30%), was added to the prepared mixture, and finally, the yogurt sauce samples were produced. To produce synbiotic yogurt sauce, the following probiotic bacteria and prebiotic compounds were individually added to the yogurt sauce samples: two prebiotic compounds (chia seeds and flaxseeds) each one at different concentrations (0%, 2%, and 4%) and two forms of *L. paracasei* (free or microencapsulated). All samples were kept at 4 ˚C for 30 days. The synbiotic yogurt sauce samples were collected at four intervals (on days 1, 10, 20, and 30) for microbiological, physicochemical, and sensory analyses.

2.7 Determination of pH, acidity, and viscosity of synbiotic yogurt sauce samples

The pH of the samples was measured by a digital pH meter (Metrohm, Switzerland) at 20 ˚C. Before use, the pH meter was calibrated with standard buffers (pH = 9 and pH = 4) at 20 ˚C (Yadav et al., 2018). To determine acidity, 10 g of each sample was dissolved in distilled water and mixed completely, followed by adding distilled water to a final volume of 100 mL. Next, 1 mL of phenolphthalein was added to 25 mL of the final solution. It was titrated against a standard sodium hydroxide solution until a pink-colored solution emerged (Yadav et al., 2018). The viscosity of the samples was determined using a Brookfield viscometer (Brookfield Engineering Lab Inc., Stoughton, MA, USA) with a No. 5 spindle at 25 rpm for 50 s at 25 ˚C (in cP) (Shaghaghi et al., 2013). The rheological characteristics of all samples were also determined at 20 ˚C by a rotating cylinder (MCR 301, Anton-Paar Co., Graz, Austria).

2.8 Color measurements of synbiotic yogurt sauce samples

The color measurements of all samples were performed using a HunterLab colorimeter (ColorFlex, HunterLab, USA), with three numerical values, namely, a*, b*, and L*. The values of a*, b*, and L* represent green/red, yellow/blue, and brightness/darkness, respectively (Krishnamurthy & Kantha, 2005). Overall, a negative value for a* indicates a green color, while a positive value for a* indicates a red color. A negative value for b* denotes a blue color, while a positive value for b* denotes a yellow color. Finally, L* = 0 represents darkness, while L* = 100 represents brightness (Flamminii et al., 2020; Krishnamurthy & Kantha, 2005).

Other indicators examined in this study included C*, h˚, ΔE, and WI. The C* parameter refers to color intensity. The h˚ parameter indicates the tonality angle (hue angle) and represents the dominant color; the hue is more similar to red when the hue angle is closer to zero. Angles of 90˚, 180˚, and 270˚ represent yellow, green, and blue colors, respectively. The ΔE value represents the overall difference in all color parameters between the samples; therefore, it can be used as a main indicator for investigating color differences. Finally, WI represents the whiteness degree of the samples; the hue is closer to white when the WI value is closer to 100.

2.9 Probiotic bacterial count

The count of probiotic bacteria was determined by plating on MRS-bile agar (Merck Co., Germany) and incubation for three days at 37 °C under anaerobic conditions (Afjeh et al., 2019).

2.10 Sensory evaluation

All sensory properties, such as color, taste, smell, texture, and overall acceptance, were examined by ten trained experts, using a five-point hedonic scale (Zanjani et al., 2012). The scores were rated as follows: 5, very good; 4, good; 3, acceptable; 2, poor; and 1, very poor (unacceptable).

2.11 Statistical analysis

The experiment was conducted in a completely randomized design consisting of four factors; the type of prebiotic (flaxseeds and chia seeds), percentage of each prebiotic (0, 2 and 4%), different forms of probiotic (free and microencapsulated *L. paracasei*), and four different storage periods. A one-way ANOVA and t-test were carried out for analyzing the effect of time and encapsulation on the survival of probiotic bacteria, respectively. A two-way ANOVA, followed by Duncan's multiple-range test at 95% confidence intervals (CIs), was used to analyze the effect of the formulation factors and storage period and their interaction on the studied quality characteristics of symbiotic yogurt sauce. Statistical analyses were performed in SPSS Version 25 (SPPSS Inc., Chicago, IL, USA). All data are presented as mean ± SD. Each test was performed in triplicate at the predefined intervals (on days 1, 10, 20 and 30)

3 Results and discussion

3.1 Survival of microencapsulated and free L. paracasei in the SGF

The survival rate of *L. paracasei* in the SGF was examined in two forms (microencapsulated and free); the results are presented in Figure 1. There was a significant difference between viable bacteria within two hours ($P < 0.05$), as it was found that after two hours, the probiotic viability reduced in

both probiotic forms (microencapsulated and free). However, the microencapsulated probiotic showed greater tolerance for gastric acidic conditions compared to the free form. As shown in Figure 1, the microencapsulation of probiotic bacteria leads to the reduced mortality of bacteria under gastric conditions compared to the free probiotic. After two hours, comparison of the two forms of probiotic bacteria only showed one logarithmic cycle reduction in the microencapsulated *L. paracasei,* while five logarithmic cycles reduction was found in the free bacteria cells; according to this finding, microencapsulation offers protection for probiotic bacteria in the SGF.

The mentioned results are in agreement with recent studies (Afzaal et al., 2019; Han et al., 2020; Qi et al., 2019; Zanjani et al., 2012), which reported the protective effect of microencapsulation with various biopolymers on probiotic bacteria against adverse conditions in the SGF. In this regard, some researchers found that sodium alginate microcapsules could be used to improve the survival of probiotic bacteria (*L. acidophilus*) in yogurt under simulated gastrointestinal conditions (Afzaal et al., 2019). Moreover, in a similar study, the effects of calcium alginate-whey protein microcapsules on two probiotic bacteria (*L. bulgaricus* and *L. paracasei*) were investigated. The results showed that the used microcapsules were beneficial for the delivery, as well as protection of bacteria under simulated gastrointestinal conditions (Han et al., 2020).

In another study, *L. casei* was encapsulated by calcium alginate and resistant starch. Next, the survival rate of microencapsulated *L. casei* was evaluated in creamy cake; the results suggested the significant viability of microencapsulated *L. casei* compared to the free probiotic (Zanjani et al., 2012). Moreover, some researchers reported the increased survival of microencapsulated *Saccharomyces boulardii* and *Enterococcus faecium,* using alginate polymers in SGF (Qi et al., 2019).

3.2 Survival of microencapsulated and free L. paracasei in SIF

The survival rate of *L. paracasei* in the SIF was studied in dissimilar forms of probiotic (microencapsulated and free); the results are shown in Figure 2. A significant difference was observed between viable bacteria within two hours ($P < 0.05$). After two hours, the viability of probiotic bacteria was reduced in both microencapsulated and free probiotics. However, the microencapsulated probiotic cells showed greater tolerance for the basic conditions of the intestines compared to the free probiotic cells. Figure 2 clearly shows that microencapsulation of probiotic bacteria reduced bacterial cell death in the SIF as compared to the free bacteria. In the microencapsulated bacteria, reduction less than one logarithmic cycle was detected, while in the free cells, four logarithmic cycles reduction was observed. This finding is consistent with the results reported by other researchers (Afzaal et al., 2019; Han et al., 2020). Besides, some researchers reported 15% and 20% improvements in the survival of *S. boulardii* and *E. faecium* in the SIF. Moreover, the increased number of viable cells of two probiotic bacteria (*L. acidophilus* and *L. rhamnosus*) in the SGF and SIF was reported (Mokarram et al., 2009; Qi et al., 2019).

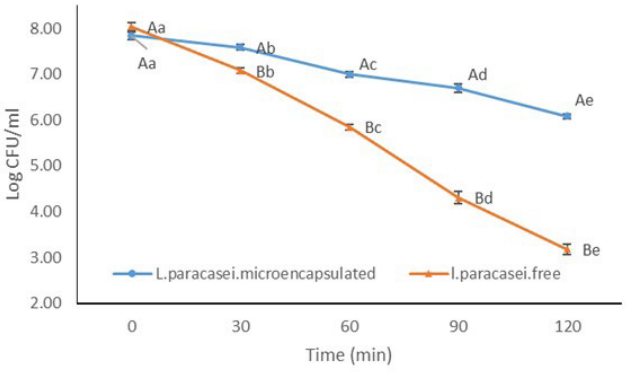

Figure 1. Survival of microencapsulated and free probiotic bacteria *(L. paracasei)* in Simulated Gastric Fluid (SGF). Different small letters represent the statistical difference within times. Different capital letters represent the statistical difference within free and microencapsulated forms at a time ($p < 0.05$).

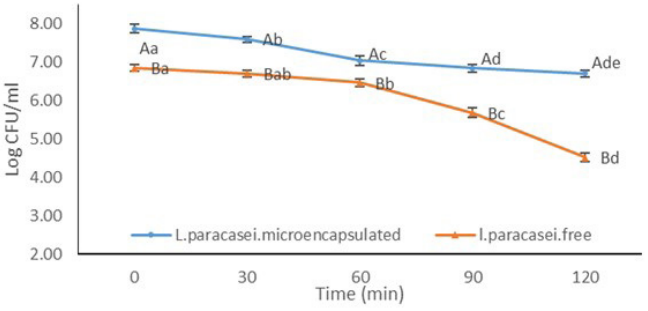

Figure 2. Survival of microencapsulated and free probiotic bacteria *(L. paracasei)* in Simulated Intestine Fluid (SIF). Different small letters represent the statistical difference within times. Different capital letters represent the statistical difference within free and microencapsulated forms at a time ($p < 0.05$).

3.3 Morphology of microencapsulated L. paracasei

The morphology of microencapsulated *L. paracasei* was determined by SEM, as shown in Figure 3. The microcapsules prepared by the emulsion method showed sizes of 37.22 and 42.54 μm, with an intact, bar, and cylindrical appearance. The resistant starch granules, used for microencapsulation, covered all pores on the surface of capsules, which were created because of the porous structure of sodium alginate, resulting in the uniform structure of the produced microcapsules that is suitable for yogurt sauce. Overall, the produced microcapsules provided great protection for probiotic bacteria in the SGF and SIF due to increased distance between the cells and gastrointestinal conditions. Therefore, the release time of probiotics in the SGF and SIF increased, resulting in the enhanced survival rate of probiotic bacteria.

It has been reported that the decreased diameter of microcapsules can reduce the protective effect of microencapsulation, while increasing the microcapsule diameter reduces the death of probiotic cells (Afzaal et al., 2019). A similar finding was reported by other researchers, using uncoated calcium alginate beads; the

beads were covered with one or two layers of sodium alginate. Among three tested microcapsules (uncoated, single-layer, and two-layer), the two-layer coating provided higher protection for probiotic bacteria (*L. acidophilus* and *L. rhamnosus*) in the SGF and SIF (Mokarram et al., 2009).

3.4 Microbial count in synbiotic yogurt sauce

The count of *L. paracasei* in the samples during storage (30 days, 4 ˚C) is presented in Table 1. As shown in Table 1, the number of viable bacteria reduced in all samples during storage compared to the first day. Expectedly, on day 30, the least viability was attributed to the sample containing the free probiotic cells. Also, there was a significant difference between samples with different prebiotic compounds ($P < 0.05$). The probiotic viability increased in the microencapsulated and free samples containing flaxseeds (2% and 4%) compared to those containing chia seeds (2% and 4%). Moreover, increasing the concentration of both prebiotics from 2% to 4% led to the increased probiotic viability. As shown in Table 1, the number of viable probiotic cells decreased during the 30-day storage. On day 30 of storage, the lowest viability of the cells (4.99 ± 0.00) was reported in the sample containing free *L. paracasei*. Besides, the death of microencapsulated probiotic cells was lower than that of free probiotic cells. Similarly, some researchers found that microencapsulation enhanced (60%) the survival rate of probiotics compared to their free form (25%) (Qi et al., 2019). These results are consistent with the findings reported by other researchers which showed that microencapsulation increased the survival rate of probiotics in dairy products (Homayouni et al., 2008). Consistently, it was reported the higher survival of microencapsulated probiotics compared to free probiotics (Iqbal et al., 2019; Muzzafar & Sharma, 2018). Similarly, it was found that microencapsulation with calcium alginate and resistant starch improved the survival of *L. casei* and *Bifidobacterium bifidum* compared to free bacterial cells in mayonnaise sauce (Fahimdanesh et al., 2013). Comparison of these two probiotic bacteria in mayonnaise sauce showed that the number of *L. casei* cells was higher than that of *B. bifidum*. Moreover, the results revealed the improvement of bacterial viability in mayonnaise containing encapsulated bifidobacteria, compared to mayonnaise containing free bifidobacteria (Khalil & Mansour, 1998). In this study, the highest viability was associated to the microencapsulated *L. paracasei* sample containing 4% flaxseeds as well as microencapsulated *L. paracasei* sample containing 4% chia seeds. In other words, viability of probiotics in sample containing microencapsulated bacteria together with prebiotic compound was higher than that of sample containing microencapsulated bacteria without prebiotic compound. The findings of similar investigation on a synbiotic jelly were revealed that viability of probiotics was higher in microencapsulated *L. rhamnosus* samples having 3% oligofructose than that of microencapsulated *L. rhamnosus* samples with no prebiotics (Karegar et al., 2022).

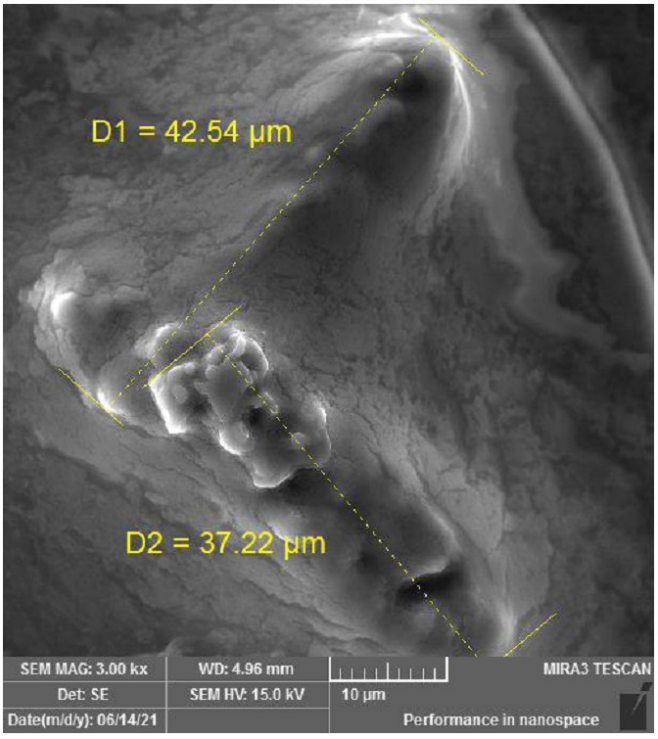

Figure 3. Micrograph of microencapsulated probiotic bacteria (*L. paracasei)*. At magnification of × 3.00 kx.

Table 1. Viable counts of *L. paracasei* in synbiotic yogurt sauce samples during storage.

Sample	Day 1	Day 10	Day 20	Day 30
	LogCFU/mL			
T1	7.86 ± 0.04$^{D, ab}$	6.90 ± 0.058$^{C, a}$	6.57 ± 0.018$^{B, a}$	6.18 ± 0.021$^{A, c}$
T2	7.86 ± 0.006$^{D, b}$	7.03 ± 0.008$^{C, c}$	6.84 ± 0.056$^{B, c}$	6.24 ± 0.021$^{A, d}$
T3	7.85 ± 0.008$^{D, a}$	6.84 ± 0.056$^{C, a}$	6.57 ± 0.014$^{B, a}$	6.12 ± 0.018$^{A, b}$
T4	7.85 ± 0.014$^{D, ab}$	7.01 ± 0.017$^{C, b}$	6.84 ± 0.057$^{B, c}$	6.20 ± 0.057$^{A, cd}$
T5	7.85 ± 0.006$^{C, a}$	6.77 ± 0.075$^{B, a}$	6.84 ± 0.057$^{B, c}$	4.99 ± 0.008$^{A, a}$
T6	7.87 ± 0.016$^{D, cd}$	7.07 ± 0.054$^{C, de}$	6.93 ± 0.029$^{B, e}$	6.63 ± 0.095$^{A, f}$
T7	7.87 ± 0.008$^{D, c}$	7.09 ± 0.037$^{C, e}$	6.94 ± 0.015$^{B, e}$	6.69 ± 0.147$^{A, fg}$
T8	7.85 ± 0.045$^{D, ab}$	7.03 ± 0.028$^{C, c}$	6.86 ± 0.017$^{B, c}$	6.57 ± 0.014$^{A, e}$
T9	7.86 ± 0.007$^{D, b}$	7.06 ± 0.056$^{C, de}$	6.89 ± 0.014$^{B, d}$	6.70 ± 0.096$^{A, fg}$
T10	7.86 ± 0.010$^{D, ab}$	7.04 ± 0.041$^{C, bcd}$	6.80 ± 0.017$^{B, b}$	6.58 ± 0.048$^{A, e}$

T1: *L. paracasei* (free) + Flaxseeds (2%); T2: *L. paracasei* (free) + Flaxseeds (4%); T3: *L. paracasei* (free) + Chia seeds (2%); T4: *L. paracasei* (free) + Chia seeds (4%); T5: *L. paracasei* (free) without prebiotic; T6: *L. paracasei* (microencapsulated) + Flaxseed (2%); T7: *L. paracasei* (microencapsulated) + Flaxseeds (4%); T8: *L. paracasei* (microencapsulated) + Chia seeds (2%); T9: *L. paracasei* (microencapsulated) + Chia seeds (4%); T10: *L. paracasei* (microencapsulated) without prebiotic; T11: Control. Data are presented as a mean ± standard deviation. Different small letters represent the statistical difference ($p < 0.05$) within a column.

3.5 Evaluation of pH, acidity, and viscosity of synbiotic yogurt sauce samples

The pH measurements of the samples during storage (30 days, 4 ˚C) are presented in Table 2. The pH of all samples decreased during the storage period. On day 30, the highest pH (4.19 ± 0.01) was measured for the microencapsulated *L. paracasei* sample (Table 2). It seems that the presence of prebiotic compounds (flaxseeds and chia seeds) did not have any significant effects on the sample pH (Table 2). A similar study revealed that encapsulated probiotic (*L. acidophilus* and *Bifidobacterium lactis*) increased the final pH to 4.25 in contrast to 3.95 in the free probiotic yogurt samples during six weeks of storage (Kailasapathy, 2006). Other studies used encapsulation in an alginate-goats' milk-inulin matrix for goat milk yogurt (Prasanna & Charalampopoulos, 2019) and encapsulated *B. breve* in whey protein (Picot & Lacroix, 2004). The results of our study demonstrated that pH decreases when prebiotic compound was added to the free *L. paracasei* sample. The same result was seen in the microencapsulated *L. paracasei* sample. The finding of present study is in accordance of a recent study which investigated on a synbiotic jelly containing microencapsulated and free *L. rhamnosus* samples (Karegar et al., 2022).

The acidity of synbiotic yogurt sauce samples, containing microencapsulated and free probiotic bacteria (stored for 30 days at 4 ˚C) are presented in Table 3. The acidity values of all samples increased during 30 days of storage. Besides, the analysis of variance of acidity in the samples indicated a steady trend up to day 10, suggesting no significant difference between the samples from day 1 to day 10 ($P > 0.05$). On the other hand, a significant difference was found between the samples on day 30 ($P < 0.05$). At the end of storage (day 30), the highest acidity was attributed to the free *L. paracasei* sample with 4% flaxseeds (0.68 ± 0.005), as well as free *L. paracasei* sample containing 4% chia seeds (0.68 ± 0.008); the lowest acidity (0.61 ± 0.01) was observed in the control sample (Table 3). These finding are consistent with the results of some researchers that found a decreasing trend in pH, besides an increasing trend in acidity in all yogurt samples during 28 days of storage (Afzaal et al., 2019). Moreover, other researchers reported a declining trend of pH, while acidity of all samples showed an increasing trend during 28 days of storage

Table 2. pH of synbiotic yogurt sauce samples during storage.

sample	Day 1	Day 10	Day 20	Day 30
	pH			
T1	$4.28 \pm 0.02^{B, a}$	$4.25 \pm 0.005^{C, d}$	$4.22 \pm 0.05^{BC, def}$	$4.01 \pm 0.01^{A, a}$
T2	$4.34 \pm 0.02^{C, bc}$	$4.32 \pm 0.04^{C, e}$	$4.19 \pm 0.01^{B, e}$	$4.02 \pm 0.01^{A, a}$
T3	$4.32 \pm 0.01^{D, b}$	$4.19 \pm 0.01^{C, bc}$	$4.15 \pm 0.01^{B, d}$	$4.02 \pm 0.01^{A, a}$
T4	$4.32 \pm 0.02^{C, b}$	$4.18 \pm 0.02^{B, ab}$	$4.14 \pm 0.02^{B, cd}$	$4.03 \pm 0.02^{A, ab}$
T5	$4.28 \pm 0.02^{C, a}$	$4.20 \pm 0.02^{B, c}$	$4.19 \pm 0.01^{B, e}$	$4.09 \pm 0.01^{A, c}$
T6	$4.32 \pm 0.01^{C, b}$	$4.16 \pm 0.02^{B, a}$	$4.08 \pm 0.02^{A, ab}$	$4.07 \pm 0.02^{A, bc}$
T7	$4.34 \pm 0.01^{D, c}$	$4.21 \pm 0.01^{C, c}$	$4.13 \pm 0.01^{A, c}$	$4.17 \pm 0.01^{B, e}$
T8	$4.32 \pm 0.005^{C, b}$	$4.17 \pm 0.01^{B, a}$	$4.04 \pm 0.02^{A, a}$	$4.14 \pm 0.02^{B, d}$
T9	$4.32 \pm 0.02^{C, b}$	$4.18 \pm 0.01^{B, ab}$	$4.07 \pm 0.02^{A, ab}$	$4.07 \pm 0.01^{A, b}$
T10	$4.32 \pm 0.01^{C, b}$	$4.24 \pm 0.01^{B, d}$	$4.23 \pm 0.005^{B, f}$	$4.19 \pm 0.01^{A, f}$
T11	$4.28 \pm 0.01^{C, a}$	$4.22 \pm 0.02^{B, c}$	$4.15 \pm 0.01^{A, d}$	$4.13 \pm 0.01^{A, d}$

T1: *L. paracasei* (free) + Flaxseeds (2%); T2: *L. paracasei* (free) + Flaxseeds (4%); T3: *L. paracasei* (free) + Chia seeds (2%); T4: *L. paracasei* (free) + Chia seeds (4%); T5: *L. paracasei* (free) without prebiotic; T6: *L. paracasei* (microencapsulated) + Flaxseed (2%); T7: *L. paracasei* (microencapsulated) + Flaxseeds (4%); T8: *L. paracasei* (microencapsulated) + Chia seeds (2%); T9: *L. paracasei* (microencapsulated) + Chia seeds (4%); T10: *L. paracasei* (microencapsulated) without prebiotic; T11: Control. Data are presented as a mean ± standard deviation. Different small letters represent the statistical difference ($p < 0.05$) within a column.

Table 3. Acidity of synbiotic yogurt sauce samples during storage.

sample	Day 1	Day 10	Day 20	Day 30
	Acidity (% in terms of acetic acid)			
T1	$0.59 \pm 0.005^{A, a}$	$0.61 \pm 0.02^{AB, ab}$	$0.64 \pm 0.02^{BC, bc}$	$0.67 \pm 0.01^{C, bc}$
T2	$0.60 \pm 0.005^{B, ab}$	$0.65 \pm 0.01^{C, c}$	$0.59 \pm 0.001^{A, a}$	$0.68 \pm 0.005^{D, c}$
T3	$0.60 \pm 0.01^{A, ab}$	$0.60 \pm 005^{A, a}$	$0.62 \pm 0.02^{AB, b}$	$0.67 \pm 0.03^{B, bc}$
T4	$0.60 \pm 0.005^{A, ab}$	$0.60 \pm 0.01^{A, a}$	$0.62 \pm 0.01^{AB, b}$	$0.68 \pm 0.005^{C, c}$
T5	$0.60 \pm 0.03^{A, ab}$	$0.61 \pm 0.01^{A, ab}$	$0.62 \pm 0.02^{AB, b}$	$0.64 \pm 0.02^{AB, ab}$
T6	$0.61 \pm 0.02^{AB, ab}$	$0.60 \pm 0.009^{A, a}$	$0.64 \pm 0.01^{BC, bc}$	$0.64 \pm 0.005^{BC, b}$
T7	$0.60 \pm 0.03^{AB, ab}$	$0.60 \pm 0.01^{A, a}$	$0.63 \pm 0.01^{B, bc}$	$0.64 \pm 0.008^{C, b}$
T8	$0.60 \pm 0.03^{AB, ab}$	$0.61 \pm 0.01^{A, ab}$	$0.64 \pm 0.02^{B, bc}$	$0.63 \pm 0.01^{B, ab}$
T9	$0.60 \pm 0.02^{AB, ab}$	$0.60 \pm 0.006^{A, a}$	$0.67 \pm 0.005^{C, d}$	$0.63 \pm 0.02^{B, ab}$
T10	$0.60 \pm 0.02^{A, ab}$	$0.62 \pm 0.02^{AB, abc}$	$0.62 \pm 0.01^{A, b}$	$0.63 \pm 0.01^{AB, ab}$
T11	$0.59 \pm 0.01^{A, ab}$	$0.60 \pm 0.01^{A, ab}$	$0.61 \pm 0.01^{AB, b}$	$0.61 \pm 0.01^{AB, a}$

T1: *L. paracasei* (free) + Flaxseeds (2%); T2: *L. paracasei* (free) + Flaxseeds (4%); T3: *L. paracasei* (free) + Chia seeds (2%); T4: *L. paracasei* (free) + Chia seeds (4%); T5: *L. paracasei* (free) without prebiotic; T6: *L. paracasei* (microencapsulated) + Flaxseed (2%); T7: *L. paracasei* (microencapsulated) + Flaxseeds (4%); T8: *L. paracasei* (microencapsulated) + Chia seeds (2%); T9: *L. paracasei* (microencapsulated) + Chia seeds (4%); T10: *L. paracasei* (microencapsulated) without prebiotic; T11: Control. Data are presented as a mean ± standard deviation. Different small letters represent the statistical difference ($p < 0.05$) within a column.

(Shaghaghi et al., 2013). Besides, it was found that the presence of probiotic bacteria (microencapsulated or free) led to the reduction of acid production in yogurt samples during storage (Kailasapathy, 2006). The results of another study showed a reduction in the post-acidification of probiotic yogurt samples due to the dual effect of *L. paracasei* encapsulation in sodium caseinate and gellan gum, along with a milk protein concentrate (MPC) with a high buffering capacity (Picot & Lacroix, 2004). Moreover, some researchers reported an increase in acidity and a decrease in pH of mayonnaise sauce, containing encapsulated bifidobacteria as compared to mayonnaise sauce containing free bacteria cells (Khalil & Mansour, 1998). The results of this study revealed that adding prebiotic compounds to the free *L. paracasei* samples lead to increasing of acidity. Moreover, in comparison between microencapsulated probiotic samples with and without prebiotic compounds, those containing flaxseeds presented higher acidity value than that of samples with no prebiotic. A similar result is found by other researchers with regards of synbiotic jelly (Karegar et al., 2022).

The results of viscosity analysis of synbiotic yogurt sauce samples during storage are presented in Table 4. A significant difference was seen in the viscosity of all samples during storage ($P < 0.05$). As shown in Table 4, on day 30 of storage, the highest viscosity was 523.3 ± 4.50 for the microencapsulated *L. paracasei* with 4% flaxseeds. Also, analysis of rheological behavior showed that the produced synbiotic yogurt sauce was a pseudoplastic fluid; nevertheless, its viscosity increased due to the presence of flaxseeds and chia seeds. The results also indicated the shear stress and shear rate of the produced synbiotic yogurt sauce, which suggested the interactive structure of the product. Also, the firmness and cohesiveness of samples containing prebiotic compounds increased the viscosity, as flaxseeds and chia seeds absorb water over time, while in samples without prebiotic compounds, a dramatic decrease occurred in viscosity. Overall, microencapsulation provided a way to control the reduction in viscosity (Rojas et al., 2019).

Table 4. Viscosity of the synbiotic yogurt sauce samples during storage.

sample	Day 1	Day 30
	Viscosity (Pascal/sec)	
T1	$193.3 \pm 0.57^{A, c}$	$276.0 \pm 2.00^{B, f}$
T2	$321.3 \pm 1.52^{A, h}$	$333.3 \pm 1.52^{B, g}$
T3	$214.6 \pm 0.57^{A, e}$	$233.6 \pm 2.51^{B, d}$
T4	$248.3 \pm 3.51^{A, f}$	$388.6 \pm 2.51^{B, i}$
T5	$94.33 \pm 3.51^{A, a}$	$92.60 \pm 2.20^{A, a}$
T6	$306.6 \pm 3.05^{B, g}$	$228.3 \pm 3.05^{A, e}$
T7	$184.6 \pm 0.57^{A, b}$	$523.3 \pm 4.50^{B, j}$
T8	$198.3 \pm 3.51^{B, d}$	$194.0 \pm 0.00^{A, c}$
T9	$390.0 \pm 6.00^{B, j}$	$381.6 \pm 2.51^{A, h}$
T10	$200.6 \pm 4.50^{B, d}$	$170.3 \pm 0.57^{A, b}$
T11	$362.6 \pm 2.51^{B, i}$	$211.3 \pm 1.52^{A, d}$

T1: L. paracasei (free) + Flaxseeds (2%); T2: L. paracasei (free) + Flaxseeds (4%); T3: L. paracasei (free) + Chia seeds (2%); T4: L. paracasei (free) + Chia seeds (4%); T5: L. paracasei (free) without prebiotic; T6: L. paracasei (microencapsulated) + Flaxseed (2%); T7: L. paracasei (microencapsulated) + Flaxseeds (4%); T8: L. paracasei (microencapsulated) + Chia seeds (2%); T9: L. paracasei (microencapsulated) + Chia seeds (4%); T10: L. paracasei (microencapsulated) without prebiotic; T11: Control. Data are presented as a mean ± standard deviation. Different small letters represent the statistical difference ($p < 0.05$) within a column.

Similar results have been reported in a number of studies. Some researchers demonstrated a non-Newtonian shear behavior in all mayonnaise samples, containing an olive leaf phenolic extract (OLE) (Flamminii et al., 2020). Their findings showed a similar trend in both control and mayonnaise samples containing free OLE, while mayonnaise samples containing free OLE showed a higher shear rate compared to the control samples. On the other hand, compared to mayonnaise samples with OLE oil droplets, the OLE-microencapsulated samples exhibited higher shear stress values compared to the samples containing free OLE (Flamminii et al., 2020). Moreover, the results of the present study are in agreement with a recent investigation, which suggested the increased viscosity of mayonnaise samples containing chia seed and pumpkin seed microcapsules; however, the presence of these oil microcapsules exerted no effects on the rheological behavior of mayonnaise samples (Rojas et al., 2019). Conversely, other researchers reported the reducing trend of viscosity in all probiotic yogurt samples during storage. The highest viscosity was attributed to the microencapsulated probiotic sample versus the free probiotic sample (Afzaal et al., 2019). Another study demonstrated an increase in the viscosity of mayonnaise samples, containing zein microcapsules loaded with fish oil (Miguel et al., 2019).

3.6 Color values of synbiotic yogurt sauce samples

Color is one of the main sensory features that can affect the acceptance or rejection of the product by the consumer (Simão et al., 2022). Table 5 presents the color parameters of yogurt sauce samples. As expected, the addition of chia seeds changed the color visually and significantly reduced the color lightness (L*) and blue-yellow index (b*) of the samples due to the dark color of the initial seeds. The luminosity (L*) of the samples ranged from 61.31 to 70.54. The lowest L* values were obtained by microencapsulated *L. paracasei* sample containing 4% chia seeds, together with microencapsulated *L. paracasei* sample containing 2% chia seeds and also free *L. paracasei* sample containing 4% chia seeds. Besides, a* values ranged from -0.22 to +2.56 in the samples. All samples had positive a* values, except the one containing microencapsulated *L. paracasei* with 4% chia seeds (-0.22) which was due to the black- brownish color of chia seeds. Sample containing microencapsulated *L. paracasei* with 4% chia seeds and sample containing free *L. paracasei* were gained the lowest and highest a* values respectively. Also, the b* parameter of the samples ranged between 9.10 and 16.33. All samples had positive b* values, which indicated the dominance of yellow color. Furthermore, microencapsulated *L. paracasei* sample containing 4% chia seeds, together with microencapsulated *L. paracasei* sample containing 2% chia seeds and also free *L. paracasei* sample holding 4% chia seeds presented the lowest b* values. This finding is consistent with the results of some researchers which indicated the slightly yellowish color of synbiotic yogurt samples due to the presence of prebiotics (fructooligosaccharides) (Madhu et al., 2012). Moreover, other researchers demonstrated the greater effect of riboflavin on the b* value of yogurt samples (Dimitreli et al., 2014). In a similar study, the color parameters (a*, b*, and L*) are not influenced by probiotics (*L. acidophilus* and *B. lactis*) or para-probiotics (inactivated forms of bacteria) in yogurt samples (Parvarei et al.,

Table 5. Colour values of synbiotic yogurt sauce samples.

Sample	Colour parameters						
	L*	a*	b*	WI	C*	H°	ΔE*
T1	69.50 ± 0.65^{bc}	1.36 ± 0.36^{bc}	13.57 ± 0.46^{bc}	66.59 ± 0.41^{bc}	13.64 ± 0.48^{b}	84.26 ± 0.28^{bc}	1.49 ± 0.07^{b}
T2	68.33 ± 2.62^{bc}	1.23 ± 0.36^{b}	12.52 ± 2.00^{bc}	65.86 ± 1.73^{bc}	12.58 ± 1.02^{b}	84.46 ± 0.76^{bcd}	2.98 ± 0.93^{c}
T3	66.92 ± 2.29^{b}	1.32 ± 0.74^{bc}	13.05 ± 2.16^{bc}	64.36 ± 1.31^{b}	13.12 ± 1.22^{b}	84.42 ± 2.33^{bcd}	2.55 ± 0.21^{c}
T4	65.54 ± 4.93^{ab}	0.39 ± 1.23^{bc}	10.79 ± 4.19^{ab}	63.67 ± 3.42^{ab}	10.83 ± 1.22^{a}	89.12 ± 6.22^{bcde}	5.49 ± 0.20^{e}
T5	68.90 ± 1.83^{bc}	2.56 ± 0.30^{e}	15.96 ± 0.74^{d}	64.95 ± 1.99^{b}	16.17 ± 0.77^{e}	80.89 ± 0.65^{a}	2.02 ± 0.51^{c}
T6	67.08 ± 1.52^{b}	1.64 ± 0.07^{bc}	14.17 ± 1.19^{bc}	64.12 ± 1.32^{ab}	14.27 ± 0.18^{bc}	83.38 ± 0.41^{bc}	1.84 ± 0.47^{bc}
T7	67.28 ± 1.69^{b}	1.13 ± 0.62^{b}	12.58 ± 2.20^{bc}	64.87 ± 0.77^{b}	12.63 ± 1.25^{bc}	85.01 ± 1.96^{d}	2.68 ± 0.83^{bc}
T8	65.62 ± 4.56^{ab}	0.63 ± 1.04^{b}	11.18 ± 4.24^{ab}	63.63 ± 3.02^{ab}	11.21 ± 1.29^{bc}	87.63 ± 4.44^{cd}	5.02 ± 0.10^{d}
T9	62.81 ± 0.55^{a}	-0.22 ± 0.05^{b}	9.10 ± 0.79^{a}	61.71 ± 1.32^{a}	9.11 ± 0.79^{a}	91.40 ± 0.49^{e}	8.43 ± 0.92^{f}
T10	69.43 ± 0.11^{bc}	1.43 ± 0.12^{bc}	13.14 ± 0.54^{b}	66.69 ± 0.35^{c}	13.22 ± 0.55^{bc}	83.80 ± 0.28^{bc}	1.67 ± 0.55^{bc}
T11	68.82 ± 0.50^{bc}	1.92 ± 0.05^{d}	14.62 ± 0.15^{c}	65.51 ± 0.38^{b}	14.74 ± 0.16^{cd}	82.53 ± 0.13^{b}	0.37 ± 0.007^{a}

T1: *L. paracasei* (free) + Flaxseeds (2%); T2: *L. paracasei* (free) + Flaxseeds (4%); T3: *L. paracasei* (free) + Chia seeds (2%); T4: *L. paracasei* (free) + Chia seeds (4%); T5: *L. paracasei* (free) without prebiotic; T6: *L. paracasei* (microencapsulated) + Flaxseed (2%); T7: *L. paracasei* (microencapsulated) + Flaxseeds (4%); T8: *L. paracasei* (microencapsulated) + Chia seeds (2%); T9: *L. paracasei* (microencapsulated) + Chia seeds (4%); T10: *L. paracasei* (microencapsulated) without prebiotic; T11: Control. Data are presented as a mean ± standard deviation. Different small letters represent the statistical difference ($p < 0.05$) within a column.

2021). Besides, other researchers found that a*, b*, and L* parameters of buffalo milk yogurt were not influenced by the starter culture type or final incubation pH (Akgun et al., 2018). Moreover, a recent study showed significant differences in the L* values of mayonnaise samples with and without alginate/pectin microcapsules containing OLE. The L* value of samples with microencapsulated OLE reduced as compared to samples with only OLE, without the microcapsules (Flamminii et al., 2020). The latter samples exhibited lower L* values because of the vast dispersion of oil particles in the system (Flamminii et al., 2020). The chroma (C*) represents the vividness or saturation of a color. Yogurt sauce samples containing free *L. paracasei*, and samples containing microencapsulated *L. paracasei* with 4% chia seeds had the lowest and highest color saturation, respectively ($p < 0.05$). Regarding the hue angle (H°), the most yellowish hue (91.40 ± 0.49) was related to the sample containing microencapsulated *L. paracasei* with 4% chia seeds, while the least yellowish hue (80.89 ± 0.65) was attributed to the sample containing free *L. paracasei*. The total color difference (ΔE*) is related to the ability of human vision to perceive the difference between two colors. Based on the results of some researchers (Cserhalmi et al., 2006) classification, ΔE* can be evaluated by consumers as non-noticeable (0-0.5), slightly noticeable (0.5-1.5), noticeable (1.5-3), visible change (3-6), and great change (6-12). Almost all yogurt sauce samples had a ΔE* value more than 1.5. The T4, T8 and T9 had the total color difference equal to 5.49, 5.02 and 8.43, respectively. However, consumers may accept the samples even if color changes are visible (Simão et al., 2022). As shown in Figure 4D, sensory evaluation panel gave a score between 3.66 and 5 to the color of the samples and the scores given to the formulated samples were better than the control sample. The whiteness is often the most critical color trait of milk and dairy products. The whiteness index (WI) indicates the degree of whiteness in a product and mathematically combines lightness and yellow-blue into a single index (Milovanovic et al., 2020). The whiteness index in T9, which contains *L. paracasei* (microencapsulated) + chia seeds (4%), was significantly ($p < 0.05$) lower than other samples. This sample had the highest amount of ΔE*. Additionally, in another study, the hue angle was close to 90°, suggesting the yellow color of mayonnaise samples, while there were no significant differences between the samples with and without alginate/pectin microcapsules containing OLE (Flamminii et al., 2020). Also, the findings of some researchers showed 15% and 20% improvements in the survival rates of *S. boulardii* and *E. faecium* in the SIF. Besides, other researchers reported the increased number of viable cells of two probiotic bacteria (*L. acidophilus* and *L. rhamnosus*) in the SGF and SIF (Mokarram et al., 2009).

3.7 Sensory characteristics

The sensory characteristics of the samples were evaluated by expert panelists; the results are presented in Figure 4. Also, the results of taste analysis, presented in Figure 4A, demonstrated minor differences between some samples during storage. It seems that sensory panelists gave higher scores to the taste of samples containing flaxseeds compared to chia seeds. At the end of storage (day 30), samples containing microencapsulated *L. paracasei* with flaxseeds (2% and 4%), and sample containing free *L. paracasei* having 4% flaxseeds were obtained the significantly ($p < 0.05$) highest scores (3.67 ± 0.57). On the other hand, samples containing free *L. paracasei* with 2% chia seeds and microencapsulated *L. paracasei* with 2% chia seeds were attributed to the lowest scores (2.00 ± 0.00). Also, it seems that based on the results of Tables 2-3 and Figure 4A, taste of yogurt sauces with lower pH and higher acidity were more desirable. The results of smell analysis are presented in Figure 4B. There was no significant difference in the smell scores of most samples during storage. On day 1, the highest score was reported for the control sample (5.00 ± 0.00), whereas the lowest score was attributed to the microencapsulated sample containing *L. paracasei* with 4% chia seeds (3.00 ± 0.10). As clearly shown in Figure 4B, the control sample had the highest score of smell analysis during storage. On day 30, following the control sample, the second highest score was reported for the sample containing microencapsulated *L. paracasei* with 2% flaxseeds (4.00 ± 0.00). Meanwhile, the free L. *paracasei* sample containing 2% flaxseeds, along with microencapsulated *L. paracasei* samples with both

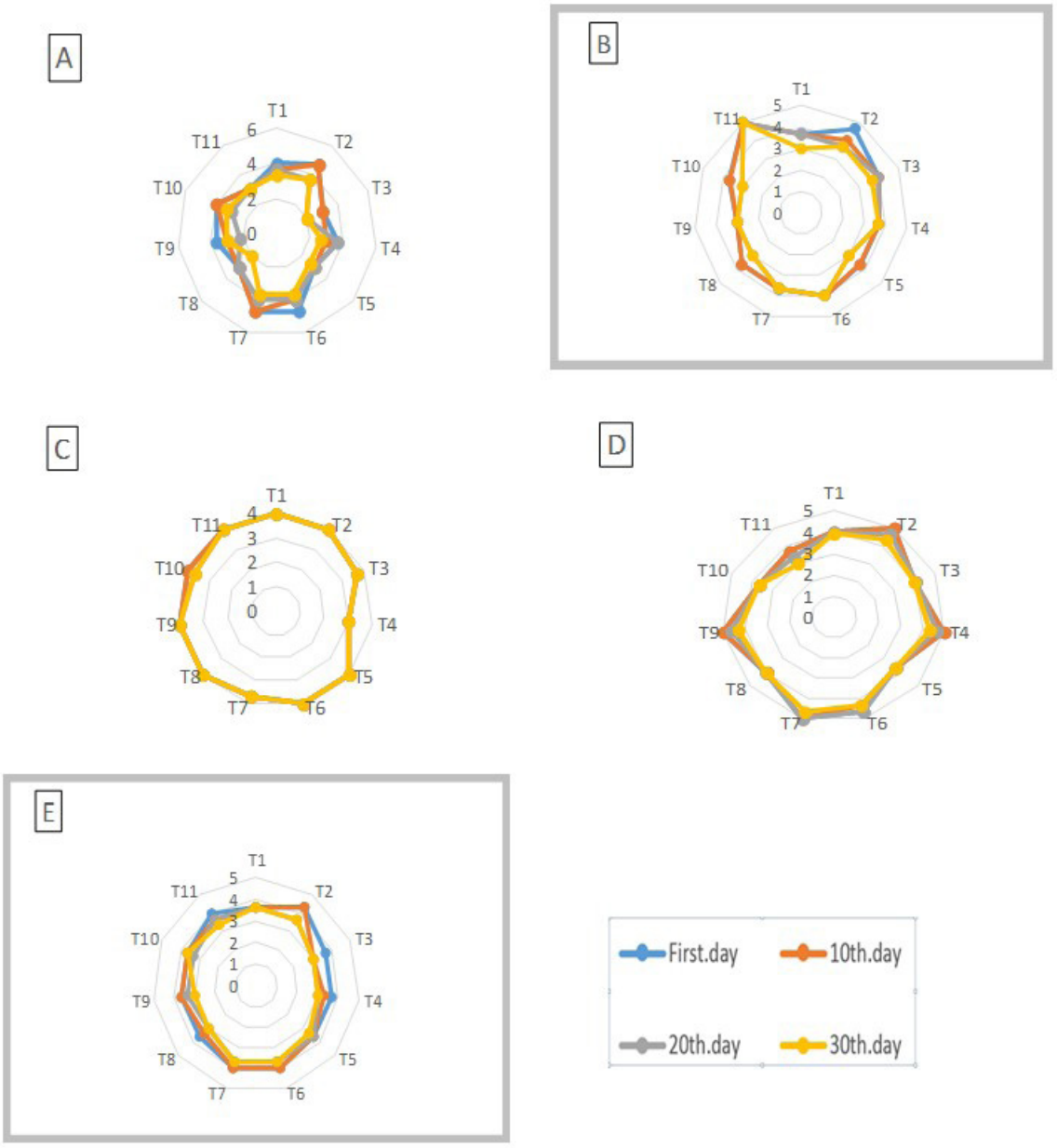

Figure 4. Sensory acceptability scores of synbiotic yogurt sauce samples during storage. A. Taste; B. Smell; C. Texture; D. Color; E. Overall acceptability. T1: *L. paracasei* (free) + Flaxseed (2%); T2: *L. paracasei* (free) + Flaxseed (4%); T3: *L. paracasei* (free) + Chia seeds (2%); T4: *L. paracasei* (free) + Chia seeds (4%); T5: *L. paracasei* (free) without prebiotic; T6: *L. paracasei* (microencapsulated) + Flaxseed (2%); T7: *L. paracasei* (microencapsulated) + Flaxseed (4%); T8: *L. paracasei* (microencapsulated) + Chia seeds (2%); T9: *L. paracasei* (microencapsulated) + Chia seeds (4%); T10: *L. paracasei* (microencapsulated) without prebiotic; T11: Control.

2% and 4% chia seeds were obtained the lowest score (3.00 ± 0.00). The results of texture analysis are presented in Figure 4C. On day 1, the highest score (4.00 ± 0.50) was reported for the sample containing microencapsulated *L. paracasei* with 2% chia seeds, whereas the lowest score (3.00 ± 0.00) was obtained for the free *L. paracasei* sample with 4% chia seeds. Conversely, at the end of storage (day 30), the control sample, together with samples containing free *L. paracasei* with 2% and 4% flaxseeds and microencapsulated *L. paracasei* samples containing 2% and 4% chia seeds were obtained the highest score (4.00 ± 0.10). On the other hand, the lowest score (3.00 ± 0.00) was attributed to the free *L. paracasei* sample with 4% chia seeds. The results of the sensory analysis of color, presented in Figure 4D, demonstrated the effect of time on the sample color. Over time, the score of color analysis reduced in the samples. On day 1, the highest score (5.00 ± 0.00) was attributed to the sample containing free *L. paracasei* with 4% chia seeds and microencapsulated *L. paracasei* containing 4% chia seeds and also 4% flaxseeds; however, the control sample together with sample containing free *L. paracasei* were obtained the lowest score. At the end of storage (day 30), the control sample had the lowest score (3.00 ± 0.00), whereas the microencapsulated *L. paracasei* sample containing 4%

flaxseeds showed the highest score (4.66 ± 0.27). The analysis of the overall acceptability of synbiotic yogurt sauce samples during storage is shown in Figure 4E. On day 1, there was no significant difference between the samples, while the effect of time on the overall acceptability of the samples emerged at the end of storage. On day 30, the sample containing microencapsulated *L. paracasei*, together with samples containing microencapsulated *L. paracasei* with flaxseeds (2% and 4%), and also samples containing free *L. paracasei* with both concentrations of flaxseeds were obtained the highest score (3.66 ± 0.57).

The analysis of the sensory features of synbiotic yogurt sauce samples during 30 days of storage showed no significant differences in the texture or color of the samples ($P > 0.05$). However, samples containing probiotics (free and microencapsulated) with chia seeds (2% and 4%) had the lowest scores due to their unpleasant taste, smell, and therefore, overall acceptability. The taste, smell, and overall acceptability analysis revealed that the addition of chia seeds had significant negative effects on the sensory properties of probiotic yogurt sauce samples.

Similar results were reported in a previous study, which showed the lower overall acceptability of mayonnaise samples with and without alginate/pectin microcapsules containing OLE due to their bitter and salty taste (Flamminii et al., 2020). Besides, in the current study, no significant differences were found in the taste or flavor of mayonnaise samples with free or microencapsulated probiotics, while there were significant differences in the texture, appearance, and color of these samples (Fahimdanesh et al., 2013). Moreover, some researchers reported that microencapsulation led to the enhancement of sensory parameters. The scores of all evaluated sensory characteristics were higher in the mayonnaise sauce samples containing microencapsulated probiotics as compared to mayonnaise samples containing free probiotics and the controls (Khalil & Mansour, 1998). The finding of an identical investigation showed no effect on color and smell parameters of functional jelly food containing *L. rhamnosus* (microencapsulated and free) as well as oligofructose samples. Furthermore, microencapsulated *L. rhamnosus* samples containing 3% oligofructose received the highest scores for taste, texture and total acceptability (Karegar et al., 2022). Recent findings showed that color was the only sensory characteristic affected by pumpkin seed oil-loaded microparticles; however, there were no significant differences in other sensory properties between mayonnaise samples containing microcapsules and the control samples (Rojas et al., 2019). Also, the results of other researchers revealed that in terms of taste and texture, symbiotic yogurt samples containing oligofructose and inulin had the highest and second highest scores, respectively, although the most satisfactory results were reported in samples containing lactulose and inulin. There was no significant difference between synbiotic yogurt samples in terms of color (Shaghaghi et al., 2013). Moreover, Portela et al. examined the expected sensitive profiling and overall liking of soursop flavored prebiotic whey beverages subjected to ultrasound processing and showed that both the prebiotic claim and the new processing technology had prominent receptivity among contributors (Portela et al., 2022). In another study, da Costa et al compared preferred attribute elicitation methodology (PAE) to conventional descriptive analysis using probiotic yogurt sweetened with xylitol and added with prebiotic components. They concluded that PAE methodology can be used to characterize sensorially and to determine the consumers' acceptance of functional yogurts (Costa et al., 2020). In addition, da Cruz developed and validated a product-specific emoji list and used that list to analyses children's emotional responses related to the consumption of probiotic fermented milks prepared with different probiotic strains. The findings suggested that the type of probiotic culture impacted the sensory characteristics of these products, supporting the use of *Bifidobacterium, L. lactis* or *L. casei* in fermented milks (Cruz et al., 2021). A similar finding was reported earlier by some researchers who showed that the addition of probiotics in free and microencapsulated forms had no significant effects on the color, taste, or texture of non-fermented synbiotic ice cream (Homayouni et al., 2008). Additionally, the results of another study demonstrated that addition of probiotics in both free and microencapsulated forms had significant negative effects on the color, taste, texture, and overall acceptability of the produced probiotic yogurt (Afzaal et al., 2019).

4 Conclusion

This study revealed the significant effect of microencapsulation with resistant starch and sodium alginate on improving the survival of probiotic bacteria in synbiotic yogurt sauce. The results also indicated the increased survival of microencapsulated *L. paracasei* compared to free probiotics cells under difficult gastrointestinal conditions. Besides, comparison of two prebiotics, namely, flaxseeds and chia seeds, showed the higher viability of probiotic cells with flaxseeds compared to chia seeds. Also, increasing the concentration of flaxseeds from 2% to 4% improved the viability of probiotic cells. The findings of this study revealed that the use of probiotic bacteria (microencapsulated and free) and prebiotic compounds (flaxseeds and chia seeds) had no negative effects on the pH, acidity, or color of synbiotic yogurt sauce. Microencapsulation with resistant starch and sodium alginate might improve the sensory properties of synbiotic yogurt sauce. Considering the simplicity and low cost of the emulsion technique, it is considered appropriate for microencapsulation in the food industry, besides the oral delivery of probiotics to the gastrointestinal system.

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