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Functional Properties of Yoghurt Fortified with *Spirulina platensis* and Milk Protein Concentrate

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ABSTRACT

Spirulina platensis powder (SPP) and milk protein concentrate (MPC) were used to produce enriched functional yoghurt, at four treatments, control T_1 skim milk powder (2% SMP), T_2 (1.5% MPC+0.5% SPP), T_3 (1%MPC+1%SPP), T_4 (0.5% MPC+1.5% SPP). The (*Spirulina platensis* powder) (SPP) was analyzed to determine the chemical, phytochemical properties and antioxidant capacity. Spirulina powder had content of protein (62.40%), fat (7.12%), total phenols (840.6 mg GAE/100g), carotenoids (482.5 mg RE/100g.), flavonoids (680.6 mg/100g) and chlorophyll pigment (1244 mg/100g). Spirulina powder had pH value (6.29). Yoghurt samples were analyzed to evaluate physicochemical properties (moisture, TS, protein, fat, ash, carbohydrates, calories, pH and acidity) Protein for control sample was 3.92%, while the value was 4.62% for T_2. Phytochemical, total antioxidant, color, physical properties were determined. The highest antioxidant values were T_4 (0.5% MPC+1.5% SPP) 57.25% for DPPH and 51.14 for ABTS. Microbial analysis resulted that the highest value of LAB was 10.12^{log10} for T_4 (0.5% MPC+1.5%SPP) with significant difference between all treatments. Sensory evaluation revealed that yoghurt t enriched with 1.0 % MPC and 1% SPP were more acceptable than other formulations.

Keyword: *Spirulina platensis*, milk protein concentrate, yoghurt, physicochemical, antioxidant capacity

INTRODUCTION

Milk and dairy products play an essential part in the human diet because of the numerous nutritional benefits provided by water-soluble vitamins, proteins, lactose and minerals (Ozturkoglu-Budak *et al*., 2016). In order to make yoghurt, *Lactobacillus bulgaricus* and *Streptococcus thermophilus* ferment lactose in milk. To produce a coagulated milk product. Lactic acid bacteria from different sources (LAB) are commonly used to make yoghurt with distinct flavors (Adolfsson *et al*., 2004). When purchasing dairy products, physico-chemical and nutritional properties are the most vital considerations. Yogurt is popular because of its flavorful and thick creamy texture as a food that supports good health (Domagla, 2005). Fermented milks are products enriched with minerals, proteins vitamins and essential fatty acids like gamma linolenic acid. (Perez *et al.*, 2007). Spirulina is high in minerals, essential amino acids, essential fatty acids, carotenoids, minerals, vitamins and other antioxidants. Powdered *spirulina platensis* contains phenolic components as well as possible flavonoids.

Spirulina platensis contains beta-carotene, which is an antioxidant. Antioxidants are molecules that neutralize free radicals in the human body. Beta-carotene is among of the most basic types of carotenoids with the formula molecular $C_{40}H_{56}$ (Khoo *et al.*, 2011). *Spirulina platensis* can be used to make functional foods since it includes bioactive chemicals that improve the food's nutritional content. Heat affects the bioactive chemicals, so processing must be carefully examined. Natural components chlorophyll and carotenoids, for example, phenolic compounds and flavonoids are found in spirulina. (Martelli *et al.*, 2014). MPC (milk protein concentrate) is a grayish-white color while the color of a skimmed milk powder (SMP) is yellowish-white. MPC has a protein content ranging from 35 to 85 percent. MPC has a number of advantages over alternative milk protein components, including the fact that caseins and whey proteins are present in their normal amounts and in their natural condition (Janki Suthar *et al*.,2017).

The current study aims to enrichment yoghurt with spirulina and milk protein concentrate with its addition in different concentrations to produce yoghurt high in bioactive compounds, and to investigate the functional, physicochemical and microbiological properties of the produced yoghurt.

MATERIALS AND METHODS

Materials

Cow's milk was received from Agriculture Faculty, Tanta University in Egypt. *Lactobacillus delbrueckii spp. bulgaricus* and *Streptococcus thermophilus* yoghurt starter (1:1) were purchased from Chr. Hansen' s, Laboratories, Copenhagen and Denmark. Skim milk powder (Grade A-low heat - spray process-pasteurized) manufactured by West farm Foods, U.S.A. Milk protein concentrate was obtained from El Nasr Pharmaceutical & chemicals (Adwic). Cairo, Egypt. *Spirulina platensis* (Food grade spirulina) powder was obtained from Aquaculture Research Center (ARC) at Arab Academy for Science, Technology, and Maritime Transport (Alexandria, Egypt).

Manufacture of yogurt

Four yogurt treatments were processed in this study. Cow's milk was separated into four parts, and the following components were added: T_1: Control (Yogurt with 2%

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SMP), T_2: Yogurt with (1.5 % MPC & 0.5 % SPP), T_3: Yogurt with (1 % MPC &1 % SPP) and T_4: Yogurt with (0.5 % MPC &1.5 % SPP). All treatments were heated for 15 minutes at 85°C after that it was cooled to 42°C. Yogurt starter cultures added as much as 3% with each treatment. The treatments were placed in 100 mL plastic cups and incubated at 42°C until the pH became 4.6, after which they were maintained at 4°C according to International Dairy Federation (1987)..The samples were analyzed for the physicochemical, phytochemical, total antioxidant, color, physical properties and sensory evaluation. The samples were produced in triplicates.

Analytical Methods:

Physicochemical analysis and energy content:

Each sample was tested in triplicate for moisture, total solids (TS), fat, protein, ash, total acidity, pH, and crude fiber. (AOAC, 2010). The total nitrogen content (TN) was determined by using the micro-Kjeldahl method, and protein content was calculated by multiplying the percentage of TN by 6.38 for milk components and 6.25 for spirulina powder. For fat content milk and yoghurt, the Gerber method was applied, whereas for spirulina powder, the Soxhelt apparatus method was used. To determine ash content, a 5g sample was heated in a muffle furnace at 550°C overnight. TS was determined using a drying oven (AOAC, 2010). Titratable acidity was calculated as a percentage of lactic acid. The total carbohydrate was determined using the difference [100 – (moisture + protein + fat + ash+ fiber) %]. The energy value was calculated using the formula provided by Osborne and Voogt (1978) applying the using the formula: Energy value (Kcal /100g) = (4×% protein) + (9×% fat) +(4× % carbohydrate).

Determination of total phenolic content

The Folin-Ciocalteau technique was used to determine the total phenolic content according to Shiri *et al.* (2011). Reagent Folin-Ciocalteau (5 ml, of 1:10 diluted sample with distilled water) was added to the samples (0.5 ml) for 5 min and aqueous sodium carbonate (4 ml, 1 M) was then added. A UV-Vis spectrophotometer (model – Systronics 2202) was used to measure the absorbance of the reaction mixture at 765 nm. The standard was gallic acid. The results were calculated as gallic acid equivalents (mg/100g sample).

DPPH radical scavenging activity:

Burits and Bucar14 described the 2, 2 diphenyl-1-picrylhydrazyl (DPPH) assays One ml algal extract (100 and 200 g/ml) was mixed with one ml DPPH reagent/methanol solution (0.002 percent w/v). The absorbance was determined at 515 nm after 30 minutes of incubation in the dark at room temperature (using jenway 6130 spectrophotometer) according to Braca *et al*. (2001) This test was performed in triplicate and the antioxidant activity was calculated as the following:

$$\textbf{Activity (\%)} = Ac - At/Ac \times 100$$

Where:

At was the absorbance of samples and Ac the absorbance of methanolic DPPH solution

Determination of total carotenoids

In order to determine the concentration of carotenoids in the *Spirulina platensis* extract. With 25 ml of acetone, 3 g of *Spirulina platensis* powder was extracted. and kept in the fridge for 24 hours. The supernatant was centrifuged for 10 minutes at 3000 rpm, and 0.5 mL was 100 times diluted. The OD was read at 450 nm (Vonshak and Borowitzka, 1991).

Total flavonoids determination

The flavonoid content was calculated using (Zilic *et al*.,2012). Basically, 500 µl of extract was combined with 250 µl of NaNO at 5%. After 6 min. 2.5 mL of a 10% AlCl3 solution was added to the mix. After 7 minutes, the mixture was centrifuged at 5000 g for 10 minutes with 1.25 ml of 1 M NaOH added. The supernatant's absorbance was measured at 510 nm in comparison to a solvent blank. Total flavonoid concentration was measured in milligrams of routin equivalent (RE) per 100 grams of sample.

Hunter color parameter:

A Chroma metre was used to analyses the color of the yoghurt. (MINOLTA CHPOMA METER CR-210). To avoid the effects of daylight, the tests were conducted under artificial light. The color parameters L* (lightness), a* (red / greenness), and b* (yellow / blueness) of the yogurt samples were measured in accordance with the International Commission on Illumination (CIE) L*a*b* system as described by Wallace and Giusti (2008).

Viscosity determination

The yoghurt's viscosity was determined by a rotational viscometer (DV-III, Brookfield, MA, USA) at 200 rpm, spindle no. 6 (Su *et al.*, 2018).

Microbial examination

The total bacterial count (TBC) was done on plate count agar (PCA) media. Lactic acid bacterial counts (LAB) were analyzed according the procedures described by (Elliker *et al*. 1956). Standard Methods for the Examination of Dairy Products were used to examine mold and yeast. (Marth, 1978), using acidified Potato Dextrose Agar (PDA).

Sensory Evaluation

A ten- trained member panel used a ten-point scoring system to assess the appearance, colour, body–texture, flavour, and overall acceptance of all yoghurt samples. (10 excellent, 1 unacceptable) according to Tamime and Robinson (1999).

Statistical Analysis

The data was analyzed using SPSS 16 for Microsoft Windows. The statistical data was evaluated by Duncan's multiple-range test at the $P < 0.05$ level of significance.

RESULTS AND DISCUSSION

Physicochemical properties of *Spirulina platensis* powder (SPP), milk (M), milk protein concentrate (MPC) and skim milk powder (SMP)

Table (1) shows the physicochemical properties of raw materials *Spirulina platensis* powder (SPP), Milk (M), milk protein concentrate (MPC) and skim milk powder (SMP). The results are in agreement with Habib *et al*. (2008) who recorded that in both cases of *Spirulina platensis* and *Spirulina maxima* the lipid content varies between 5.6 and 7%. (Mistry,2002**)** Milk protein concentrate (MPC) has the highest value of protein (80.20%) followed by *Spirulina platensis* (SPP) (62.40%). This result was in agreement with Becker (2007) and Belay (2008**)** who states that protein content of SPP varies between 50 and 70% of its dry weight and this is in agreement with Sharoba (2014) who resulted that the content of protein of (SPP) was (62.84%) and

Vijayarani *et al.* (2012) and Salmeàn *et al.* (2015), who stated high amount of protein (65-70%) in Spirulina powder. Spirulina powder had high value of protein. The protein in meat, dried milk, eggs, soybeans, and grains are less than protein in *spirulina platensis*. The highest value of ash was for SPP (8.10%) and the lowest value of ash was for M (0.78%) with significant differences between samples. Fiber content of SPP was 5.54% but not detected in M, MPC and SMP. Fiber considered an important for public health and improve both metabolic and overall health. This result was in agreement with Sharoba (2014) who found that the content of fiber was (8.12%) whereas, Saharan and Jood (2017) found that the amount of crude fiber (9.70%). The biochemical composition depends upon the Spirulina source, culture conditions and season of production (Habib *et al*., 2008). The highest value of pH is for SPP (6.82) and the lowest value was for M (6.62) This result is agreement with Sharoba (2014) who found that pH value was (6.84).

Table 1. Physicochemical properties (on wet weight) of *Spirulina platensis* powder (SPP), milk (M), milk protein concentrate (MPC) and skim milk powder (SMP)

Chemical composition	SPP	M	SMP	MPC
Moisture	6.01^{b}	87.17^{a}	3.80^{d}	4.32^{c}
T.S	93.99^{c}	12.83^{d}	96.20^{a}	95.68^{b}
Protein	62.40^{b}	3.25^{d}	33.40^{c}	80.20^{a}
Fat	7.12^{a}	4.28^{b}	0.50^{d}	1.76^{c}
Total solids not fat (%)	86.87^{c}	8.55^{d}	95.70^{a}	93.92^{b}
Ash	8.10^{a}	0.78^{d}	8.01^{b}	7.2^{c}
Fiber	5.54	-	-	-
Carbohydrate	10.74^{b}	4.52^{d}	54.29^{a}	6.52^{c}
Energy (kcal)	356.64^{c}	69.60^{d}	443.44^{a}	362.72^{b}
Total acidity	0.12^{ab}	0.16^{a}	0.09^{b}	0.07^{b}
pH value	6.29^{d}	6.62^{c}	6.76^{b}	6.80^{a}

$^{a-d}$ The different superscript letter have a significant difference in the same raw. (Duncan's test P<0.05)
SPP= *Spirulina platensis* powder
M=Milk
SMP=Skim milk powder
MPC=Milk protein concentrate

Phytochemical and antioxidant properties of *Spirulina platensis* powder (SPP)

The phytochemical screening of *Spirulina platensis* powder represented in Table (2) the results revealed of total phenols (840.60 mg GAE/100g.), total carotenoids (482.50 mg RE/100g.), total flavonoids (680.20 mg/100g) and chlorophyll (1244 mg/100G).The results are in agreement with those of earlier research that have found phenolic and other bioactive compounds. (Bhavisha and Parula, (2010), Deasy *et al.* (2019).

Total antioxidant activity values (DPPH & ABTS) for SPP were 48.50 and 53.20, respectively. Spirulina has been shown to have high antioxidant properties in several studies. (Manoj *et al*.,1992) reported that spirulina alcohol extract reduced lipid peroxidation 65 percent better than chemical anti-oxidants such as -tocopherol (35 percent), butylated hydroxy anisol (45 percent), and -carotene (48 percent). The antioxidant activity of spirulina water extract (76%) is greater than gallic acid (54%) and chlorogenic acid (56%). Phycocyanin also reduced lipid peroxidation in liver microsomes. (Zhi-Gang *et al.*,1997).

Table 2. Phytochemicals and antioxidant of *spirulina platensis* powder (SPP)

Constituents	Values
Total phenols (mg GAE/100g)	840.60
Total carotenoids (mg RE/100g)	482.50
Total flavonoids (mg/100g)	680.20
Chlorophyll (mg/100g)	1244
DPPH (%)	48.50
ABTS (%)	53.20

GAE= Galic acid equivalent
RE= Routin equivalent

Physicochemical composition of yoghurt fortified with *spirulina platensis* and milk protein concentrate

Table (3) illustrates the physicochemical analysis of fortified yogurt treatments. The total solids content varied from 15.97 to 16.24 per cent (control sample) and T_4 (0.5%MPC+1.5% SPP), respectively. There are significant differences between all treatments. The highest fat content (4.25%) in yogurt containing (0.5%MPC+1.5 %SPP) and the lowest fat content value is for control yoghurt because of the content of fat for SPP (7.12%). The highest value of protein is for yoghurt (1.5% MPC+0.5%SPP) with significant differences between all samples.

Table 3. Physicochemical composition of yoghurt fortified with *spirulina platensis* powder (SPP) and milk protein concentrate (MPC)

Treatments	Moisture (%)	TS (%)	Protein (%)	Fat (%)	Ash (%)	CHO (%)	Calories Kcal/100g	pH	Acidity (%)
T_1	84.03^{a}	15.97^{d}	3.92^{d}	3.89^{d}	0.89^{a}	7.26^{a}	79.73^{d}	4.68^{a}	0.74^{b}
T_2	83.92^{b}	16.08^{c}	4.62^{a}	4.04^{c}	0.90^{a}	6.52^{c}	80.92^{c}	4.60^{b}	0.76^{b}
T_3	83.87^{c}	16.13^{b}	4.56^{b}	4.17^{b}	0.90^{a}	6.50^{c}	81.77^{b}	4.53^{c}	0.78^{b}
T_4	83.73^{d}	16.24^{a}	4.49^{c}	4.25^{a}	0.92^{a}	6.63^{b}	82.73^{a}	4.41^{d}	0.82^{a}

$^{a-d}$ The different superscript letter have a significant difference in the same raw. (Duncan's test P<0.05)
Where: T_1 (control), T_2 (1.5% MPC+0.5% SPP), T_3 (1%MPC+1%SPP), T_4 (0.5%MPC+1.5% SPP)

That is because of the high content of protein for milk protein concentrate (80.20%). Milk protein concentrate and spirulina are known to be a major source of protein. The natural composition of spirulina and protein milk concentrate may be responsible for the yoghurt's compositional change. Spirulina-enriched yoghurt has a higher carbohydrate and ash content. Spirulina powder increased the calorie content of the yoghurt. Similar data were revealed by (Shin *et al*., 2008) and (Malik *et al*., 2013) on yogurts containing up to 0.5% of Spirulina. The results indicate that, acidity values ranged from 0.74 to 0.82 per cent lactic acid (LA) for control sample and yoghurt fortified with (0.5% MPC+1.5% SPP), respectively. Decrease in corresponding pH from 4.68 to 4.41 for control sample and yoghurt fortified with (0.5% MPC+1.5% SPP) with significant differences. (Table 3). Because of the buffering action the extra proteins, phosphates, citrates, lactates, and other miscellaneous milk ingredients, the titrable acidity of milk rose as a result of raising the level of SNF in milk. (Walstra and Jenness, 1984). However, at all degrees of spirulina incorporation, the acidity and pH of the yoghurt were within BIS standards. The results were in agree with (Szigeti *et al*., 2003). Ash, carbohydrates and calories values were in significant differences.

Phytochemical compounds composition of yoghurt fortified with *spirulina platensis* powder (SPP) and milk protein concentrate (MPC)

As shown from results (Table 4), the TPC (Total phenolic compounds) of fortified yogurt ranged between 7.12 to 18.78 mg GAE/ 100 g for T_1 and T_4, respectively. The Data illustrated that T_4 (0.5%MPC+1.5%SPP) had the highest content (18.78 mg GAE /100 g) of TPC followed by T_3 (1%MPC+1% SPP) (17.56 mg GAE /100 g), T_2 (16.80 mg GAE /100 g) and control sample was the lowest value (7.12 mg GAE /100 g). Contents of TPC were significant differences ($P < 0.05$) in different treatments of fortified yogurt. Phenolic chemicals are beneficial to human health and have a variety of therapeutic actions, such as antioxidant activity. (López *et al*.,2011). In fortified yoghurt, spirulina enhanced the amount of phenolic components. This could be attributed to spirulina's high levels of beta-carotene, vitamin E, and oligo-elements. (Agustini *et al*., 2016). The results indicate that, increase of spirulina from 0 to 1.5 per cent resulted in increase in total carotenoids from 0.82 to 7.43 mg RE/100 with significant differences between all treatments. According to the Food and Agriculture Organization, consuming 6 mg of -carotene per day can reduce the incidence of cancer in humans (FAO, 2008). According to these findings, Minea *et al*., 2006, vitamin A levels were found to be highest in yoghurt with spirulina powder. Flavonoids and chlorophyl increased with increasing SPP levels with significant differences between all samples.

Table 4. phytochemical compounds composition of yoghurt fortified with *spirulina platensis* powder (SPP) and milk protein concentrate (MPC)

Compounds Treatments	Total phenolic (mg GAE/100g)	Total carotenoids(mg RE/100g)	Total flavonoids (mg/100g)	Chlorophyl (mg/100g)
T_1	7.12^d	0.82^d	5.45^d	0.00^d
T_2	16.80^c	4.01^c	8.43^c	5.15^c
T_3	17.56^b	5.81^b	9.11^b	11.32^b
T_4	18.78^a	7.43^a	10.41^a	18.55^a

a-d The different superscript letter have a significant difference in the same column. (Duncan's test P<0.05)
Where: T_1 (control), T_2 (1.5% MPC+0.5% SPP), T_3 (1%MPC%+1%SPP) and T_4 (0.5%MPC+1.5% SPP)

Antioxidant properties of yoghurt fortified with *spirulina platensis* powder (SPP) and milk protein concentrate (MPC)

The antioxidant activity of fortified yogurt with *Spirulina platensis* powder (SPP) and MPC (milk protein concentrate) are described in (Table 5). The TA (total antioxidant) concentrations of the yogurt ranged from 22.56% to 57.25%. The highest level ($P < 0.05$) was for T_4 sample, which had high TPC content. Antioxidant activity was increased by adding *spirulina platensis* powder significantly when compared to the other yoghurt treatments; this could be because of the high TA concentration in spirulina powder. Increased levels of chlorophylls and carotenoids in spirulina powder may be responsible for the increase in free radical scavenging. (Ismaiel *et al*.,2016). These results are in agreement with given by Barkallah *et al*.,2017 for fermented milk's antioxidant properties. Spirulina powder contains additional antioxidant chemicals, including beta-carotene, vitamin E, oligo-elements, and an undetermined number of bioactive substances. (Guan *et al*.,2009).

Table 5. Total antioxidant activity of yoghurt fortified with *spirulina platensis* powder (SPP) and milk protein concentrate (MPC)

Treatments	T_1	T_2	T_3	T_4
DPPH %	22.56^d	34.2^c	40.39^b	57.25^a
ABTS %	32.12^d	43.29^c	$46,43^b$	51.14^a

a-d The different superscript letter have a significant difference in the same raw. (Duncan's test P<0.05)
Where: T_1(control), T_2(1.5% MPC+0.5% SPP), T_3(1%MPC+1%SPP) and T_4 (0.5%MPC+1.5% SPP)

Color attributes of fortified yoghurt with *spirulina platensis* powder (SPP) and milk protein concentrate (MPC)

The color of yoghurt is an important factor in determining its acceptability. (Bchir *et al*., 2018). Color parameters of yoghurt fortified yoghurt with spirulina platensis powder (SPP) and milk protein concentrate (MPC)are shown in Table (6). A statistical comparison of these formulations found a significant difference. ($p < 0.05$). This difference could be attributed to the high chlorophyll content of SPP (Ghaeni and Roomiani, 2016; Priyadarshani and Muthumuniarachchi 2018). Indeed, Spirulina is a good source of carotenoids and chlorophylls. (Tang and Suter, 2011). Increasing the concentration of spirulina, significantly decreased L* value ($p < 0.05$) from 112 for control to 58 for T_4 (yoghurt fortified with 0.5%MPC+1.5%SPP), respectively. In addition, During the drying of spirulina, enzymatic and non-enzymatic browning could cause coloring. Moreover, the samples prepared with 0.5%MPC+1.5% SPP (T3) changed color from yellow to greenish (low a* and b*) (Table 6). The quantity of chlorophyll in spirulina powder could explain this color change. (Ghaeni and Roomiani, 2016). As a result, spirulina pigments could be employed in the production of natural milk colorants. Similar results were found by Lee and Lucy 2010. Consumers generally accept natural colorants. Moreover, they are safe non-chemical products

Table 6. Color attributes of yoghurt fortified with *spirulina platensis* powder (SPP) and milk protein concentrate (MPC)

Treatment	T_1	T_2	T_3	T_4
L	112^a	65^b	60^c	58^d
A	-5^a	-8^b	-11^c	-14^d
B	34^a	25^b	19^c	12^d

a-d The different superscript letter have a significant difference in the same raw. (Duncan's test P<0.05)
Where: T_1(control), T_2(1.5% MPC+0.5% SPP), T_3(1%MPC+1%SPP) andT_4(0.5%MPC+1.5% SPP)

Viscosity of fortified yoghurt with *spirulina platensis* powder (SPP) and milk protein concentrate (MPC)

The viscosity of new fermented dairy products is important to its development. The apparent viscosity ranged from 2835 to 3901 mPs (Fig.1). There was a significant difference ($P < 0.05$) for the viscosity values of yogurt samples with the addition of SPP and MPC. T_2 sample (1.5% MPC+0.5% SPP) had the highest viscosity values compared with other treatments. These results in accordance with those reported by Patel and Sapan K. (2011), who resulted that fortified yogurt with MPC had a higher viscosity than that of fortified yogurt with SMP. Agustini et al. (2017) who reported that enriched yoghurt with 1% spirulina had a higher viscosity than that for control yoghurt.

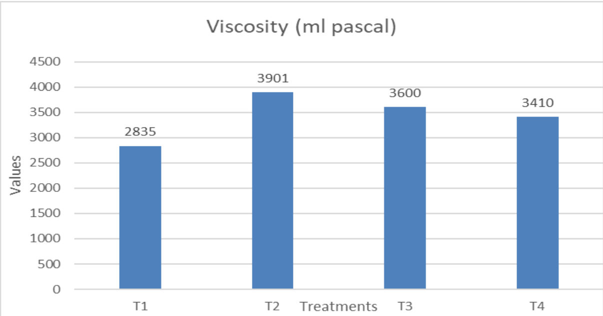

Fig. 1. Viscosity of yoghurt fortified with *spirulina platensis* powder (SPP) and milk protein concentrate (MPC)Where: T_1(control), T_2(1.5% MPC+0.5% SPP), T_3(1%MPC+1%SPP) and T_4 (0.5%MPC+1.5 %SPP).

Microbial count of yoghurt fortified with *spirulina platensis* powder (SPP) and milk protein concentrate (MPC)

Table (7), shows total bacterial count, lactic acid bacterial count (LAB) and molds & yeast count for yoghurt fortified with spirulina and milk protein concentrate.

The number of total count ranged from 94.0 log10 cfu /g for control(T_1) treatment to 57.0 log^{10} cfu /g for T_4. The count of LAB varied from 8.23 to 10.12 log^{10} cfu /g in all treatments.

Increasing the amount of Spirulina powder in fortified yoghurt led in a higher LAB count and faster development. T_4 (0.5%MPC+1.5%SPP) had the highest value of LAB (10.12 log^{10} cfu/g) while, the lowest value of LAB was for T_1(control sample). The LAB can utilize the nutritional value of spirulina to help them grow. Similar trends were recorded by Fadaei et al. (2013) and Agustini et al. (2017) showed that The addition of Spirulina platensis to yoghurt increased the survival rate of LABs in yoghurt, probably due to its high fat, protein, vitamins, and dietary fiber contents.

Data showed that, molds& yeast counts were not detected in all treatments of yogurt. These findings could be attributed to the high hygienic and sanitary standards used in the preparation of yoghurt treatments. Also, because of the importance of LAB in product preservation, as well as their ability to create lactic acid. (Atallah et al., 2020).

Table 7. Microbial count of yoghurt fortified with *spirulina platensis* powder (SPP) and milk protein concentrate (MPC) (log^{10}cfu/g)

Treatments	TBC	LAB	M&Y
T_1	94^a	8.23^d	-
T_2	80^b	8.74^c	-
T_3	74^c	9.50^b	-
T_4	57^d	10.12^a	-

a-d The different superscript letter have a significant difference in the same column. (Duncan's test P<0.05)
Where: T_1(control), T_2(1.5%MPC+0.5% SPP), T_3(1%MPC+1%SPP) and T_4(0.5%MPC+1.5% SPP)

Sensory evaluation of yoghurt fortified with *spirulina platensis* powder (SPP) and milk protein concentrate

Table 8 shows the sensory evaluation of yoghurt treatments. The significant differences ($P < 0.05$) were found in scores of different sensory properties (Color and appearance, body & texture, whey separation, flavor and over all acceptability) among samples of yogurt. The highest scores of over all acceptability ($P < 0.05$) were observed for T_3 (1%MPC+1%SPP) yogurt which is no significant with T_1(control sample). In contrary, the lowest scores ($P < 0.05$) were obtained in T_4(0.5%MPC+1.5% SPP). The addition of spirulina to the yoghurt changed the color from white to green, which could be attributable to the spirulina powder. Panelists evaluated this property on its unsuitable colour and appearance.

Table 8. Sensory evaluation of yoghurt fortified with *spirulina platensis* powder (SPP) and milk protein concentrate

Treatments	Color and appearance (20)	Body and texture (30)	Whey separation (10)	Flavor (40)	Over all acceptability (100)
T_1	18.0^a	27^{bc}	8.0^a	35^a	88.0^a
T_2	16.0^b	28^{ab}	7.5^b	32^c	83.5^b
T_3	18^a	29^a	7.1^c	34^b	88.1^a
T_4	14^c	26^c	8.2^a	30^d	78.2^c

a-d The different superscript letter have a significant difference in the same column. (Duncan's test P<0.05)
Where:T_1 (control),T_2 (1.5%MPC+0.5%SPP),T_3(1%MPC+1%SPP)andT_4 (0.5%MPC+1.5%SPP)

CONCLUSION

Spirulina platensis powder and milk protein concentrate improved nutritional value of fortified yoghurt. Spirulina platensis powder and milk protein concentrate are rich sources of protein. Spirulina platensis powder is a good source of antioxidant which neutralize free radical substances, total phenols, carotenoids, flavonoids and chlorophyll pigment which are important for health.

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الخصائص الوظيفية للزبادى المدعم بطحلب الاسبريولينا ومركز بروتين اللبن

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تهدف الدراسة الحالية الى معرفة تاثير مسحوق طحلب الاسبريولينا ومركز بروتين اللبن وانتاج أربع عينات من الزبادى الوظيفى وهى: T_1 (العينة الكنترول) باستخدام ٢% لبن فرز مجفف ، T_2 (١,٥% مركز بروتين اللبن ، ٠,٥% مسحوق طحلب الاسبريولينا) ، T3 (١% مركز بروتين اللبن، ١% مسحوق طحلب الاسبريولينا) ، T4 (٠,٥% مركز بروتين اللبن ، ١,٥% مسحوق طحلب الاسبريولينا) وتم تحليل مسحوق الطحلب وتقدير الخصائص الكيميائية والكيميائية الحيوية ونشاط المضاد للاكسدة. وكان محتوى مسحوق الطحلب من البروتين (٦٢,٤٠%) والدهن (٧,١٢%) والمواد الفينولية (٨٤٠,٦ مجم GAE / ١٠٠ جم) والكاروتينيدات (٤٨٢,٥ مجم RE /١٠٠ جم) والفلافونويدات (٦٨٠,٦ مجم/١٠٠ جم) وصبغة الكلوروفيل (١٢٤٤ مجم/١٠٠ جم) . والرقم الهيدروجينى لمسحوق الطحلب (٦,٢٩) . وتم تحليل الخواص الفيزوكيميائية لعينات الزبادى والتى تشمل (الرطوبة- المواد الصلبة- البروتين – الدهن – الرماد- الكروهيدرات- السعرات الحرارية- الرقم الهيدروجينى- الحموضة) والخواص الطبيعية . وأوضح نتائج الميكروبيولوجى أن أعلى قيمة لبكتريا حمض اللاكتيك كانت ١٠,١٢ log10 للعينة T_4(0.5 % مركز بروتين اللبن ، ١,٥% مسحوق طحلب الاسبريولينا) ووجد فروق معنوية بين جميع المعاملات وتم التقييم الحسى للعينات وكانت العينة T_3 (١% مركز بروتين اللبن ، ١% مسحوق طحلب الاسبريولينا) أكثر قبولا بين العينات.

الكلمات المفتاحية: طحلب الاسبريولينا، مركز بروتين اللبن، الزبادى، الخصائص الفيزو كيميائية، مضادات الاكسدة