International Food Research Journal 31(1): 157 - 168 (February 2024)
Journal homepage: *http://www.ifrj.upm.edu.my*

Effect of *Spirulina platensis* on probiotic, nutritional, and quality properties of yogurt

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Article history

Received:
14 January 2022
Received in revised form:
10 July 2023
Accepted:
3 November 2023

Keywords

Bifidobacterium animalis, Lactobacillus acidophilus, probiotic bacteria, Spirulina platensis, yogurt

Abstract

The present work was carried out to investigate the changes in the microbiological, physicochemical, textural sensory properties, and antioxidant profile of probiotic yogurt enriched with different concentrations (0, 0.5, 0.75, and 1%) of *Spirulina platensis*. Results indicated that when the amount of *S. platensis* was increased, there was a corresponding increase in water retention ability. This could have been attributed to a significant increase in fibre content ($p < 0.05$). The ratio of the soluble/insoluble dietary fibre of the yogurt samples depended on the concentration of algae. Towards the end of storage, there was a decrease in pH which resulted from the combined effect of the starter culture and probiotics working together. The amounts of *Lactobacillus acidophilus* and *Bifidobacterium animalis* increased in groups with *S. platensis* throughout storage, which is essential for a product to possess probiotic properties. In the sensory evaluation, group D which contained 1% (w/v) *Spirulina* was ranked as the most preferred. Additionally, this group exhibited the highest DPPH activity. The addition of *Spirulina* to yogurt had a significant impact on its quality characteristics, and exhibited potent prebiotic properties.

DOI
https://doi.org/10.47836/ifrj.31.1.14

Introduction

Due to their excellent nutritional composition, dairy products are an essential source of nutrients in a healthy and balanced diet. Among dairy products, yogurt, a fermented product, represents a good source of nutrients in a more concentrated form in the absence of lactose. Due to the high-quality nutritious profile, yogurt represents a valuable candidate to be transformed into a functional food. Functional foods have become more popular as the awareness of the relation between nutrition and health increases. Foods that contain active ingredients (*i.e.*, phytochemicals, pro and prebiotics) have numerous health benefits such as reducing blood cholesterol, improving immunity, and treating diarrhoea (Shah, 2000).

Among a variety of functional yogurts, probiotic yogurts were found to be the preference of choice worldwide. To further increase the nutritional value, different components such as herbs, extracts, or microalgae, are integrated to probiotic yogurts (Sah *et al*., 2016; Zhang *et al*., 2019).

S. platensis, a nutrient-rich microalgae with high bioavailability, has been the focus of many studies that aimed to integrate it into different food matrices. *S. platensis*, also known as *Arthrospira platensis*, belongs to cyanobacteria phylum, and has been used as food source since antiquity (Pulz and Gross, 2006; Borowitzka *et al*., 2009).

Nowadays, *S. platensis* is available as a commercialised product. The composition of commercial powders of *Spirulina* biomass generally contains 60 - 70% protein consisting of essential amino acids, 20% carbohydrate as a source of dietary fibre, 5% fat, 7% mineral, and 3 - 6% moisture (Belay *et al*., 1993; Gutiérrez-Salmeán *et al*., 2015). Regarding its valuable composition, *S. platensis* has been the focus of studies that intended to utilise it in production of various functional dairy products, emulsion products, confectionery, and beverages. Similar studies have reported that the addition of *S. platensis* to various foods not only increased the nutritional value but also had very important role in improving their textural and rheological properties

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(Suzery *et al*., 2018; Nourmohammadi *et al*., 2020). Fermented dairy products are both good source of nutrition and bioactive peptide (Donkor *et al*., 2007). The bioactive peptides that the fermented dairy products such as yogurt contain confer them antioxidant activity (Power *et al*., 2013).

Taking into consideration the aforementioned benefits, the present work aimed at assessing the effect of *S. platensis* on the nutritional, physicochemical, microbial, textural, and sensorial qualities of probiotic yogurts throughout storage.

Materials and methods

Chemicals and reagents

Polypropylene plastic containers (Coveris™ Rigid, Turkey) were utilised for packaging the yogurts produced in the experiment. Powdered *S. platensis* was purchased from EGERT Inc., a spin-off company from Ege University (Izmir, Turkey), and was stored at 4°C until further analyses. Skimmed milk powder was supplied by Sutas Dairy Products (Bursa, Turkey). Freeze-dried yogurt starter cultures composed of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* at a ratio of 1:1 (w/w) were purchased from Maysa Gıda Sanayi Ticaret Anonim Sirketi (Istanbul, Turkey). Probiotic bacteria (*Lactobacillus acidophilus* and *Bifidobacterium animalis*) were obtained from Chr. Hansen (Hørsholm, Denmark). Violet red bile agar (VRBA), de Man Rogosa Sharpe (MRS) agar and broth, M17 agar, L-cysteine hydrochloride, and Anaerocult A were purchased from Merck (Darmstadt, Germany). Nalidixic acid and neomycin sulphate were obtained from PhytoTechnology Laboratories (Lenexa KS, USA). Plate count agar (PCA), potato dextrose agar (PDA), and paromomycin sulphate were supplied from Oxoid (Basingstoke, England), Difco (Sparks, USA), and Cayman Chemical Company (Ann Arbor, MI, USA), respectively.

Activation of microbial cultures

The commercial cultures used in the production were activated in 12% (w/v) reconstituted skimmed milk supplemented with 2% (w/v) glucose and 1.2% (w/v) yeast extract, and then the mixture was incubated at 37°C for 18 h. *Bifidobacterium animalis* were propagated twice using 1% (w/v) inoculum MRS broth supplemented with 0.05% (w/v) of L-cysteine-HCl, and were incubated at 37°C for 18 h under anaerobic conditions. Cultures that were stored at 4°C were sub-cultured three times in the same media before experiments.

Preparation of probiotic yogurts

Cow milk (free of antibiotics and other chemical preservatives) was used for yogurt production. Initially, raw milk was pasteurised (85°C/30 min), and then was cooled to 45°C. To enhance the textural properties, skimmed milk powder was added at a ratio of 3% (w/v). *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* were added as starter culture at a ratio of 3% (w/v). To confer probiotic properties, *Lactobacillus acidophilus* and *Bifidobacterium animalis* strains were inoculated at a ratio of 0.01% (w/v). The whole mixture was divided into four equal portions, and *Spirulina platensis* powder was added to each group at different ratios (0, 0.5, 0.75, and 1% (w/v)). The final mixture was transferred into sterile polypropylene containers, and incubated at 42°C for ~ 4 h until the final product reached a pH value of 4.3 ± 0.02. Subsequently, samples were coded, and the corresponding groups were stored at 4°C for 28 d. The samples were subjected to microbiological, physicochemical, rheological, and sensorial evaluation on days 0, 7, 14, 21, and 28 of storage. Production and analysis were performed in triplicates.

Physicochemical analysis

Titratable acidity, pH, fat content, ash, dry mater, total nitrogen, total protein content, and water activity (a_w) were evaluated according to AOAC (2019). Total dietary fibre amount was determined according to ISO 6541:1981 (ISO, 1981).

Microbiological analysis

Yogurt samples (10 g) were transferred aseptically to stomacher bags containing 90 mL of peptone water, and homogenised (IUL Instruments Masticator, Spain) for 60 s at ambient temperature. Appropriate serial dilutions were made with 0.1% (w/v) peptone water solution, and bacterial populations were counted using selective media following spread-plating. After incubation, only the plates that contained 30 - 300 colonies were counted. Microbial counts of samples were expressed as log colony forming units per gram (log CFU/g) (ISO, 2003a).

The total aerobic mesophilic counts were enumerated on PCA incubated at 30 ± 1°C for 48 h (ISO, 2003a). The yeast and mould counts were enumerated on PDA. The plates were incubated at 22 ± 1°C for 5 d, and then were evaluated (ISO, 2009). Coliform counts were enumerated on VRBA. Plates were incubated at 30 ± 1°C for 24 h, and then were evaluated (ISO, 2006).

Lactobacillus delbrueckii subsp. *bulgaricus* counts were enumerated on MRS agar (pH 5.8). The plates were incubated under anaerobic conditions at 42 ± 1°C for 72 h. At the end of incubation, distinct typical colonies were evaluated as *L. delbrueckii* subsp. *bulgaricus* (de Man *et al*., 1960). *Streptococcus thermophilus* counts were enumerated on M17 agar. The plates were incubated at 37 ± 1°C for 72 h. At the end of incubation, distinct typical colonies were evaluated as to be *S. thermophilus* colonies (ISO, 2003b).

MRS agar was used for the enumeration of *Lactobacillus acidophilus*. The plates were incubated at 37 ± 1°C for 72 h under anaerobic conditions (ISO, 2006). *Bifidobacterium animalis* was identified on MRS agar supplemented with NNLP. It consisted of MRS agar ISO formulation, supplemented with filter sterilised solutions of nalidixic acid (15 mg/L), neomycin sulphate (100 mg/L), lithium chloride (3.0 g/L), paromomycin sulphate (200 mg/L), and L-cysteine hydrochloride (0.5 g/L). Incubation was done under anaerobic conditions at 37°C for 72 h (Dave and Shah, 1996).

Sensorial analysis

Sensory evaluation was performed following the international standard (ISO, 2016). The panel group consisted of ten trained members (university staff members, post graduate students, 25 - 45 years old, experienced male and female). Sessions were held in an odourless, white-lighted room, and panellists were allowed to drink water and eat breadcrumbs to remove the taste left between samples. Samples were served in sterile polypropylene cups (50 g). The tested sensory parameters were colour, odour, taste, texture, and general acceptability. Evaluation was done using the hedonic type scale from 1 to 9 (from 9 = very pleasant to 1 = very unpleasant).

Textural analysis

Textural analyses were performed *via* a texture analyser (TexturePro CT V1.8 Build 31, Brookfield Engineering Labs, Inc. USA). Textural properties (*i.e.*, hardness, adhesiveness, and resilience) of yogurt samples were evaluated under the following settings: target value 4500 g, trigger load 6.8 g, test speed 1 mm/s, probe TA3/100 (acrylic cylinder-35 mm diameter), and probe penetration 10 mm (Romeih *et al*., 2002).

Determination of antioxidant activity

The free radical scavenging activity of the samples during 28-d storage was determined according to Bersuder *et al*. (1998) based on the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical. Ethanol was used as the blank solution and ascorbic acid was used as the reference antioxidant to determine the scavenging activity of the DPPH free radical. Then, the absorbance was measured at 517 nm using a UV-visible spectrophotometer (Perkin Elmer, L60000CC, Massachusetts, USA). Radical scavenging activity tests were performed in triplicates. DPPH radical scavenging activity was calculated using Eq. 1:

$$I\ (\%) = [(A_0 - A_1) / A_0] \times 100 \qquad \text{(Eq. 1)}$$

where, A_0 = absorbance of the control, and A_1 = absorbance of the yogurt sample.

Statistical analysis

The production and analyses of yogurt samples were carried out in triplicates. The results were subjected to One-way analyses of variance (ANOVA) using the Statistical Package for Social Sciences (SPSS) (Version 25.0; SPSS, Chicago, IL, USA). Statistical procedure Tukey's test was used to determine significant differences between mean values at the $p < 0.05$ level. Results were expressed as mean ± standard deviation.

Results and discussion

Physicochemical changes in probiotic yogurts

The results of the analysis of the physicochemical parameters (*i.e.*, dry matter, ash, fat, protein content, water activity, insoluble and soluble dietary fibres) of probiotic yogurts enriched with *Spirulina* are presented in Table 1. Dry matter values of the yogurt samples produced experimentally ranged from 12.33 to 13.14%. It was observed that the dry matter content increased as the *Spirulina* concentration increased. These findings agreed with

similar studies. Prakash and Kumari (2011) found similar results for frozen yogurt samples supplemented with *Spirulina* and 10% (w/w) papaya. The ash content values obtained from groups B and D on day 1 ranged from 0.69 to 0.86%. The differences in ash content were related with the concentration of *S. platensis*. No significant difference was observed in the fat content among groups. The protein content of the groups enriched with *S. platensis* was found to be higher than that of the control group ($p < 0.05$). On day 1, it was observed that the protein content ranged from 4.63 to 4.83%. These were related with high protein content (50 - 60%) of *S. platensis* (Lupatini *et al*., 2017). The presence, variety, and quantity of essential amino acids (*i.e*., lysine, leucine, phenylalanine, and valine) play important role on the nutritional value of the final product, and on preserving the viability of the starter cultures and probiotics used in the yogurt production.

Table 1. Physicochemical properties of yogurts throughout 28-day storage.

		A	B	C	D
Dry mater (%)		12.42 ± 0.09^{A}	12.33 ± 0.07^{A}	13.14 ± 1.14^{A}	12.81 ± 0.24^{A}
Ash (g/100 g)		0.73 ± 0.01^{C}	0.69 ± 0.01^{D}	0.81 ± 0.01^{B}	0.86 ± 0.02^{A}
Fat content (%)		3.63 ± 0.10^{AB}	3.52 ± 0.26^{B}	3.73 ± 0.04^{AB}	3.80 ± 0.06^{A}
Total protein content (%)		4.63 ± 0.05^{C}	4.74 ± 0.02^{B}	4.77 ± 0.02^{B}	4.83 ± 0.02^{A}
Water activity		47.05 ± 1.11^{BC}	45.55 ± 2.39^{C}	48.30 ± 1.34^{AB}	50.26 ± 1.52^{A}
Insoluble dietary fibre (%)		0.09 ± 0.01^{C}	0.14 ± 0.01^{B}	0.15 ± 0.02^{B}	0.19 ± 0.02^{A}
Soluble dietary fibre (%)		0.92 ± 0.02^{D}	1.55 ± 0.04^{C}	1.73 ± 0.02^{B}	1.96 ± 0.01^{A}
Storage day		**A**	**B**	**C**	**D**
pH	**0**	5.11 ± 0.03^{Ca}	5.17 ± 0.03^{Ca}	5.50 ± 0.06^{Aa}	5.38 ± 0.03^{Ba}
	7	4.91 ± 0.01^{Ab}	4.76 ± 0.02^{Cb}	4.92 ± 0.01^{Ab}	4.84 ± 0.02^{Bb}
	14	4.06 ± 0.03^{Ac}	3.90 ± 0.01^{Cc}	4.00 ± 0.02^{Bc}	3.96 ± 0.03^{Be}
	21	3.94 ± 0.02^{Bd}	3.87 ± 0.02^{Cc}	3.98 ± 0.03^{Bc}	4.05 ± 0.04^{Ad}
	28	3.84 ± 0.02^{Ce}	3.82 ± 0.02^{Cd}	3.89 ± 0.01^{Bd}	4.13 ± 0.03^{Ac}
Titratable acidity (LA%)	**0**	0.65 ± 0.05^{Ad}	0.62 ± 0.02^{ABe}	0.55 ± 0.03^{Cd}	0.58 ± 0.03^{BCc}
	7	0.84 ± 0.04^{ABc}	0.88 ± 0.01^{Ad}	0.79 ± 0.02^{Bc}	0.78 ± 0.08^{Bb}
	14	0.99 ± 0.02^{Ab}	0.97 ± 0.01^{Ac}	0.81 ± 0.03^{Bc}	0.88 ± 0.07^{Ba}
	21	1.01 ± 0.03^{Ab}	1.01 ± 0.01^{Ab}	0.96 ± 0.03^{Bb}	0.85 ± 0.02^{Cab}
	28	1.08 ± 0.03^{Aa}	1.1 ± 0.03^{Aa}	1.01 ± 0.01^{Ba}	0.83 ± 0.02^{Cab}

Values are mean ± standard deviation. Means with different uppercase superscripts in the same row are significantly different ($p < 0.05$). Means with different lowercase superscripts in the same column are significantly different ($p < 0.05$). **(A)**: control; **(B)**: *S. platensis* 0.5%; **(C)**: *S. platensis* 0.75%; and **(D)** *S. platensis* 1%.

Based on the data presented in Table 1, the water holding capacity (WHC) values increased for all samples throughout 28-d storage ($p < 0.05$) with the increase in the concentration of *S. platensis*. WHC is related with the water retaining ability of proteins and dietary fibres in the structure of yogurt. In addition, the production of many capsular polysaccharides and exopolysaccharides by *B. animalis* may be another reason for this increase (Shazly *et al*., 2022).

The control group had the lowest insoluble dietary fibre content 0.09% ($p < 0.05$). In Table 1, the values for soluble and insoluble fibres obtained on day 1 are given. As the concentration of *S. platensis* increased, the fibre content also increased significantly ($p < 0.05$). The soluble/insoluble dietary fibre ratio of the yogurt samples also increased as the algae concentration increased. The fibre content acts as physical stabiliser as it increases the visible viscosity of yogurt.

pH changes in probiotic yogurts

In fermented dairy products, pH is of major importance in terms of quality and consumer acceptability. The pH of the yogurt decreased by the end of 28-d storage due to the synergistic activity of the starter culture and probiotics. Since factors such as low pH and temperature can negatively affect the viability of probiotics; foods containing probiotics need to be fortified with prebiotics. Group D was observed to have the highest pH ($p < 0.05$). The presence of probiotics (*L. acidophilus* and *B. bifidum*) was observed to have profound effect on the decrease in pH over the 28-d storage. However, the addition of *S. platensis* to yogurt caused a more controlled change in pH. This indicated that samples containing *S. platensis* exhibited higher buffering capacity. It is known that the higher the buffering capacity, the slower the pH decreases (Barkallah *et al*., 2017). In the present work, probiotics were used together with *Spirulina* to preserve their viability at a level approximately 10^9 CFU/g, which is essential for a product to guarantee positive health benefits (Table 2).

Table 2. Starter cultures and probiotic counts of yogurts throughout 28-day storage (log CFU/g).

Storage day	A	B	C	D
		L. bulgaricus		
0	8.37 ± 0.28^{Ac}	8.24 ± 0.20^{Ac}	8.29 ± 0.29^{Ac}	8.37 ± 0.28^{Ac}
7	9.08 ± 0.08^{Aa}	8.87 ± 0.10^{Ab}	9.03 ± 0.13^{Ab}	9.08 ± 0.08^{Aa}
14	8.70 ± 0.29^{Bbc}	9.21 ± 0.09^{Aa}	9.31 ± 0.12^{Aab}	8.70 ± 0.29^{Bbc}
21	8.73 ± 0.19^{Bab}	9.23 ± 0.06^{Aa}	9.37 ± 0.04^{Aa}	8.73 ± 0.19^{Bab}
28	7.59 ± 0.09^{Cc}	8.68 ± 0.09^{Bbc}	8.98 ± 0.33^{Bab}	9.28 ± 0.13^{Aab}
		S. thermophilus		
0	9.17 ± 0.06^{Ad}	9.21 ± 0.16^{Ab}	8.92 ± 0.22^{ABb}	9.17 ± 0.06^{Ad}
7	9.52 ± 0.38^{Bcd}	10.03 ± 0.45^{Aba}	10.30 ± 0.13^{Aa}	9.52 ± 0.38^{Bcd}
14	10.22 ± 0.03^{Aa}	10.28 ± 0.08^{Aa}	10.35 ± 0.05^{Aa}	10.22 ± 0.03^{Aa}
21	9.03 ± 0.65^{Cab}	9.73 ± 0.42^{ABbc}	10.26 ± 0.13^{ABa}	10.38 ± 0.06^{Aa}
28	10.06 ± 0.24^{Aab}	10.26 ± 0.05^{Aa}	10.40 ± 0.07^{Aa}	10.06 ± 0.24^{Aab}
		L. acidophilus		
0	8.82 ± 0.44^{Ac}	8.36 ± 0.05^{Bd}	8.83 ± 0.23^{Ad}	8.82 ± 0.44^{Ac}
7	9.65 ± 0.22^{Aa}	9.21 ± 0.08^{Bc}	9.21 ± 0.08^{Bc}	9.65 ± 0.22^{Aa}
14	8.75 ± 0.06^{Cb}	9.35 ± 0.09^{Bab}	9.61 ± 0.27^{Bb}	10.03 ± 0.16^{Ab}
21	9.25 ± 0.06^{Cb}	9.67 ± 0.10^{Bb}	9.97 ± 0.06^{Ab}	9.25 ± 0.06^{Cb}
28	9.27 ± 0.08^{Bab}	10.15 ± 0.13^{Aa}	10.29 ± 0.08^{Aa}	9.27 ± 0.08^{Bab}
		B. animalis		
0	8.17 ± 0.33^{Bb}	8.79 ± 0.71^{ABc}	9.04 ± 0.11^{Ac}	8.17 ± 0.33^{Bb}
7	9.07 ± 0.17^{Ba}	9.24 ± 0.15^{Aba}	9.38 ± 0.05^{Abc}	9.11 ± 0.14^{Bc}
14	8.85 ± 0.25^{Dab}	9.19 ± 0.08^{Ca}	9.93 ± 0.14^{Bab}	10.27 ± 0.10^{Aab}
21	8.52 ± 0.25^{Cbc}	8.99 ± 0.15^{Ba}	10.18 ± 0.11^{Aa}	10.39 ± 0.08^{Aa}
28	7.84 ± 0.33^{Cd}	8.14 ± 0.23^{Cb}	9.51 ± 0.50^{Bb}	10.14 ± 0.04^{Ab}

Values are mean ± standard deviation. Means with different uppercase superscripts in the same row are significantly different ($p < 0.05$). Means with different lowercase superscripts in the same column are significantly different ($p < 0.05$). **(A)**: control; **(B)**: *S. platensis* 0.5%; **(C)**: *S. platensis* 0.75%; and **(D)** *S. platensis* 1%.

Total titratable acidity changes in probiotic yogurts

Titratable acidity is an important parameter in yogurt gel formation, and determines the structural quality of the fermented dairy products. Group B with 0.5% *Spirulina* had the highest acidity level compared to the control, 0.75, and 1% *S. platensis* on the last day of storage. Titratable acidity values of the yogurt samples, except group D ($p > 0.05$), increased steadily during storage (Table 1). At the end of the 28-d storage, the lowest value was observed IN group D ($p < 0.05$).

Microbiological changes in probiotic yogurts

On day 1 of storage, coliform, total aerobic mesophilic, and yeast and mould counts of yogurt samples belonging to groups A, B, C, and D were determined in the range of 4.01 - 3.92, 7.98 - 8.43, and 3.73 - 3.79 log CFU/g, respectively. For the groups containing *S. platensis* at different concentrations, approximately a 2-log decrease in coliform counts were observed on the following days of the evaluation. The results were related with the antimicrobial activity of *Spirulina* (Barkallah *et al*., 2017). These properties are attributed to different components of *S. platensis* (*i.e*., γ-linolenic acid, active fatty acid, synergetic effect of lauric and palmitoleic acid) (da Silva *et al*., 2019). The results obtained on day 1 of the microbiological evaluation indicated that there was no statistically significant difference among the groups regarding yeast and mould counts ($p > 0.05$). Although for the *S. platensis* supplemented groups, TAMC were higher than that for the control group ($p < 0.05$); it was observed that it did not depend on the microalgae concentration ($p > 0.05$).

Changes in starter culture and probiotic counts in probiotic yogurts

No significant difference was observed in the results of *L. bulgaricus* counts in the first two days of storage ($p > 0.05$). On the other evaluation days, counts of the samples containing *S. platensis* were found to be higher compared to that of the control group ($p < 0.05$). In the last day of storage, the highest counts of *L. bulgaricus* were observed in group D (Table 2). The fact that *Spirulina* significantly increases the viability of lactic acid bacteria can be explained by the presence of nitrogenous substances such as free amino acids and peptone in the structure of *S. platensis* (Fadaei *et al*., 2013). A similar study reported different results, and that the addition of *Ascophyllum nodosum* (brown algae) had no significant effect on the counts of *L. bulgaricus* (O'Sullivan *et al*., 2016).

Except for day 7 of the analyses ($p > 0.05$), it was determined that the counts of *S. thermophilus* in *Spirulina* containing groups (B, C, and D) were higher than that of the control group ($p < 0.05$). The counts in the last day of storage appeared to be higher compared to the initial evaluation ($p < 0.05$) (Table 2). Bersuder *et al*. (1998) showed that yogurt groups that contained *Ascophyllum nodosum* had lower amounts of *S. thermophilus* than the control groups (O'Sullivan *et al*., 2016). Considering these results, it was deduced that *S. platensis* had positive effect on *S. thermophilus*. The prebiotic effect of *Spirulina* is due to its polysaccharide and oligosaccharide content (Cai *et al*., 2022).

In order to benefit from the health-promoting effects of probiotics, it is necessary to keep cell viability at a minimum of 10^6 CFU/g until the probiotic enriched products are consumed. The results of the present work showed that both *L. acidophilus* and *B. animalis* counts did not decrease below the above-mentioned levels until the last day of storage. As shown in Table 2, on day 14, 21, and 28 of the evaluation, the *L. acidophilus* counts in the control group were observed to be less than that of the *S. platensis* supplemented groups. Similar studies have reported different results. It has been reported that the *L. acidophilus* counts in yogurt enriched with the microalgae *Arthrospira platensis* and *Chlorella vulgaris* did not enhance the viability of *L. acidophilus* (Beheshtipour *et al*., 2012). As compared to other microalgae, *S. platensis* had more positive effect on the viability of *L. acidophilus*.

L. acidophilus exploits peptides and free amino acids for its own growth and acidification, but it can also generate low molecular mass nitrogen species, particularly peptides, *via* the hydrolysis of milk caseins which sustains the growth of *Bifidobacterium* (Timmerman *et al*., 2004). *B. animalis* counts, especially on day 14 and 28 of storage, increased in parallel with the increase in algae concentration ($p < 0.05$). A similar study reported that the *B. animalis* counts decreased steadily over storage in all groups when different types of microalgae were added (Beheshtipour *et al*., 2012). Khaledabad *et al*. (2020) stated that when the amount of *S. platensis* was increased, it was observed that the lactic acid bacteria populations were above 10^8 CFU/g during storage. Most of the probiotics do not preserve their viability

at very low pH levels. In the present work, the increase in the *B. animalis* counts was related to the presence of certain compounds in *Spirulina* such as pectin, arabinoxylan, β-glucan, and essential amino acids.

Textural changes in probiotic yogurts

Texture is an important parameter that affects the product quality and consumer acceptance (Abou-Soliman *et al*., 2017). Proteins, water holding capacity, fibres, pH, and acidity are related with texture. Except for the first day of evaluation ($p > 0.05$), for groups A and B, the hardness values were observed to be higher than that of other groups ($p < 0.05$) (Figure 1). While the highest resilience values were determined for groups B and C, the lowest value was observed for group A. Resilience values decreased as the concentration of *S. platensis* increased (Figure 1). Similar findings were also reported by Barkallah *et al*. (2017). These studies outlined that the highest elasticity values were found for the groups that contained the highest concentration of *S. platensis*. The higher solid and fibre contents of *S. platensis* may be associated with the increase in viscosity and consequently improved textural properties of yogurt gels. In the evaluation of adhesiveness (Figure 1), the lowest value was detected for group A. On the last evaluation day, group B (0.5%) had the highest score. Although similar results were presented in other studies (Barkallah *et al*., 2017; Shazly *et al*., 2022), better results were obtained in the present work. As the amount of seaweed in yogurt increased, the stickiness values decreased.

Sensorial changes in probiotic yogurts

The acceptability of a product among consumers is not solely determined by its health-promoting properties but also by the quality of its taste, appearance, and aroma. Although it was reported that the use of microalgae in fermented dairy products may cause a green or bluish colour in the product, such a colour problem was not observed in the present work. Regarding colour (Figure 2), although all groups received similar scores on the first and second days of the analysis, group D had the highest scores at the end of storage ($p < 0.05$). Regarding structure, especially on day 14, 21, and the 28 of evaluation, the highest scores were observed with the groups that contained *S. platensis*. Although generally all groups received similar scores regarding their aroma, at the end of their storage (except the last day of analysis), group D received the highest scores ($p < 0.05$). Initially, groups A and B were the most preferred groups, and group D became preferred after day 14 of the evaluation ($p < 0.05$).

Groups containing *S. platensis* got higher scores for general acceptability ($p > 0.05$). Contrary scores were obtained at the end of the 28-d storage compared to the initial evaluation ($p < 0.05$). On the last day of analysis, it was observed that the general acceptability increased with the increase in the *Spirulina* concentration ($p > 0.05$). According to Barkallah *et al.* (2017), the general acceptability scores of yogurts decreased as the *S. platensis* concentration increased. In a similar way, Khaledabad *et al*. (2020) outlined that as the *Spirulina* concentration increased, the overall acceptability scores decreased.

Antioxidant activity changes in probiotic yogurts

DPPH radical scavenging activity is one of the most widely used method for determining the antioxidant activity. Figure 3 illustrates the antioxidant activities of the samples throughout 28-d storage, as measured by evaluating their capability to scavenge DPPH radicals. The analysis showed that while the inhibition value (%) for group D was 77.13 - 56.27%, for group C it was 65.22 - 48.66%, for group B was 56.67 - 40.81%, and for group A it was 47.16 - 30.64%. The obtained results revealed that from day 7, except for group D, the inhibition values (%) for all the yogurt samples ($p > 0.05$) decreased steadily over the period of storage. For ascorbic acid, the inhibition value (%) was found to be 96.27%.

Fermented dairy products and yogurt are well-known for their content of bioactive peptides that possess antioxidant effects (Power *et al*., 2013). The results showed that the antioxidant activity depended on the strain used, and it generally increased during fermentation. It is hypothesised that the bacteria involved in the fermentation process, as well as the metabolic compounds generated during the breakdown of milk proteins, produced agents capable of scavenging the hydroxyl radical (Hernández-Ledesma *et al*., 2005; Virtanen *et al*., 2007). It was reported that the natural components added to the prebiotic yogurt increased the antioxidant effect (Şengül *et al*., 2012; Azizkhani and Parsaeimehr, 2018). Many studies on the antioxidant effects of

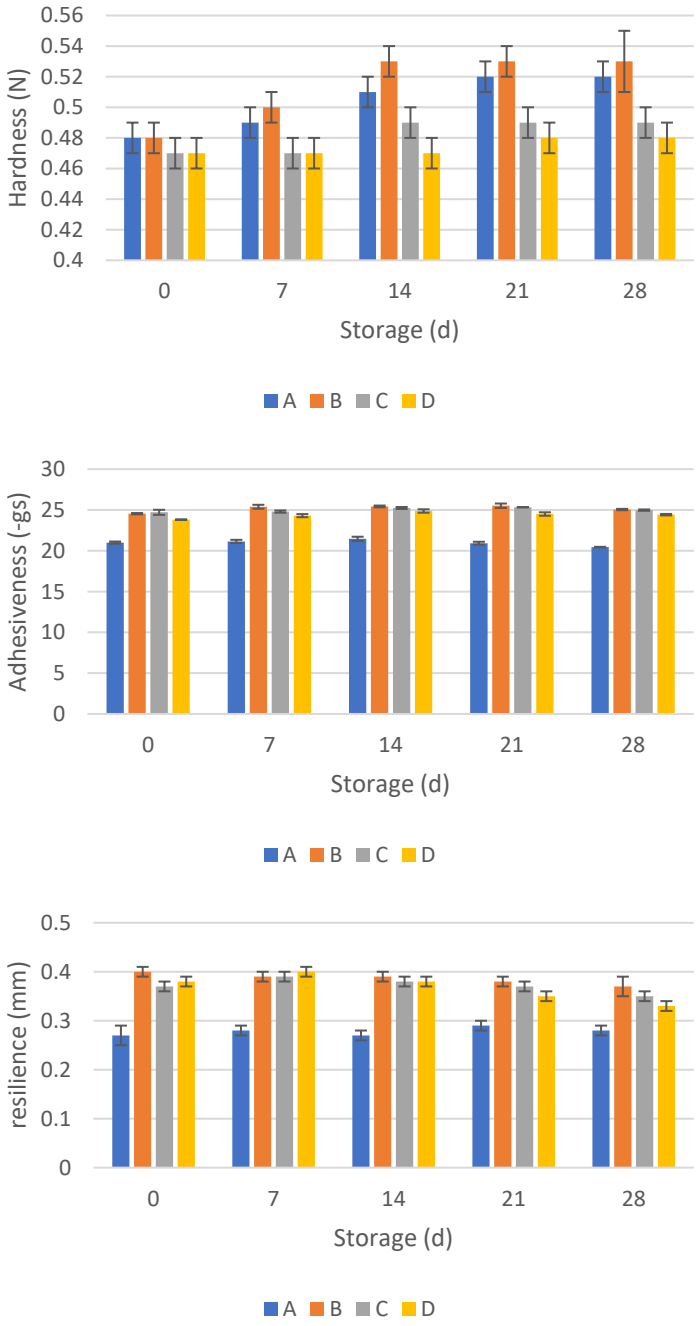

Figure 1. Textural properties of yogurts throughout 28-day storage. **(A)**: control; **(B)**: *S. platensis* 0.5%; **(C)**: *S. platensis* 0.75%; and **(D)** *S. platensis* 1%.

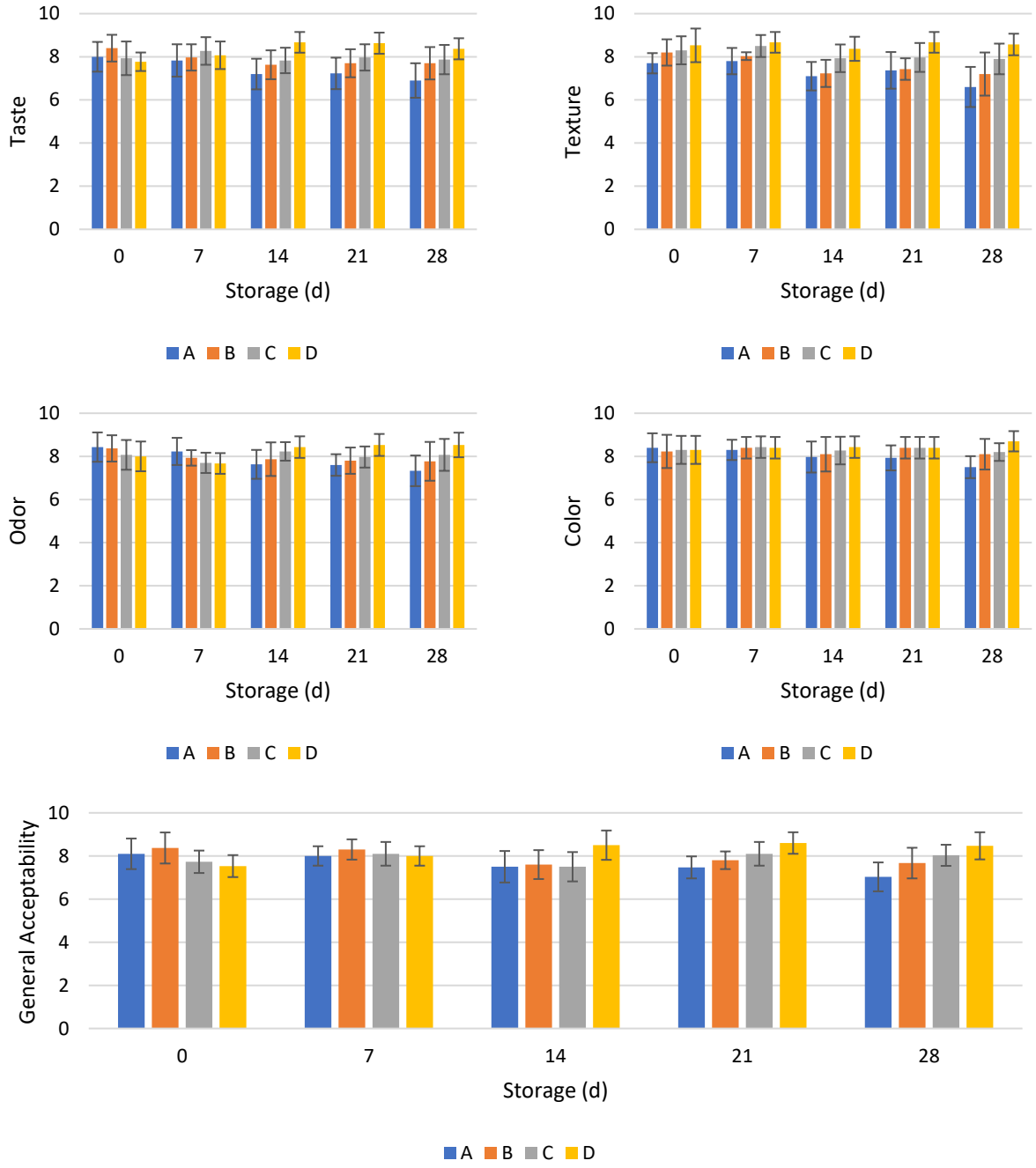

Figure 2. Sensorial properties of yogurts throughout 28-day storage. **(A)**: control; **(B)**: *S. platensis* 0.5%; **(C)**: *S. platensis* 0.75%; and **(D)** *S. platensis* 1%.

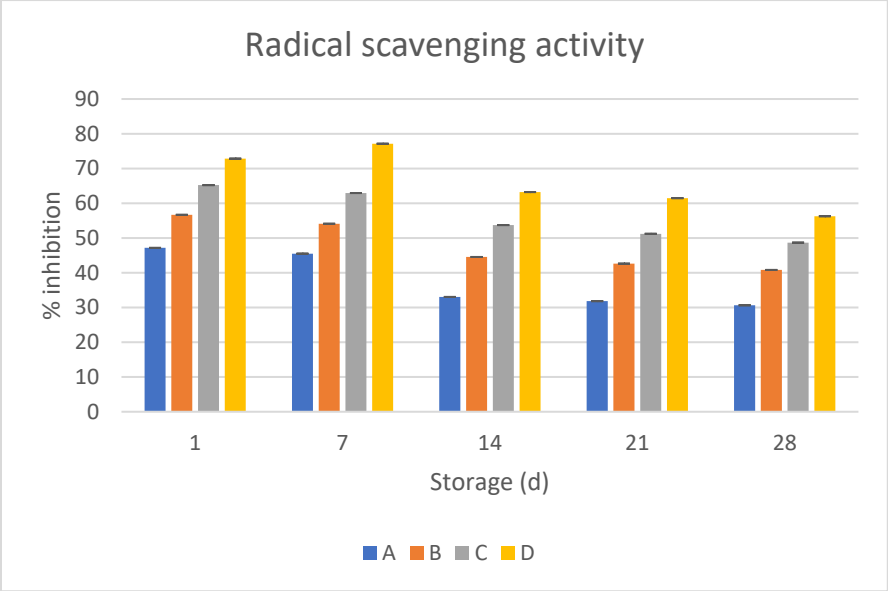

Figure 3. Radical scavenging activity of yogurts throughout 28-day storage. **(A)**: control; **(B)**: *S. platensis* 0.5%; **(C)**: *S. platensis* 0.75%; and **(D)** *S. platensis* 1%.

Spirulina have reported that the content (especially chlorophyll, carotenoid, phycocyanin) of *Spirulina* prevent or delay the negative effects of free radicals (Goiris *et al*., 2012; Ismaiel *et al*., 2016).

Conclusion

Although there are some studies on the combined use of *S. platensis* and probiotics in yogurt production, none of them were carried out during long storage period as performed the present work. The results presented in the present work are important in terms of showing that *S. platensis* has a positive effect on the quality parameters of probiotic yogurts, and that this effect can sustain during long storage period. The incorporation of different concentrations of *S. platensis* powder into probiotic yogurt can also be considered as a 'healthy alternative' to produce functional dairy products. In addition to increasing the nutritional profile of yogurt, it also had an important role on preserving the stability throughout storage as water holding capacity increased. Adding 1% (w/w) of *S. platensis* was observed to be sufficient for enhancing probiotic viability, preserving metabolic activity of the yogurt starter cultures, and conserving the sensory and quality parameters. Due to its rich content of dietary fibres and proteins, along with its ability to enhance the preservation process of the product, *S. platensis* is being considered as a promising substitute for synthetic chemicals that could potentially harm consumers' health.

Acknowledgement

The authors gratefully acknowledge the financial support received from Burdur Mehmet Akif Ersoy University Scientific Research Projects Coordinator (grant no.: 0521-YL-18).

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