



## Fortification of Iranian Traditional Cookies with *Spirulina platensis*

Saeede Shahbazizadeh<sup>1</sup>, Kianoush Khosravi-Darani<sup>2\*</sup> and Sara Sohrabvandi<sup>2</sup>

<sup>1</sup>Department of Food Science and Technology, Shahr-e-Qhods Branch, Islamic Azad University, Tehran, Iran.

<sup>2</sup>Research Department of Food Technology, National Nutrition and Food Technology Research Institute, Faculty of Nutrition Sciences and Food Technology, Shahid Beheshti University of Medical Sciences, P.O.Box: 19395-4741, Tehran, Iran.

### Authors' contributions

This work was carried out in collaboration between all authors. Author S Shahbazizadeh performed preliminary data analysis, gathered the initial data and produced the initial draft. Author KKD designed the study, anchored the field study, and interpreted the data. Author S Sohrabvandi managed the literature searches. All authors read and approved the final manuscript

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### ABSTRACT

**Background:** Nowadays, there is a great interest towards medical and nutritional properties of *Spirulina platensis* due to its beneficial compounds. The purpose of this study was to incorporate Iranian traditional cookies with *S. platensis* biomass as a supplement ingredient.

**Materials and Methods:** The wheat flour was replaced with microalga powder at 0, 0.5, 1 and 1.5% w/w and the impacts of incorporation on physical, nutritional, antioxidant, antistaling and organoleptic characteristics of cookies were evaluated.

**Results:** Results showed that iron, protein and  $\gamma$ -linolenic acid content of fortified cookies increased as a result of *S. platensis* incorporation, coupled antioxidant properties. According to organoleptic evaluation by hedonic tests, samples containing 1-1.5% *S. platensis* received highest scores after control.

\*Corresponding author: E-mail: [kiankh@yahoo.com](mailto:kiankh@yahoo.com), [k.khosravi@sbm.ac.ir](mailto:k.khosravi@sbm.ac.ir);

**Conclusion:** It was possible to produce fortified cookies with suitable nutritional and organoleptic properties by addition of *S. platensis*.

**Keywords:** *Spirulina platensis*; cookies; nutritional; antistaling; organoleptic aspects.

## 1. INTRODUCTION

Cookies are widely consumed confectionary products, appreciated for their organoleptic properties, versatility, convenience, texture, and appearance. Application of natural ingredients with functional properties beyond traditional nutrients is an attractive way to design new products [1]. Microalgal biomass is a valuable source of fine chemicals such as carotenoid pigments, vitamins, proteins, fatty acids and other biologically active compounds, presenting potential health benefits [1,2]. *Spirulina platensis* is a microscopic and filamentous cyanobacterium that has a long history in human nutrition [2]. Potential health properties of *S. platensis* due to its nutritional and therapeutic ingredients are the fields of interests for future research [3]. Early interest in *S. platensis* focused mainly on its rich content of protein, vitamins, minerals, essential amino acids fatty acids. *S. platensis* comprises 60-70% protein by dry weight. Also, this microalgae is a rich source of vitamin B<sub>12</sub>, provitamin A ( $\beta$ -carotene), iron and  $\gamma$ -linolenic acid (GLA) [2,3]. In 1997, the FDA based on several studies conducted on rodents and human historical usage of *S. platensis* microalgae, confirmed its safety and announced that its maximum daily intake is 1.35 gr. Therefore, there is not any limitation to use dried whole *S. platensis* microalga in foods [4,5]. *S. platensis* and other microalgae have already been used experimentally in feed and food products [6].

Mixture of *Chlorella* and *Scenedesmus* in gingerbread, chocolate cookies and cake (0.25% w/w) [7]; *S. platensis* in manioc based bakery products [8]; *C. vulgaris* and *S. maxima* (0.5-2.0% w/w) [9] as well as *Sargassum marginatum* in pasta (1.0, 2.5, 5.0% w/w) [10]; *S. maxima* and *Diacronema vlkianum* in vegetable gelled desserts (0.1-1.0% w/w) [11]; *Isochrysis galbana* in biscuits (1-3% w/w) [12]; *C. vulgaris* in cookies (0.5-3.0% w/w) [13]; *C. vulgaris* and *Haematococcus pluvialis* in oil-in-water pea protein stabilized emulsions (0.75, 1.25% w/w) [14]; and *Enteromorpha compressa* in common traditional snack food in India (0-15% w/w) [1] have been applied and improved nutritional and organoleptic properties of the products are reported.

*S. platensis* represents a very large, relatively unexploited reservoir of novel compounds, many of which are likely to show biological activity, presenting unique and interesting structures and functions. Until 1996 only 208 cyanobacterial compounds with biological activity had been discovered while in 2001 the number of compounds screened was raised to 424, including lipoproteins (40%), alkaloids, amides etc. The reported biological activities include cytotoxic, antitumor, antibiotic, antimicrobial (antibacterial, antifungal, antiprotozoa), antiviral (e.g. anti-HIV) activities as well as biomodulatory effects like immunosuppressive and anti-inflammatory. The cytotoxic activity, important for anticancer drugs development, is likely related to defense strategies in the highly competitive marine environment, since usually only those organisms lacking an immune system are prolific producers of secondary metabolites such as toxins [15]. Recently comprehensive reviews on medical and nutraceutical properties of *S. platensis* are reported [3,16,17] and dairy products as suitable food matrix for delivery of this healthy biomass are introduced [6, 18-29].

In some countries, snacks such as biscuits, cakes, cookies, which are consumed widely by children, have been fortified with iron and other nutrients and are used in the feeding program affairs of schools. According to the findings of this study, fortification of cookies with *Spirulina platensis* microalgae is a highly effective method for increasing the nutrients in diet of children, due to its simplicity, low cost and feasibility. So it can be a suitable carrier for fortification. Moreover, due to its high availability, the cost of production becomes lower.

The aim of this work was to use *S. platensis* as a nutritional supplement (e.g. protein, iron, fatty acids) in Iranian traditional cookies (called 'Koloocch'eh') in order to eliminating malnutrition caused by shortage of nutrients. The effects on protein and iron content, fatty acid profile, organoleptic characteristics, peroxide value, texture and color intensity of the cookies were determined as a function of pigment concentration along 12 weeks of storage time.

## 2. MATERIALS AND METHODS

### 2.1 Microalga

The microalga used in this study was purchased from Sina Rizjorbake Qeshm (Iran). It was a homogenous mixture of *S. platensis* cultured by the open commercial farms. After harvesting and spray drying, the final product was a green powder.

### 2.2 Cookies Preparation

Traditional Iranian cookies (as control) were prepared using the following formulation (% w/w): 50 flour, 12.5 sugar, 13.75 shortening oil, 4.4 egg, 7.87 water, 0.375 baking powder and allowed spices such as nutmeg and cinnamon. Fortification was conducted by replacement of wheat flour by *S. platensis* biomass powder at 0.5%, 1% and 1.5% concentration levels (w/w). The cookies were baked in an oven at 180°C for 30 min. The cabinet oven was manufactured by Mobtaker industrial workshop. After cooling, cookies were kept inside sealed Cellophane packages (with low permeability to air, oils, greases, bacteria and water) at room temperature for 3 months.

### 2.3 Color Measurement

Color of cookies was determined instrumentally using a Hunter Color Lab colorimeter, model Color flex (Hunter Associates Labinc. Model No. co4-1005-6310 REV.E. USA) [1,12]. The instrument was calibrated using a white standard plate ( $L^*=92.22$ ,  $a^*=-1.3$ ,  $b^*=1.21$ ). The results were reported in terms of  $L^*$ ,  $a^*$  and  $b^*$  which respectively accounts for the lightness (0-100),  $a^*$ , redness/greenness (+ to -),  $b^*$ , yellowness/blueness (+ to -) according to the CIELAB system. The color changes during storage were expressed as  $\Delta E^*$  (color of the fresh product after 1 day of storage as reference sample), which is calculated from the equation [1]:

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad (1)$$

Measurements were performed in triplicate after storage for 0, 1, 2, 3, 4, 8 and 12 weeks (in constant lighting and room temperature 25±5°C).

### 2.4 Texture Analysis

The texture of cookies was measured objectively (after 0, 1, 2, 3, 4, 8 and 12 week of storage) using a texturometer (H5KS, Hounsfield, UK) in penetration mode with a cylinder probe of 3.2 mm in diameter, at 15 mm depth and at 60

mm/min speed. The driving force of the motor was used to move the probe at constant speed. Measurements were replicated 3 times, at room temperature (25±5°C). By connecting the probe to the tip of the texturometer an auto-force-distance curve was plotted: In a short interval, when the probe tip moved to the sample of cookie, an initial rapid increment of force was observed and cookie sample was deformed without any hole in it. This stage was finished with penetration of cylinder probe to the cookie, which was accompanied with a sudden change of curve slope called yield point. Yield point actually represents the moment when the cylinder begins to penetrate into the texture of cookie and caused an irreversible crushing. In other hand, yield point is the maximum force (N) required until penetration is observed on the peak of texturogram curve and corresponds to firmness value. After the yield point, the required force decreased and the slope of curve became negative [1,12].

### 2.5 Organoleptic Evaluation

Organoleptic evaluation of cookies was done by 14 trained panelists, after baking. Panelists were used clean white plastic dishes to serve 40 gram of labeled samples randomly on the palates, in individual booths at room temperature. Cookies were evaluated in terms of different attributes such as flavor, odor, color, texture uniformity, non mouth texture and mouth texture according to 5 point hedonic test\* (5; like extremely and 1; dislike extremely). Finally, total acceptability was calculated according to equation 2 [3, 26].

Equation 2:

$$\text{Total acceptability} = (\text{flavor+odor}) \times 6 + (\text{color}) \times 2 + (\text{texture uniformity}) \times 2 + (\text{mouth texture}) \times 3.5 + (\text{non mouth texture}) \times 1$$

### 2.6 Nutritional Evaluation

Iron and protein content of *S. platensis* powder, wheat flour and cookies were measured 3 times,

\* Hedonic scales are used to help assessors respond to questions about the complacency of one or more attributes or overall palatability of a tagged or labeled food. First, the person reads the question and after evaluation of food according to his preferences, will select the appropriate response from classes or degrees of Hedonic scale. It should be noted that the Hedonic scales used are often classified. It means that the respondent can just select one among the ranks or classes and there is no possibility to choose between two consecutive classes. As a result, the effect of usage this scale of discrete data is created. In Hedonic scale, the concept of words which are selected for each class or grade should have a logical increment or decreasing compared to the previous and next [3,27].

after 3 months of storage as following. Iron content of above mentioned foods was determined using AACC 40-70.01 method. The titrisol containing 1000 mg of iron was desolved in deionized water (1000 mL) to prepare stock solution of Fe (III). 10 mL of the stock iron solution was diluted to get intermediated iron solution (Fe (III) 100 ppm). Finally, the standard iron solutions (Fe (III) 1, 5 and 10 ppm) were prepared by dilution of 1, 5 and 10 mL of the intermediated iron solution to 100 mL. 1 g of dried food (1 h at 90°C) was weighted and slowly turned to ash by furnace at 550°C. The ash was desolved by 8 mL of the HCl:HNO<sub>3</sub> mixture (3:1 v/v) and diluted in distilled water to reach the volume of 25 mL. The blank solution was prepared with the same method. Thermo Scientific-Solaar M-5 atomic absorption spectrometry system was used. Calibration curve of absorbance vs. concentration of standard iron solutions was obtained. Protein content of cookies was determined using AACC 46-12.01 method.

Further more, the fatty acid profile of *S. platensis* and the lipid fraction of cookies were determined by using AACC 58-18.01 method. Extraction of lipid phase was carried out by addition of 10 mL of n-hexan to 10 g of food. After solvent removal, preparation of fatty acid methyl esters was carried out (0.2 g of extracted oil and methanolic KOH 20:100% v/v). Then, esterification was done at 40-50°C (1.5 h). After solvent removal (under nitrogen), 1 µL of dried organic phase was injected to GC HP-5890 equipped with 30 m capillary column (0.25 mm internal diameter, and 0.25 µm film thickness). Then pressure of carrier phase (nitrogen) was 10 psi, and temperature of injector and detector were adjusted to 250°C.

## 2.7 Peroxide Value

Lipidic phase of cookies was extracted using the AACC 58-16.01 method. The peroxide value, which indicates the initial occurrence of primary oxidation compounds, was determined on the 12th weeks of the study according to Gouveia et al., 2008 [14]. This value shows the level of active oxygen (expressed in miliequivalents) and is related to primary deterioration of the lipidic phase.

## 2.8 Statistical Analysis

Physical, organoleptic and nutritional characteristics were subjected to ANOVA-Post Hoc Comparisons-Scheffe test, at 0.05 probability level, using Stat Soft STATISTICA

program (version 6.0). All experiments were conducted in triplicate.

## 3. RESULTS AND DISCUSSION

### 3.1 Color Analysis

#### 3.1.1 Effect of microalgal biomass addition

*S. platensis* cookies presented an appealing appearance, showing green tonality with algae concentration. These characteristics are shown in Fig. 1. As Fig. 1a shows, lightness parameter (L\*) significantly decreased with microalgal coloring agent concentration in biomass, as previously observed in similar products with the addition of other microalgae biomass [1,11-13] (P<0.05). The evaluation of cookies' chromaticity coordinate parameters (a\* and b\*) are observed in Fig. 1b and 1c, respectively. The incorporation of microalgal biomass resulted an increased green color (negative a\* values) and decreased yellow tonality (b\* values) of incorporated cookies comparing to control which presented a dominant yellow chromaticity (positive b\*) with only a very slight contribution from the a\* parameter (P<0.05). Similar results were observed in previous studies by Gouveia and et al. [1,12,15].

*S. platensis* microalgae contains carotenoids, chlorophyll, and phycocyanin as three major pigments with amount of 0.4, 1.0 and 14% dry wt, respectively. So by replacing some part of the flour containing yellow Xanthophyll pigment with microalgae powder containing green chlorophyll and green-blue phycocyanin pigments leads to decrease of L\* and negative values of a\* and b\* in microalgae cookies compared with control [2].

#### 3.1.2 Effect of storage time

Cookies coloration underwent changes during the 12 weeks storage, with ( $\Delta E^*$ ), within 0, 1, 2, 3, 4, 8 and 12 weeks after cooking, shown in (Fig. 1d). Using Duncan test, mean total color differences ( $\Delta E^*$ ) were 5.16±3.53 for 0% *S. platensis*, 2.63±2.38, 3.73±1.80 and 2.97±2.26 for 0.5, 1 and 1.5% *S. platensis* cookies, respectively (P<0.05). Consequently, color stability of *S. platensis* cookies were more stable than control during 12 weeks as previously reported by Gouveia et al. [13]. It seems that the main cause of more color stability in microalgae cookies, was slow and gradual decomposition and oxidation process of pigments due to the presence of multi-component antioxidant systems in microalgal biomass and their synergistic or additive interactions. Not only

carotenoids but also phenolic compounds should be considered in microalgae as a source of natural antioxidants<sup>†</sup>. [1,12]. The color of *Spirulina* powder, in products with low moisture such as cookies, even after three months of storage at room temperature, without special storage conditions (temperature, light, and atmosphere), has a good stability. Therefore, when the goal is use of a natural green dye with high stability, the *spirulina* powder can be helpful. A similar result is reported for comparing the stability of three blue pigments of Gardenia Blue, Indigo and C-Phycocyanin to light and temperature in aqueous solutions such as soft drinks, chewing gum, gel, hard candy and dried preparations such as sugar cover for soft candy. It was reported that the blue color of C-Phycocyanin has much higher stability in the dried preparations than the rest [16].

### 3.2 Texture Analysis

#### 3.2.1 Effect of microalgal biomass addition

The firmness of the cookies generally decreased by enhancing microalgal biomass content as data are presented in Table 4 ( $P < 0.05$ ). This is in accordance with previous studies which reported the formation of weaker gels when *S. platensis* biomass was added to batter [1]. Cookies are considered solid emulsions of sucrose, lipids and gelatinized starch, and this morphology leads to its structure and texture. The main factors affecting texture properties are the moisture content and water mobility, which are greatly influenced by the interaction with hydroxyl groups present in the matrix [12]. Batista et.al noted large particles of *Spirulina* microalgae could imprint discontinuities at dough network, resulting to softer structure [22]. *Spirulina* is a cyanobacteria with lack of rigid cell wall which lead to higher water absorption rates (by its cellular components mainly proteinaceous structure). In fact *S. platensis* protein molecules due to having a hydrophilic property, compete for water binding sites with starch molecules, destabilizing and delaying gelation of starch, leading to a more fragile gel structure [20].

#### 3.2.2 Effect of storage time

As Table 4 shows, the texture of the *S. platensis* incorporated cookies did not change significantly (especially for cookies incorporated by 1.5 % *S. platensis*). But firmness of the control cookies

increased significantly during the 12 weeks of the study ( $P < 0.05$ ). The observed anti-staling effect of incorporation on the texture of product associated with the previous color stability show that shelf life of the product can be enhanced by the incorporation of microalgae, with a positive commercial impact. Similar results have been reported about anti-staling effect in bread crumb due to addition of microalgal hydrocolloids [17].

#### 3.2.3 Effect of microalgal incorporation on organoleptic properties

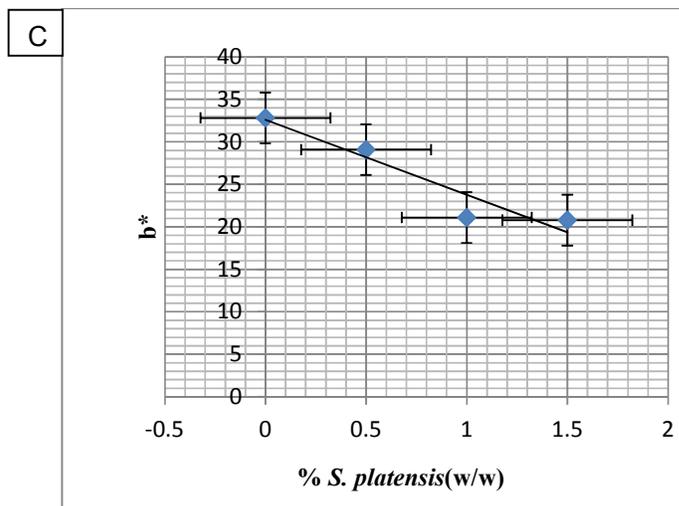
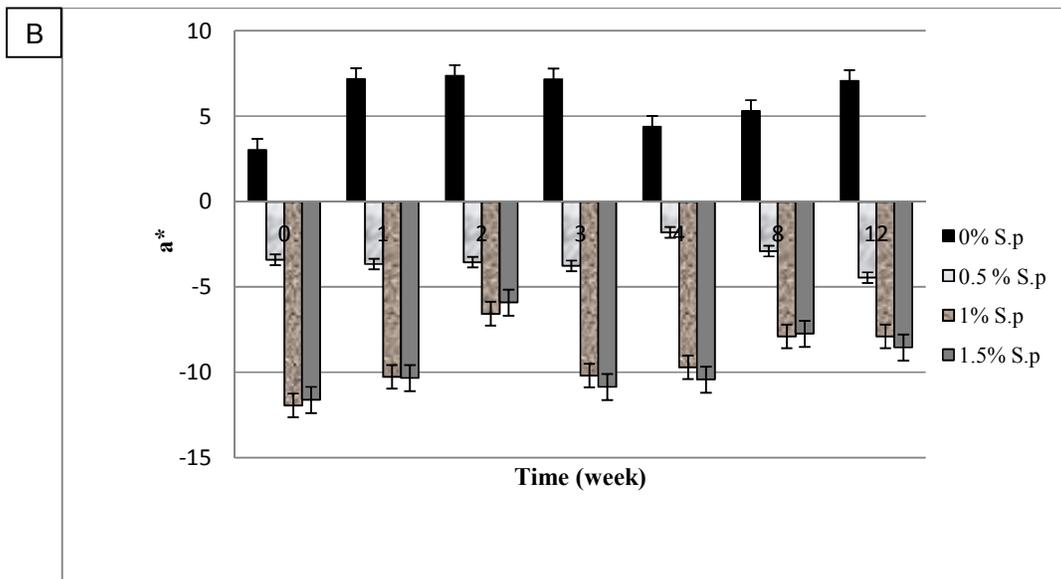
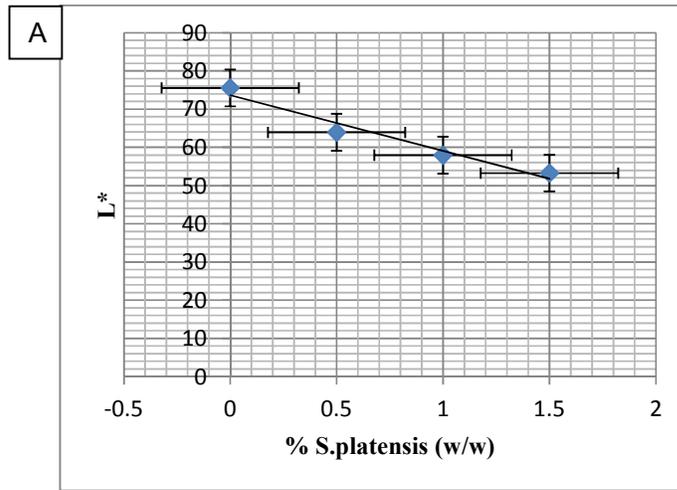
Organoleptic evaluation of cookies included color, texture uniformity, taste, flavor, and mouthfeel was done just after baking and its results are presented in Table 1. The color of *S. platensis* cookies turned to green, which was dominant in all fortified cookies. The greenish discoloration of cookies was considered unpleasant. The taste of microalgal cookies was a little bitter, but scores of fortified cookies were not significantly different with each other. The texture uniformity, flavor, the mouth-texture scores in microalgal cookies did not differ significantly with each other. Cookies containing 1 and 1.5% *S. platensis* cookies received maximum scores after control (without *S. platensis*) according to equation [2].

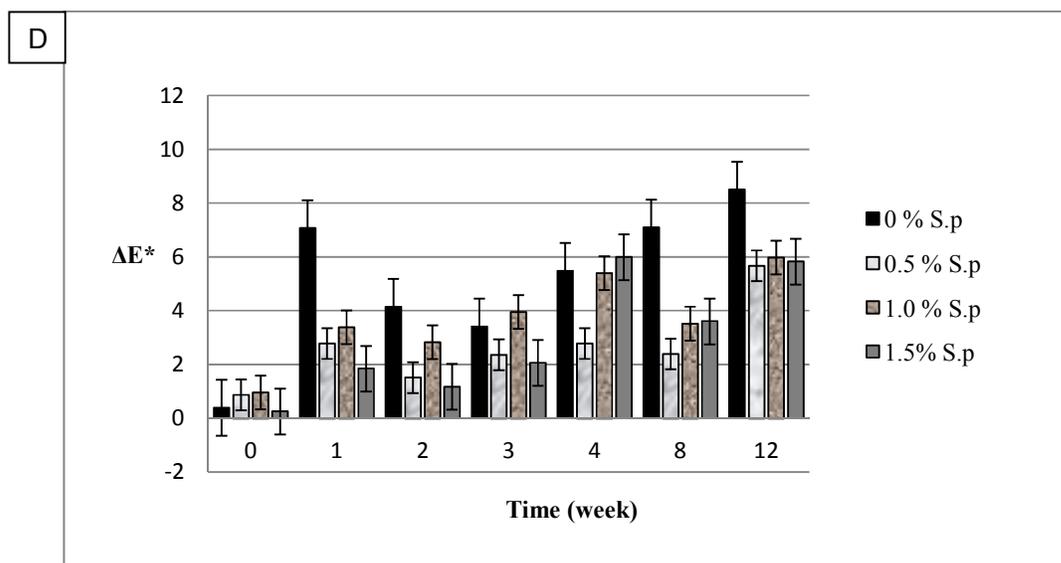
### 3.3 Nutritional Evaluation

Results showed that the iron content of *S. platensis* microalga is 0.125% w/w dry weight which is more than wheat flour (Table 2). Replacing the wheat flour by *S. platensis* increased iron content of cookies, as presented in Table 2. In a similar study, incorporation of *Enteromorpha compressa* (green seaweed) at 7.5% w/w increased the iron content in snacks [17].

Table 2 shows that protein content in *S. platensis* biomass was 62.9% w/w dry weights which was more than wheat flour. Furthermore, protein content of fortified cookies was more than control (Table 2). These results are in agreement with previous study which reported increase of protein content in Manico based bakery products by incorporating of *S. platensis* microalga [9]. The protein ingredient chosen for incorporation in cookies should have suitable flavor, low water absorption capacity (to inhibit microbial contamination), and high protein efficiency without significant changes in dough elasticity and consistency [19].

<sup>†</sup> Dried and fresh *Spirulina* powder can be stored in anti-oxygen special containers for 5 years or more without losing its  $\beta$ -carotene content [13].





**Fig. 1. Color parameters L\*: lightness (A), a\*: redness/greenness (B), b\*: yellowness/blueness (C) and  $\Delta E^*$  (D) of cookies incorporated by 0, 0.5, 1 and 1.5% *S. platensis* during 3 months of storage at room temperature**

The fatty acid profile of *S. platensis* includes saturated fatty acids (46.9%), monounsaturated (7.8%) and polyunsaturated fatty acids (42.8%) with  $\gamma$ -linolenic acid as the most abundant PUFA (Table 3). The fatty acids profile of the fortified cookies was clearly related to shortening, with a predominance of saturated (~36%, mainly palmitic acid 29.5%) and polyunsaturated fatty acids (27%, mainly included linoleic acid, 18:2 $\omega$ 6, 24.8%), monounsaturated fatty acids correspond to ~34% which mainly contains 33.7% oleic acid. *S. platensis* cookies presented  $\gamma$ -linolenic acid contents of 2.54, 2.78%, 2.80% and 2.73% for 0, 0.5, 1 and 1.5% *S. platensis* microalgal biomass incorporation, respectively (Table 3). The concentration of  $\gamma$ -linolenic acid was increased in fortified cookies, even after baking, whereas all the other fatty acids, mainly provided by shortening, showed large variations. The authors suggest that the microalgae cells could resist thermal treatment, encapsulating the fatty acid molecules, thus protecting them from oxidation [11]. Gouveia et al. also reported that incorporation of 0.1-1% *S. maxima* in preparing vegetable gelled desserts led to improvement of  $\gamma$ -linolenic acid level [12]. Also, results show that  $\gamma$ -linolenic acid presented good stability maintaining high nutritional levels even after 3 months of storage at room temperature without any changes in packaging or modified atmosphere, temperature or light.

### 3.4 Peroxide Value

Fig. 2 shows that addition of microalgal biomass to the cookies, yielded smaller values of primary oxidation products. The incorporation of *S. platensis* green microalgal biomass provided a higher oxidation stability over time, in comparison with control (Fig. 2). *S. platensis* cookies with 1.5% microalgal biomass presented lower peroxide values than other. It seems that reduction of peroxide value in microalgal cookies is due to the presence of antioxidant pigments including phycocyanin,  $\alpha$  and  $\beta$ -carotene, vitamin C, tocopherols, cryptoxanthin, astaxanthin, xanthophylls and polar polyphenolic compounds [1]. Antioxidant activity of phycocyanin is attributed to its prosthetic group of phycocyanobilin and high oxidation stability of carotene and carotenoids is related to their conjugated double bounds [2]. *S. platensis* pigments, as an Ingredient of fat products can retard lipid oxidation.

In the other hand, this biomass is an effective multi-component antioxidant system due to synergistic or additive interactions between their different antioxidant components [1].

Table 1. Organoleptic evaluation of cookie incorporated with various contents of *S. platensis*<sup>1</sup>

<i>S. platensis</i> (%)	Color	Texture harmony	Taste	Flavor	Mouth texture	Non-mouth texture
0	7.00±1.03 <sup>a</sup>	7.14±1.29 <sup>a</sup>	19.28±4.19 <sup>a</sup>	21.42±3.87 <sup>a</sup>	12.50±2.26 <sup>a</sup>	3.28±0.61 <sup>b</sup>
0.5	3.71±1.89 <sup>c</sup>	5.28±2.01 <sup>b</sup>	14.14±5.05 <sup>b</sup>	13.71±5.48 <sup>b</sup>	10.00±3.02 <sup>b</sup>	2.64±0.62 <sup>a</sup>
1	4.85±1.29 <sup>bc</sup>	5.57±1.94 <sup>b</sup>	15.85±4.46 <sup>ab</sup>	15.43±5.11 <sup>b</sup>	10.50±2.74 <sup>ab</sup>	2.64±0.63 <sup>a</sup>
1.5	5.28±2.01 <sup>b</sup>	4.57±1.82 <sup>b</sup>	15.42±6.94 <sup>ab</sup>	15.43±7.33 <sup>b</sup>	11.00±3.22 <sup>ab</sup>	3.00±0.78 <sup>ab</sup>

<sup>1</sup> Non-identical letters in each column show significant difference (P<0.05)Table 2. Protein and iron contents in bleach wheat flour, *Spirulina platensis* powder and cookies incorporated with *S. platensis*

Treatments	Protein (% w/w) dry weight (g/100 g)	Iron (% w/w) dry weight (mg/100 g)
<i>S. platensis</i> powder	62.9±0.10	125±0.05
Bleach wheat flour	9±0.003	3.9±0.02
Cookies with 0.0 <i>S. platensis</i>	6.2±0.02 <sup>d</sup>	2.46±0.05 <sup>d</sup>
Cookies with 0.5 <i>S. platensis</i>	6.5±0.05 <sup>c</sup>	3.20±0.10 <sup>c</sup>
Cookies with 1.0 <i>S. platensis</i>	7.2±0.14 <sup>b</sup>	3.36±0.05 <sup>b</sup>
Cookies with 1.5 <i>S. platensis</i>	7.4±0.02 <sup>a</sup>	4.00±0.10 <sup>a</sup>

\* Total nitrogen × 6.25

Table 3. Fatty acids concentration in *Spirulina platensis* powder and cookies fortified with *S. platensis*<sup>1</sup>

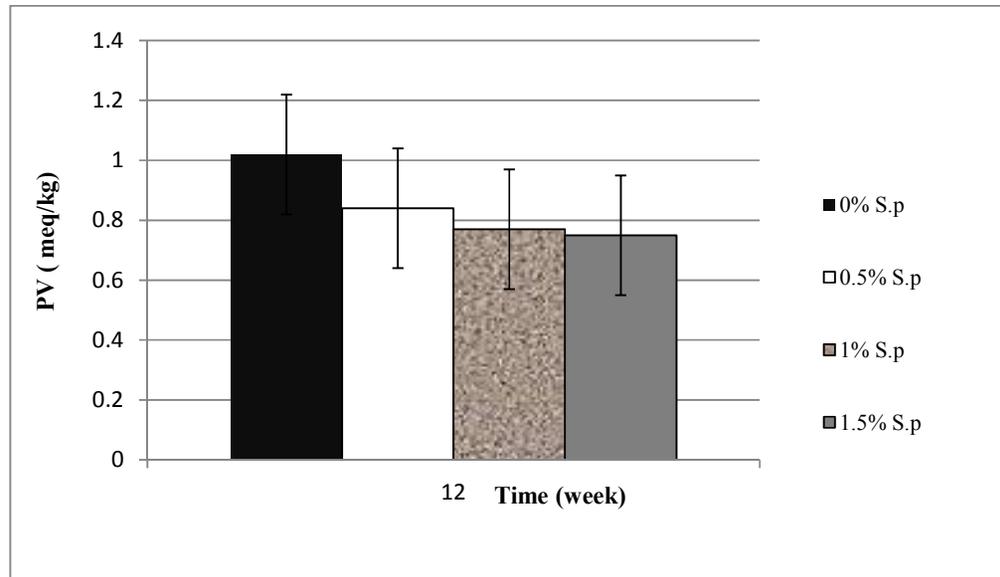
Trials	Fatty acid concentration (%)							
	C14:0 Miristic	C16:0 Palmetic	C16:1 Palmitoleic	C18:0 Stearic	C18:1ω6 Oleic	C18:2ω6 Linoleic	C18:3ω3 α-Linolenic	C18:3Cω6 γ-Linolenic
0.0%	0.67±0.0 <sup>c</sup>	29.50±1.50 <sup>a</sup>	0.62±0.01 <sup>b</sup>	6.77±0.12 <sup>a</sup>	33.7±0.12 <sup>c</sup>	0.16±0.01 <sup>a</sup>	0.16±0.01 <sup>a</sup>	2.54±0.05 <sup>a</sup>
0.5%	0.64±0.01 <sup>b</sup>	32.2±0.30 <sup>b</sup>	0.64±0.06 <sup>b</sup>	7.08±0.18 <sup>b</sup>	31.48±0.12 <sup>a</sup>	0.16±0.00 <sup>a</sup>	0.16±0.00 <sup>a</sup>	2.78±0.08 <sup>b</sup>
1.0%	0.54±0.00 <sup>a</sup>	29.50±0.50 <sup>a</sup>	0.61±0.01 <sup>b</sup>	7.16±0.04 <sup>b</sup>	32.60±0.12 <sup>b</sup>	0.16±0.00 <sup>a</sup>	0.16±0.00 <sup>a</sup>	2.80±0.09 <sup>b</sup>
1.5%	0.68±0.00 <sup>d</sup>	30.93±0.47 <sup>ab</sup>	0.55±0.01 <sup>a</sup>	7.59±0.00 <sup>b</sup>	24.63±0.17 <sup>a</sup>	0.16±0.01 <sup>a</sup>	0.16±0.01 <sup>a</sup>	2.73±0.06 <sup>b</sup>
<i>S. platensis</i>	0.2±0.00	45.0±0.03	5.6±0.08	0.3±0.00	1.4±0.09	2.2±0.01	17.9±0.01	24.9±0.15

<sup>1</sup> Non-identical letters in each column show significant difference, data are come from triplications (P<0.05)

**Table 4. Firmness values of cookies with different concentrations of *S. platensis* biomass, during three months storage<sup>1</sup>**

<i>S. platensis</i> % (w/w)	Time (weeks)						
	0	1	2	3	4	8	12
0	0.89±0.97 <sup>edc</sup>	0.68±0.05 <sup>defg</sup>	0.70±0.04 <sup>defg</sup>	0.82±0.11 <sup>cdef</sup>	0.75±0.02 <sup>cdetg</sup>	0.81±0.08 <sup>cdef</sup>	1.18±0.13 <sup>ab</sup>
0.5	0.73±0.03 <sup>cdefg</sup>	0.69±0.05 <sup>defg</sup>	0.87±0.15 <sup>cde</sup>	0.60±0.08 <sup>efg</sup>	0.91±0.07 <sup>cd</sup>	0.85±0.08 <sup>cdef</sup>	1.31±0.14 <sup>a</sup>
1.0	0.85±0.06 <sup>cdef</sup>	0.76±0.17 <sup>cdetg</sup>	0.50±0.03 <sup>g</sup>	0.60±0.03 <sup>efg</sup>	0.82±0.11 <sup>cdef</sup>	0.85±0.06 <sup>cdef</sup>	1.00±0.02 <sup>bc</sup>
1.5	0.56±0.05 <sup>fg</sup>	0.73±0.03 <sup>cdetg</sup>	0.56±0.05 <sup>fg</sup>	0.66±0.03 <sup>defg</sup>	0.68±0.04 <sup>edgf</sup>	0.63±0.05 <sup>defg</sup>	0.72±0.03 <sup>cdetg</sup>

<sup>1</sup>All treatments were compared to each other, in order to evaluate both the effect of microalgae amount and storage time on the texture of cookies ( $P<0.05$ )



**Fig. 2. Primary oxidation products concentration—peroxide value (PV), from cookies, incorporated 0, 0.5, 1 and 1.5% *S. platensis* after 12 weeks of storage at room temperature**

#### 4. CONCLUSION

Sweet cookies, an Iranian traditional and nutritious food, can be fortified with addition of a natural microalgal biomass of *S. platensis* (rich in iron, protein and PUFAs, particularly GLA). Regardless of lower overall acceptability of *S. platensis* cookies compared to control, increment of antioxidant effects beside to anti-staling properties revealed a new niche food market.

The future trend is incorporation of *S. platensis* into center cream of Iranian traditional cookies instead of replacing flour and study on storability of the cookie, antistaling, antioxidation, and antimicrobial effects of *Spirulina platensis* microalgae. Also the examination of the influence of traditional cookies containing microalgae on probiotics strains is of special priority. Sensory studies on masking effects of different types and concentrations of flavors on the taint of microalgae-enriched cookie can be considered.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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