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Research Article

Development, quality assessment and nutritive valorization of *Spirulina platensis* in yogurt spread

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Abstract

Dairy products are an excellent source of lactose, water-soluble vitamins, proteins and minerals. They are not considered a key source of bioactive compounds, despite their positive nutritional benefits. The main objective of this study was to develop yogurt spread fortified with Spirulina platensis (SP) and to valorize its impact on the physicochemical properties, shelf life, and quality assessment of the yogurt spread during storage yogurt spread at 0, 14, and 28 d. SP is threadlike blue-green cyanobacteria or microalgae containing polyphenols, phytochemicals like flavonoids and carotenoids which act as antioxidants. Yogurt was developed by using low-fat milk fortified with SP by 1.5, 2.5, and 3.5% respectively to assess the shelf life of spread. The acquired data was inspected by using two-factor factorial designs (two-way analysis of variance) and LSD (Least significant difference) test was used for relative comparison of treatments. Physicochemical characteristics in yogurt spread were 75.6% moisture, 0.68% ash, 4.20 pH, 1.16% acidity, and 0.24mg of iron. Sensory evaluation of yogurt spread was done at different storage periods (0, 14, and 28 d). The current study revealed that SP can be fortified in yogurt spread for utilization of its bioactive substance's benefits which in return can boost human health.

Keywords yogurt spread, bioactive substances, Spirulina, flavonoids, carotenoids

Abbreviations SP – *Spirulina platensis;* LSD – least significant difference; TVC – total viable count; TSS – total soluble solids; LAB – lactic acid bacteria; WHC – water holding capacity; TPC – total phenolic compound; TCC – total carotenoid content

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Introduction

Milk and milk products are a fine source of lactose, water-soluble vitamins, proteins, and minerals. They have an important role in the human diet as they improve the health status (Ozturkoglu-Budak et al. 2016). Despite their beneficial health effects, milk and milk products are not recognized as a primary source of bioactive substances. They are widely produced and consumed throughout many countries (Caleja et al. 2016). Recently, nutritional scientists have reported that the supplementation of dairy by-products using organic environment is now one of the best effective methods to alleviate the total food intake with reduced adverse outcomes (Gahruie et al. 2015). In this way, yogurt has begun to attract the attention of consumers because of its pleasantly sour and creamy taste, and increased health benefits. Yoghurt spread is a fermented milk product prepared by separating whey, lactose, and salts from yoghurt. In the Middle East Region, it is known as Labneh. It has a somewhat acidic flavor and a lower fat level than standard cream cheese (El-Sayed et al. 2017).

In the agri-food industries, several synthetic additives have been used for the purposes of fortifying, coloring, flavoring and extending the useful shelf-life of yogurt (Caleja et al. 2016). However, many studies have confirmed that the excessive consumption of synthetic food additives is related to respiratory, dermatological, gastrointestinal and neurological adverse reactions (Randhawa and Bahna 2009; Carocho et al. 2014). As a result of the requirement to utilize safe chemicals, researchers have developed compounds derived from natural sources that are suitable for use in dairy products (Beheshtipour et al. 2012; Ranadheera et al. 2012; Donmez et al. 2017).

SP filamentous blue-green microalgae or cyanobacteria are popular microalga specie because of its extraordinary nutritional profile (Beheshtipour et al. 2012). SP is said to have multiple potential health benefits in preventing and treating different illnesses like renal failure (Ghaeni and Roomiani 2017), male infertility, cancers, hypertension (Suliburska et al. 2016). The chemical composition of SP contains fatty acids, essential amino acids, many essential minerals as well as vitamins and enzymes. In addition to its nutritional value, antifungal and antibacterial actions against some human pathogens have also been found (Ahsan et al. 2015) and it has been analyzed that it assists the growth of health promoting bacteria in milk and milk products (Beheshtipour et al. 2012). SP is a native of Soda lakes of East Africa, subtropical alkaline lakes and China (Mahmoud et al. 2018). The studies have not historically struggled with the nutritional and sensory characteristics of yogurt.

Although the technological impacts of SP on the growth performance of probiotics in fermented milk products have been investigated (Beheshtipour et al. 2012), research determining its impact on the sensorial qualities and valorization of yogurt spread has not been addressed before. The main objectives of the current study were to develop a yogurt spread fortified with SP, which is rich in bioactive compounds, and valorize its impact on the physicochemical, shelf life, and quality of the yogurt spread during storage at 0, 14, and 28 d.

Materials and Methods

Materials. Cow milk was procured from the Department of Livestock Management, University of Agriculture Faisalabad, Pakistan (UAF). Starter culture mixture was obtained from Institute of Microbiology, UAF. Spirulina platensis powder, salt, black pepper, sugar, and other spices were procured from the local market of Faisalabad.

Yogurt spread production. The milk was pasteurized (75°C for 20 min) and rendered free from microbial contaminants followed by homogenization at 4000 bar pressures using FT9 (single stage) homogenizer and then was cooled at 40°C. This was injected with 2% starter culture (*Lactobacillus delbruckii, Streptococcus thermophilus*) and kept for 4h before hitting 4.6 pH. The yoghurt produced was placed into cloth bags and was left overnight at 4°C to drain by gravity. The yogurt spread obtained was packaged in containers made up of PVC (500 g). In refrigerated trucks, the samples of spread (50 g packages) were transported to the laboratory and stored at refrigerated temperature. Yogurt spread with 0% SP was served as control (T_0) while three other experimental group containing SP powder at concentrations of 1.5, 2.5 and 3.5% were formulated and entitled T_1, T_2 and T_3 respectively (Table 1).

Table 1. Treatment plan for yogurt spread

Treatment	Concentration of spirulina powder, %
T_0	0
T_1	1.5
T_2	2.5
T_3	3.5

Physicochemical analysis of yogurt spread. The physicochemical analyses for yoghurt spread like acidity, fat, crude protein, pH, moisture and total solids were carried out at storage intervals of 0, 14, and 28 d.

All experiments including control were performed in triplicate and mean were quoted.

Moisture. Hot air kiln was used to assess the moisture level of yoghurt taster as per directed by (AOAC 2016). The amount of humidity of the spread trial was measured by desiccating the taster in the rotisserie at 105°C. The spread taster was weighed and poured in a china dish and sited in the kiln at 105°C till a persistent mass was acquired. The trial was put out after 24 h and preserved in the desiccator for 15 min. The specimen was measured, and the interpretation was logged.

Ash content. Five g of sample were measured in porcelain containers and then put in a muffle incinerator at 550-600°C for 3 h till carbon free ashes were obtained (AOAC 2016). The porcelain containers were then chilled in dryer and measured.

Fat content. According to AOAC (2016) the amount of fat was calculated by using Gerber technique. Ten ml of sulphuric acid was weighed and poured in the butyrometer. Later on, 10 ml of spread specimen and 1ml of isoamyl alcohol was poured in butyrometer. The butyrometer then sealed tightly. The blend in butyrometer stirred mildly and reserved for centrifugation process at 1100 rpm for 300 s at 65°C. The fat concentration of the taster was logged from the advanced gauge of butyrometer.

Total solids content. The overall solid concentration was obtained using (AOAC 2016) methods. Three grams of the tester was measured into a dried furnace with a plain aluminum dish and warmed to a water bath for 10 - 15 min. The plate was put in the oven at 105°C for three hours and then chilled in the muffle furnace instantly. Measurement was replicated until the gap was < 0.1 mg between the two readings.

Protein determination. The Kjeldahl's technique was utilized to assess the protein according to the (AOAC 2016). Nitrogen content was then multiplied with factor in order to obtain protein percentage. 1g of sample was weighed in the butter paper and cautiously poured into the digestion container. Digestion blend (5 g) was also applied to the flask for digestion. Ultimately, 35 ml of sulphuric acid was applied and the digestive flask was put in the test tube for digestion before the mixture turns into light green color. The mixture was then cooled for some time and then 150ml of deionized water was poured in it. Digestion flask was rinsed for 2 - 3 times to extract the digested tasters from the digestion flask completely. Protein content was obtained by multiplying N,% with factor (6.38).

Microbiological analysis. The TVC of prepared yoghurt spread was determined according to (Sengupta et al. 2014). A regular saline mixture of sodium chloride of 0.9/100 g composition was developed for the attenuation of taster. 14g of plate count agar was dissolved in 1L of purified water and disinfected at 121°C for 15 min. Nutrient agar (17 g) was measured and dissolved in 500 ml of water. Petri dishes and sampling vessels were disinfected at 171°C for 30 min. Six attenuations of each specimen were prepared in the six test tubes. Taster (1 g) was diluted in the 9 ml saline water in the first test tube; six dilutions were prepared by transporting ml to the succeeding sampling vessel. After disinfection nutrient agar was relocated to the petri dish as a layer. Alter compacting nutrient agar into the petri plate the specimen was dispersed to the media in the form of a thin layer. Petri dishes was reversed and placed into the kiln at 37°C, for 24 h. The colonies were measured with the aid of colony counter after 24 h. The petri dishes those comprised of colonies from 30 - 300 were calculated and multiplied with dilution factor.

pH value. pH of yoghurt was measured through a pH meter given in AOAC (2016). Prior to computing pH, the gadget was standardized by utilizing buffers of pH 4 and 7. After adjustment, the milk taster was positioned in the tumbler, and then pH of yogurt taster was recorded by injecting the shaft of pH meter in the beaker.

Titratable acidity. Phenolphthalein index was prepared by immersing 1g phenolphthalein in ethyl alcohol (95% v/v) to make an ultimate volume of 100 ml. Four g NaOH was liquefied in deionized water and volume was makeup to 100 ml. The spread acidity was measured using the titrimetric process proposed in (Sengupta et al. 2014). The acidity of each sample was computed by summing up 10 ml of the sample in the titration container. Titrate the solution against 0.1N NaOH utilizing phenolphthalein as a predictor. The titration proceeded till the light pink color emerged. The amount of NaOH used was recorded and the acidity was determined.

Viscosity measurement. Yogurt viscosity determined by RV Brookfield viscometer at room temperature in 100 ml sample. Before viscosity measurement, sample was stirred for 45 s. The results were identified in centipoise units (cP). Viscosity results were measured at 10rpm with spindle (Sengupta et al. 2014).

Sensory analysis. Sensory analysis was done by using nine points hedonic scale according to the method of (Land and Shepherd 1984). Sensual outlining of the yogurt sample was directed, by utilizing predictable describing, by untrained jury. The jury were provided with a hedonic form to assess color, taste, flavour, and odour and the overall acceptability of coded trials with diverse treatments. Those stowed for diverse interval of time (0, 14, 28 d).

Minerals analysis. Yogurt spread containing SP was probed for calcium, magnesium, iron and zinc after wet digestion following the guidelines of (AOAC 2016). They were determined by using the atomic absorption spectrophotometer. The sample (0.5 g) firstly digested at temperature of (60-70°C) for 20 min with 10 ml HNO_3 on hot plate in a conical flask. Then it was digested at high temperature (190°C) with 5 ml $HClO_4$ till mixture became clear. After that, the sample was transferred into a 100 ml volumetric flask and then added distilled water to make volume double and filtered it. Sample of recorded strength then ran for minerals to obtain standard curve.

Total phenolic compounds and carotenoids. The amount of carotenoids and phenolic compounds was determined in the product using a spectrophotometer. The choice of solvent has a significant impact on the absorption maxima of extracted pigments. While carotenoids red absorption maximum raises with increasing solvent polarity, their blue absorption maximum shifts from 400 to 500 nm. Total carotenoids were quantified using a spectrophotometer at 470 nm Selvamuthukumaran and Farhath (2014), while total phenolic compounds were measured using a spectrophotometer at 653 nm (Zheng and Wang 2001). This was done by pipetting exactly 50 ml of newly produced extracts (10 mg sample in 1 ml of acetone) through each hole in the plate, followed by the addition of 150 ml of acetone to each hole. It was diluted 4 times to achieve the maximum absorbance of 1.00 absorbance unit from the origin extract in this manner.

Statistical analysis. The acquired data was inspected by using two factor factorial designs (two-way analysis of variance) and LSD test was used for relative comparison of treatments. Statistics 8.1 software was utilized for application of statistical parameters. Trials were done in triplicate sequence for calculating the standard deviation and mean values (Montgomery 2017).

Results and Discussion

This study was carried out to examine the impact of SP on quality and shelf life along with its effect on storage, physicochemical, sensory characteristics and minerals contents of yogurt spread incorporating with varying concentration of its powder. Physicochemical and sensory characteristics were carried out using various procedures to determine the impact of spirulina on shelf life and quality of yogurt spread.

Physicochemical analysis of yogurt spread. The results symbolize the mean value for physicochemical analysis for yogurt spread supplemented with SP is delineate in Table 2. The significant changes were measured in physicochemical properties of yogurt spread during spread storage and different concentration of SP. The moisture content among concentrations of SP varied non-significantly ($p > 0.05$) and a declining trend in moisture was observed in all treatments. The duration of days exhibited significant decreasing trend in moisture content but values were statistically at par ($p < 0.05$). Significantly high SP content of fiber, protein and fat may also a cause of decreasing moisture content (Barkallah et al. 2017). It has been demonstrated that storage at low temperatures has no significance whereas SP improves the shelf life of yoghurt (Amellal-Chibane and Benamara 2011; Ani et al. 2018). An increasing trend in ash content of fortified yogurt spread with SP was 0.78% and computed significantly higher than treatment T_0 (0.63%) ($p < 0.05$). Outcome is similar as studies on quality parameter measured by adding SP into ice cream. Ice cream fortified with SP contain high ash content ($p < 0.01$) at the termination of storage which is possibly due to high minerals and protein content of SP (Malik et al. 2013). The results after statistical analysis indicated that the fat content varied non-significantly as storage days progressed and with the proportion of change in concentrations of SP. There was non-significant difference between fortified control sample yogurt spread ($p > 0.05$). Results of this study associated with products obtained by Barkallah et al. (2017), they also perceived a non-significant decrease in fat content with storage and it can be suspected this decreasing to lipolysis. TSS of yogurt spread fortified with SP and control were 27.56% and 24.33% respectively. There was perceived a significant difference in TSS among control and fortified yogurt spread ($p < 0.05$). The duration of days exhibited the decreasing trend in total solids and had a non-significant impact on the total solids of spread supplemented with SP. TSS were significantly high in enriched ice cream with different SP concentration compared to control at the end of storage might be due to the syneresis of LAB (Malik et al. 2013). Likewise, total protein average was higher in formulation T_2 yogurts spread fortified with SP ($p < 0.01$). Significant increasing trend in protein content was noticed when different concentration of chickpea added in yogurt (Zare et al. 2011). Data published against storage of yogurt exhibits the decreasing trend in protein which may be due to the proteolytic activity and milk quality (Andleeb et al. 2008).

TVC of yoghurt spread differ from 3.15 to 4.20 log CFU/g as depicted in Table 2. Non-significant increase was perceived among the different formulation of yogurt spread fortified with SP (T_0 - T_3). However, increase in total viable count (log CFU/g) was noticed to be significant as the days of storage advanced ($p < 0.05$). In treatment T_2 maximum upsurge was observed at the terminal stage of storage span of yogurt spread. Similar narration was published in which it was observed an increase in TVC (log CFU/g) with the passage of time (Eker and Karakaya 2020).

The increase in SP concentration at levels ranging from 0 to 3.5% evolved in non-significantly decrease in pH from 4.47 to 3.96 while pH significantly decreased with days and increasing trend was seen in acidity with duration from 1.08 to 1.38% (Table 2). Similar significant increasing trend was (Zare et al. 2011) directed in a study in which it was used different chickpea concentrations in yogurt and was found that the pH with storage periods decreased significantly and increased yogurt acidity and closed out that the addition of proteins caused the upsurge in solid content of yogurt spread due to which the buffering magnitude increased that mandatory further acid change by starter cultures to accomplish a similar pH goal (Barkallah et al. 2017).

Viscosity of yogurt spread is one of the crucial physical characteristics that supports in upholding all of the components of spread in homogeneous state. The current study (Table 2) depicted increase in viscosity with rise in concentration of SP. The viscosity increased up-to treatment T_2 and then decreased at 0 d of storage. Viscosity decreased as storage day progressed. Similar relation in viscosity was observed in yogurt spread because of high content of ash, fiber, total solids and protein in SP which showed the water holding capacity of 1.45 g water/g of protein (Malik et al. 2013). WHC of casein is about 2.5g water/g protein (Parveen 2022).

Sensory analysis. The sensory score of the products for color, taste, flavor, texture, and overall acceptability are depicted in Table 3. The 9-point hedonic scale outcome displayed the maximum scores for yogurts spread containing 2.5% Spirulina ($p < 0.05$). Yogurt spread containing concentration 1.5 and 3.5% depicted minimum score and overall acceptability. Flavor scores showed that there were no significant differences between the control yogurts and yogurts fortified microalgae ($p > 0.05$).

Table 2. Impact of treatment and day's storage on physicochemical analysis of yogurt spread

Treatments	Storage days	Moisture, %	Ash, %	Fat, %	Total soluble solids, Brix°	Protein, %	TVC, log CFU/g	pH	Acidity, %	Viscosity, cP
T_0	0	77.59 ± 1.01^{a}	0.65 ± 0.02^{c}	3.85 ± 0.01^{a}	24.53 ± 0.30^{b}	3.47±0.05^{fg}	3.15 ± 0.15^{c}	4.47±0.6^{a}	1.08±0.02^{b}	12066±757.1^{ab}
	14	76.1 ± 1.72^{ab}	0.65 ± 0.01^{c}	3.67 ± 0.21^{a}	24.53 ± 0.25^{b}	3.09 ± 0.05^{gh}	3.74 ± 0.17^{b}	4.22±0.7^{ab}	1.22±0.20^{ab}	12051±635.32^{ab}
	28	75.53 ± 3.55^{b}	0.63 ± 0.01^{c}	3.44 ± 0.31^{a}	24.33 ± 0.45^{b}	2.74 ± 0.05^{h}	4.21 ± 0.23^{a}	4.02±0.06^{b}	1.26±0.52^{a}	12001±649.02^{ab}
T_1	0	77.04 ± 0.73^{a}	0.71 ± 0.02^{b}	3.93 ± 0.04^{a}	25.40 ± 0.2^{b}	4.95 ± 0.02^{abc}	3.34 ± 0.18^{c}	4.44±0.26^{a}	1.12±0.06^{b}	13067± 665.8^{ab}
	14	77.12 ± 0.37^{ab}	0.68 ± 0.03^{b}	3.72 ± 0.30^{a}	25.07 ± 0.80^{b}	4.33 ± 0.06^{de}	3.81 ± 0.17^{b}	4.20±0.59^{ab}	1.21±0.06^{ab}	13120± 648.12^{ab}
	28	75.10 ± 1.38^{b}	0.69 ± 0.01^{b}	3.67 ± 0.36^{a}	24.73 ± 0.15^{b}	3.95 ± 0.09^{ef}	4.14 ± 0.26^{a}	4.04±0.2^{b}	1.26± 0.08^{a}	13151±405.2^{ab}
T_2	0	76.83 ± 0.45^{a}	0.75 ± 0.13^{ab}	4.09 ± 0.07^{a}	27.56 ± 0.54^{ab}	5.42 ± 0.09a	3.36 ± 0.15^{c}	4.39±0.22^{a}	1.11± 0.06^{b}	13892± 814.64^{ab}
	14	74.60 ± 1.61^{ab}	0.69 ± 0.05^{ab}	3.57 ± 1.33^{a}	25.84 ± 0.41^{ab}	4.55 ± 0.41^{cd}	3.84 ± 0.83^{b}	4.12±0.09^{ab}	1.22±0.06^{ab}	11707± 504.71^{b}
	28	74.10 ± 0.42^{b}	0.78 ± 0.07^{ab}	3.64 ± 0.35^{a}	25.49 ± 0.85^{ab}	4.23 ± 0.05^{de}	4.20 ± 0.38^{a}	4.04±0.10^{b}	1.31±0.07^{a}	14862± 753.89^{ab}
T_3	0	75.60 ± 2.25^{a}	0.77 ± 0.02^{a}	4.17 ± 0.07^{a}	26.60 ± 0.26^{a}	5.19 ± 0.04^{ab}	3.40 ± 0.14^{c}	4.36±0.23^{a}	1.13±0.04^{b}	12003±439.55^{ab}
	14	74.53 ± 2.1^{ab}	0.71 ± 0.01^{a}	3.80 ± 0.44^{a}	26.40 ± 0.21^{a}	4.96 ± 0.06^{abc}	3.88 ± 0.29^{b}	4.15±0.30^{ab}	1.24±0.06^{ab}	14342±150.69^{ab}
	28	74.08 ± 0.33^{b}	0.73 ± 0.12^{a}	3.39 ± 0.96^{a}	25.90 ± 0.39^{a}	4.71 ± 0.43^{bcd}	4.19 ± 0.27^{a}	3.96±0.19^{b}	1.38±0.07^{a}	15212±553.9^{a}

T_0 - Control group;
T_1 - 1.5% Spirulina yogurt spread;
T_2 - 2.5% Spirulina yogurt spread;
T_3 - 3.5% Spirulina yogurt spread;
Means having the same letters within a column differ significantly ($P > 0.05$)

Table 3. Impact of treatment and days storage on sensory analysis of yogurt spread

Treatments	Storage days	Color	Flavor	Taste	Texture	Overall acceptability
T_0	0	8.83 ± 0.10^{a}	7.38 ± 0.03^{a}	7.39 ± 0.07^{a}	7.90 ± 0.49^{a}	7.50 ± 0.01^{a}
	14	7.73 ± 0.83^{ab}	7.39 ± 0.02^{a}	6.86 ± 0.13^{a}	6.70 ± 0.02^{a}	7.32 ± 0.17^{a}
	28	6.73 ± 0.14^{b}	7.36 ± 0.03^{a}	6.27 ± 0.21^{a}	5.90 ± 0.03^{a}	7.19 ± 0.06^{a}
T_1	0	8.46 ± 0.09^{a}	7.36 ± 0.03^{a}	6.64 ± 0.26^{a}	7.85 ± 0.01^{a}	7.48 ± 0.27^{a}
	14	6.80 ± 1.32^{ab}	7.35 ± 0.02^{a}	6.44 ± 0.12^{a}	6.42 ± 0.02^{a}	7.04 ± 0.21^{ab}
	28	6.72 ± 0.68^{b}	7.39 ± 0.03^{a}	6.78 ± 0.08^{a}	5.76 ± 0.03^{a}	6.56 ± 0.14^{abc}
T_2	0	6.99 ± 1.69^{a}	7.38 ± 0.04^{a}	6.21 ± 0.04^{ab}	7.78 ± 0.02^{a}	6.10 ± 0.2^{bcd}
	14	6.83 ± 1.31^{ab}	7.35 ± 0.01^{a}	5.73 ± 1.46^{ab}	6.34 ± 0.73^{a}	5.66 ± 0.35^{cd}
	28	6.63 ± 0.36^{b}	7.32 ± 0.02^{a}	5.62 ± 1.08^{ab}	5.63 ± 0.02^{a}	6.63 ± 0.72^{abc}
T_3	0	8.05 ± 0.04^{a}	7.37 ± 0.01^{a}	5.79 ± 0.16^{b}	7.65 ± 0.03^{a}	5.82 ± 0.2^{cd}
	14	7.07 ± 0.10^{ab}	7.33 ± 0.02^{a}	5.37 ± 0.83^{b}	6.25 ± 0.02^{a}	5.37 ± 0.83^{d}
	28	5.44 ± 0.91^{b}	7.32 ± 0.03^{a}	4.46 ± 0.82^{b}	5.51 ± 0.07^{a}	5.17 ± 0.15^{d}

T_0 - Control group;
T_1 - 1.5% Spirulina yogurt spread;
T_2 - 2.5% Spirulina yogurt spread;
T_3 - 3.5% Spirulina yogurt spread;
Means having the same letters within a column differ significantly ($P > 0.05$)

A decreasing trend in color acceptability was observed in the treatments for different SP concentrations. The storage span also exhibited the decreasing trend in color score and had a significant impact on the color of yoghurt spread supplemented with different concentration of SP ($p < 0.01$). The color of yogurt spread changed from whitish yellow to greenish due to SP fortification. However, the differences were remarkable from the point of view of oral-texture ($p < 0.01$). Treatments containing 1.5 and 3.5% of SP had the non-significant impact on the spread texture and mouthfeel ($p > 0.05$). Color value decrease throughout the storage, but storage conditions were hypothesized to have no consequential effect at all by using Moringa seed oil (Zhang et al. 2019). Similar outcomes were noticed for color as concentration of SP alters and storage span increased. They worked on yogurt physicochemical and textural properties and found significant decreasing effect (Barkallah et al. 2017). The results obtained by Mahdian and Tehrani (2007) also support the outcomes of sensory assessment of the study.

Similar outcomes were noticed for flavor as concentration of spirulina alters and storage span increased (Barkallah et al. 2017). Taste value increases throughout the storage, but storage conditions were hypothesized to have no consequential effect at all by using Moringa seed oil (Zhang et al. 2019). Similar outcomes were noticed for taste as concentration of SP alters and storage span decrease non-significantly. They worked on yogurt physicochemical and textural properties and found significant decreasing effect on taste (Barkallah et al. 2017). Results of this study are inconsistent with outcomes attained by Ahmad et al. (2013). They worked on qualitative assessment of labneh cheese having coating of olive oil and supplementation of culinary herbs. They noticed a decrease in sensory scores for texture with the passage of time. Similar description was available (El-Syed et al. 2017). They added *Moringa oleifera* oil in labneh to ameliorate its nutritional and physicochemical values. They stated that as the storage time increases, decrease in texture is monitored. The results obtained by Mahdian and Tehrani (2007) also support the results of sensory evaluation of the current study. Overall acceptability value decreases throughout the storage, but storage conditions were hypothesized to have no consequential effect at all by using Moringa seed oil (Zhang et al. 2019). Similar outcomes were noticed for overall acceptability as concentration of SP alters and storage span increased. They worked on yogurt physicochemical and textural properties and found significant decreasing effect (Barkallah et al. 2017).

Total phenolic compounds and carotenoids. The results discussed in Table 4 showed that the TPC and TCC of yogurt spread significantly ($p < 0.01$) alter by altering the concentration of SP. The duration of days exhibited non-significantly ($p > 0.05$) impact on TPC and TCC of yogurt supplemented with SP. The maximum value of TPC and TCC were recorded after 0 d of storage. The treatment containing 2.5% spirulina showed the highest value for TPC and TCC at the end of storage. The findings are consistent with the findings of a research on SP enriched ice cream (Malik et al. 2013). Results regarding the ratio of carotenoids content agree with the outcomes of (Gies et al. 2019), a significant increase occurred in the ratio of carotenoids with the application of concentration of maize into yogurt. SP was shown to be a rich source of antioxidants and therefore it may change the very low-density lipoprotein in human blood (Habib et al. 2013; Maghu et al. 2017). In reality, microalgae's antioxidant action stems from their abundance of free radical scavengers. So, in order to assess the contribution of Spirulina to the TPC and TCC level of yoghurt measurements were taken. SP inclusion improves TCC and TPC concentration substantially when compared to control yoghurts (Barkallah et al. 2017).

Minerals Analysis. When SP concentration was changed, a significant ($p < 0.05$) rising trend for magnesium was seen among the treatments from T_0 - T_3 (Table 5). As the storage days progressed, non-significant declining trend for magnesium was noticed. For calcium and zinc, non-significant ($p > 0.05$) increasing trend was perceived as the concentration of SP altered. Likewise, storage span also left a non-significant impact for calcium and zinc. Results indicated that iron content of yoghurt spread increase significantly ($p < 0.01$) as concentration of SP changed and non-significant changes perceived

Table 4. Impact of treatment and days storage on phenolic and carotenoids contents of yogurt spread

Treatments	Storage days	TPC, µg GAE/ml	TCC, mg/g
T_0	0	4.87 ± 0.51[b]	0.33 ± 0.016[b]
	14	4.74 ± 0.38[b]	0.29 ± 0.02[b]
	28	5.03 ± 0.25[b]	0.23 ± 0.005[b]
T_1	0	15.41 ± 2.02[a]	4.49 ± 1.18[ab]
	14	12.05 ± 1.4[a]	4.49 ± 1.42[ab]
	28	11.37 ± 2.8[a]	4.35 ± 0.65[ab]
T_2	0	28.28 ± 1.44[a]	6.19 ± 1.09[a]
	14	25.26 ± 2.13[a]	6.69 ± 0.36[a]
	28	19.78 ± 1.78[a]	4.40 ± 2.71[a]
T_3	0	16.55 ± 1.17[a]	8.84 ± 1.56[a]
	14	13.93 ± 2.80[a]	4.18 ± 2.44[a]
	28	14.54 ± 0.56[a]	4.30 ± 1.26[a]

T_0 - Control group;
T_1 - 1.5% Spirulina yogurt spread;
T_2 - 2.5% Spirulina yogurt spread;
T_3 - 3.5% Spirulina yogurt spread;
Means having the same letters within a column differ significantly ($P > 0.05$)

Table 5. Impact of treatment and days storage on minerals analysis of yogurt spread

Treatments	Storage days	Magnesium, mg/100g	Calcium, mg/100g	Zinc, mg/100g	Iron, mg/100g
T_0	0	11.84 ± 0.37^{b}	94.06 ± 4.41^{a}	0.61 ± 0.07^{a}	0.05 ± 0.02^{c}
	14	11.87 ± 0.31^{b}	95.60 ± 6.62^{a}	0.62 ± 0.07^{a}	0.05 ± 0.02^{c}
	28	11.85 ± 0.07^{b}	96.04 ± 6.55^{a}	0.68 ± 0.06^{a}	0.06 ± 0.02^{c}
T_1	0	12.25 ± 0.10^{ab}	96.48 ± 6.15^{a}	0.65 ± 0.07^{a}	0.15 ± 0.03^{bc}
	14	12.33 ± 0.51^{ab}	97.26 ± 6.51^{a}	0.70 ± 0.07^{a}	0.19 ± 0.01^{bc}
	28	12.45 ± 0.98^{ab}	100.46 ± 7.14^{a}	0.72 ± 0.07^{a}	0.16 ± 0.07^{bc}
T_2	0	13.04 ± 0.71^{ab}	99.30 ± 6.75^{a}	0.67 ± 0.06^{a}	0.27 ± 0.06^{ab}
	14	11.32 ± 1.53^{ab}	98.34 ± 5.75^{a}	0.71 ± 0.07^{a}	0.29 ± 0.02^{ab}
	28	13.13 ± 0.50^{ab}	102.37 ± 8.66^{a}	0.75 ± 0.08^{a}	0.26 ± 0.02^{ab}
T_3	0	13.92 ± 0.06^{a}	99.81 ± 6.38^{a}	0.69 ± 0.06^{a}	0.38 ± 0.03^{a}
	14	12.88 ± 0.20^{a}	104.78 ± 6.19^{a}	0.74 ± 0.08^{a}	0.32 ± 0.02^{a}
	28	12.65 ± 1.43^{a}	106.21 ± 7.61^{a}	0.76 ± 0.08^{a}	0.37 ± 0.01^{a}

T_0 - Control group;
T_1 - 1.5% Spirulina yogurt spread;
T_2 - 2.5% Spirulina yogurt spread;
T_3 - 3.5% Spirulina yogurt spread;
Means having the same letters within a column differ significantly ($P > 0.05$)

in iron as storage days progressed. Similar narration was published in which different SP concentration in yogurt formulated and found that iron with storage periods slightly decreased and increased yogurt ash with change in concentration of SP and closed out that addition of SP caused the upsurge in ash content of yogurt spread. The calcium and zinc showed the non-significant impact (Barkallah et al. 2017).

Conclusion

Shelf life, nutritional value, and functional properties of yogurt spread supplemented with SP were improved. They can further improve product quality by extending the shelf life of spread. The fortification of SP in yogurt spread could be beneficial as it promoted the viability of beneficial bacteria. Microbiota improves gut health and improves the nutrients absorption. T_2 (with 2.5% spirulina powder) was mainly liked by the consumer. Viscosity and spread ability increased with increasing SP concentration. SP fortification did not improve the shelf life of yogurt but also enhanced the bioactive component and iron content of yogurt spread. SP improved the protein quality and micronutrients of spread; therefore, they can combat the micronutrient and protein-energy malnutrition in growing children and pregnant women. SP appears to be significant as a fortification substance in yogurt since they deliver a handy meal that fulfills customer desire while also providing health advantages.

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