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# Evaluation of Physicochemical, Microbiological and Sensory Properties of Croissants Fortified with *Arthrospira platensis* (Spirulina)

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**Abstract**

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The major physical, chemical, microbiological, and sensory properties of croissants enriched with Spirulina at concentrations ranging between 0.5 and 1.5% were evaluated. The results showed that the use of *A. platensis* biomass for the production of croissants improved the textural and organoleptic properties of the final products. Spirulina fortification also increased the protein and moisture levels and water-holding capacity, whereas it decreased the crumb firmness and lightness of croissants. Optimum sensory results were obtained when Spirulina was applied at a rate of 1%. Besides these benefits, the *A. platensis* biomass enhanced the levels of biologically active substances (i.e. essential amino acids, chlorophyll, phycocyanin, carotenoids, minerals, vitamins, and essential fatty acids) in croissant samples. To our knowledge, this is the first scientific study on Spirulina-fortified croissants.

**Keywords**: fortification; cyanobacteria; organoleptic; product shelf life

Spirulina is the common term for a dietary and feed supplement made from two species of cyanobacteria previously known as blue-green algae: *Arthrospira platensis* and *A. maxima*. It is known to have therapeutic properties, which include hypocholesterolaemic, antiviral, immunological, and antiglutagenic effects (McCarty 2007; Soheili & Khosravi-Darani 2011; Hoseini *et al.* 2013). *Arthrospira platensis* is an extremely rich source of protein (55–70%, w/w). It contains 47% of essential amino acids, e.g. methionine, which is usually absent in other algae, and cyanobacteria, 15–25% carbohydrates, 8–13% minerals, 3–7% fat, 8–10% fibre, and 6–7% moisture. In contrast to other microbial biomasses, Spirulina possesses a polysaccharide cell wall with high digestibility and low nucleic acid content. It also contains chlorophyll, phycocyanin, carotenoids, minerals, vitamins, essential fatty acids, and other bioactive components (Khan *et al.* 2005; Spolaore *et al.* 2006; Iyer *et al.* 2008). Spirulina's high protein content and its natural capability of chelating vitamins exert protective effects against many toxic metals, e.g. copper and mercury (Constantinescu *et al.* 2014).

Spirulina has been legally approved for human consumption for almost four decades and, as a result, Spirulina-based foods are currently produced and commercialised in approximately 80 countries (Varga *et al.* 2002; Hoseini *et al.* 2013). Spirulina is increasingly incorporated into bread and other bakery products such as cassava cake (Navacchi *et al.* 2012), sweet bread (Minh 2014), cassava doughnuts

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(Rabelo *et al.* 2013), biscuits (Sharma & Dunkwal 2012), hot dog rolls (Constantinescu *et al.* 2014), and pasta (De Marco *et al.* 2014).

Croissant is a popular bakery product usually eaten for breakfast or lunch in many parts of the world. The objective of this research was to evaluate the physicochemical, microbiological, and sensory properties of croissants enriched with *A. platensis* biomass at various concentrations. To our knowledge, this is the first scientific study on Spirulina-fortified croissants.

## MATERIAL AND METHODS

***Manufacture of croissant samples***. On a wheat flour basis (100%), the following ingredients were used to produce croissants: 50–57% of margarine, 5.5% of eggs, 6.5% of milk, 1.8% of salt, 6.1% of sugar, 1% of baking powder, and up to 1.5% of Spirulina powder. Wheat flour (Akbari, Tehran, Iran), which contained 11.5% of moisture, 0.53% of ash, 12% of protein, and 31.2% of gluten, was mixed thoroughly with margarine (Kalin, Tehran, Iran). Then eggs, salt, sugar, and baking powder were added and whisked. Spirulina-enriched croissant samples contained powdered *A. platensis* (Rizjolbak Qeshm, Tehran, Iran) at the rate of 0.5, 1, or 1.5%. Milk was added at the rate of 1.5% (w/w) to the mixture in a bowl and mixed to form dough. The dough was divided into pieces, with each piece being rolled up. The rolled pieces were put on a baking tray and baked at 315°C for 30 minutes. Finally, croissant samples were wrapped in polypropylene stretch film.

***Chemical analysis***. Protein content of the final products was determined by the reapproved AACC 46-12.01 method (AACCI 1999), and moisture content was measured as described by Danesi *et al.* (2011).

***Texture evaluation***. The maximum shearing force to cut the croissants was determined using a TA-XTIIi texturometer (Stable Micro Systems, Surrey, UK) applying the force perpendicular to the sample contact area, with compression of up to 80% deformation, considering the initial sample height. The Warner Bratzler rectangular steel blade probe (HDP/BSW; Stable Micro Systems, UK) was used. The parameters applied to determine the compression force were as follows: pre-test speed 3 mm/s; test speed 1 mm/s; post-test speed 10 mm/s. The trigger force limit used was 0.1 N (to allow the sample to accommodate itself under the blade at the beginning of the test). The rate of data acquisition was 200 points/s (Rosenthal 1999).

***Lightness evaluation***. One of the most important factors in sensory evaluation is lightness ($L^*$), which ranges from 0 (black) to 100 (white) along a grey scale. This parameter was determined using a HunterLab colorimeter (Model D25 with DP-9000 processor; Hunter Associates Laboratory, Reston, USA) in three replications during storage (Attia *et al.* 1993).

***Enumeration of yeasts and moulds***. Yeast Extract Glucose Chloramphenicol (YGC) agar (Merck, Darmstadt, Germany) was used to enumerate yeasts and moulds. The inoculated plates were incubated aerobically at 25°C for up to 5 days. Yeast and mould colonies were then counted and reported as colony-forming units (CFU)/g of product (ISO 21527-2:2008 – Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of yeasts and moulds – Part 2: Colony count technique in products with water activity less than or equal to 0.95).

Table 1. Protein and moisture contents* of Spirulina-enriched croissant samples monitored over a 3-week storage period

| Component | Spirulina (%, w/w) | Day 1 | Day 7 | Day 14 | Day 21 |
|---|---|---|---|---|---|
| Protein | 0 | $10.27 \pm 0.08^{b}$ | $10.29 \pm 0.06^{b}$ | $10.35 \pm 0.05^{ab}$ | $10.41 \pm 0.03^{a}$ |
| | 0.5 | $10.33 \pm 0.01^{ab}$ | $10.34 \pm 0.05^{ab}$ | $10.34 \pm 0.02^{ab}$ | $10.35 \pm 0.07^{ab}$ |
| | 1.0 | $10.34 \pm 0.02^{ab}$ | $10.35 \pm 0.01^{ab}$ | $10.35 \pm 0.04^{ab}$ | $10.36 \pm 0.05^{a}$ |
| | 1.5 | $10.35 \pm 0.05^{ab}$ | $10.36 \pm 0.03^{ab}$ | $10.36 \pm 0.04^{a}$ | $10.37 \pm 0.08^{a}$ |
| Moisture | 0 | $17.80 \pm 0.23^{b}$ | $17.82 \pm 0.25^{b}$ | $17.83 \pm 0.35^{ab}$ | $17.86 \pm 0.27^{ab}$ |
| | 0.5 | $17.77 \pm 0.35^{b}$ | $17.80 \pm 0.23^{b}$ | $17.81 \pm 0.15^{b}$ | $17.82 \pm 0.17^{b}$ |
| | 1.0 | $17.83 \pm 0.43^{ab}$ | $17.84 \pm 0.45^{ab}$ | $17.84 \pm 0.25^{ab}$ | $17.85 \pm 0.21^{ab}$ |
| | 1.5 | $18.23 \pm 0.25^{a}$ | $18.29 \pm 0.53^{a}$ | $18.29 \pm 0.23^{a}$ | $18.31 \pm 0.35^{a}$ |

*values are percentage means ± SD based on 6 observations (3 samples × 2 replicates); $^{a,b}$values within the same component followed by different lowercase superscripts differ ($P < 0.01$)

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Table 2. Texture lightness* of Spirulina-enriched croissant samples monitored over a 3-week storage period

| Spirulina (%, w/w) | Day | | | |
|---|---|---|---|---|
| | 1 | 7 | 14 | 21 |
| 0 | 71.68 ± 0.97[a] | 67.04 ± 0.63[a] | 65.44 ± 0.64[a] | 61.08 ± 0.36[ab] |
| 0.5 | 51.29 ± 0.65[ab] | 51.65 ± 0.48[ab] | 47.39 ± 0.35[b] | 45.33 ± 0.14[b] |
| 1.0 | 49.50 ± 0.77[b] | 47.61 ± 0.37[b] | 45.19 ± 0.24[b] | 41.45 ± 0.29[bc] |
| 1.5 | 45.25 ± 0.25[b] | 43.65 ± 0.69[bc] | 40.51 ± 0.75[c] | 38.19 ± 0.37[c] |

*values are *L** means ± SD based on 6 observations (3 samples × 2 replicates); [a–c]values followed by different lowercase superscripts differ ($P < 0.01$)

***Sensory analysis***. Sensory evaluation was conducted using a 10-member panel and a 5-point hedonic rating scale, with 0 = inconsumable, 1 = unacceptable, 2 = acceptable, 3= satisfactory, and 4 = excellent (Fradique *et al.* 2010). The degree of acceptance was estimated with respect to the attributes of colour, flavour, taste, texture, and mouthfeel.

***Statistical analysis***. Each formulation was produced three times and each experiment was performed in duplicate. Experiments were set up using a completely randomised design. Data were subjected to analysis of variance, and comparison of the means was done using two-way ANOVA test from Minitab software (Version 17.1.0) at a significance level of 0.01.

## RESULTS AND DISCUSSION

***Chemical characteristics***. The protein content of croissants increased with advancing storage time and increasing Spirulina concentration (Table 1). The latter finding is in agreement with the results reported by Rabelo *et al.* (2013), Selmo and Salas-Mellado (2014), and Shahbazizadeh *et al.* (2014), who have found that Spirulina enrichment increases the protein content of the final products. The Spirulina powder used in our trials contained 62.9% (w/w) of protein, thereby increasing the protein content of croissants (Ravelonandro *et al.* 2011). Besides being a rich source of protein, Spirulina also contains several minerals and vitamins, including vitamin $B_{12}$ (Danesi *et al.* 2010; Minh 2014).

The highest moisture contents were measured in the samples supplemented with 1.5% of *A. platensis* biomass (Table 1). As a general rule, the more Spirulina the croissants contained, the higher their moisture content. A plausible explanation for this finding is that, because of its starch and dietary fibre contents, Spirulina increases the water-holding capacity of the dough (Rabelo *et al.* 2013; Shahbazizadeh *et al.* 2014).

***Physical characteristics***. Table 2 shows that there were significant differences in lightness (*L**) between control and Spirulina-enriched croissant samples during storage ($P < 0.01$). Lightness was highest in control samples at the beginning of the 3-week storage period, whereas, the lowest *L** value was measured on day 21 in the product containing 1.5% of *A. platensis* biomass ($P < 0.01$). These results are consistent with those of

Table 3. Yeast and mould counts* of Spirulina-enriched croissant samples monitored over a 3-week storage period

| Microbial group | Spirulina (%, w/w) | Day | | | |
|---|---|---|---|---|---|
| | | 1 | 7 | 14 | 21 |
| Yeast | 0 | 70 ± 3.2[a] | 55 ± 1.7[ab] | 40 ± 5.2[b] | 32 ± 1.5[bc] |
| | 0.5 | 50 ± 2.6[ab] | 40 ± 1.0[b] | 35 ± 4.1[b] | 25 ± 2.1[c] |
| | 1.0 | 50 ± 1.3[ab] | 32 ± 2.5[bc] | 30 ± 6.3[bc] | 22 ± 3.4[c] |
| | 1.5 | 30 ± 2.5[c] | 25 ± 3.3[c] | 22 ± 3.5[c] | 20 ± 5.2[c] |
| Mould | 0 | 21 ± 11.2[b] | 37 ± 15.2[ab] | 50 ± 14.7[a] | 65 ± 12.8[a] |
| | 0.5 | 19 ± 14.1[b] | 35 ± 13.5[ab] | 47 ± 15.6[ab] | 56 ± 14.2[a] |
| | 1.0 | 17 ± 12.4[b] | 30 ± 14.4[ab] | 42 ± 17.0[ab] | 50 ± 17.4[a] |
| | 1.5 | 15 ± 18.1[b] | 25 ± 11.4[b] | 38 ± 15.3[ab] | 47 ± 16.5[ab] |

*values are CFU/g means ± SD based on 6 observations (3 samples × 2 replicates); [a–c]values within the same microbial group followed by different lowercase superscripts differ ($P < 0.01$)

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Table 4. Effects of storage time and Spirulina addition on sensory properties of croissants

| Source | DF | Colour | Flavour | Taste | Texture | Mouthfeel |
|---|---|---|---|---|---|---|
| A | 3 | $0.60^{ns}$ | $0.11^{ns}$ | $0.14^{ns}$ | $0.37^{ns}$ | $3.00^{ns}$ |
| B | 4 | $0.90^{ns}$ | $1.71^{ns}$ | $1.40^{ns}$ | $1.12^{ns}$ | $2.62^{ns}$ |
| A × B | 12 | $0.32^{ns}$ | $0.64^{ns}$ | $0.31^{ns}$ | $0.51^{ns}$ | $2.06^{ns}$ |

ns – not significant ($P > 0.01$); A – Shelf life; B – Spirulina concentration; A × B – interaction effect of shelf life and Spirulina concentration

Selmo and Salas-Mellado (2014) and Shahbazizadeh *et al.* (2014), who have reported that decreased lightness is generally observed in Spirulina-fortified bread and cookies because the deep green coloured microalgal biomass makes the products darker.

***Microbiological properties***. The *A. platensis* biomass inhibited the growth of both yeasts and moulds in croissants throughout the entire storage period ($P < 0.01$) (Table 3). Similarly, Varga and Szigeti (1998) found that Spirulina added to yogurts at 0.3% (w/w) completely suppressed the growth of yeasts and moulds up to 4 weeks of refrigerated storage at 4°C. Interestingly, yeast counts decreased with the progress of time, whereas mould counts increased as storage time advanced (Table 3). Mendiola *et al.* (2007) studied the antimicrobial activity of *A. platensis* extracts against *Staphylococcus aureus* (Gram-positive bacteria), *Escherichia coli* (Gram-negative bacteria), *Candida albicans* (yeast) and *Aspergillus niger* (mould). The results showed that *C. albicans* was the most sensitive test organism to all Spirulina fractions obtained by supercritical fluid extraction. The antifungal activity observed in our trials may be related to antimicrobially active lipids (e.g. γ-linolenic acid), fatty acids, and other bioactive substances found in *A. platensis* cells (Ördög *et al.* 2004; Bhowmik *et al.* 2009; Mala *et al.* 2009; Guldas & Irkin 2010).

***Sensory properties***. Figure 1 illustrates the impact of Spirulina content on organoleptic properties including colour, flavour, taste, texture, and mouthfeel of croissants. It is worth noting that there were no significant differences ($P > 0.01$) between the sensory results of samples fortified with 0.5, 1.0, and 1.5% of *A. platensis* biomass (Table 4). All things considered, the control product was only rated acceptable by sensory panellists, whereas the croissants containing 0.5 or 1.5% of Spirulina proved to be satisfactory, and the samples supplemented with 1% of *A. platensis* biomass were even superior to the other three products in terms of sensory scores (Figure 1).

***Hardness of texture***. Changes in the texture firmness of Spirulina-enriched and control croissant samples over 3 weeks of storage are shown in Figure 2. The highest texture hardness was measured in the control product on day 21, whereas the lowest mean value was observed on day 1 in croissants enriched with 1.5% of *A. platensis* biomass. Firmness decreased with increasing Spirulina levels ($P < 0.01$)

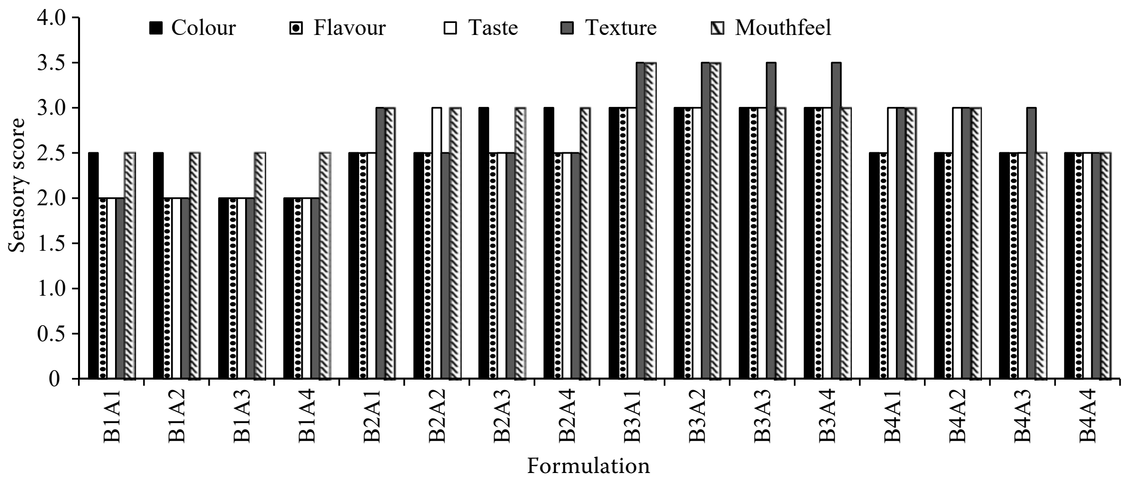


Figure 1. Sensory properties of Spirulina-enriched croissant samples following 1 to 21 days of storage

Sensory score: 0 – inconsumable; 1 – unacceptable; 2 – acceptable; 3 – satisfactory; 4 – excellent

A– Shelf life: A1 = day 1, A2 = day 7, A3 = day 14, A4 = day 21; B – Spirulina concentration: B1= 0%, B2 = 0.5%, B3 = 1%, B4 = 1.5%

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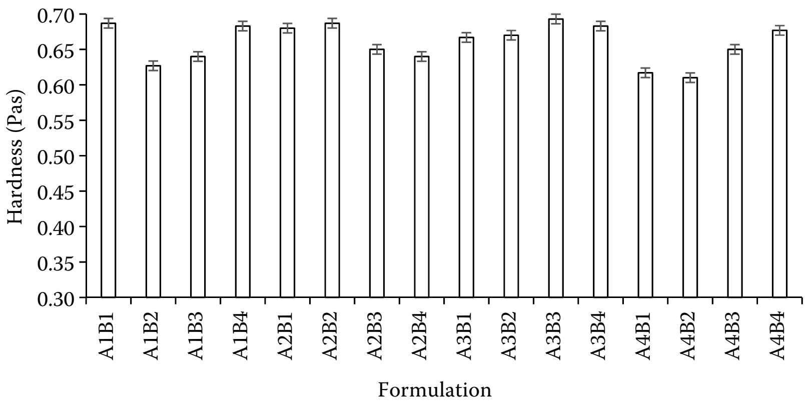


Figure 2. Hardness of samples enriched with *Arthrospira platensis* biomass at concentrations ranging between 0 and 1.5% (w/w), following 1 to 21 days of storage (SD = 0.01)

A – Shelf life: A1 = day 1; A2 = day 7; A3 = day 14; A4 = day 21; B – Spirulina concentration: B1 = 0%; B2 = 0.5%; B3 = 1%; B4 = 1.5%

because, as mentioned previously, the microalgal biomass is capable of increasing the water-holding capacity and, thus, improving the softness of bakery products (Danesi *et al.* 2010; Batista *et al.* 2011; Rabelo *et al.* 2013; Selmo & Salas-Mellado 2014; Shahbazizadeh *et al.* 2014) (Figure 2). *Arthrospira platensis* produces hydrocolloids (Raposo *et al.* 2013), and these types of polysaccharides are known to reduce the degree of moisture loss during storage of bakery products, thereby lowering the rate of crumb dehydration and hardening (Guarda *et al.* 2004). Similarly, the carbohydrates and, especially, the proteins of microalgae were reported by Gouveia *et al.* (2007) to reinforce the dough system through improving its water absorption properties.

Up to 21 days of storage, time had no influence ($P > 0.01$) on the hardness of samples fortified with *A. platensis* biomass (Figure 2). It was also observed that Spirulina incorporation beneficially affected the texture stability of croissants.

## CONCLUSIONS

The use of Spirulina at 0.5%–1.5% for the manufacture of croissants considerably improves the textural and sensory properties of the final products. Due to its non-starch polysaccharides, the *A. platensis* biomass is capable of enhancing the water-retention capacity of bakery products. Spirulina fortification also increases the protein content and decreases the crumb firmness and lightness of croissants. Optimum sensory results are obtained when the cyanobacterial biomass is added to the product at the rate of 1%. In addition to these benefits, Spirulina increases the levels of vitamins and essential amino acids in croissants. The abundance of biologically active substances in *A. platensis* (e.g. essential amino acids, chlorophyll, phycocyanin, carotenoids, minerals, vitamins, essential fatty acids) is of paramount importance from a nutritional viewpoint and Spirulina thus provides a novel opportunity for the manufacture of healthy bakery products.

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