International Food Research Journal 26(5): 1565-1576 (October 2019)

Journal homepage: *http://www.ifrj.upm.edu.my*

Investigation of physicochemical, nutritional, textural, and sensory properties of yoghurt fortified with fresh and dried Spirulina (*Arthrospira platensis*)

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Article history

Received: 10 March, 2019
Received in revised form: 24 July, 2019
Accepted: 6 August, 2019

Keywords

Fresh spirulina,
Yoghurt,
Physicochemical,
Sensory properties,
Principal component analysis

Abstract

The present work investigated the effect of incorporating fresh spirulina as compared to dried spirulina into yoghurt, at three different concentrations (0.1%, 0.3% and 0.5%). The samples were analysed to determine the physicochemical, rheological and sensory properties, and viability of *Streptococcus thermophilus* during storage. Interrelationships between the parameters were investigated using the principal component analysis. Supplementing yoghurt with both *spirulina* forms increased the protein content, acidity, and apparent viscosity, and lessened the syneresis rate. The supplementation of fresh *spirulina* slightly reduced the viability of bacterium during the storage. Yoghurts fortified with fresh *spirulina* yielded different colour parameters, a low amount of protein, ash, and viscosity as compared to yoghurt supplemented with dried *spirulina*. Grouping the variables in the principal component analysis plot indicated that each yoghurt has specific characteristics. Panellists appreciated the yoghurt that was enriched with 0.3% of fresh *spirulina* more than other formulations and attributed the lowest score to appearance. Overall, it can be concluded that untreated spirulina can be exploited as a natural ingredient to develop novel yoghurt with high nutritional properties.

Introduction

Consumers have become more cautious about their diet and health. Their demand for food products with higher nutritional values and health benefits has increased. In fact, protein is considered as the most expensive component of any diet and is an essential ingredient of any balanced diet (Schönfeldt and Gibson Hall, 2012). According to Food and Agriculture Organization (FAO) and World Health Organization (WHO), the single most important nutrient deficiency corresponds to protein (Akalin *et al.*, 2009; Stokes *et al.*, 2018). Indeed, more than two thirds of low-income countries' burden of disease is essentially related to nutritional deficiencies (Akalin *et al.*, 2009). Furthermore, according to WHO, population average requirement is 0.66 g protein/kg body weight per day. Therefore, adding an amount of protein to food could improve the health benefits.

Arthrospira platensis (*spirulina*) has been widely used as a source of protein and for many other nutritional requirements. Research has shown that *spirulina* is rich in protein (~61.57%) and contains a high proportion of essential amino acids (~38.81% of the protein) (Danesi *et al.*, 2010; Beheshtipour *et al.*, 2012; Ghaeni and Roomiani, 2016). *Spirulina* is highly suitable for children and their growth, thanks to its high calcium and iron contents (1043.62 and 338.76 mg/100 g, respectively) (Ghaeni and Roomiani, 2016). It also contains selenium (~0.0488 mg/100 g) and phytopigments (chlorophyll: 1.4% and phycocyanin: 14.1%). These elements are

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powerful antioxidants (Ghaeni and Roomiani, 2016). Therefore, *spirulina* can ensure the whole food and alkaline balance of the human body. Due to its chemical composition, *spirulina* has various health benefits. It slows the development of several diseases, such as cancer, renal failure and high blood pressure (Danesi *et al.*, 2010).

On the other hand, the characteristics of *spirulina* are linked to many parameters such as, its nature, raw material, pre-processing, processing, and storage. Some bioactive compounds inherent in *spirulina* are heat-sensitive. Therefore, processing techniques need to be strictly considered. Desmorieux and Hernandez (2004) showed a decrease of protein and total sugar content after convective drying, oven drying and infrared drying. These treatments could modify the physicochemical, nutritional, rheological, sensory and technofunctional properties of *spirulina*. The characteristics of *spirulina* also depend on whether it is fresh or treated.

Spirulina is often used for human consumption in various forms: tablets, capsules, or food additives such as candy (Christki *et al.*, 2012), gel dessert (Guarda *et al.*, 2004), bakery products (Gouveia *et al.*, 2008), confectionary (Varga *et al.*, 2002) and dairy products (Deepak *et al.*, 2016). As far as the human diet is concerned, adding *spirulina* to dairy products is essential due to its multiple nutritional benefits from proteins, minerals and water-soluble vitamins. Yoghurt is consumed by large segments of population as a part of diet (Felfoul *et al.*, 2017; Martínez-Hernández *et al.*, 2018; Su *et al.*, 2018). Reinforcing these products will effectively decrease diseases linked to nutritional deficiencies. Nevertheless, the current industry and researchers use only dried *spirulina* powder and never a fresh form in the food formulation.

Therefore, the main objectives of the present work were firstly to investigate the effect of incorporating fresh *spirulina* into yoghurt as compared to dried *spirulina*, at three different concentrations (0.1%, 0.3% and 0.5%) after 24 h of storage, and secondly to determinate the impact of different forms (fresh and dried) and concentrations of *spirulina* on the viability of *Streptococcus thermophilus* for 15 d of storage.

Materials and methods

Materials

The basic ingredients used for yoghurt formulations are cow milk, dairy starter culture, sucrose and *spirulina*. Cow milk provided from the morning milking, was cooled down to 4°C in order to be transported to a laboratory within 2 h, where it was used to produce yoghurt. Sucrose was obtained from the local market (Monastir, Tunisia). Mixed commercial starter cultures of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (PAL YOG 3-30 D, Laboratoires STANDA, F-14050 CAEN CEDEX) were also used. *Spirulina* was used in two forms; (1) fresh *spirulina* directly harvested from the cultivation area in Monastir, Tunisia; and (2) oven-dried *spirulina* (Memmert, Germany) at 40°C until getting a final water content below 10%. Then, the powder was ground in a Fritsch laboratory mill with a 1 mm mesh sieve (Haan, Germany).

Preparation of yoghurt

Yoghurt samples were made from cow milk. First, the sucrose (11% w/v milk) was dissolved in the raw milk. Then, *spirulina* was added at the rate of 0.1%, 0.3% and 0.5% (w/v). Milk without the addition of *spirulina* served as control. The mix was subjected to heat treatment (at 85°C for 30 min) to dissolve the microalgae and to pasteurise the mixture. Following pasteurisation, milk was cooled down to 42°C. Then, 2% (w/v) commercial starter culture (10^6 - 10^7 CFU/mL) containing *S. thermophilus* and *L. bulgaricus* was added to the milk. Next, approximately 100 mL of milk were poured into different glass cups covered with plastic covers. The samples were incubated at 42°C for 4 h to reach a pH of 4.3. Then, the yoghurts were stored in a refrigerator (4°C) for 24 h until analyses.

All analytical determinations were performed in triplicate (n = 3). Data were expressed as mean ± standard deviation.

Physicochemical analysis

Chemical analysis

Total solid content of yoghurt was quantified by drying 5 g of yoghurt at 103°C during 7 h in a capsule containing sand (IDF, 1987; Su *et al.*, 2018). According to AFNOR (1989); method NF V04-208, ash contents of yoghurts were determined (incineration at 550°C in a muffle furnace). The total protein contents of yoghurt samples were determined by the Kjeldahl method followed by its conversion by a factor of 6.25., and the milk fat of yoghurts was estimated (IDF, 1997) using Gerber method. Compositional analyses of fortified yoghurts were made 24 h after yoghurt preparation. The Soxhlet solvent extraction method was used to determine the total lipid of yoghurts. The total carbohydrate of yoghurts was determined by the difference of mean value, 100 – (sum of percentage of protein ash, moisture, and lipid) (AOAC, 1995). Titratable acidity of yoghurt was calculated using the potentiometric method according to International Dairy Federation standard (IDF, 1991) and expressed in degree Dornic (°D).

All analytical determinations were performed in triplicate (n = 3). Data were expressed as mean ± standard deviation.

Physical analysis

The pH values of the yoghurt were determined using a pH meter (Kallang, Singapore) at 20°C.

The colour of the *spirulina* and yoghurt was measured using a colorimeter (Lab Scan II, Hunter Associate Laboratory Inc., Reston, VA, USA). Different parameters (L*, a*, b*, ΔE ,C, h°) were determined according to Fermenia *et al.* (1997) and Bchir *et al.* (2018).

Water activity (a_w) was measured using an Aqualab (Switzerland) instrument at 20°C.

Functional properties

Water-holding capacity

The water-holding capacity (WHC) of *spirulina* was measured following a method described by Bchir *et al.* (2018). Briefly, 15 mL of distilled water was stirred with 1 g of sample in a 50 mL centrifuge tube and left overnight at room temperature. The solution was then centrifuged at 15,000 *g* for 20 min and the absorbed water was weighed. WHC was given a gram of water per gram of dry powder.

Oil-holding capacity

The oil-holding capacity (OHC) of *spirulina* was measured following a method reported in the literature (Guillon and Champ, 2000). Briefly, 1 g of samples was added to 15 mL of sunflower oil in a 50 mL centrifuge tube and left overnight at room temperature. The solution was then centrifuged at 15,000 g for 20 min. Free oil was decanted and the absorbed oil was weighed. OHC was calculated as gram of oil per gram of dry powder (AOAC, 1995).

Swelling capacity

The swelling capacity (SWC) of *spirulina* was determined according to Guillon and Champ (2000). Briefly, 1 g of the sample was weighed in a glass cylinder and put to swell at room temperature overnight after adding 15 mL of distilled water. The swelling capacity was indicated as millilitre of swollen sample per gram of initial dry matter.

Sensory properties

Texture measurement

Texture was determined according to Felfoul *et al.* (2017) and Barkallah *et al.* (2017). Instrumental Texture Profile Analysis (TPA) was analysed using the universal Texture Analyser (LLOYD instruments, England) controlled by a computer. All measurements were expressed in a controlled room at 25°C. The TPA technique is a two-cycle compression test, and can quantify texture characteristics of hardness, cohesiveness, chewiness and springiness. The instrument gives two upward positive areas (1 and 2) and two downward negative curves (3 and 4). Areas 3 and 4 were detected just after the first (Area 1) and the second compression (Area 2) (Bouaziz *et al.*, 2010). A fixed quantity of the sample was put in a plastic food container in order to have a constant sample thickness (30 mm). The test was done using a cylindrical probe of 25 mm diameter. The probe was aligned in the middle of the yoghurt. The instrument parameters were set with a pretest speed of 1 mm/s, a test speed of 1 mm/s and a post-test speed of 2 mm/s. The trigger detection force was of 0.005 kg. The probe compressed the yoghurt sample by 50% of its original height (30 mm). The following texture parameters: hardness, adhesiveness, cohesiveness and gumminess were assessed. All measurements were determined in a controlled room at 25°C.

Hardness is defined as a force which is necessary for obtaining the deformation of the probe (Salvador and Fiszman, 2004). It represents the peak force (N) of the first compression for the product. Cohesiveness is defined as a force of internal bonds, which keeps the product as a whole, and was calculated as the area 2 divided by the area 1. Adhesiveness (N/mm) is the force needed to take away the material that adsorbs to a specific surface (area 3). Gumminess (N) is energy necessary for crumbling semi-solid consistency product into a state ready for swallowing (hardness × cohesiveness) (Salvador and Fiszman, 2004).

Syneresis and viscosity of yoghurt samples

Yoghurt syneresis was determined by the centrifugation method of Felfoul *et al.* (2017). Syneresis was determined as the volume of separated whey per 100 mL of yoghurt.

Viscosity of the yoghurt was expressed by a rotational viscometer (DV-III, Brookfield, MA, USA) with spindle no. 6 at the speed of 200 rpm (Su *et al.*, 2018).

Sensory analysis

The sensory acceptance of yoghurt samples was evaluated by a panel of 80 volunteer consumers (40 males and 40 females). The panel consisted of students and staff of the Laboratory of Analysis. Their ages ranged from 23 to 50 years. Then, yoghurt samples were given in a homogeneous way to the panellists. Each studied sample was identified with a three-digit random number and was randomly presented to the panel. Panel members were later asked to rate the likeness on appearance, flavour (odour and taste), after taste, texture and on whey separation of the samples. The rating was done through a seven-point hedonic scale, with 1 = dislike extremely, 4 neither like nor dislike, and 7 = like extremely. The overall acceptability was calculated as the mean value of these sensory proprieties. Yoghurt enriched with *spirulina* (fresh and dried forms) was compared to control to predict the acceptance of the enriched yoghurt by consumers.

Microbiological analysis

The microbiological analysis of yoghurt was carried out for a total viable count of *S. thermophilus* by using yeast glucose agar (YGA) at 37°C anaerobically. The total viable counts of *S. thermophilus* colonies in yoghurts were determined at the zero time, and at each 5 d during storage (15 d) following the procedure previously described by Salvador and Fisczman (2004).

Statistical analysis

Statistical analyses were carried out using a statistical software programme (SPSS for Windows, version 14.0). Analysis of variance (ANOVA) was performed using Duncan test to determine significant differences between the samples ($p < 0.05$). To classify the experimental samples of enriched yoghurt, the principal compound analysis (PCA) was run using XLSTAT software 2018. PCA transforms the original measured variables into new uncorrelated variables called principal components. The first component describes most of the variation in the data. The second principal component is orthogonal and covers much of the remaining variation.

Results and discussion

Chemical characteristics of yoghurt

The effect of enriching yoghurt with fresh and dried *spirulina* on physicochemical composition of yoghurt is shown in Table 1. Results showed that solid content, protein, fat, ash and carbohydrate contents were found to be the highest in *spirulina*-fortified yoghurts. Solid content of control and yoghurts fortified with fresh and dried *spirulina* were ~18%, ~19% and ~20%, respectively. Similarly, Lee and Lucey (2010) showed that the addition of *spirulina* increases the solid contents of yoghurt. Statistical analyses showed a significant difference in the protein content of the enriched samples as compared to control ($p < 0.05$). The protein content significantly increased ($p < 0.05$) in yoghurt with increasing concentration of dried *spirulina* from 2.94% to 3.50% and from 2.16 to 2.67% for fresh *spirulina*. The compositional change of yoghurt might be attributed to the inherent composition of *spirulina*. Table 2 reveals the predominance of protein content in the different *spirulina* forms cultivated in Tunisia (dried: 57% and fresh: 7%). Several authors reported very high protein content (~45%) in *spirulina* (Malik *et al.*, 2013; Barkallah *et al.*, 2017). Table 1 does not show a significant difference in fat content of yoghurt before and after the supplementation of *spirulina* ($p > 0.05$). Ash content of the yoghurt fortified with fresh and dried *spirulina* was ~0.70% and ~0.80%, respectively, which was higher than control. Based on Table 1, the increase in carbohydrate content in *spirulina*-enriched yoghurt was insignificant.

The change in the composition of yoghurt might be caused by the composition of *spirulina*. This finding is in accordance with the results of several studies in the literature conducted with dried *spirulina* (Malik *et al.*, 2013; Kavimandan, 2015).

The chemical composition of yoghurt enriched with fresh *spirulina* was slightly different from that found in yoghurt enriched with dried *spirulina*. A possible solution to increase the consumption of natural substances is fortifying yoghurt with fresh *spirulina*. In fact, Agustini *et al.* (2015) revealed that fresh *spirulina* shows exceptional qualitative and quantitative results in bioactive compounds as compared to that of the dried *spirulina*.

Physical characterisation

Acidity and pH analysis

The results showed that adding *spirulina* at different concentrations (0.1%, 0.3% and 0.5%) to yoghurt increased titratable acidity from 81°D for control to 90°D and 89°D for the yoghurt fortified with 0.5% of dried and fresh *spirulina*, respectively. The titratable acidity of milk increased due to the buffering effect of the additional *spirulina* constituents such as proteins, phosphate, citrate and lactate (Malik *et al.*, 2013). The increase in acidity indicates the reduction of pH from 4.7 for control to 4.6 and 4.5 for

Table 1. Physicochemical parameters of different yoghurts.

		Control	Yoghurt with dried *spirulina*			Yoghurt with fresh spirulina		
		Spirulina concentration						
		0%	0.1%	0.3%	0.5%	0.1%	0.3%	0.5%
Solid content (%)		18.26^{a} ± 0.08	19.00^{b} ± 0.22	19.48^{c} ± 0.33	20.63^{c} ± 0.66	18.81^{b} ± 0.02	19.14^{bc} ± 0.33	19.80^{c} ± 0.69
Protein (g/100 g DM)		2.10^{a} ± 0.05	2.92^{c} ± 0.12	3.10^{d} ± 0.14	3.50 ^{d} ± 0.15	2.16^{b} ± 0.02	2.45^{c} ± 0.05	2.67^{c} ± 0.14
Lipid (g/100 g DM)		3.60^{a} ± 0.14	3.69^{a} ± 0.06	3.72^{a} ± 0.11	3.75^{a} ± 0.10	3.61^{a} ± 0.09	3.65^{a} ± 0.21	3.67^{a} ± 0.11
Ash (g/100 g DM)		0.65^{a} ± 0.02	0.79^{ab} ± 0.07	0.80^{b} ± 0.05	0.82^{b} ± 0.02	0.68^{a} ± 0.06	0.69^{a} ± 0.03	0.71^{a} ± 0.03
Carbohydrate (g/100 g DM)		11.90^{a} ± 0.13	11.98^{a} ± 0.52	12.04^{a} ± 0.69	13.05^{ab} ± 0.79	12.36^{a} ± 0.15	12.35^{a} ± 0.15	12.80^{ab} ± 0.28
Titratable Acidity (°D)		81.67^{a} ± 2.52	88.33^{b} ± 1.22	89.50^{bc} ± 1.40	90.67^{c} ± 1.88	85.33^{b} ± 1.52	87.00^{b} ± 1.36	89.67^{bc} ± 2.06
Syneresis (%)		66.16^{c} ± 3.83	55.93^{b} ± 3.15	53.32^{b} ± 0.81	51.05^{a} ± 1.01	59.31^{b} ± 2.36	57.76^{b} ± 3.81	54.22^{a} ± 2.25
pH		4.70^{a} ± 0.07	4.63^{a} ± 0.02	4.61^{a} ± 0.03	4.58^{a} ± 0.02	4.58^{a} ± 0.03	4.52^{a} ± 0.11	4.50^{a} ± 0.08
a_w		0.980^{a} ± 0.00	0.975^{a} ± 0.005	0.974^{a} ± 0.004	0.972^{a} ± 0.005	0.970^{a} ± 0.005	0.969^{a} ± 0.005	0.968^{a} ± 0.005
Cie-Lab coordinate	*L**	117.22^{f} ± 0.07	113.13^{e} ± 1.79	109.03^{d} ± 1.71	105.74^{c} ± 1.2	106.46^{c} ± 1.32	99.13^{b} ± 2.13	97.31^{a} ± 2.11
	*a**	-5.12^{b} ± 0.03	-5.55^{b} ± 0.42	-6.49^{a} ± 0.29	-7.12^{a} ± 0.53	-6.63^{d} ± 0.34	-7.71^{c} ± 0.32	-7.74^{c} ± 0.01
	*b**	14.42^{bc} ± 0.29	13.80^{b} ± 0.21	13.29^{b} ± 0.39	12.70^{b} ± 0.56	12.05^{b} ± 0.86	11.50^{b} ± 0.6	10.37^{a} ± 0.52
	ΔE	-	4.18^{a} ± 0.42	8.49^{b} ± 1.85	12.03^{c} ± 1.51	13.34^{c} ± 1.90	20.77^{d} ± 2.83	24.14^{d} ± 1.44
	C*	15.3^{a} ± 0.1	16.31^{b} ± 1.09	17.55^{c} ± 1.21	17.69^{c} ± 1.16	20.96^{d} ± 0.85	23.67^{e} ± 0.6	25.37^{f} ± 0.52
	h°	70.44^{a} ± 0.02	78.66^{b} ± 2.49	77.86^{b} ± 3.53	71.91^{b} ± 2.88	87.26^{c} ± 1.02	88.74^{c} ± 0.78	89.08^{c} ± 0.31
Texture parameters	Hardness (N)	0.64^{a} ± 0.02	0.65^{a} ± 0.02	0.67^{ab} ± 0.02	0.68^{b} ± 0.01	0.64^{a} ± 0.02	0.64^{a} ± 0.02	0.65^{a} ± 0.01
	Adhesiveness (N/mm)	0.21^{a} ± 0.03	0.20^{a} ± 0.06	0.19^{a} ± 0.06	0.20^{a} ± 0.05	0.23^{a} ± 0.02	0.22^{a} ± 0.02	0.20^{a} ± 0.04
	Cohesiveness (n.a)	0.33^{a} ± 0.04	0.35^{a} ± 0.09	0.37^{a} ± 0.03	0.38^{a} ± 0.05	0.30^{a} ± 0.02	0.32^{a} ± 0.07	0.33^{a} ± 0.06
	Gumminess (N)	0.21^{a} ± 0.03	0.21^{a} ± 0.05	0.21^{a} ± 0.05	0.20^{a} ± 0.06	0.18^{a} ± 0.04	0.18^{a} ± 0.02	0.19^{a} ± 0.03
Apparent viscosity (mPa.s)		703^{a} ± 4	795^{c} ± 7	851^{e} ± 1	903^{f} ± 4	748^{b} ± 4	788^{c} ± 4	823^{d} ± 4

Means with different letters are significantly different ($p < 0.05$). DM: Dry Matter.

the yoghurt fortified with dried and fresh *spirulina*, respectively (Table1). However, pH value did not fall below pH 4.0, a rate detrimental to probiotic organisms (Varga *et al.*, 2002; Beheshtipour *et al.*, 2012; Ranadheera *et al.*, 2012). These results are in concordance with the findings of Ozturkoglu-Budak *et al.* (2016) who reported a decrease in the pH from 4.53 to 4.0 for the yoghurt samples fortified with *spirulina*. This decrease in pH induces an increase of titratable acidity (95 to 114.5°D). In addition, Szigeti *et al.* (2003) showed that adding cyanobacteria significantly enhances the acid production of yoghurt due to the increased proliferation rate of certain thermophilic dairy cultures.

The stability of the final yoghurt product is assured by the combined effects of pH and water activity (Lee and Lucey, 2010).

Water activity (a_w)

Water is the most abundant component in food and the most significant parameter of food safety (Barkallah *et al.*, 2017). Data demonstrfated that the *spirulina*-enriched yoghurts had similar aw values to that of control (Table 1). In fact, statistical analysis did not show a significant ($p > 0.05$) difference between all the samples.

Colour analysis

The colour of yoghurt is a critical parameter to determine its acceptability (Bchir *et al.*, 2018). Table 1 shows colour parameters of yoghurt with and without *spirulina*. The colour difference or ΔE* (taking the control as reference) showed the influence of *spirulina* on the colour of yoghurt. Statistical analysis revealed a significant difference between these formulations ($p < 0.05$). This difference could be mainly due to the richness of the *spirulina* powder in chlorophylls (Ghaeni and Roomiani, 2016; Priyadarshani and Muthumuniarachchi, 2018). Indeed, *spirulina* is a source of chlorophylls and carotenoids (Tang and Suter, 2011; Barka *et al.*, 2018). In addition, the sample supplemented with *spirulina* presented the highest h° value as compared to control (Table 1). The variation of h° indicated that the colour of yoghurt leaned towards green. Otherwise, we observed a significant increase in C* values, from 15.3 for control to 17.69 and 25.37, respectively for the yoghurt enriched with 0.5% of dried and fresh *spirulina*. Yoghurts enriched with fresh *spirulina* had the highest ΔE*, C*, and h°. In fact, natural *spirulina* has a higher amount of chlorophylls, carotenoids especially provitamin A carotenoids and other nutrients than treated *spirulina* (Ghaeni and Roomiani, 2016). Increasing the concentration of *spirulina*, significantly decreased *L** value ($p < 0.05$) from 117 for control to 105 and 97 for yoghurt fortified with 0.5% of dried and fresh *spirulina*, respectively. Fresh *spirulina* has a less dark colour as compared to dried *spirulina*. In addition, coloration could result from enzymatic and non-enzymatic browning during the drying of *spirulina*. Moreover, the samples prepared with 0.5% of fresh *spirulina* changed colour from yellow to greenish (lowest *a** and *b** values) (Table 1). This colour change could be explained mainly by the abundance of chlorophyll in *spirulina* powder (Ghaeni and Roomiani, 2016). Therefore, the *spirulina* pigments may be used for preparing natural colorants in milk products. Indeed, similar results were showed by Lee and Lucey (2010). Natural colorants are readily accepted by consumers. Moreover, they are safe non-chemical products.

Table 2. Physicochemical parameters of fresh and dried spirulina.

		Fresh *spirulina*	Dried *spirulina*
Dry matter g/100 g		$14.02^a \pm 0.22$	$91.30^b \pm 0.12$
Protein g/100 g DM		$7.39^a \pm 0.33$	$57.28^b \pm 9.42$
Lipid g/100 g DM		$0.55^a \pm 0.01$	$2.08^b \pm 0.36$
Ash g/100 g DM		$3.58^a \pm 0.01$	$12.07^b \pm 0.05$
Carbohydrate g/100 g DM		$3.05^a \pm 0.22$	$19.87^b \pm 9.19$
a_w		$0.959^a \pm 0.00$	$0.410^b \pm 0.00$
Cie-Lab coordinate	L*	$32.03^a \pm 0.30$	$30.73^b \pm 0.21$
	a*	$-3.78^a \pm 0.23$	$-5.74^b \pm 0.07$
	b*	$7.51^a \pm 0.64$	$7.34^a \pm 0.07$
	C*	$8.41^a \pm 0.68$	$9.31^b \pm 0.10$
	h°	$63.23^b \pm 0.61$	$51.98^a \pm 0.10$
	ΔE	$42.21^a \pm 0.58$	$43.74^b \pm 0.13$
WHC (%)		$10.50^a \pm 0.50$	$17.30^b \pm 0.20$
OHC (%)		$3.30^a \pm 0.05$	$7.70^b \pm 0.10$
SWC (%)		$1.30^a \pm 0.05$	$3.30^b \pm 0.05$

WHS: Water-Holding Capacity; OHC: Oil-Holding Capacity; SWC: Swelling Capacity. Means with different letters are significantly different ($p < 0.05$).

Syneresis

Syneresis is a common problem in fermented milk products like yoghurt (Ghaeni and Roomiani, 2016). Serum release is considered as one of the most important parameters that indicates the quality of yoghurt during the different stages of storage (Malik *et al.*, 2013). The increase in the concentration of *spirulina* reduced the syneresis rate in yoghurt. The lowest syneresis was observed with yoghurt supplemented with 0.5% of dried (51.05%) and fresh (54.22%) *spirulina* followed by the other concentrations in a descending order. This could be

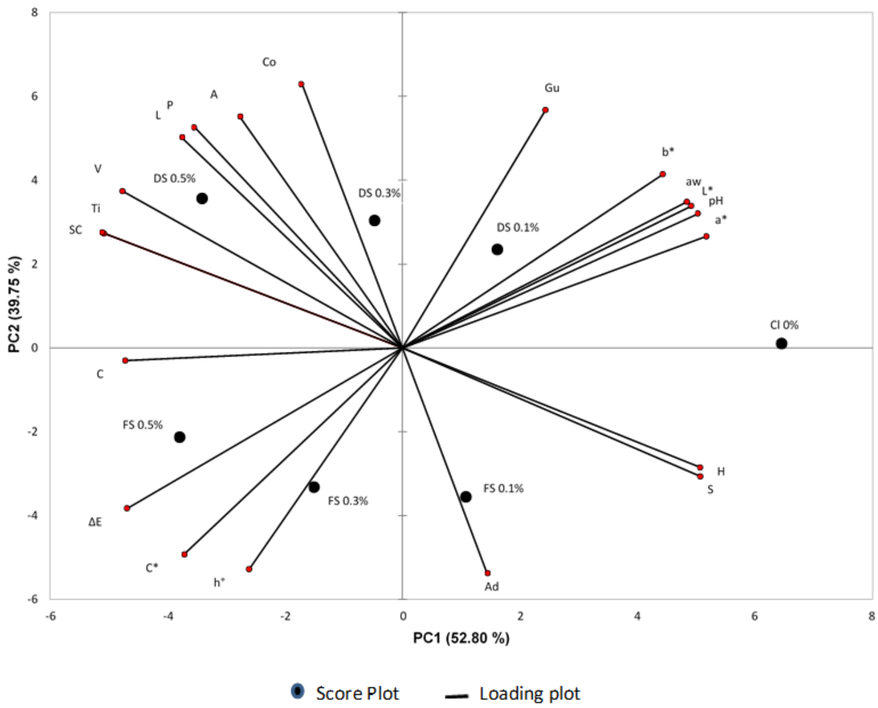

Figure 1. The score and loading plots for PC1 versus PC2. SC: Solid Content; P: Protein; L: Lipid; A: Ash; C: Carbohydrate; Ti: Titrable Acidity; S: Syneresis; *L**, *a**, *b**, ΔE, C*, h°: Cie-lab coordinate; H: Hardness, Ad: Adhesiveness; Co: Cohesiveness; Gu: Gumminess; V: Viscosity; DS: Dried *Spirulina*; FS: Fresh *Spirulina*; Cl: Control.

due to the high carbohydrate and protein contents of *spirulina*. Many authors showed that high dietary fibre and milk fat globules found in yoghurt are correlated with lower syneresis (Hanou *et al.*, 2016; Barkallah *et al.*, 2017). Consequently, dried or fresh *spirulina* could be used as a stabiliser in yoghurt to reduce whey separation and improve texture. Yoghurt fortified with fresh *spirulina* had higher syneresis value as compared to the other formulations. This could be due to the different technofunctional properties of various forms of *spirulina*. Dried *spirulina* had higher water-holding capacity (WHC: 17.3%), oil-holding capacity (OHC: 7.7%) and swelling capacity (SWC: 3.3%) as compared to fresh *spirulina* (Table 2). Malik *et al.* (2013) showed that a high protein content plays an important function in reducing syneresis rate for an ice cream enriched with *spirulina* powder.

Viscosity

Table 1 shows that the resistance of a fluid to deformation under shear stress varied with the supplementation of *spirulina*. The viscosity value significantly increased ($p < 0.05$) with increasing concentration of *spirulina*. Yoghurt without *spirulina* had the lowest viscosity value (700 mPa.s). Hanou *et al.* (2016) revealed that the growing interactions between the constituents of *spirulina* such as protein is the principal factor responsible for the increase of the viscosity of yoghurt enriched with dried *spirulina*. The addition of 0.5ff% of dried *spirulina* had the most marked effect on the viscosity values (903 mPa.s) as compared to the viscosity of yoghurt enriched with fresh *spirulina* (823 mPa.s). This result could be due to the high solid content in dried *spirulina* as compared to the fresh *spirulina*.

Texture profile analysis

Texture is considered as the most determining parameter to assess the quality of a product. Indeed, sensory properties are highly correlated with textural parameters (Meullenet *et al.*, 1997; Ben Halima *et al.*, 2015). In addition, textural modification is one of the most existent forms of assessment and treatment of swallowing difficulties (dysphagia) (Meullenet and Gross, 1999). Textural parameter values (hardness, adhesiveness, cohesiveness and gumminess) of unfortified and yoghurts enriched with different concentrations of *spirulina* are shown in Table 1. The texture profiles of the yoghurts were taken after 24 h storage at 4°C. Increasing the concentration of *spirulina* resulted in an increase in hardness values. This shows that the gel network of yoghurt was affected by the addition of *spirulina*, which indicates an increase in the curd strength of yoghurt. Malik *et al.* (2013) demonstrated the increase in curd strength

Table 3. Sensory attribute ratings of the 24-h-yoghurts.

	Control	Yoghurt with dried spirulina			Yoghurt with fresh spirulina		
	Spirulina concentration						
	0%	0.1%	0.3%	0.5%	0.1%	0.3%	0.5%
Appearance	$4.93^{b} \pm 0.34$	$4.8^{b} \pm 0.15$	$4.70^{b} \pm 0.08$	$3.77^{a} \pm 0.38$	$4.75^{b} \pm 0.06$	$4.70^{b} \pm 0.08$	$4.42^{a} \pm 0.10$
Taste	$5.10^{b} \pm 0.22$	$5.08^{b} \pm 0.21$	$5.12^{b} \pm 0.42$	$4.39^{a} \pm 0.69$	$5.05^{b} \pm 0.12$	$5.25^{b} \pm 0.2$	$4.88^{ab} \pm 0.38$
Aftertaste	$4.95^{c} \pm 0.21$	$4.90^{c} \pm 0.18$	$4.98^{c} \pm 0.27$	$3.95^{a} \pm 0.55$	$4.85^{c} \pm 0.13$	$4.93^{c} \pm 0.26$	$4.58^{b} \pm 0.28$
Texture	$5.10^{b} \pm 0.15$	$5.05^{b} \pm 0.17$	$5.10^{bc} \pm 0.26$	$4.63^{a} \pm 0.43$	$4.95^{b} \pm 0.27$	$5.20^{bc} \pm 0.05$	$4.33^{a} \pm 0.37$
Odour	$4.75^{b} \pm 0.04$	$4.69^{b} \pm 0.19$	$4.76^{b} \pm 0.25$	$3.78^{a} \pm 0.25$	$4.82^{b} \pm 0.06$	$5.08^{c} \pm 0.20$	$4.63^{b} \pm 0.19$
Whey separation	$3.48^{a} \pm 0.13$	$3.45^{a} \pm 0.14$	$4.20^{b} \pm 0.16$	$4.38^{b} \pm 0.16$	$3.61^{a} \pm 0.21$	$4.42^{b} \pm 0.12$	$3.90^{a} \pm 0.15$
Overall acceptability	$4.72^{b} \pm 0.07$	$4.66^{b} \pm 0.15$	$4.87^{b} \pm 0.08$	$4.10^{a} \pm 0.19$	$4.67^{b} \pm 0.15$	$5.03^{c} \pm 0.14$	$4.45^{a} \pm 0.29$

Means with different letters are significantly different ($p < 0.05$).

with increasing level of *spirulina* powder, which could be attributed to the acidification by lactic acid cultures which strengthen the 3D network by entrapping water and also due to the higher protein content of *spirulina*. The increase in protein content in yoghurt enhances protein interaction, which results in the formation of the firm gel (Guarda *et al.*, 2004). However, statistical analysis did not show a significant difference between the hardness of control and those enriched with dried and fresh *spirulina*.

On the other hand, the incorporation of *spirulina* into yoghurt did not significantly change ($p > 0.05$) the adhesiveness, cohesiveness and gumminess of the samples, which were still similar to that of control.

In general, the enrichment of yoghurt by *spirulina* improved the functional and nutritional quality as well as the health benefits without a significant modification in the textural properties. The textural parameters of yoghurt enriched with fresh and dried *spirulina* were slightly different.

Principal component analysis

The physico-chemical results were subjected to principal component analysis (PCA). The PCA plot of yoghurt samples is shown in Figure 1 which demonstrates: (a) interrelation among analysed parameters; and (b) positioning of analysed yoghurt in comparison to each other. The first axis (PC1) accounted for 52.80% of the total variance and the second (PC2) for 39.75%, accounting for 92.55% of the total variance. From the third axis or principal compounds, the accumulated variance did not satisfactorily increase to justify adopting it. In this plot, we can observe that PC1 axis was negatively correlated (localised in the negative axis of PC1) with five variables (solid content, carbohydrate, ΔE, titratable acidity and viscosity) and positively correlated (localised in the positive axis of PC1) with syneresis, pH, aw, L^*, a^*, b^* and hardness. On the other hand, the variables C*, h° and adhesiveness were negatively loaded with the second axis, whereas protein, lipid, ash, cohesiveness and gumminess were positively loaded.

According to the results of the PCA, the yoghurt samples appeared to be separated into five well-defined groups: (1) yoghurt enriched with 0.5% and 0.3% of dried *spirulina*, (2) yoghurt enriched with 0.1% of dried *spirulina*, (3) yoghurt enriched with 0.5% and 0.3% fresh *spirulina*, (4) yoghurt enriched with fresh *spirulina* 0.1%, (5) control (0% *spirulina*). This confirms our previous results showing that different formulations have different physicochemical parameters. However, yoghurt fortified with 0.3% and 0.5% of *spirulina* had closer results. The addition of *spirulina* involves a modification of the physicochemical characteristics of the yoghurt.

The PCA results revealed that: (1) cohesiveness, ash, protein, lipid, viscosity, solid content, and titratable acidity were responsible for the separation of the first group (yoghurt enriched with 0.5% and 0.3% dried *spirulina*), and (2) gumminess, colour (a^*, b^*, L^*), a_w, and pH contributed to the separation of the second group (yoghurt enriched with 0.1 % dried *spirulina*). These results indicate that the increase of *spirulina* content increased the number of correlated variables. Additionally, the colour coordinates (L^*, a^*, b^*) were grouped together on the right side of the PCA plot, and the yoghurts enriched with dried *spirulina* (0.3% and 0.5%) were grouped on the left side of PCA plot. The visualisation of these factors indicates that samples with higher *spirulina* content were darker and more colourful to attract consumers.

Concerning the yoghurts fortified with fresh *spirulina*, similar results were found. Indeed, yoghurt enriched with 0.5% and 0.3% of fresh *spirulina* were characterised by descriptors such as carbohydrate and

Table 4. Viability of Streptococcus thermophilus during storage at 4°C of yoghurt samples (CFU/mL).

Day	Control	Yoghurt with dried spirulina			Yoghurt with fresh spirulina		
	Spirulina concentration						
	0%	0.1%	0.3%	0.5%	0.1%	0.3%	0.5%
0	$408.18^{b} \times 10^{4} \pm 18.36$	$410^{b} \times 10^{4} \pm 16.81$	$406.81^{b} \times 10^{4} \pm 24.54$	$405.91^{b} \times 10^{4} \pm 18.68$	$390^{ab} \times 10^{4} \pm 21.25$	$381.81^{a} \times 10^{4} \pm 41.38$	$365.45^{a} \times 10^{4} \pm 16.81$
5	$300^{a} \times 10^{4} \pm 63.77$	$310.9^{a} \times 10^{4} \pm 36.31$	$384.54^{b} \times 10^{4} \pm 13.3$	$388.63^{b} \times 10^{4} \pm 13.62$	$355.45^{c} \times 10^{4} \pm 42.91$	$348.18^{b} \times 10^{4} \pm 19.7$	$318.18^{a} \times 10^{4} \pm 3.27$
10	$233.63^{a} \times 10^{4} \pm 28.34$	$225.45^{a} \times 10^{4} \pm 22.20$	$237.27^{a} \times 10^{4} \pm 7.18$	$244.54^{a} \times 10^{4} \pm 15.00$	$336.81^{c} \times 10^{4} \pm 77.09$	$292.72^{ab} \times 10^{4} \pm 36.6$	$283.62^{ab} \times 10^{4} \pm 77.75$
15	$130^{a} \times 10^{4} \pm 10.63$	$200.27^{b} \times 10^{4} \pm 11.27$	$233.18^{b} \times 10^{4} \pm 13.65$	$255.54^{b} \times 10^{4} \pm 7.33$	$258.18^{b} \times 10^{4} \pm 5.72$	$249^{b} \times 10^{4} \pm 12.19$	$264.45^{b} \times 10^{4} \pm 36.02$

Means with different letters are significantly different ($p < 0.05$).

colour (ΔE, c* and h°) showing that the increase of *spirulina* concentration in yoghurt greatly influenced the colours of samples.

Concerning the yoghurt enriched with 0.1% of fresh *spirulina*, it was characterised by other different parameters (adhesiveness, syneresis and hardness). The addition of a small amount of *spirulina* modified a correlated variable of each formulation. However, the untreated yoghurt was not attached to any variable.

Sensory evaluation

Sensory analyses were obtained using 80 untrained participants. Each participant consumed six yoghurt samples per week as reported in the demographic questionnaire. The average scores for the overall acceptability, appearance, taste, aftertaste, texture, odour and whey separation are given in Table 3. Results showed that the addition of *spirulina* with different forms and concentrations to yoghurt modified the scores of all sensory parameters. Table 3 reveals that appearance score for all formulation ranged from 3.77 to 4.93 and was higher in control. The decrease in appearance score with the increase in the rate of incorporated *spirulina* from 0.3 to 0.5% may be attributed to the light green colour of *spirulina*. Similar results were recorded by Malik *et al.* (2013) who reported that higher level of replacement resulted in intense green colour and sedimentation of *spirulina* which might be the reason for the lower scores for appearance and overall acceptability. Indeed, yoghurt enriched with 0.5% dried or fresh *spirulina* received the lowest score for all the sensory characteristic parameters. Therefore, consumers did not appreciate yoghurt containing a high *spirulina* content. Statistical analysis showed a significant difference ($p < 0.05$) between yoghurt enriched with 0.5% fresh or dried *spirulina* and the other formulations (Table 3). Duncan's test shows mean scores with different letters. The decrease in score with increasing concentration could probably be due to the increase in acidity (Barkallah *et al.*, 2017; Agustini *et al.*, 2017). However, using a low level of *spirulina* in yoghurt did not have a significant impact in all sensory parameters scores. Table 3 reveals a similar score between control and the yoghurt fortified with 0.1% of fresh or dried *spirulina*. Statistical analysis did not show a significant difference between control and yoghurt fortified with 0.1% of *spirulina*. Nevertheless, the scores obtained for appearance, taste, aftertaste, texture, whey separation and odour of the sample supplemented with 0.3% of fresh and dried *spirulina* were higher than control. The overall acceptability score reflected in the scores obtained by all sensory parameters of yoghurt supplemented with 0.3% of fresh *spirulina* was significantly ($p < 0.05$) higher. As a conclusion, the addition of 0.3% of fresh *spirulina* to yoghurt was most appreciated by panellists and was judged a superior product in comparison to other treated yoghurt products. On the other hand, sensory characteristics showed that appearance and whey separation had the lowest score and were the limiting factors for consumer acceptability.

Viability of yoghurt culture

Yoghurts were analysed for the viability of *S. thermophilus* at a regular interval of 5 d (Table 4) during storage (15 d). During the initial stage, viable count of *S. thermophilus* significantly decreased ($p < 0.05$) with the supplementation of *spirulina*. Initially, the supplementation of yoghurt with *spirulina* decreased the viability of S. thermophilus from 408.18 × 10^4 CFU/mL in control to 405.91 × 10^4 CFU/mL in the yoghurt enriched with 0.5% of dried *spirulina*, and 365.45 × 10^4 CFU/mL in the yoghurt fortified with 0.5% of fresh *spirulina*. With the progress of storage up to 15 d, the viable count decreased to 130 × 10^4 CFU/mL in control and to 255.54 × 10^4 – 264.45 × 10^4 CFU/mL in dried and fresh *spirulina* yoghurt (0.5% level), respectively. Results are consistent with the international recommendations. In fact, worldwide agreement has been reached on recommended levels; generally, the values of 10^6 CFU/mL and 10^7 or 10^8 CFU/mL are accepted as minimum and satisfactory levels (Sohrabvandi *et al.*, 2010; Azizkhani and Parsaeimehr, 2018). Yoghurt fortified with 0.5% fresh *spirulina* had the highest viability of bacteria after 15 d of storage as compared to the other formulations. Statistical analysis did not show a significant difference ($p > 0.05$) between viability of *S. thermophilus* observed in yoghurt enriched with fresh and dried *spirulina*. The decrease in the viability of the yoghurt culture could mainly be due to the increased acidity during storage. Law *et al.* (1993) and Pittaya (2015) observed that the increase in acidity during yoghurt preparation reduces the viability of *S. thermophilus* and *L. bulgaricus*. Table 4 shows that the viability of bacterium in enriched yoghurt was higher (up to 15 d of storage) than that of control. This could be due to the beneficial contribution of *spirulina*. *Spirulina* is known to have beneficial influence on the survival of the starter bacterial culture due to its high protein, essential fatty acids such as gamma linolenic acid, vitamins and minerals (Beheshtipour *et al.*, 2012).

Conclusion

The supplementation of *spirulina* into food products produces functional food and improves its nutritional value. Fresh *spirulina* proved its efficient use as innovative and attractive additive in yoghurt processing. Addition of fresh or dried *spirulina* to yoghurt at concentrations of 0.5% increased the nutritional quality (protein), the viscosity, and affected the sensory attributes of the final product. The application of PCA explored the data more efficiently revealing patterns that indicate grouping tendency among the samples. In fact, the yoghurts supplemented with 0.3% and 0.5% fresh *spirulina* corresponded to one of five groups and were essentially correlated with colour parameters. The grouping of variables and parameters in PCA plots indicated specific yoghurts properties, which are important for their potential commercial or industrial use. Moreover, fresh *spirulina* addition had a beneficial influence on the survival of the bacterial starter culture. Fresh *spirulina* could be used successfully as a food ingredient to develop new formulations of yoghurt. Based on the obtained results, yoghurt fortified with fresh *spirulina* meets satisfactory levels of physicochemical properties and nutritional values as compared to yoghurt enriched with dried *spirulina*. In addition, from hedonic analysis results, the panellists appreciated more the yoghurt that was enriched with 0.3% of fresh *spirulina* than the other formulations. Sensory characteristics showed that appearance and whey separation, having the lowest score, were the limiting factors for consumer acceptability. Thus, the use of coating elements such as caramel, chocolate or fibre is recommended to enhance these parameters.

The use of fresh *spirulina* in yoghurt formulation would firstly lead to a more convenient food that satisfies consumers and provides health benefits due to its great nutritional contents as compared to dried *spirulina*. Secondly, it eliminates the need for the drying, which is a very costly process.

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