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Evaluation of the effect of *Spirulina platensis* on the viability of *Lactobacillus acidophilus* (LA-5) in yogurt and its properties

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Abstract

Dairy products are known as the best carriers of probiotics and prebiotics, among which yogurt has a special place due to its ability to preserve probiotics. The aim of this study was to evaluate the prebiotic effect of *Spirulina platensis* extract (SPE) at a rate of 0.5 and 1.5% on the viability of probiotic bacteria *Lactobacillus acidophilus* (CFU/ml 1 × 10^8) in yogurt during storage at refrigerator (4 ± 1°C). Samples were designed in four groups including control, probiotic, probiotic + 0.5% SPE and probiotic + 1.5% SPE. Then, the viability of probiotic, chemical (titratable acidity and pH) and sensory tests were done. The findings showed that the highest growth of probiotic bacteria was related to the treatment containing 1.5% SPE (5.68 log CFU/g), which compared to 0-day and the end of the study. This treatment had a lower decrease than the probiotic treatment and the control. Also, it was found that the viability rate of probiotic bacteria increases in the presence of SPE. In addition, during the study, titratable acidity and pH increased and decreased, respectively ($p < 0.05$). According to the findings of the present study, it was found that the addition of SPE improves the viability of *L. acidophilus*, which indicates the desirability of using this combination to increase the viability of probiotic bacteria in probiotic products.

Introduction

Probiotics are living microorganisms that leave their health-giving effects by maintaining, improving and balancing the intestinal microbiota and are useful and effective in the health of the consumer (Al-Sheraji *et al*., 2013) and accordingly, various studies in the past years until now regarding lactic cultures in products different foods have been tested and the results indicate the health-enhancing effects of different probiotic food products and their benefits have been proven (Dave and Shah, 1997).

In the beginning, most of these studies were about the treatment of various diseases such as intestinal infections with the help of probiotics, but today, according to the concerns and challenges of researchers, the focus of the study is on the health-giving and preventive effects of these products (Pimentel-González *et al*., 2009) and based on the favorable results of these studies. The production and demand of probiotic products among world consumers is expanding (Samira *et al*., 2007; Madhu *et al*., 2012). Most of the microorganisms used for probiotics belong to the category of lactic acid bacteria (the most important microorganisms associated with the human digestive system) (Saarela *et al*., 2000). *Lactobacillus acidophilus* (*L. acidophilus*) is considered the most important probiotic species (Vrese and Schrezenmeir, 2008) which is a microaerophilic, gram-positive, rod-shaped bacterium with a round end, which is single-celled, paired or short-chained, non-motile, non-sporous, and homofermentative, and cannot tolerate salt, and its optimal growth temperature is 35-40°C

(Hood and Zoitola, 1998). This bacterium is effective in helping to reduce blood cholesterol levels, preventing cancer, increasing the body's immunity against intestinal infections, and improving the use of lactose (Gupta *et al*., 1996). Microorganisms that are used as probiotics must have a human source be resistant to bile and stomach acids, have the ability to settle in the human digestive system, be non-pathogenic, and have proven effectiveness and safety (Saarela *et al*., 2000). Adding prebiotics to probiotic compounds produces synbiotic products that are in the category of super-beneficial foods (Shigwedha *et al*., 2016).

Spirulina platensis (*S. platensis*) is a kind of blue-green microalgae, spiral filament, photosynthesizer and prokaryote, which has two species *Arthrospira platensis* and *A. maxima* and belongs to the cyanobacteria branch and the Oscillatoraceae family (Louis-Jeune *et al*., 2012). It is rich in all kinds of vitamins, minerals, proteins and amino acids, essential fatty acids. Its benefits include antioxidant, anticancer properties, helping to reduce body cholesterol, anti-inflammatory properties, increasing the body's immune system, and antimicrobial properties, effective in treating high blood pressure and allergy (Madhu *et al*., 2012; da Silva *et al*., 2019). Today, *S. platensis* is widely used in the food industry and various industries such as the pharmaceutical industry (Gouveia *et al*., 2006; Masoumizadeh, 2015).

Dairy products are known as the best carriers of probiotics, among them yogurt, due to its ability to preserve probiotics and its popularity due to its special sensory properties, it is a suitable environment for the transfer of probiotics to the body (Kaur *et al*., 2014). Yogurt is the most well-known and popular fermented product made from milk, in which starters are used to create flavor. In yogurts that are beneficial, in addition to starter bacteria (*Streptococcus thermophilus* and *L. bulgaricus*), probiotic microorganisms have also been used (Criscio *et al*., 2010; Pourahmad *et al*., 2015). Yogurt has favorable organoleptic properties, which is one of the reasons for its popularity. With changes such as adding additives to yogurt and creating a new taste, the demand for this product increases.

Materials and Methods

Preparation of SPE: At first, 150 g of dried seaweed with the Green Sea brand was purchased from a reliable supply center and crushed by a grinder (Moulinex Co., Spain). Then, it was mixed with water, placed on a shaker (T&N Co., China) for 48 hours, and filtered. This step was repeated by adding 1 liter of water. To concentrate and dry, the extract was placed in an oven device at 40 ± 1°C for one week. The dried extract was stored at 4 ± 1°C in light -proof glasses.

Probiotic preparation: The culture of lyophilized *L. acidophilus* (LA-5) prepared by CHR Hansen (Horsholm Co., Denmark) was placed in sterile MRS Broth (Merck Co., Germany) culture medium at 37°C for 24 hours in anaerobic conditions. Re-cultivation was carried out for 48 hours to obtain the maximum number of active probiotics. Then, the cell mass was collected by a refrigerated centrifuge (Eppendorf AG Co., Germany) at 4000 rpm for 15 minutes and in duplicate with sterile physiological serum 0.9% was washed and the bacterial suspension was calculated with a concentration of 1×10^8 CFU/ml (Pimentel-González *et al*., 2009).

Viability of *L. acidophilus* in yogurt samples: *L. acidophilus* was counted on the study days. After preparing the series of dilutions, it was cultured in MRS bile agar medium (Merck Co., Germany) in three replicates and stored at 37°C for 72 hours. The counting results were reported in terms of CFU/g (Golestani and Pourahmad, 2017; Yadav *et al*., 2018).

pH measurement: The pH of the samples was measured using a pH meter. The amount of 10 g of the homogenized samples were poured into the beaker and measured by a pH meter (Metrohm Co., Switzerland) which was initially calibrated at 20°C by buffers 4 and 9 (Yadav *et al*., 2018).

Acidity measurement: The amount of titratable acidity of the samples was done with the help of titration method. The amount of 10 g of the sample was dissolved with distilled water at 45°C and brought to a volume of 100 ml. Then, 10 ml of solution was titrated with sodium hydroxide in the presence of phenolphthalein reagent until a stable pink color appeared and the acidity was determined in terms of lactic acid percentage (Yadav *et al*., 2018).

Sensory evaluation: The samples were prepared according to the treatment table and randomly coded. Sensory characteristics (taste, texture, and color) were evaluated by 10 trained evaluators using a 5-point hedonic rating scale, average, bad, and very bad.

Statistical analysis: Statistical analysis of data was done with SPSS software. First, the normality of the data was checked using the Kolmogorov-Smirnov test, and then the homogeneity of the variance of the data was performed using the Leven test.

Results and Discussion

Viability of *L. acidophilus* in yogurt during storage: The viability of *L. acidophilus* bacteria in yogurt under storage conditions (28 days at 4 ± 1°C) was shown in Fig. 1. The number of live bacteria showed a significant decrease during the storage period compared to the first day ($p < 0.05$). The treatment containing *S.*

platensis showed a significant decrease in the viability of the probiotic bacteria *L. acidophilus* compared to the probiotic treatment and the control ($p < 0.05$). In a similar study by Fadaei *et al*. (2013), the effect of SPE on the

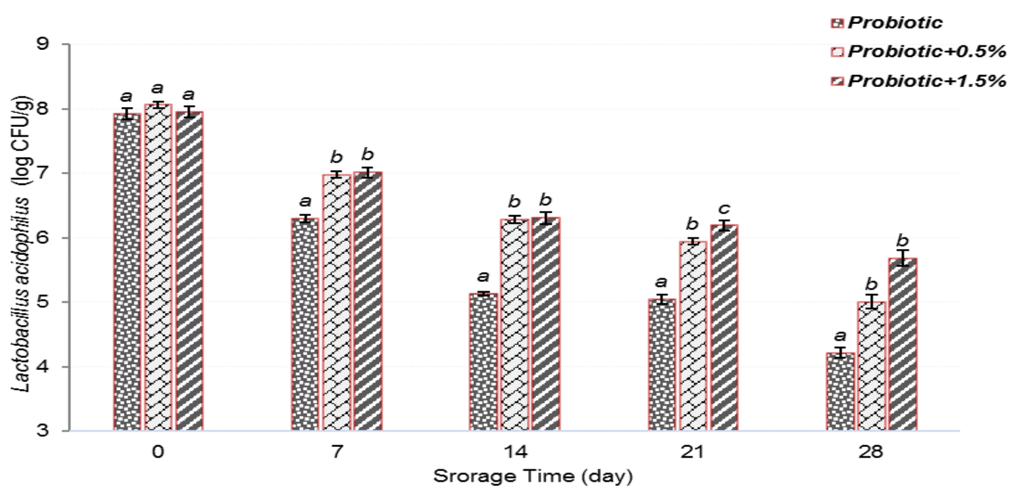

Fig. 1. Viability of *Lactobacillus. acidophilus* in yogurt during storage. Small and non-similar English letters indicated significant differences between groups ($p < 0.05$).

viability of *L. acidophilus* added to Spanish yogurt stored at refrigerator and the starter bacteria *L. delbrueckii* subspecies *bulgaricus* and *S. thermophilus* were investigated in 21 days. It was observed that SPE was significantly effective on the growth of lactic acid bacteria in probiotic spinach yogurt ($p < 0.01$) and provided a suitable environment for the growth of this group of bacteria, which is consistent with the results of this study (Fadaei *et al*., 2013).

pH and acidity of yogurt containing *L. acidophilus*: In Table 1, the changes in pH and in Table 2, the changes in the acidity of synbiotic yogurt containing the probiotic *L. acidophilus* were shown in storage conditions (28 days at 4 ± 1°C). As can be seen, the trend of pH and acidity in all treatments was decreasing and increasing, respectively. There was no significant difference between treatments until day 21 in terms of acidity. At the end of the study, the samples containing SPE had a lower pH than the samples containing probiotic bacteria alone. This could be due to the nitrogenous substances in SPE and stimulation of the treatments containing SPE in acid production (Varga *et al*., 1999). As we expected from the results of this study, the treatment containing 1.5% SPE and probiotic bacteria had the lowest pH (3.80), the highest acidity (0.98), and a significant difference with other treatments containing lower concentrations of SPE. According to the study of Beheshtipour *et al*. (2012), it was due to the buffering capacity in samples containing microalgae. In this study, the effect of adding two species of microalgae, *Arthrospira platensis* and *Chlorella vulgaris*, on the biochemical properties (pH, and titratable acidity) of yogurt during a 28-day storage period at refrigerator was investigated. The treatments included seven groups of yogurt containing control and three concentrations for each microalga. According to the results, it was found that pH decreased and acidity increased during the study, which is consistent with the findings of this study (Beheshtipour *et al*., 2012). In comparison of the treatment containing probiotic bacteria and the control, it was also found that at the end of the study, the treatment containing probiotics showed lower pH and higher acidity, which according to the study of Talwalkar *et al*. (2004). The reason is lactose fermentation and acetic acid production by probiotic bacteria.

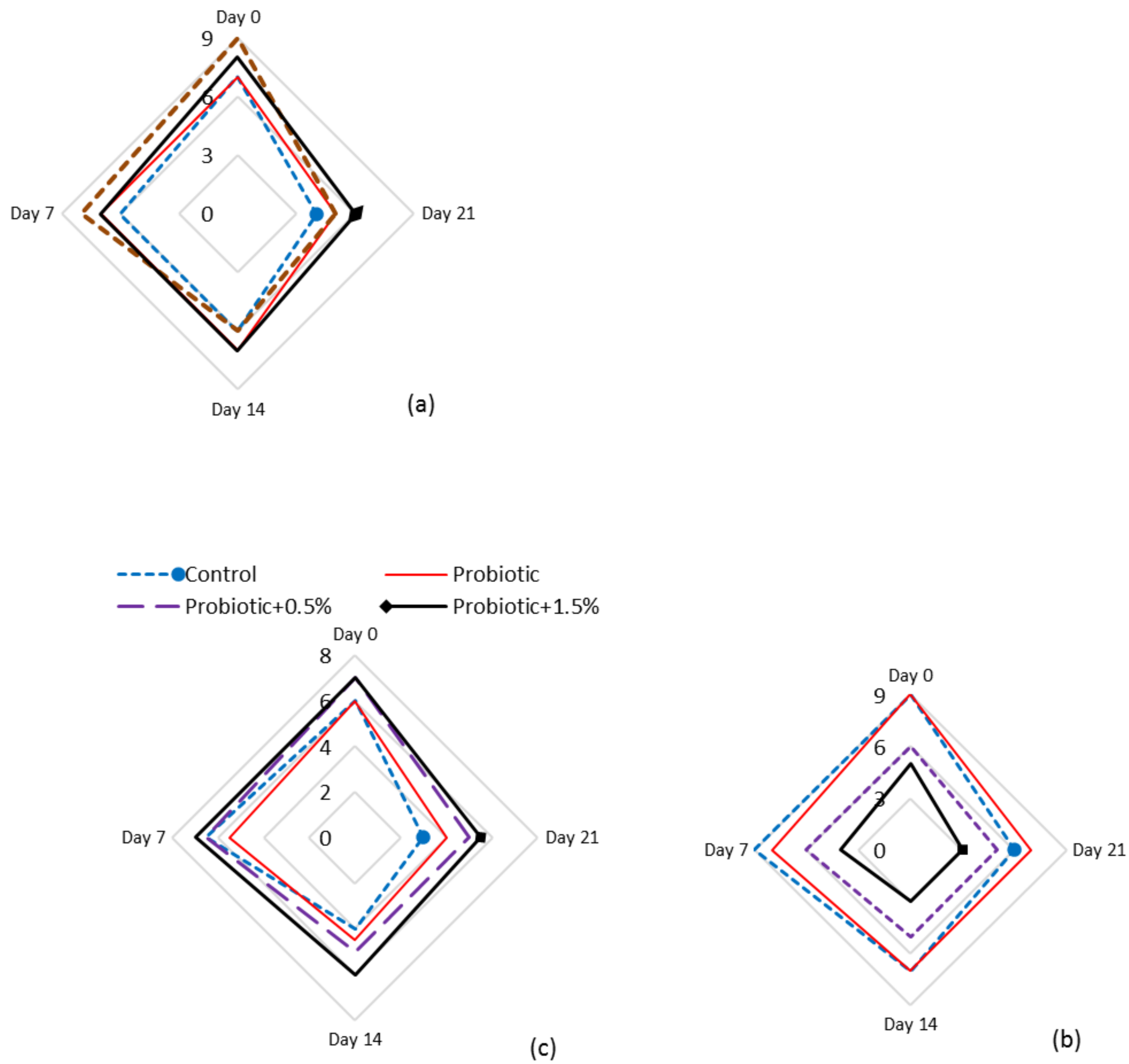

Fig. 2. Sensory properties; taste (a), color (b)and texture (c) of different treatments stored in cold storage (4 ± 1°C).

Table 1. Change in pH of yogurt during storage.

Day / Treatment	0	7	14	21	28
Control	4.52 ± 0.03^{a}	4.40 ± 0.01^{a}	4.30 ± 0.01^{b}	4.18 ± 0.02^{b}	4.08 ± 0.02^{b}
Probiotic	4.55 ± 0.01^{a}	4.40 ± 0.02^{a}	4.25 ± 0.03^{c}	4.15 ± 0.02^{b}	4.03 ± 0.01^{c}
Probiotic + 0.5%	4.56 ± 0.03^{a}	4.37 ± 0.01^{a}	4.24 ± 0.01^{c}	4.12 ± 0.00^{c}	4.00 ± 0.02^{c}
Probiotic + 1.5%	4.54 ± 0.02^{a}	0.03^{b}± 4.30	0.05^{d}± 16.4	01 ± 0.02^{d}.4	3.91 ± 0.01^{d}

- Small and non-similar English letters indicated significant differences between groups ($p < 0.05$).

Table 2. Change in acidity of yogurt during storage.

Day / Treatment	0	7	14	21	28
Control	0.01[a] ± 0.74	0.02[a] ± 0.76	0.01[a] ± 0.77	0.00[a] ± 0.77	0.02[a] ± 0.78
Probiotic	0.75 ± 0.02[a]	0.78 ± 0.02[a]	0.82 ± 0.01[a]	0.85 ± 0.01[b]	0.86 ± 0.01[b]
Probiotic+0.5%	0.73 ± 0.02[a]	0.78 ± 0.01[a]	0.84 ± 0.00[a]	0.86 ± 0.02[b]	0.88 ± 0.01[b]
Probiotic+1.5%	0.74 ± 0.01[a]	0.80 ± 0.02[a]	0.85 ± 0.01[a]	0.86 ± 0.02[b]	0.89 ± 0.01[b]

- Small and non-similar English letters indicated significant differences between groups ($p < 0.05$).

Sensory evaluation: The results of sensory indicators (taste, color, and texture) of probiotic yogurt stored at refrigerator condition on days 0, 7, 14, and 21 were shown in Fig. 2. In the evaluation of the taste parameter, at the end of the study, the treatment containing 0.5% SPE received the highest score. On the other hand, in color and texture parameters, the scores decreased with the increase of SPE percentage. In a study by Golmakani *et al*. (2019), the effect of SPE on the sensory characteristics of a type of feta cheese was investigated over a period of 60 days. The results showed a softer texture in the samples containing SPE compared to the control samples. However, the samples containing 0.5 or 1.5% SPE did not differ significantly from the control samples, which is consistent with the results of this study. The findings indicated the desirability of using this combination to increase the viability of probiotic bacteria in probiotic products.

According to the findings of the present study, the enrichment of yogurt sauce with *S. platensis* microalgae has been effective in improving the texture of yogurt and has not had a negative effect on its chemical properties.

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Conflict of Interest

The authors report no conflicts of interest.

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