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Enhancing *Bifidobacterium* and lactic acid bacteria activity, and improving oxidative stability in functional algal concentrated yoghurt with *Spirulina platensis* powder

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Abstract. The purpose of this study was to examine the growing demand for healthy functional dairy products by investigating the incorporation of *Spirulina platensis* into concentrated yoghurt and evaluating its impact on starter cultures and product properties. A comprehensive analysis demonstrated the potential of spirulina to enhance bifidobacteria growth, acidity, PUFA content, and antioxidant activity in

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yoghurts, indicating its potential to improve the nutritional quality and oxidative stability of dairy products. The study investigated the impact of different *Spirulina* ratios on the activity of yoghurt starter cultures, including *Lb. casei*, *Lb. plantarum, Lb. acidophilus*, and *Bifidobacterium* mix. Three concentrated yoghurt samples were prepared, including a control, a probiotic sample, and a sample incorporating *Spirulina* powder. The findings suggest that while *Spirulina* did not significantly affect lactic acid bacteria, it noticeably stimulated the growth of *Bifidobacterium*. Treated samples showed increased acidity and TVFAs, with pH values displaying an inverse trend compared to the control. Clear differences in colour parameters and sensory evaluations distinguished control from treated samples. Additionally, *Spirulina* supplementation demonstrated a pronounced effect in enhancing antioxidant activity, as observed through DPPH radical scavenging activity, and influencing the peroxide value, reflecting improved oxidative stability in the yoghurt. This successful utilisation of *Spirulina* suggests its potential application in diverse edible algae in the food sector, especially in dairy products, enhancing both nutritional and sensory aspects, as well as contributing to improved oxidative stability

Keywords: Edible algae; functional foods; antioxidant activity; useful bacteria; dairy products

INTRODUCTION

The demand for functional foods that offer both nutritional benefits and enhanced health properties has surged in recent years, driven by an increasingly health-conscious consumer base. As the global population grows, so does the need for sustainable and innovative food solutions. In this context, the exploration of new ingredients with potential health-promoting properties becomes crucial. One such ingredient garnering attention is *Spirulina platensis*, a type of green algae known for its rich nutritional profile and potential health benefits. Incorporating *Spirulina* into dairy products such as yoghurt, presents an opportunity to create functional foods that not only provide essential nutrients but also contribute to improved gut health and oxidative stability.

Spirulina is great for children's development due to its high calcium and iron content (1,043.62 and 338.76 mg/100 g, respectively). It also includes selenium (0.0488 mg/100 g) and plant pigments (phycocyanin: 14.1% and chlorophyll: 1.4%) (Ali *et al.,* 2022). Strong antioxidants, physical chemistry, sensory qualities, fresh *spirulina*, and yoghurt are some of these ingredients. The components of the *spirulina* cell wall include lipids, carbs, and protein. As a result, *spirulina* arguably has a better nutritional bioavailability than other food sources, especially plant food sources (Hassan *et al.,* 2021). More protein (62%) may be found in *spirulina* than any other natural food. The whole flavonoid is present in *spirulina*. It has the highest natural concentration of carotene, phycocyanin, and vitamin E (Darwish *et al.,* 2020).

M. Golmakani *et al.* (2019) evaluated the effects of *Spirulina (Arthrospira platensis)* addition on the growth of probiotic *Lactobacillus casei* in bacteriologically acidified feta-type (BAF) cheese, along with assessing its chemical, textural, and sensorial characteristics. After 60 days of storage, significantly higher viable counts of *Lb.casei* (9.10-9.35 log CFU g^{-1}) were observed in *Spirulina* samples compared to the control (8.68 log CFU g^{-1}), indicating a more successful maintenance of *Lb. casei* counts in the probiotic BAF cheese containing *Spirulina*.

P. Thiviya *et al.* (2022) noted that macroalgae, or seaweeds, are becoming more popular as an alternative source of protein. They have an outstanding amino acid profile that is on par with other conventional protein sources and are rich in protein. Bioactive substances found in seaweed protein include lectins, free amino acids, peptides, and phycobiliproteins, which include, among others, phycocyanin, and phycoerythrin. The antihypertensive, antidiabetic, antioxidant, anti-inflammatory, antitumoral, antiviral, antibacterial, and several other advantageous functional qualities of seaweed proteins have been proved. As a result, proteins from seaweed may provide a healthy substitute for other sources for developing functional foods (Shazly *et al.,* 2022). Due to its distinct chemical composition, which includes vital amino acids, vitamins, natural colours, and fatty acids, spirulina has been used traditionally as a food supplement and provides a plethora of health benefits (Alshafei *et al.*, 2022).

H. Zahran *et al.* (2022) mentioned thar preserving yoghurt's oxidative stability is imperative for sustaining its quality and nutritional value over its shelf life. Oxidation processes can compromise yoghurt's flavour, texture, and overall quality, primarily due to the presence of unsaturated fatty acids and reactive compounds, influenced by storage conditions, ingredients, and microbial activity (Silva *et al.,* 2022). By assessing yoghurt's oxidative stability under varied conditions, insights into the potential role of *Spirulina platensis* in bolstering resistance to oxidative changes are elucidated, promising extended shelf life and enhanced product quality (da Silva *et al.,* 2019; Mesbah *et al.,* 2022).

Furthermore, there is curiosity in the as-yet-uncharted region of *Spirulina platensis's* impact on *Bifidobacterium* and lactic acid bacteria development in probiotic algal concentrated yoghurt. Thus, the purpose of this study was to investigate the effects of *Spirulina* supplementation on the activity of yoghurt starter cultures and the overall quality of concentrated yoghurt, thereby contributing to the development of healthier and more functional dairy products.

MATERIALS AND METHODS

At Cairo University in Egypt's Faculty of Agriculture, fresh raw cow milk was taken from the herd with a fat percentage of 3.5% and a Total Solids (TS) of 12%. One of the starting cultures utilised was a freeze-dried direct vat set kind of yoghurt starter culture that came from Chr. Hansen's Laboratories in Copenhagen, Denmark. It contained *Str. salivaricus subsp. thermophilus* and *Lb. delbrueckii ssp. bulgaricus*. Furthermore, strains of *Lactobacillus casei, Lactobacillus plantarum,* and *Lactobacillus acidophilus* were obtained from the National Research Centre (NRC) Dairy Microbiology Laboratory in Egypt. The probiotic culture was obtained from the identical facility as well. Through Walmart.com/Spring Valley USA, the powdered algal biomass was acquired from Wal-Mart Stores, Inc., Bentonville, AR 72716. Table 1 lists the nutrients included in *Spirulina platensis* powder.

***Table 1**. The composition per 100 g of Spirulina platensis powder*

Parameter	***Amount***
Energy	1.213 k j
Carbohydrates	23.9 g
Fat	7.72 g
Protein	57.47 g
Vitamins and minerals	***mg***
B_1	2.36
B_2	3.65
B_3	12.80
B_5	3.46
Iron	28.54
Manganese	1.89

***Note:** data mentioned on the label of the package*
***Source:** compiled by the authors of this study*

According to the methods described by D. Mohamed *et al.* (2017), MRS medium was used to count lactic acid bacteria in *Lactobacillus bulgaricus*, and M17 medium was utilised in *Streptococcus thermophilus* following the instructions provided by A. Hussein *et al.* (2017). Colony-forming units (CFU/g) were used to express the measurements of the findings. *Bifidobacterium* counts were determined by activating 1 ml of *Bifidobacterium* mix in a tube containing 10 ml MRS broth medium supplemented with 3% lithium chloride and 2% sodium propionate, in line with the protocols outlined by H. Abbas *et al.* (2017) and M. El-Shenawy *et al.* (2019). Colonies were counted to ascertain the growth kinetics after a 48-hour incubation period at 37 °C; the results were expressed as CFU/ml. *Staphylococcus* aureus was counted using Baird-Parker media (Oxoid) supplemented with egg yolk and potassium tellurite. The plates were incubated at 37°C for 48 hours. The tryptone-bile-glucuronic medium (TBX) (Oxoid) was used to count *E. coli*. The yeast and mould were counted using potato dextrose agar medium (pH 3.50), and they were subsequently cultured for 3-5 days at 25°C. The solid medium approach was used to identify the coliform group on the violet-red bile agar media plates after they were incubated for 24 hours at 30°C. Sterilised distilled water was used to prepare a suspension of *Spirulina platensis* at concentrations of 1, 3, and 5 mg/ml in media, which was then introduced to MRS broth medium tubes holding various bacterial cultures. Next, the tubes were incubated for 24 and 48 hours, respectively, at 37°C, according to D. Bhowmik *et al.* (2009). Additionally, a control was made without *S. platensis*.

A commonly used technique in the Middle East was employed to make samples of concentrated yoghurt (Zaky *et al.,* 2013). The milk sample was heated to 85°C for 15 minutes and then cooled to 40°C. From the sample, three sections were removed. Three product groups were developed: T1, a plain yoghurt with only 2% yoghurt starter (*Str. thermophilus* + *Lb. bulgaricus*, 1:1); T2, probiotic yoghurt with 2% Bifidobacterium mix + yoghurt starter; and T3, the algal sample with 2% yoghurt culture plus 2% *Bifidobacterium* mix with 3% *S. platensis* powder. Each cultured milk was produced following the yoghurt manufacturer's directions and then poured into sterilised plastic containers. The samples were incubated at 40°C until total coagulation was achieved. To make concentrated yoghurt, all the yoghurt samples were then salted with 2% NaCl, hung, and allowed to drain the whey. Samples were refrigerated and kept for 21 days at 5°C. The gross chemical composition of fresh samples was examined, as presented in Table 2.

***Table 2**. Total chemical makeup of samples of fresh and concentrated yoghurt*

Gross composition	**T1**	**T2**	**T3**
Total solids (%)	24.37	25.04	27.12
Fat content (%)	8.03	8.19	9.04
Protein content (%)	11.70	11.67	12.89
Ash content (%)	1.44	1.48	1.51

***Notes:** T1 –control, 2% of yoghurt starters in the basic sample; T2 – probiotic sample with 2% Bifidobacterium mix + yoghurt starter; T3 – the algal sample containing 5% S. platensis powder, 2% yoghurt culture, and 2% Bifidobacterium mix*
***Source:** compiled by the authors of this study*

According to AOAC (2012) recommendations, the samples' gross chemical composition, peroxide value, and acidity % were evaluated. A digital laboratory pH-meter device with a glass electrode (HI 94 1400, Hanna Instruments) was used to test pH readings. The methodology described by F. Cabrera *et al.* (2019) was used to determine the total volatile fatty acids (TVFAs) in the samples, which were then expressed as millilitres of 0.1 N NaOH per 100 mg sample.

The measurement of DPPH radical scavenging activity (RSA%) and free radical scavenging activity in the samples followed a modified methodology based on W. Mettwally *et al.* (2022). Using this approach, 2.9 mL of 1,1-diphenyl-2-pycrylhydrazyl (DPPH) dissolved in methanol was combined with 100 µL of the sample solution. A UV-visible spectrophotometer was used to measure the mixture's absorbance at 517 nm after vigorously shaking it for 30 minutes. The following formula was used to obtain the RSA%:

$$\mathrm{RSA\%} = \frac{Abs.B - Abs.S}{Abs.B} x100, \quad (1)$$

where *Abs. B* and *Abs. S* are the absorbance values of the blank and sample, respectively. All measurements were conducted in threefold replication for accuracy and consistency.

The colour of the samples was assessed using a Hunter colourimeter Model D2s A-2 (Hunter Assoc. Lab. Inc., VA, USA). Before any measurements were made, the instrument was calibrated using a black tile at the bottom and a white tile at the top of the scale. The tri-stimulus colour values (L*, a*, and b*) were then recorded when the sample's flat layer specimen was positioned at the specimen port. Here, colour is represented by a*, which goes from red (+) to green (-), yellow (+) to blue (-), and darkness, represented by L*, which goes from black (0) to white (100).

The present study involved the sensory evaluation of yoghurt samples that were refrigerated for 7, 14, and 21 days, as well as fresh samples. Twenty-five panellists, who were members of the Dairy Sciences Laboratory, National Research Centre, Egypt, evaluated the samples' flavour, colour and appearance, body and texture, and overall acceptability. The samples were measured in 100 ml cups at 10°C and each sample was evaluated by three different individuals. The parameters were ranked on a scale from 1 to 7.

Mean values and standard deviations for physical properties and chemical composition were computed, followed by an assessment of formulation differences using analysis of variance (ANOVA) with a significance level of $p < 0.05$. Statistical analyses were performed using the SPSS 20.0 software package (SPSS Inc., Chicago, USA). Post-hoc analysis using Duncan's multiple range tests at a significance level of 5% was then conducted to find specific differences between the formulations (Steel & Torrie, 1960).

RESULTS AND DISCUSSION

Table 3 illustrates the counts of lactic acid bacteria and *Bifidobacterium* mix (log CFU/mL), portraying the influence of different concentrations of *Spirulina platensis* powder in pure media prior to its incorporation into yoghurt samples. The findings suggest that varying levels of *S. platensis* did not distinctly enhance the tested lactic acid bacteria, hinting at the potential necessity for algae concentrations exceeding 5 mg/mL to yield a more pronounced effect. However, noticeable enhancement was observed in the growth of *Bifidobacterium* mix, particularly evident when *Spirulina platensis* was utilised at a concentration of 5%. Specifically, *Bifidobacterium* counts reached 8.86 (log CFU/mL) after 24 hours, rising to 9.40 after 48 hours of incubation, suggesting that this concentration is more conducive to preparing the aforementioned probiotic yoghurt.

***Table 3**. Growth kinetics of lactic acid bacteria (log CFU/mL) added with S. platensis*

Concentration of *S. platensis*	*Lb. casei*		*Lb. plantarum*		*Lb. acidophilus*		*Bifidobacterium mix.*	
	Time (h)							
	24	48	24	48	24	48	24	48
0 (Control)	6.90	7.33	7.00	7.80	7.20	8.25	7.30	8.60
1 mg/ml	6.50	6.80	6.88	6.50	7.00	6.80	7.50	8.70
3 mg/ml	6.70	6.95	6.80	6.75	6.90	7.00	7.60	8.55
5 mg/ml	5.60	6.40	6.00	6.20	6.55	6.30	8.86	9.40

***Source:** compiled by the authors of this study*

Previous research has repeatedly documented *Spirulina's* enhancing effect on lactic acid bacteria. *Str. thermophilus* was found to be promoted by *S. platensis* powder in milk by G. de Caire *et al.* (2000), a finding that was confirmed by L. Varga *et al.* (2002) with ABT-fermented milk containing *S. platensis* powder. After a period of refrigerated storage, the viable counts of *Str. thermophilus* were significantly greater in the probiotic and plain yoghurt samples, with variations of up to 1.0 and 1.6 log CFU/g, respectively. A. Akalin *et al.* (2009) also reported comparable results.

The microbiological analysis of yoghurt samples encompassed assessments for *Lb. bulgaricus, St. thermophilus, Bifidobacterium* mix, Coliform bacterial group,

mould and yeast, *E. coli*, and *Staphylococcus aureus* at the initial stage and storage for 7, 14, and 21 days (Table 4). The enumeration of *Str. thermophilus*, *Lb. bulgaricus*, and *Bifidobacterium* mix in fresh yoghurt samples varied between 24×10^7 to 89×10^7 CFU/g. Subsequently, these counts increased during storage. Notably, T2 and T3 exhibited higher counts, particularly at 7 days of storage, reaching 65×10^7 and 21×10^8 CFU/g, respectively. However, the control group experienced a slight decrease to 7×10^7 CFU/g at 21 days, and analogous decreasing trends were observed in all samples at 21 days, recording counts of 25×10^7, 41×10^7, and 54×10^7 for T1, T2, and T3, respectively. This decline may be associated with acidity development over time (Chen *et al.,* 2017).

***Table 4**. The bacterial counts of concentrated-yoghurt samples during 21 days of storage*

Samples		*Str. thermophilus*	*Lb. bulgaricus*	*Bifidobacterium* mix.	Coliform bacterial group	M.Y	*E. coli*	*Staphylococcus aureus*
Fresh	T_1	24×10^7	36×10^7					
	T_2	51×10^7	59×10^7	60×10^7				
	T_3	37×10^7	47×10^7	89×10^7				
7 (d)	T_1	36×10^7	52×10^7			9		
	T_2	73×10^7	94×10^7	95×10^7		8		
	T_3	65×10^7	75×10^7	21×10^8				
14 (d)	T_1	20×10^7	38×10^7			16		
	T_2	47×10^7	76×10^7	80×10^7		10		
	T_3	36×10^7	58×10^7	9×10^8		4		
21 (d)	T_1	7×10^7	13×10^7			32		
	T_2	22×10^7	41×10^7	54×10^7		17		
	T_3	25×10^7	38×10^7	83×10^7		10		

***Notes:** T1 –control, 2% of yoghurt starters in the basic sample; T2 – probiotic sample with 2% Bifidobacterium mix + yoghurt starter; T3 – the algal sample with 5% S. platensis powder, 2% yoghurt culture, and 2% Bifidobacterium mix; = Nil*

***Source:** compiled by the authors of this study*

S. platensis did not significantly raise the viable counts of *Str. thermophilus* in yoghurts, with a maximum rise of 0.5 log CFU/g in plain yoghurt compared to the control, according to a study by N. Kearney *et al.* (2008). After storage, viable counts in plain and probiotic yoghurts were 6.5 and 7.7 log CFU/g following the inclusion of 1% *Spirulina* powder. While *Lb. bulgaricus* increased by 1 and 1.3 log CFU/g in plain yoghurt compared to the control, viable counts of *Str. thermophilus* were slightly lower than in earlier investigations, at about 8 log CFU/g in algal yoghurts (Varga & Szigeti, 1998). After seven days of storage, the counts of moulds and yeasts, which were not present in the fresh control yoghurt, increased to 9 CFU/g. In samples enhanced with 2% yoghurt culture + 2% *Bifidobacterium* mix + 3% *S. platensis* powder, these microorganisms stayed undetected until 7 days of storage. Small counts (16 CFU/g) were observed for T1 at 14 days, and in other samples at the same interval, counts were recorded as 10 and 4 CFU/g for T2 and T3, respectively. These microorganisms, undesirable in yoghurt, pose potential risks to public health (Gallegos-Acevedo *et al.,* 2018). Such systems can enhance the bioavailability of certain compounds, affecting the penetration of bioactive compounds into cells (Pérez-Soto *et al.,* 2021). Notably, all bacterial pathogens, including the coliform bacteria, *Escherichia coli* and *Staphylococcus aureus*, were not detected in any yoghurt samples. N. Kearney *et al.* (2008) suggested that yoghurt starter bacteria and probiotic bacteria require nutrients for growth and survival, and *Spirulina platensis* powder could serve as a unique nutrient source due to its significant concentrations of vitamins, minerals, amino acids, and nucleic acid precursors.

Additionally, M. Guldas and R. Irkin (2010) found that at the conclusion of the storage period, the viable counts of lactic acid bacteria in all samples containing *Spirulina* powder were greater than 6 CFU/g, while the samples of yoghurt with control had lower counts of lactic acid bacteria. This suggests that *S. platensis* powder helps lactic acid bacteria survive when yoghurt is being stored. In contrast to the findings of A. Akalin *et al.* (2009), the counts of *L. bulgaricus* did not surpass those of other bacteria. According to earlier research (Varga *et al.,* 2002; Donkor *et al.,* 2006), the higher survival rate of *L. bulgaricus* and *L. acidophilus* in probiotic yoghurt may be related to competitive interactions among bacteria. At the conclusion of the storage period, *L. acidophilus* counts increased by 2.4 and 3.1 log CFU/g in samples containing 0.5% and 1.0% *Spirulina* powder, respectively. Growth curves showed that *L. acidophilus* outlived *S. thermophilus* and *L. bulgaricus*.

The presented acidity results, expressed as a percentage, showed variations across different samples (T1, T2, and T3) and storage times (fresh, 7 days, 14 days, and 21 days) (Fig. 1). The acidity values are integral to the sensory profile and quality of yoghurt, reflecting the fermentation process and metabolic

activities of the microbial cultures present. For control – plain yoghurt, the acidity levels in T1 showed a gradual increase over the storage period. From fresh to 21 days, acidity increased from 1.40 to 1.50%. This incremental pattern is expected as lactic acid-producing bacteria, particularly *Lb. bulgaricus* and *St. thermophilus*, continue their metabolic activities during storage, leading to the accumulation of acids. T2 showed slightly higher acidity compared to the control (T1) at each time point. This increase can be attributed to the metabolic activity of the additional probiotic strain *Bifidobacterium* mix, which produces lactic acid along with other beneficial compounds. The acidity progression from fresh to 21 days follows a trend comparable to T1, with values ranging within 1.80-2.26%. On the other hand, the T3 sample, enhanced with *Spirulina platensis* powder, showed the highest acidity levels among the samples. The incorporation of *S. platensis* may contribute to the acidity through its metabolic byproducts or by influencing the activity of lactic acid bacteria. The acidity increased from 1.40 (fresh) to 1.50% (21 days), reflecting a trend comparable with T1 and T2.

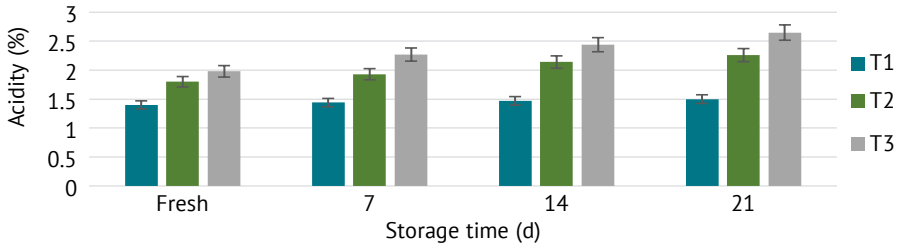

Figure 1. *Acidity (%) contents of yoghurt samples during the storage period*
Notes: *T1 – control, 2% of yoghurt starters in the basic sample; T2 – probiotic sample with 2% Bifidobacterium mix + yoghurt starter; T3 – the algal sample with 5% S. platensis powder, 2% yoghurt culture, and 2% Bifidobacterium mix*
Source: *compiled by the authors of this study.*

The acidity levels tended to increase progressively with the extension of storage time across all samples. This aligns with the natural fermentation process in yoghurt, where lactic acid bacteria consume lactose and produce lactic acid, contributing to the characteristic tangy taste. T3 consistently showed higher acidity compared to T1 and T2. The additional contribution from *Spirulina platensis* may enhance the fermentation process or influence the production of organic acids (Son *et al.*, 2023). T2, containing *Bifidobacterium* mix, showed slightly elevated acidity compared to T1. The presence of probiotic strains contributes to the overall acidification, supporting findings from microbiological analyses indicating increased counts of *Bifidobacterium* in T2. The acidity trend in T3 does not indicate a disproportionate surge despite the higher concentration of *Spirulina platensis*. This suggests that while *Spirulina* may influence acidity, its impact might be subtle and balanced within the microbial ecosystem.

The presented pH values represent the acidity levels in yoghurt samples for three different samples (T1, T2, and T3) at various storage times (fresh, 7 days, 14 days, and 21 days) (Fig. 2). The pH of yoghurt is a crucial parameter influencing its taste, texture, and overall quality. The pH values for T1 showed a gradual decrease from fresh (4.72) to 21 days (4.14). This decline was expected during yoghurt fermentation, reflecting the conversion of lactose to lactic acid by lactic acid bacteria. The pH values fall within the typical range for yoghurt. T2 followed a trend comparable to T1, with a decrease in pH from 4.76 (fresh) to 4.51 (21 days). The addition of Bifidobacterium mix may have contributed to the acidity, influencing the pH values during the storage period. Therewith, T3 showed a distinctive pattern, starting with a pH of 4.51 (fresh) and decreasing to 4.23 at 21 days. The incorporation of *Spirulina platensis* powder appears to have a more pronounced effect on pH reduction compared to T1 and T2.

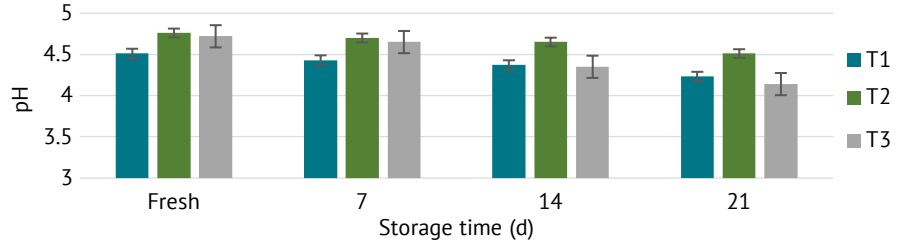

Figure 2. *pH values contents of yoghurt samples during the storage period*
Notes: *T1 – control, with 2% of yoghurt starters in the basic sample; T2 – probiotic sample with 2% Bifidobacterium mix + yoghurt starter; T3 – the algal sample with 5% S. platensis powder, 2% yoghurt culture, and 2% Bifidobacterium mix*
Source: *compiled by the authors of this study*

The observed decrease in pH values aligns with the findings of M. Guldas and R. Irkin (2010), who reported a slight decrease in pH with an increase in *Spirulina platensis* powder amount. The pH values reaching approximately 4.20 during the storage period are consistent with the current findings. L. Varga and J. Szigeti (1998) and L. Varga *et al.* (2002) also noted analogous trends in pH reduction caused by *Spirulina platensis* powder, supporting the idea that the addition of Spirulina promotes the growth of lactic acid bacteria, leading to increased acidity and a lower pH. The decrease in pH over the storage period is indicative of ongoing fermentation and acidification, essential for yoghurt development and preservation. T3 sample, containing *Spirulina platensis* powder, showed a more significant pH reduction, suggesting that *Spirulina* contributed to the overall acidity of the yoghurt. This could be attributed to the unique nutritional composition of *Spirulina*, which may enhance lactic acid bacteria activity.

The presented results show the Total Volatile Fatty Acids (TVFAs) content in millilitres of 1.0 N NaOH per 100 mg sample for three different samples (T1, T2, and T3) at various storage times (fresh, 7 days, 14 days, and 21 days) (Fig. 3). TVFAs are important indicators of the organic acid composition in yoghurt, providing insights into the metabolic activities of fermenting microorganisms. The TVFAs content in T1 showed a slight increase from fresh to 21 days, starting at 0.54 ml/100 mg and reaching 1.26 ml/100 mg. This rise is consistent with the progression of fermentation, where lactic acid bacteria convert lactose to lactic acid. The increase in TVFAs aligns with the expected organic acid production during the storage period. In addition, T2 showed a pattern comparable to T1, with a gradual increase in TVFAs over the storage period. The TVFAs content increased from 0.60 ml/100 mg (fresh) to 0.78 ml/100 mg (21 days). The addition of *Bifidobacterium* mix in T2 may contribute to the overall production of volatile fatty acids, enhancing the fermentation process. Therewith, T3 sample, enriched with *Spirulina platensis* powder, showed a more pronounced increase in TVFAs compared to T1 and T2. The TVFAs content started at 0.65 ml/100 mg (fresh) and reached 1.26 ml/100 mg at 21 days. The higher TVFAs levels in T3 may be attributed to the metabolic activities of both lactic acid bacteria and the unique characteristics of *Spirulina platensis*.

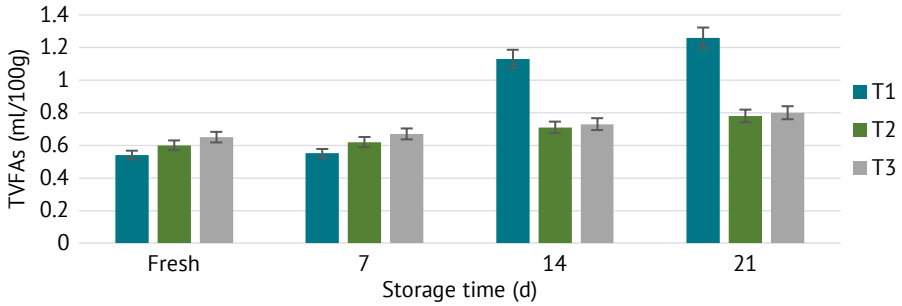

Figure 3. *TVFAs contents of yoghurt samples during the storage period*
Notes: *T1 – control, with 2% of yoghurt starters in the basic sample; T2 – probiotic sample with 2% Bifidobacterium mix + yoghurt starter; T3 – the algal sample with 5% S. platensis powder, 2% yoghurt culture, and 2% Bifidobacterium mix; TVFAs are measured as millilitres (ml) of 1.0 N NaOH per 100 milligrams of sample*
Source: *compiled by the authors of this study*

The overall trend across all samples indicates a progressive increase in TVFAs during the storage period. This aligns with the continued metabolic activities of lactic acid bacteria and probiotic strains. T3 consistently showed higher TVFAs levels compared to T1 and T2. The incorporation of *Spirulina platensis* may contribute additional organic acids, influencing the TVFAs profile in the yoghurt. T2, containing *Bifidobacterium* mix, showed slightly higher TVFAs levels compared to T1. This supports the fact that probiotic strains contribute to the overall production of volatile fatty acids. T3's noticeable increase in TVFAs, especially at 21 days, suggests that *Spirulina platensis* may influence the organic acid composition. The unique nutritional profile of *Spirulina* may contribute to the production of specific volatile fatty acids. The antioxidant activity, expressed as inhibition percentage through DPPH radical scavenging activity, revealed distinctive patterns in the studied yoghurt samples over different storage periods (Fig. 4). In the fresh state, T3, the algal sample containing 2% yoghurt culture + 2% *Bifidobacterium* mix + 5% *S. platensis* powder, showed the highest antioxidant activity at 87.1%, followed by T2 (probiotic sample) at 58.2%, and T1 (plain sample) at 35.4%. As the storage period progressed, there was a general decline in antioxidant activity across all samples. However, T3 consistently maintained the highest inhibition percentage, even at 21 days, indicating its sustained antioxidant potential. T2 showed moderate antioxidant activity, while T1 showed the lowest values. These findings underscore the potential of *Spirulina platensis* in enhancing the antioxidant profile of yoghurt, with implications for product stability and health benefits.

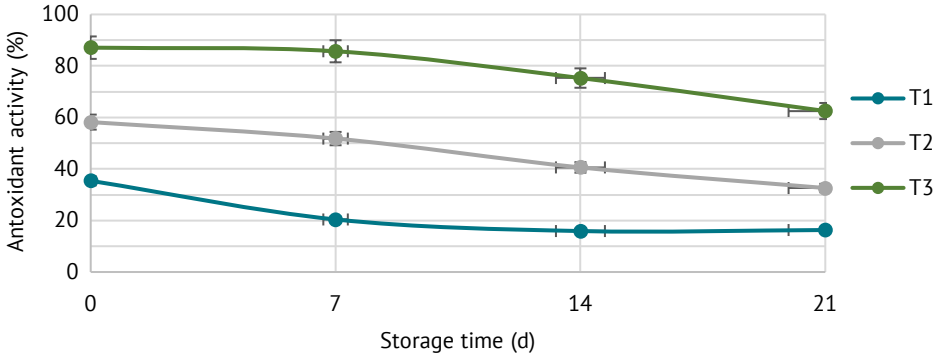

***Figure 4**. Changes in antioxidant activity of concentrated yoghurt samples during storage*
***Notes:** T1 – control, with 2% of yoghurt starters in the basic sample; T2 – probiotic sample with 2% Bifidobacterium mix + yoghurt starter; T3 – the algal sample with 5% S. platensis powder, 2% yoghurt culture, and 2% Bifidobacterium mix*
***Source:** compiled by the authors of this study*

S. da Silva *et al.* (2019) demonstrated the efficacy of spray-dried *Spirulina platensis* as a beneficial component for enhancing yoghurt formulations. The study emphasised that this ingredient not only preserved the nutritional composition of yoghurt but also exhibited a sustained improvement in antioxidant activity over the storage duration. The heightened antioxidant potential of *Spirulina platensis* can be attributed to the abundance of bioactive compounds, including α-tocopherol and phycocyanin. These compounds are known for their synergistic antioxidant properties, as highlighted by I. Santiago-Morales *et al.* (2018). While *Spirulina* contains various tocopherol forms, the predominant presence of α-tocopherol is primarily responsible for its antioxidant activity, with β- and δ-tocopherols contributing to anti-inflammatory effects, as outlined by A. Azzi (2018). This underscores the multifaceted antioxidant benefits associated with incorporating *Spirulina platensis* into yoghurt formulations, with implications for product quality and potential health advantages.

The peroxide values of the yoghurt samples, as presented in the provided data, reveal notable changes during the storage periods of fresh, 7 days, 14 days, and 21 days, across different samples (T1, T2, and T3). In the control sample (T1) with only 2% yoghurt starters, a gradual increase in peroxide values was observed (Fig. 5), suggesting a potential oxidation process occurring over time. In contrast, the probiotic sample (T2) containing 2% *Bifidobacterium* mix and yoghurt starter showed a more controlled increase in peroxide values, suggesting a moderated impact on oxidative changes. Interestingly, the algal sample (T3) containing 2% yoghurt culture, 2% *Bifidobacterium* mix, and 5% *S. platensis* powder showed the most effective mitigation of peroxide value elevation, showcasing the potential antioxidative properties of *Spirulina platensis*. This observation aligns with the known antioxidant capabilities of *Spirulina*, as discussed in previous studies by S. da Silva *et al.* (2019), I. Santiago-Morales *et al.* (2018). The progressive reduction in peroxide values with *Spirulina* incorporation suggests its role in enhancing the oxidative stability of yoghurt during storage, emphasising its potential as a functional ingredient for extending product shelf life.

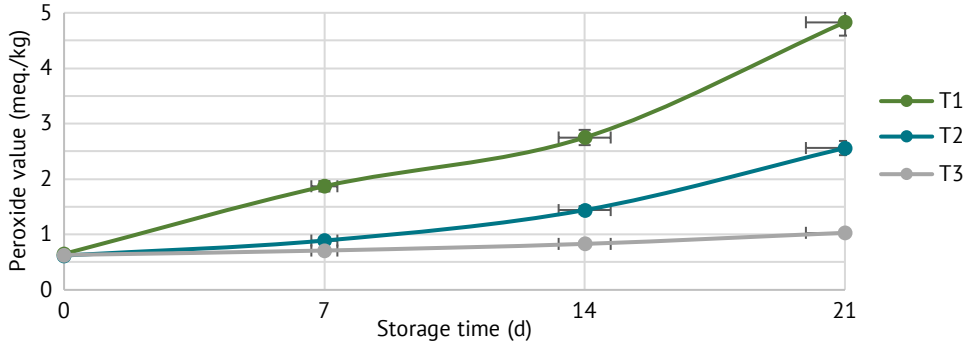

***Figure 5**. Changes in peroxide value of concentrated yoghurt samples during storage*
***Notes:** T1 – control, with 2% of yoghurt starters in the basic sample; T2 – probiotic sample with 2% Bifidobacterium mix + yoghurt starter; T3 – the algal sample with 5% S. platensis powder, 2% yoghurt culture, and 2% Bifidobacterium mix*
***Source:** compiled by the authors of this study*

The colour parameters (L*, a* and b*) of concentrated yoghurt samples (T1, T2 and T3) were evaluated over different storage periods (Table 5). These observations provide insights into the dynamic colour changes influenced by storage time and sample variations. T3 showed the highest L* value, indicating a lighter appearance compared to T2 and T1. This aligns with the addition of *S. platensis* powder, known for its green pigment. Over time, all samples show a decrease in L* values, suggesting a gradual darkening. The reduction in lightness may be attributed to a series of factors, including interactions between ingredients or storage-related processes. T3 The increase in b* values signifies a shift towards a slightly bluer colour during storage, highlighting the dynamic nature of colour changes in concentrated yoghurt (Flores-Mancha *et al.,* 2021).

The data presented in Table 6 shows a detailed sensory evaluation of concentrated yoghurt samples (T1, T2, and T3) over various storage periods (Fresh, 7 days, 14 days, and 21 days), using a ranking scale from 1 to 7 for attributes related to Body & Texture, Flavour, Appearance & Colour, and Overall Acceptability (Folkenberg & Martens, 2003). In the initial assessment (Fresh), all samples showed high rankings, reflecting favourable body and texture attributes. Over time, a consistent decline was observed in the rankings across all samples, suggesting a potential textural change during the storage period. This decline might be attributed to factors such as moisture loss or alterations in the structural integrity of the yoghurt. Initial Flavour rankings were relatively high across all samples, with T1 (plain sample) displaying a slight advantage. The 21-day evaluation showed a noticeable decrease in Flavour rankings for all samples. This decline could be associated with the development of acidity or changes in microbial activity during prolonged storage. T3 consistently secured higher rankings for Appearance & Colour across all storage periods, indicating that the inclusion of yoghurt culture, *Bifidobacterium* mix, and *Spirulina* powder contributes positively to visual appeal. The 21-day evaluation showed a slight decrease in rankings for all samples, which may be linked to colour changes or settling effects during storage. Initial Overall Acceptability rankings were relatively high for all samples, emphasising the overall positive reception of the concentrated yoghurt samples. While T3 maintained a favourable ranking throughout, other samples experience a decline in overall acceptability over the storage period, potentially due to cumulative changes in texture, flavour, or appearance.

***Table 6**. Sensory evaluation of samples during storage*

Parameters	Storage period (day)	T1	T2	T3
Body & Texture	Fresh	6.90	6.80	6.84
	7	6.82	6.78	6.82
	14	6.68	6.75	6.80
	21	6.65	6.70	6.73
Flavour	Fresh	6.65	6.75	6.68
	7	6.60	6.66	6.65
	14	6.33	6.50	6.64
	21	6.20	6.55	6.60
Appearance & Colour	Fresh	6.82	6.80	6.50
	7	6.71	6.68	6.48
	14	6.35	6.39	6.44
	21	6.15	6.25	6.40
Overall Acceptability	Fresh	6.90	6.90	6.82
	7	6.65	6.75	6.70
	14	6.41	6.55	6.50
	21	6.00	6.30	6.20

***Notei:** T1 – control, with 2% of yoghurt starters in the basic sample; T2 – probiotic sample with 2% Bifidobacterium mix + yoghurt starter; T3 – the algal sample with 5% S. platensis powder, 2% yoghurt culture, and 2% Bifidobacterium mix*
***Source:** compiled by the authors of this study*

The observed decline in sensory attributes, particularly in Body & Texture and Flavour during the storage period was consistent with the natural evolution of yoghurt products. Factors such as fermentation by-products, microbial activity, and physicochemical changes contributed to these alterations. T3 consistently outperformed other samples in terms of Appearance & Colour and Overall Acceptability, suggesting that the combination of yoghurt culture, *Bifidobacterium* mix, and Spirulina powder contributes positively to the visual appeal and overall consumer preference. The 21-day evaluation indicated the need for careful consideration

of storage conditions and formulation adjustments to maintain optimised sensory attributes over an extended period. M. Barkallah *et al.* (2017) emphasised the useful application of *Spirulina* powder as a novel and enticing yoghurt-processing addition. *Spirulina* has other nutritional benefits in addition to being a natural colouring and flavouring ingredient. Its high content of dietary fibres and proteins also serves as a physical stabiliser, boosting mouthfeel, syneresis, and perceived viscosity, all of which help to maintain textural features. Interestingly, it was discovered that adding merely 0.25% of *Spirulina* was a substantial way to accelerate these positive benefits.

CONCLUSIONS

In this comprehensive study investigating the incorporation of *Spirulina platensis* powder into concentrated yoghurt, noticeable effects were observed across microbial, chemical, and sensory parameters during various storage periods. While the addition of *Spirulina* showed a more pronounced impact on *Bifidobacterium* growth, its enhancing effect on lactic acid bacteria was less clear, suggesting a potential need for higher concentrations. Microbial counts, particularly for *Str. thermophilus, Lb. bulgaricus*, and *Bifidobacterium* mix, showed dynamic changes during storage, with higher counts in *Spirulina*-containing samples. *Spirulina*-enriched samples demonstrated effectiveness in controlling moulds and yeasts, highlighting potential preservative qualities. Chemical parameters, including acidity and pH values, showed slight increases in treated samples, while the pronounced effect was noted in *Bifidobacterium*-supplemented samples. Colour properties showed noticeable changes, emphasising the need for careful consideration of *Spirulina* concentrations for colour stability. Sensory evaluation indicated that samples with a combination of *Bifidobacterium* and *Spirulina* achieved the highest acceptability scores.

Overall, the presented findings offer insight into the intricate interplay of *Spirulina platensis* in concentrated yoghurt, providing opportunities for both microbial enhancement and improved product quality. Future studies should focus on optimised *Spirulina* concentrations and delve into the mechanisms underlying its influence on microbial communities in yoghurt production. Furthermore, additional investigations could explore the specific compounds contributing to this antioxidant activity and optimise formulations for prolonged efficacy during storage. These avenues of research will contribute to the ongoing development of functional dairy products with enhanced nutritional and sensory properties, meeting the demands of health-conscious consumers in the modern food industry. Further research endeavours could explore innovative processing techniques to maximise the bioavailability of *Spirulina*'s bioactive compounds in concentrated yoghurt, paving the way for the development of novel functional dairy products with enhanced health benefits.

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None.

CONFLICT OF INTEREST

The authors of this study declare no conflict of interest.

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Підвищення активності біфідобактерій та молочнокислих бактерій, а також покращення окислювальної стабільності у функціональному водоростевому концентрованому йогурті з порошком *Spirulina platensis*

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Анотація. Це дослідження спрямоване на задоволення зростаючого попиту на здорові функціональні молочні продукти шляхом вивчення включення Spirulina platensis до складу концентрованого йогурту та оцінки її впливу на закваски та властивості продукту. Комплексний аналіз продемонстрував здатність спіруліни посилювати ріст біфідобактерій, кислотність, вміст ПНЖК та антиоксидантну активність у йогуртах, що вказує на її потенціал для покращення поживної якості та окислювальної стабільності молочних продуктів. У дослідженні вивчався вплив різних співвідношень спіруліни на активність йогуртових заквасок, включаючи Lb. casei, Lb. plantarum, Lb. acidophilus та суміш біфідобактерій. Було приготовано три концентровані йогурти, включаючи контроль, зразок пробіотика та зразок з додаванням порошку спіруліни. Результати показали, що хоча спіруліна не мала значного впливу на молочнокислі бактерії, вона помітно стимулювала ріст біфідобактерій. Оброблені зразки демонстрували підвищену кислотність і вміст TVFAs, причому значення pH мали зворотну тенденцію порівняно з контролем. Чіткі відмінності в параметрах кольору та сенсорних оцінках відрізняли контрольні зразки від оброблених. Крім того, добавка спіруліни продемонструвала помітний ефект у підвищенні антиоксидантної активності, що спостерігається через активність поглинання радикалів DPPH, і вплинула на значення пероксиду, що відображає покращену окислювальну стабільність йогурту. Таке успішне використання спіруліни свідчить про її потенційне застосування в різноманітних їстівних водоростях у харчовому секторі, особливо в молочних продуктах, покращуючи як поживні, так і сенсорні аспекти, а також сприяючи покращенню окислювальної стабільності

Ключові слова: їстівні водорості; функціональні продукти харчування; антиоксидантна активність; корисні бактерії; молочні продукти