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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