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Effect of *Spirulina platensis* fortification on physicochemical, textural, antioxidant and sensory properties of yogurt during fermentation and storage

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ABSTRACT

Due to the high consumption rate of fermented milk products such as yogurt, the fortification of these products will effectively reduce diseases associated with nutritional deficiencies. In the present study, after incorporating *Spirulina* into yogurt at four different concentrations (0.25, 0.5, 0.75 and 1%), we studied its effect on the fermentation process, texture, nutraceutical and sensory characteristics of yogurt. The addition of 0.25% of *Spirulina* was significantly sufficient to accelerate the end of fermentation ($p < 0.05$) and conserve the textural properties and sensory acceptability of the final milk product. This treatment also exhibited significant better water holding capacity and lower whey syneresis during 28 days of storage. During this period, the colored yogurt showed negligible variations for the L*, a* and b* indices, reflecting the strong stability of *Spirulina* color. Thanks to its high content in pigments, *Spirulina* considerably improve the antioxidant activity of the new formulated yogurt. Overall, it can be concluded that *Spirulina* can be used as a natural ingredient to develop a novel yogurt with high nutritional properties.

1. Introduction

Milk and dairy products have an important role in human diet due to their many nutritional benefits from proteins, lactose, minerals and water-soluble vitamins (Ozturkoglu-Budak, Akal, & Yetisemiyen, 2016). They are produced and consumed massively in many countries (Caleja et al., 2016). Despite their beneficial effects on health, these products are not usually considered as an important source for bioactive substances (Gahruie, Eskandari, Mesbahi, & Hanifpour, 2015). Recently, scientists of nutrition have mentioned that the fortification of milk products using natural resources is one of the best ways to ameliorate the overall dietary intake of food with minimal undesirable effects (Gahruie et al., 2015). In this way, yogurt has begun to attract the attention of consumers because of its pleasant creamy taste and increased health benefits.

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 (Carocho, Barreiro, Morales, & Ferreira, 2014; Randhawa & Bahna, 2009). Therefore, the need to use safe additives has prompted researchers to prepare additives from natural sources that would be appropriate for the application on dairy products (Beheshtipour, Mortazavian, Haratian, & Darani, 2012; Dönmez, Mogol, & Gökmen, 2017; Senaka Ranadheera, Evans, Adams, & Baines, 2012).

Microalgae have been commercially exploited, and the mainly used genera are *Dunaliella, Chlorella* and *Arthrospira* for functional food (Beheshtipour et al., 2012). *A. platensis*, also known as *Spirulina*, is the most popular microalgal species because of its high protein content (65%) and its great nutritional value (Beheshtipour et al., 2012). It has been proved that *Spirulina* has divers possible

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health-promoting beneficial effects in the prevention and treatment of various diseases, such as cancers, renal failure (Ghaeni & Roomiani, 2016), hypertension (Suliburska, Szulińska, Tinkov, & Bogdański, 2016) and male infertility. This is due to its chemical composition which includes compounds like essential amino acids, fatty acids, vitamins and many essential minerals and enzymes. Besides its nutritional value, it has been found to exhibit antibacterial and antifungal activities against some human pathogens (Ahsan et al., 2015; Usharani, Srinivasan, Sivasakthi, & Saranraj, 2015) and promote growth of lactic acid bacteria in milk and dairy products (Beheshtipour et al., 2012).

Although the technological impacts of *Spirulina* on the growth performance of probiotics in fermented milk products have been investigated (Beheshtipour et al., 2012), a research determining its effects on the nutritional and sensorial qualities of yogurt has not been addressed before.

The main objectives of the current study was to produce a new Tunisian yogurt fortified with *Spirulina*, which is rich in bioactive compounds, and to evaluate its effects on the functional, physicochemical, instrumental textural, and microbiological properties of the obtained dairy dessert during 28 days of storage.

2. Material and methods

2.1. The study design and yogurt production

The whole yogurt manufacturing process was carried out according to the production chain of Tunisian industries. Four yogurt treatments containing different concentrations (0.25, 0.50, 0.75 and 1.00%) of *Spirulina* powder (Bio Algues, Mahdia, Tunisia), were produced using the analyzed cow milk and subjected to heat treatment (at 85 °C for 30 min) to dissolve the microalgae powder and to pasteurize the mix. Zero % concentration of *Spirulina* was defined as control. Prepared milks with or without *Spirulina*, were inoculated with a mixed commercial starter culture YC-X11 (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) according to the manufacturer's instructions (Chr. Hansen, Denmark). The mixtures were transferred into 100 ml plastic cups and incubated at 42 °C for 4 h to achieve a pH of 4.3 ± 0.02. Biochemical parameters including pH drop and acidity increase were monitored throughout the fermentation period (every 1 h). After fermentation, yogurt samples were cooled and transferred to a refrigerator at 4 °C. These samples were analyzed directly after production and after 7, 14, 21 and 28 days of refrigerated storage.

2.2. Physicochemical measurements

The pH of unfortified and *Spirulina*-fortified yogurt samples was measured after the calibration of the pH meter (HACH, Loveland, Colo., U.S.A.) with standardized pH buffer solutions (4.0, 7.0 and 10.0) prior to analysis. The titratable acidity (TA) of yogurts was determined by titrating 9 g of sample with 0.1 N NaOH solution using phenolphthalein as the indicator. TA was expressed as a percentage of lactic acid produced. The total solids of samples were determined by drying samples at 105 °C overnight to constant weight using an air oven (Thermoline Scientific, Australia). Ash content was measured by ignition of dried samples in an electric furnace (Labec Laboratory Pty Ltd, Marrickville, NSW, Australia) to burn off all the organic matter at 550 °C. Concentrations of calcium and iron were measured by atomic absorption spectroscopy. The protein content was analyzed by the Kjeldhal method using a nitrogen conversion factor of 6.38. The total lipids of yogurt samples were extracted according to the modified procedure of Bligh and Dyer (1959) with chloroform/methanol/water (2/1/1).

The fatty acid methyl esters (FAMEs) of total lipids were obtained by adding 500 μL of KOH (1N)–CH_3OH (2N) to the extracted lipids and heating for 10 min at 40 °C. 500 μL of *n*-hexane was added to the reaction mixture. The FAMEs in the supernatant were analyzed using gas chromatography (GC, Shimadzu GC-17A, Shimadzu Scientific Instruments, Columbia, Maryland, USA).

The total soluble sugar content was determined through the method described by Dubois, Gilles, Hamilton, Rebers, and Smith (1956).

The determination of insoluble, soluble and total dietary fibers in yogurt samples was effectuated by the nonenzymatic-gravimetric method based on the precipitation of fibers with ethanol.

For the determination of chlorophylls and carotenoids content, 1 ml of each sample was centrifuged at 5000 rpm for 10 min. The pellet was dissolved and then sonicated in 1 ml of ethanol at 65 °C for 30 min. After sonication, the solutions were centrifuged at 10000 rpm for 5 min. The pigments content was estimated by measuring the supernatant absorbance (A) at 666, 653 and 470 nm and calculated using the following equations (Kumar, Ramakritinan, & Kumaraguru, 2010; Lichtenthaler & Wellburn, 1985);

(1) [Chlorophyll *a*] (mg L^{-1}) = 15.65 × A_{666}–7.340 × A_{653}
(2) [Chlorophyll *b*] (mg L^{-1}) = 27.05 × A_{653}–11.21 × A_{666}
(3) Total Chlorophylls (mg L^{-1}) = Chlorophyll *a* + Chlorophyll *b*
(4) Carotenoids (mg L^{-1}) = (1000 × A_{470} − 2.860 × [Chlorophyll *a*] − 85.9[Chlorophyll *b*])/245

The determination of soluble pigment c-phycocyanin was done according to the protocol described by Bennett and Bogorad (1973).

Physicochemical parameters were measured after one week of storage, except for pH and titrable acidity which were measured after 2, 3 and 4 weeks of storage.

2.3. Susceptibility to syneresis (STS) and water-holding capacity (WHC)

The yogurt STS was evaluated according to the method of Isanga and Zhang (2009) by placing 100 ml of each sample in a funnel lined with Whatman filter paper number 1 (Whatman International Ltd., Maidstone, England). After 6 h of drainage, the volume of whey was measured and used as an index of syneresis. The following formula was used to calculate STS:

$$STS\ (\%) = (V1/V2) \times 100$$

where: V1 = Volume of whey collected after drainage; V2 = Volume of yogurt sample.

The WHC of yogurts was measured by the centrifugation of 5 g at 4500 × g for 15 min at 4 °C. The WHC was calculated as follows:

$$WHC\ (\%) = (1 - W1/W2) \times 100$$

where, W1 = Weight of whey after centrifugation, W2 = Yoghurt weight (Isanga & Zhang, 2009).

2.4. Dairy dessert properties

2.4.1. Sensory evaluation

The sensory evaluation of standard and *Spirulina* fortified yogurts (stored at 4 °C) was conducted by 32 panelists aged 20–40 years, 8 days after production. The tasting panel consisted of researchers and staff recruited in the Tunisian industry. Each panelist received 2 samples of yogurt to evaluate and comment on the sensory characteristics. The panelists were asked to evaluate the

taste, color and appearance, body and texture, flavor and overall acceptability of the final product depending on a nine-point hedonic scale, ranging from 9 (like extremely liked) to 1 (extremely disliked) for each organoleptic characteristic.

2.4.2. Color evaluation

The evaluation of the color samples was carried out using a colorimeter (Konica Minolta, Chroma Meter, CR400, Japan). The instrument was standardized using standard white plates. An average value was determined by taking observations from five different points (three on the top and two on bottom) of the same sample, and the CIE L*a*b* color space values were registered.

2.4.3. Instrumental texture analysis

All instrumental texture analyses were done on dairy samples stored at least for 24 h at 4 °C, using a texture analyzer (Texture Analyzer, TA Plus, LLOYD instruments, England). The test was carried out directly in a 100-g plastic sample cup, using a 12-mm back extrusion probe. Then, samples were compressed to 50% of their original thickness in a double cycle with a rate of 40 mm/min. The texture profile parameters, namely elasticity (mm), firmness (N), and cohesiveness were computed from the resulting force-deformation curves. Three different cups were measured for each sample.

2.5. Evaluation of antioxidant properties by DPPH and ferric reducing antioxidant power (FRAP) methods

The free radical scavenging activity of yogurt samples was evaluated according to the method described by Bersuder, Hole, and Smith (1998) using as indicator the 1,1-diphenyl-2-picrylhydrazil (DPPH). The reducing power of freeze dried yogurt samples was determined according to the method of Yildirim, Mavi and Kara (2001). All reactions were carried out in triplicates.

2.6. Microbial analyses

Testing for Coliforms, Yeast and Mold was according to Standard Methods for the Examination of milk Products (Marth, 1978), using the Violet Red Bile Agar (VRB) and acidified Potato Dextrose Agar (PDA) mediums respectively.

2.7. Statistical analysis

All analytical determinations were performed at least in triplicates and values were expressed as the mean ± standard deviation (SD). One-way ANOVA and Duncan's multiple comparison tests were used to compare results with significant differences ($P < 0.05$). IBM SPSS statistics software version 19 (IBM Corp., USA) was used to perform all statistical analyses.

3. Results and discussion

3.1. Preliminary results

The level of adding *Spirulina* to yogurt was optimized. Fig. 1 shows changes in pH and acidity during the fermentation period (4 H) in different yogurt treatments. For all treatments, the minimum decrease rates of pH as well as the minimum increase rates of acidity were observed within the initial fermentation steps that could be affected to the being in the early stages of bacterial growth and also to the relatively high buffering capacity of milk products (Beheshtipour et al., 2012). The time of maximum pH drop (tmax-pH-D) in treatments containing *Spirulina* was 60–120 (min–min) (Fig. 1B, C, D, E), while this time for the control was 120–180 (Fig. 1A). For example, the treatment with 0.75% of *Spirulina* showed 0.83 decline in pH (5.73–4.9) at the minutes 60–120 of fermentation (Fig. 1D), while this pH decline value was 0.08 (6.18–6.1) in control (Fig. 1A) ($p < 0.05$). In other words, in treatments with *Spirulina*, the fastest pH-decline period (60–120 during fermentation) finished at a pH less than 5, except for *Spirulina*-0.25%. However, there was no significant difference between samples containing 0.25, 0.5, 0.75 and 1% microalgae powder. Similarly, pH values in the milk inoculated with the mixed culture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* and containing *Spirulina* decreased more rapidly than in control samples during the period of fermentation (Varga, Sziget, & Ördög, 1999). These treatments also showed significantly greater mean acidity increase rates ($p < 0.05$). These characteristics can be attributed to the different buffering capacity effects of the treatments. Samples containing *Spirulina* exhibited lower buffering capacity during the fermentation process. Furthermore, *Spirulina* stimulates significantly the viability and growth rates of lactic acid bacteria (Beheshtipour et al., 2012).

Textural modification is one of the most existent forms of assessment and treatment of swallowing difficulties (dysphagia) (Ben Halima et al., 2015). It is a key criteria used to determine the organoleptic quality of milk products. Indeed, textural parameters highly correlated with sensory properties supply more information than the classic evaluation (Steele et al., 2015). The values of the textural parameters (firmness, cohesiveness and elasticity) of unfortified and fortified yogurts with different percentages of *Spirulina* are shown in Table 1. It was found that the maximal firmness values were attributed to control yogurts. The firmness of the control yogurt (0.67 N) was similar to that supplemented with 0.25% *Spirulina* (0.62 N) ($p = 0.302$), but the firmness was significantly lower in yogurts made with 0.5% ($p < 0.05$), 0.75% ($p < 0.001$) and 1% ($p < 0.001$) of microalgae powder. The increased concentration of *Spirulina* affected the firmness of the final product by disrupting the gel network. On the other hand, the incorporation of *Spirulina* in yogurts didn't change significantly the cohesiveness of samples, which was still similar to that of control samples ($p > 0.05$). Moreover, there was no significant difference between controls and samples containing 0.5, 0.75, and 1% of microalgae in terms of elasticity, while treatment with 0.25% of *Spirulina* exhibited the greater elasticity attributes ($p = 0.03$).

The sensory ratings of individual products for color, flavor, taste, mouth texture, and overall acceptability are presented in Table 2. The result of hedonic scale showed the highest scores for yogurts containing 0.25% of *Spirulina*. As represented, treatments with higher microalgae concentrations (0.75 and 1%) possessed lower sensory acceptability for all organoleptic properties compared to the control ($p < 0.001$). The unsuitable flavor caused by the supplementation of *Spirulina* is linked to the generated compounds from the oxidation of lipids as well as minerals that not only act as pro-oxidant molecules but also might produce metallic off-flavors (Shimamatsu, 2004). Flavor scores show that there were no significant differences between the control yogurts and yogurts fortified with 0.25% of microalgae ($p = 0.145$) (Table 2). The treatment with 1% of *Spirulina* had the lowest flavor score (5.45; $p < 0.001$). The addition of *Spirulina* into the yogurt changed the color of this milk product from green to blue due to the added microalgae concentration. This characteristic was admitted as an unsuitable sensory propriety (appearance) by panelists ($p < 0.001$). Moreover, graininess produced by insoluble *Spirulina* particles was noted mostly in treatments of 0.75 and 1% of microalgae. However, differences were remarkable from the point of view of oral-texture ($p < 0.001$). Treatments containing 0.75 and 1% of microalgae have the weakest scores for the parameters of texture and mouthfeel ($p < 0.001$). Overall, there was no significant difference

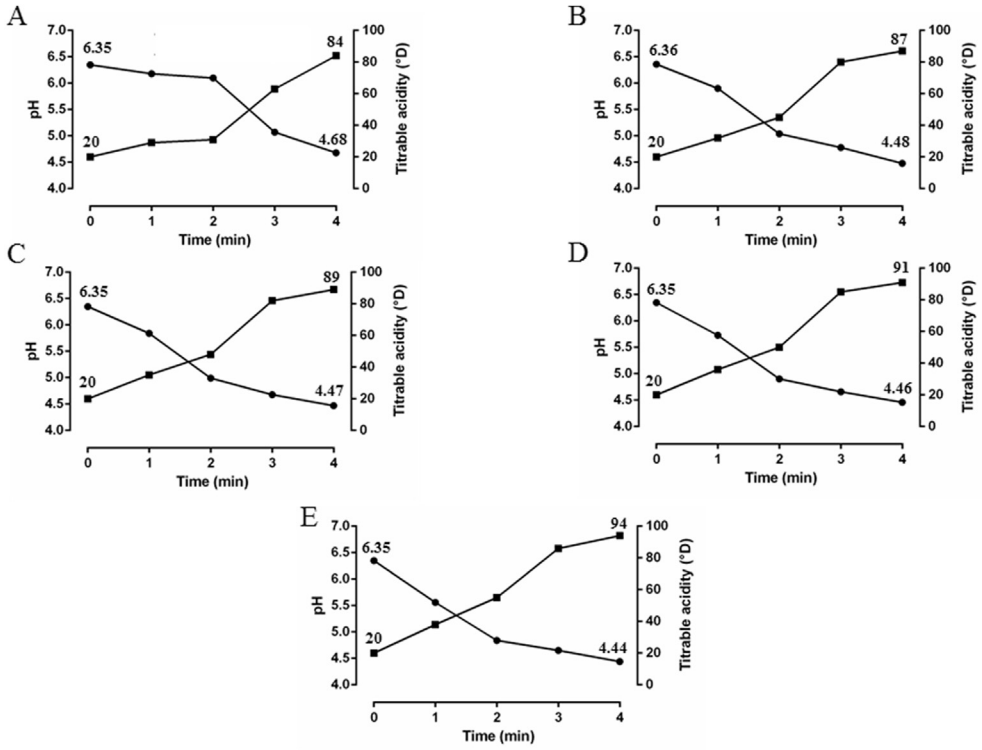

Fig. 1. Changes in pH drop and titrable acidity increase in control (A) and 0.25 (B), 0.5 (C), 0.7 (D) and 1% (E) *Spirulina*-fortified yogurts during fermentation period (4H).

Table 1
Parameters obtained by texture analyzer for control and fortified yogurts with different concentrations of *Spirulina* after 24 h of storage.

	Control yogurt	0.25% *Spirulina* yogurt	0.5% *Spirulina* yogurt	0.75% *Spirulina* yogurt	1% *Spirulina* yogurt
Firmness (N)	0.67 ± 0.02	0.62 ± 0.04	0.58 ± 0.05[a]	0.47 ± 0.01[b, d, e]	0.47 ± 0.01[b, d, e]
Cohesiveness	0.32 ± 0.04	0.41 ± 0.03	0.45 ± 0.19	0.41 ± 0.12	0.36 ± 0.1
Elasticity (mm)	19.94 ± 0.29	22.58 ± 1.81[a]	21.29 ± 0.887	21.74 ± 0.678	19.87 ± 1.16[c]

Control vs *Spirulina*-fortified yogurts (0.25%, 0.5%, 0.75% and 1%):[a] $p < 0.05$; [b] $p < 0.001$.
0.25% *Spirulina* yogurts vs 0.75 and 1% *Spirulina* yogurts: [c] $p < 0.05$; [d] $p < 0.01$.
0.5% *Spirulina* yogurts vs 0.75 and 1% *Spirulina* yogurts: [e] $p < 0.05$.

Table 2
Mean scores of tasting panellists (n = 32) for sensory properties of control and *Spirulina*-fortified yogurts (0.25%, 0.5%, 0.75% and 1%) at one week after production.

	Control yogurt	0.25% *Spirulina* yogurt	0.5% *Spirulina* yogurt	0.75% *Spirulina* yogurt	1% *Spirulina* yogurt
Color	9	7.15[b]	6.25 [b, c]	5.95 [b, c]	4.65 [b, c]
Flavor	7.7	7.54	6.46 [b, c]	6.22 [b, c]	5.45 [b, c]
Taste	7.18	7.35	6.2 [b, c]	5.29 [b, c]	4.17 [b, c]
Texture	7.94	7.01 [a]	6.21 [b, c]	5.91 [b, c]	5.45 [b, c]
Overall acceptability	7.63	7.25	6.45 [b, c]	5.64 [b, c]	5.05 [b, c]

Control vs fortified yogurts (0.25%, 0.5%, 0.75% and 1%): [a] $p < 0.05$; [b] $p < 0.001$.
0.25% *Spirulina* yogurts vs 0.75 and 1% *Spirulina* yogurts: [c] $p < 0.001$.

between the treatments without (controls) and with 0.25% of *Spirulina*. This treatment was significantly sufficient to accelerate the end of fermentation ($p < 0.05$) and conserve the textural properties and sensory acceptability of the final milk product. Yogurt supplemented with 0.25% of *Spirulina* was chosen as the best final product and selected for further analyzes to evaluate its physicochemical, microbiological and antioxidant properties.

3.2. Physicochemical characteristics

Total solids (TS), protein, fat and ash contents were found to be the highest in *Spirulina*-fortified yogurts (Table 3). Total solids of the control and fortified yogurts with *Spirulina* were 22.47% and 22.62%, respectively. There was a significant difference in TS between these 2 yogurt formulations ($p = 0.048$). Similarly, total protein values were higher in the yogurts fortified with *Spirulina* ($p = 0.014$). This was probably due to the high protein content of *Spirulina* (Lupatini, Colla, Canan, & Colla, 2017). The High protein content of fortified yogurt may have played an important role in lesser whey separation (Meyer, Bayarri, Tárrega, & Costell, 2011) compared with control yogurt at the end of storage. There is no significant difference in fat content between control (3.38) and fortified (3.4) yogurt samples ($p > 0.05$). The ten most abundant fatty acids detected in the yogurt samples are presented in Fig. 2.

Table 3
Mean values for physicochemical characteristics of control and 0.25% *Spirulina*-fortified yogurts at one week after production (n = 3).

	Control yogurt	0.25 *Spirulina* yogurt
Moisture (g/100 g on dry weight basis)	77.52 ± 0.03	77.38 ± 0.06 [a]
Total solids (g/100 g on dry weight basis)	22.47 ± 0.03	22.62 ± 0.07 [a]
Protein (g/100 g on dry weight basis)	3.63 ± 0.03	3.87 ± 0.07 [a]
Fat (g/100 g on dry weight basis)	3.38 ± 0.77	3.4 ± 0.68
Carbohydrate (g/100 g on dry weight basis)	14.49 ± 0.02	14.66 ± 0.03
Total dietary fiber (g/100 g on dry weight basis)	1.21 ± 0.02	1.82 ± 0.04 [c]
Soluble dietary fiber (g/100 g on dry weight basis)	1.12	1.62
Insoluble dietary fiber (g/100 g on dry weight basis)	0.1	0.19
Sugar (g/100 g on dry weight basis)	13.55	13.59
Ash (g/100 g on dry weight basis)	0.551 ± 0.004	0.685 ± 0.005 [c]
Iron (mg/100 g on dry weight basis)	3.363 ± 0.58	9.01 ± 0.044 [c]
Calcium (mg/100 g on dry weight basis)	96.13 ± 1.33	107.7 ± 3.34 [b]
Magnesium (mg/100 g on dry weight basis)	48,71	62.31[b]

Control vs 0.25% *Spirulina* fortified yogurt: [a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.001$.

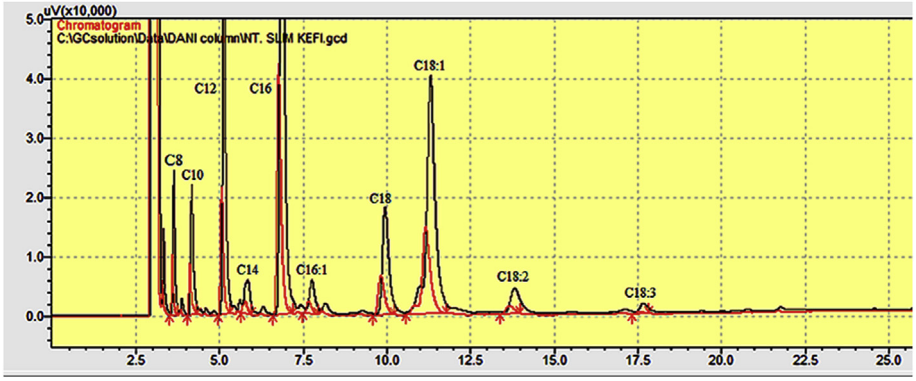

Fig. 2. Fatty acids profile by gas chromatography with flame-ionization detection of control (in black) and 0.25% *Spirulina*-fortified yogurts (in red) after one week of storage. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The most abundant ones in control yogurts were palmitic acid (38.39%), followed by oleic acid (24.75%), lauric acid (13.69%) and stearic acid (8.57%). As expected, saturated fatty acids (70.56%) prevailed over unsaturated ones (29.19%), with the mono-unsaturated showing higher amounts than polyunsaturated. Caleja et al. (2016) described the same trends in terms of abundances (SFA > MUFA > PUFA), but identified a higher number of fatty acids. *Spirulina*-fortified yogurts showed values close to that of control yogurts ($p > 0.05$) (Fig. 2). Ash content of the fortified yogurt with *Spirulina* was 0.68%, and significantly higher than any of the control yogurts (0.55%) ($p < 0.01$). The treatment containing 0.25% micro-algae had the highest ash content because of the presence of significant amount of essential minerals such as magnesium ($p = 0.027$), iron ($p < 0.001$) and calcium ($p < 0.01$). This finding is in accordance with the results of study conducted on the ice cream enriched with *Spirulina* (Malik, Kempanna, & Paul, 2013). In addition, the increase of total dietary fiber content in *Spirulina*-fortified yogurts was also found to be significant ($p < 0.001$). Changes in these parameters, especially protein, fat and dietary fiber contents may affect certain other physicochemical properties such as pH, titrable acidity and STS.

Fig. 3 illustrates the pH, titrable acidity, a_w, STS and WHC of the unfortified and 0.25% *Spirulina*-fortified yogurt samples during the 28 days of storage. In the present study, the pH of the fortified yogurt samples decreased (4.53–4.04) during storage, indicating the development of acidity (95–114.5 °D) over the storage period, but did not fall below pH 4.0, which is generally considered detrimental to the survival of probiotic organisms (Senaka Ranadheera et al., 2012). These pH values were slightly higher than that of control yogurt during the whole storage period (28 days) at 4 °C. These results were in agreement with the findings of Ozturkoglu-Budak et al. (2016) who reported a decrease in the pH over the course of 21 days. Similarly, Lee and Lucey (2010) concluded that additional proteins had led to an increase in solid content of yogurt milk, thereby increasing the buffering capacity that required additional acid development by starter cultures to achieve a similar pH target.

Determining a_w parameter is essential to evaluate food safety. Indeed, the presence of huge available water quantities in the components is the principal factor inducing the development of microorganisms, which affects the quality and safety of food. Data demonstrated that *Spirulina*-added yogurts were characterized by similar a_w values compared to the control formulations during the storage period ($p = 0.397$) (Fig. 3). The stability of the final yogurt product is assured by the combined effects of a_w and pH.

Serum release, known as syneresis, an undesirable property in yogurt products, is considered as one of the most important parameters indicating the quality of yogurt during storage (Senaka Ranadheera et al., 2012). Syneresis was found to be significantly higher in control yogurt than in the siprulina fortified yogurts ($p = 0.045$) (Fig. 3), probably due to its high protein ($p = 0.014$), fat and dietary fiber ($p < 0.001$) contents. High dietary fiber content in yogurt has been associated with lower syneresis values in previous experiments (Senaka Ranadheera et al., 2012). Wu, Hulbert, and Mount (2001) demonstrated that WHC was related to the ability of the proteins and dietary fibers to retain water within the structure of yogurt. They also suggested that milk fat globules may also play an important role in water retention. In this study, 0.25%

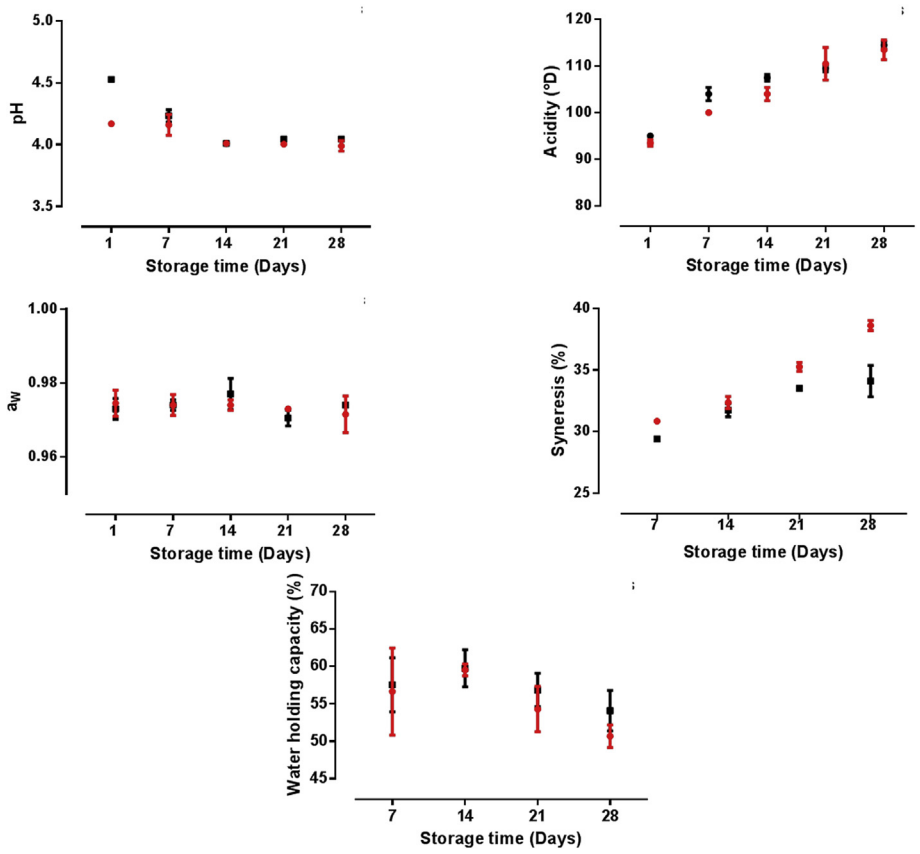

Fig. 3. Changes in the pH, acidity, a_w, syneresis and water holding capacity rates for control (in red) and 0.25% *Spirulina*-fortified yogurts (in black) during cold storage (28 days). The error bars represent SD of the mean. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Spirulina-fortified yogurts demonstrated significantly higher WHC ($p = 0.048$) compared to control yogurts, possibly reflecting the higher protein ($p = 0.014$) and fat ($p < 0.001$) contents of the fortified yogurts (Fig. 3).

3.3. Microbiological quality

There was no mold, yeast, or coliform bacteria detected in any of the samples during storage. The absence of these microorganisms indicated that the yogurts were safe and clean even after storage at 4 °C for 4 weeks. These data indicate that the processing of the yogurt was conducted under good hygienic-sanitary conditions.

3.4. Color measurement of yogurt samples

Due to the important influence of dairy product colors on consumer acceptance (Dönmez et al., 2017), color parameters (L^*, a^* and b^*) of yogurt samples were measured (Fig. 4). Fig. 4A shows the variations of the L^* index (lightness) with storage time (28 days) for control and *Spirulina*-fortified yogurts. The L^* attributes show that control yogurts have higher lightness values than those of 0.25% of *Spirulina* fortified yogurts ($p < 0.01$). For these samples, the differences related to the L^* index were so low they could not be visually perceived on the graph in Fig. 4A, indicating that the colorant evaluated in this work had no tendency to lighten the color of the samples during 28 days of storage. In addition, the values of SD were too low to be perceived on the graphs (Fig. 4). Moreover, samples prepared with 0.25% of *Spirulina* powder were characterized by the lowest a^* and b^* values ($p < 0.001$), which indicates that yellow color decreased toward a greenish color (Fig. 4B and C). This could be explained mainly by the richness of *Spirulina* powder in chlorophylls. The statistical analysis indicated that there was no significant difference related to a^* and b^* values ($p > 0.05$) between 1 and 28 days for the control and *Spirulina*-fortified yogurts. In conclusion, based on the evaluation of the acquired data concerning the color parameters studied, we can argue that the *Spirulina* pigments may be useful as natural colorants in milk products.

3.5. Antioxidant properties

Actually, much recent interest has been focused on the potential role of the enriched foods with bioactive compounds, generally, derived from microalgae thanks to their safeness and effectiveness in the treatment of many human diseases. In fact, the antioxidant activity of microalgae derived from their richness in numerous free radical scavengers. So, in order to evaluate the contribution of *Spirulina* to the yogurt antioxidant capacities, chlorophyll and carotenoid contents, ferric reducing power and DPPH-radical scavenging activity have been measured (Table 4). Compared to the control yogurts, *Spirulina* incorporation improve significantly the chlorophyll ($p < 0.001$), carotenoid ($p < 0.001$) and Phycocyanin ($p < 0.001$) levels, besides the enhancement of anti-DPPH ($p < 0.001$) and the reducing power ($p < 0.001$) activities. Furthermore, a positive correlation between the total antioxidant activity, determined by DPPH and FRAP methods, and pigment contents was clearly observed. The concentration required to obtain 50% DPPH radical scavenging activity for the yogurt incorporated with 0.25% of *Spirulina* was 30 mg, while it was superior to

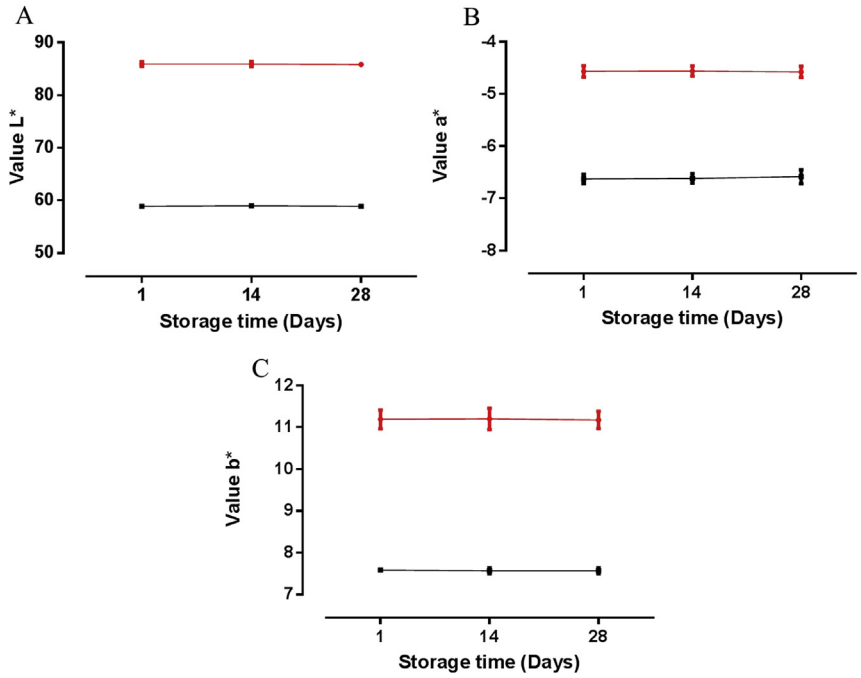

Fig. 4. Variation of (A) L* index (lightness), (B) a* index (redness) and (C) b* index (yellowness) in control (red) and *Spirulina*-fortified yogurts (black), stored for 28 days (mean ± standard deviation). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 4
Pigments content and antioxidant activities of control and 0.25% *Spirulina*-fortified yogurts after one week of storage.

	Control yogurt	0.25% *Spirulina* yogurt
Chlorophylls (mg/g on dry weight basis)	0	27.6 ± 0.84 [a]
Carotenoids (mg/g on dry weight basis)	0	10.86 ± 0.25 [a]
Phycocyanin (mg/g on dry weight basis)	0	0.297 ± 0.005 [a]
Scavenging activity (%)[A]	43.6 ± 1.4	52.41 ± 2.61 [a]
Reducing power[A]	0.781	1.35 [a]

Control vs 0.25% *Spirulina*-fortified yogurt: [a] $p < 0.001$.
[A] DPPH radical-scavenging activity (%) and reducing power (absorbance at 700 nm) were determined at a concentration of 50 mg of yogurts/ml.

50 mg in the case of control (Table 4). Moreover, a comparable trend was also noticed for ferric-reducing power assay. In fact, ferric reducing power for control and *Spirulina* fortified yogurts were 0.781 and 1.35 respectively, at a concentration of 50 mg of yogurts/ml (Table 4). Similar studies describe that the antioxidant activity of yogurts was enhanced by the presence of microalgae, for example, in studies with yogurts fortified with *Chlorella vulgaris and Dunaliella* sp. powders. The increase in the free radical scavenging may be attributed to the increase in the content of chlorophylls (Ismaiel, El-Ayouty, & Piercey-Normore, 2016), carotenoids (Goiris et al., 2012) and phycocyanin (Anbarasan, Kishor Kumar, Satheesh Kumar, & Venkatachalam, 2011) as a result of incorporation of spirulina. These results suggested also that yogurt processing did not have an impact on the antioxidant compounds.

4. Conclusions

Spirulina powder proved its efficient use as innovative and attractive additive in yogurt processing. In addition to its nutritional role, *Spirulina* serves as a good source of natural coloring and flavoring agent. Moreover, *Spirulina*, rich in dietary fibers and proteins, plays the role of a physical stabilizer in the textural product maintenance by improving mouth feel and enhancing syneresis and apparent viscosity. The addition of 0.25% of *Spirulina* was significantly sufficient to accelerate the end of fermentation and conserve the sensory acceptability of the final milk product. This treatment increased not only the nutritional quality of the final product but also increased the nutraceutical property by increasing its antioxidant activity. The evaluation of the instrumental color parameters (L*, a* and b*) demonstrated higher stability of the color indices in the yogurt during 28 days of storage. These results could be used to develop a novel yogurt fortified with *Spirulina* as a source of bioactive compounds, which might be an alternative to the fortification with synthetic chemical compounds that may cause adverse effects in consumers. The proper selection and use of *Spirulina* as a fortification material in yogurt appears to be important, as it provides a convenient food that satisfies consumer interest and provides health benefits.

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