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Microalgae enriched breadsticks: analysis for vitamin C, carotenoids, and chlorophyll a

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Microalgae enriched breadsticks: analysis for vitamin C, carotenoids, and chlorophyll a

Abstract

Microalgae are a potential ingredient that can enhance the nutritional value of food. There are already various products made from microalgae such as pasta, cookies, breadstick, crackers, and extrudates. Moreover, these products have a typical green colour, provided from microalgae pigments. This study aimed to evaluate the effect of the addition of *Chlorella vulgaris* and *Arthrospira platensis* biomass on vitamin C, total carotenoids, and chlorophyll a levels in breadsticks and its doughs. Microalgae addition in breadstick formulations is a viable alternative, because they presented a greater content of carotenoids and chlorophyll a than control breadsticks. Consequently, microalgae enriched breadsticks can provide health benefits to consumers. Here, Chlorella enriched breadsticks showed the highest studied pigments content. Despite microalgae powder containing vitamin C, breadstick dough did not present vitamin C and therefore nor the breadstick.

Keywords: *Spirulina platensis*, *Chlorella vulgaris*, breadstick, carotenoids, chlorophyll

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1. INTRODUCTION

Chlorella vulgaris is a spherically shaped single-cell organism that has a diameter between 2-10 μm (Safi et al., 2014). This green algae have a cellulose-based cell wall, which varies in thickness and composition according to growth conditions (Safi et al., 2015). From a nutritional perspective, these microalgae produce high amounts of xanthophylls and carotenes (Inbaraj et al., 2006). Spirulina (*Arthrospira platensis*) is cyanobacteria characterised by a filamentous multicellular morphology and ability to thrive in harsh environments (Madkour et al., 2012). Spirulina is well known in the industrial and commercial community because of the large number of companies investing in Spirulina-based supplementary products. Large-scale culture and industrial processing of this cyanobacterium is attributed to their rich medical and nutritional values as antioxidant, antimicrobial, and anticancer compounds (El-Kassas et al., 2015). Further, it is a valuable source for vitamins and several carotenoids such as carotenes, xanthophyll, and chlorophyll a (Jaime et al., 2005). Carotenoids are lipid-soluble pigments produced by plants and some microorganisms and result in a variety of colours observed in many foods. Carotenoids are classified to carotenoid hydrocarbons, also known as carotenes, and oxygenated carotenoids, also known as xanthophylls. Carotenoids play a role in regulation of plant growth and development, serving as photo-protectors, photosynthesis pigments, hormone precursors, and attractants for other species to help pollination and seed distribution (Rivera et al., 2012). The consumption of carotenoids has been linked with several health benefits, including cancer chemoprotection, prevention of heart and vascular disease, and prevention of other chronic and degenerative diseases (Amorim-Carrilho et al., 2014). Chlorophyll a is the major pigment that converts photons into chemical energy. In the centre of the chlorophyll molecule, magnesium (Mg^{2+}) has ionic and hydrophilic properties, surrounded by hydrophobic porphyrin rings with a polar carbonyl group.

Currently, there are numerous commercial applications for microalgae in food and animal feed. For example, in food, microalgae can enhance the nutritional value of

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pasta (Fradique et al., 2010), cookies (Batista et al., 2017), [10] breadstick (Uribe-Wandurraga et al., 2019) crackers (Batista et al., 2019), and extrudates (Uribe-Wandurraga et al., 2020). These products presented a typical green colour of microalgae, provided from their pigments. Therefore in this study, we aimed to evaluate the addition effect of *Chlorella vulgaris* and *Arthrospira platensis* biomass on vitamin C, total carotenoids, and chlorophyll a content in breadsticks and its doughs.

2. MATERIAL AND METHODS

2.1. Raw materials

Freeze dried *Arthrospira platensis* (Spirulina) and *Chlorella vulgaris* were supplied from Alga Energy S.A. (Madrid, Spain). Wheat flour, salt, yeast, and sunflower oil were purchased from a local supermarket (Alcampo, Valencia, Spain).

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2.2. Dough formulation and breadstick preparation

Dough formulation and breadsticks was prepared according to Uribe- Wandurraga et al. (2019). “Three dough samples of 60 g were prepared by mixing microalgae and wheat flour, the ratio was calculated by percentage; control dough contains 0% microalgae and 100% wheat flour. Spirulina dough comprised 1.5% *Arthrospira platensis* and 98.5% wheat flour. Chlorella dough comprised 1.5% of *Chlorella vulgaris* and 98.5% wheat flour. The other components of the dough were 14 g of sunflower oil, 7 g of fresh yeast, 0.4 g of salt and 18.6 g of water. The ingredients were mixed in a food processor (Kenwood chef classic, KM400/99 plus, Kenwood Corporation, Tokyo, Japan), kneaded for 15 min at speed 2 (200 rpm). Dough samples were fermented for 1 hour at 33 °C in a controlled temperature oven (Convotherm OES 6.06 mini CC, CONVOTHERM Elektrogeräte GMBH, Eglfing, Germany). The dough-breadsticks were weighed at 10 g and shaped by hand to a 10 cm length; they were fermented again for 30 min at 33 °C. After the second fermentation, samples were baked on rectangular baking sheets at 180 °C for 28 min in a steamer oven (Convotherm OES 6.06 mini CC, CONVOTHERM Elektrogeräte GMBH, Eglfing, Germany). Breadsticks were cooled at 25 ºC for 2 hours and placed in heat-resistant polyethylene plastic pouches (Cryovac® HT3050) and stored at room temperature (25 °C) for 15 days."

2.3. Analysis

2.3.1. Vitamin C

To determine vitamin C dehydroascorbic acid (DHAA) was reduced to ascorbic acid (AA), using DL-dithiothreitol the as reducing reagent according to Igual et al. (2016). Sample vitamin C content was determined by high performance liquid chromatography HPLC with a Jasco equipment (Italy) against the AA standard solution (Panreac, Spain). The detection was at 243 nm and at 25 °C. Results were expressed as mg of AA per 100g of sample.

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2.3.2. Total carotenoids

The total carotenoids (TC) in the samples was extracted with a solvent hexane/acetone/ethanol mixture following the Olives et al. (2006) method. The spectrophotometric reference method of AOAC (2000) was used for quantification. Sample absorbance was measured at 446 nm in an UV-visible spectrophotometer (Thermo Electron Corporation, USA). The TC content was expressed as mg of β-carotene (Fluka-Biochemika) per 100 g of sample.

2.3.3. Chlorophyll a

From the TC extract, chlorophyll a was determined. Sample absorbance was measured at 663 nm in a UV-visible spectrophotometer (Thermo Electron Corporation, USA). The chlorophyll a content was expressed as mg/100g of sample, calculated according to Lichtenthaler and Buschmann (2001) and Zvezdanovic and Markovic (2008).

2.3.4. Colour measurement

Colour measurement was carried out according Uribe- Wandurraga et al. (2019). "The dough and breadstick samples colour was measured using a colourimeter (Konica Minolta CM-700d/600d series, Tokyo, Japan) with a standard illuminant D65 and a visual angle of 10°. Results were obtained in terms of L* (brightness: L* = 0 (black), L* = 100 (white)), a* (-a* = greenness, +a* = redness), and b* (-b* = blueness, +b* = yellowness), according to the CIELab system (CIE, 1986). Chroma, C^*_{ab} (saturation) and hue angle, h^*_{ab} were also calculated, using equations 1 and 2, respectively." Colour coordinates were compared to chlorophyll a and TC content, as the responsible components for samples colour.

$$C^*_{ab} = \left[\left(a^{*2} + b^{*2}\right)\right]^{1/2} \quad (1)$$

$$h^*_{ab} = arctan\left(\frac{b^*}{a^*}\right) \quad (2)$$

2.4. Statistical analysis

Analysis of variance (ANOVA) was applied with a confidence level of 95% ($p < 0.05$), using Statgraphics (Centurion XVII Software, version 17.2.04) to evaluate the differences among samples. Furthermore, a correlation analysis among pigments content and colour coordinates, with a 95% significance level, was conducted (Statgraphics Centurion XVII).

3. RESULTS AND DISCUSSION

Microalgae biomass is used in food because of their high nutritional value and potential health benefits. Table 1 shows the mean values and standard deviation of vitamin C, TC, and chlorophyll a content in Chlorella and Spirulina powder used to formulate breadsticks. Vitamin C in Spirulina was like the value found by Babadzhanov et al., (2004) while the vitamin C content in Chlorella was slightly higher than the value of Pratt and Johnson (1967). The chlorophyll a and total carotenoid detected in

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microalgae were in the same range of values reported by other authors (Seyfabadi et al., 2011; Hynstova et al., 2018; Park et al., 2018). Spirulina presented significantly higher ($p < 0.05$) vitamin C and Chlorophyll a values than Chlorella. However, there are no significant TC content differences between Spirulina and Chlorella with respect.

Before dough samples were fermented for 1 hour at 33 °C, they were analysed. Moreover, breadsticks were analysed at the end of processing, when samples had cooled to 25 °C. Figures 1 and 2 show the mean values and standard deviation of TC and chlorophyll a content, respectively, in the control, Chlorella, and Spirulina dough and breadsticks. After dough fermentation, vitamin C was not detected in any sample. Doughs were maintained at 33 °C for 1 hour and provoked the total loss of vitamin C, because of oxidation of AA forming DHAA and finally 2,3-diketogulonic acid (Belitz et al., 2009). As seen in Figure 1, the addition of Chlorella and Spirulina significantly increased ($p < 0.05$) the TC in the dough than the control. Moreover, Spirulina dough showed significantly higher ($p < 0.05$) TC content than Chlorella dough. Considering TC content in Chlorella and Spirulina powder (Table 1) and microalgae concentration in the doughs, after fermentation, the Chlorella and Spirulina doughs lost 41.4 and 34% of TC, respectively. Breadsticks presented a significant decrease ($p < 0.05$) in TC than the Chlorella and Spirulina dough samples, however, this effect was not observed in control. As documented by numerous studies, carotenoids in food suffer thermal degradation during processing (Ahmed et al., 2002; Fratianni et al., 2010; Sampaio et al., 2013). In this study, samples were baked at 180 °C for 28 min, therefore carotenoids of breadsticks were lost in baking. Moreover, Spirulina breadsticks contained significantly lower ($p < 0.05$) TC than Chlorella breadsticks.

Figure 2 shows chlorophyll a content of studied doughs and breadsticks. Doughs were ranged from highest to lowest chlorophyll a content as Spirulina > Chlorella > control, with significant differences ($p < 0.05$). After dough fermentation, Chlorella and Spirulina doughs lost 42.4 and 33% chlorophyll a content, respectively, when considering

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chlorophyll a content of Chlorella and Spirulina powder (Table 1), and microalgae concentration in dough.

Spirulina and Chlorella breadsticks did not show significant differences ($p < 0.05$) in chlorophyll a content. Comparing microalgae doughs and breadsticks, we see a decrease in chlorophyll a content because of baking, as chlorophylls in food suffer thermal degradation during processing (Steet and Tong, 1996; Van Loey et al., 1998; Weemaes et al., 1999). Control breadsticks presented chlorophyll a values significantly lower than samples enriched with Spirulina and Chlorella.

Here, pigments analysed (carotenoids and chlorophyll a) were less stable in Spirulina samples, since that breadstick suffered the highest pigment loss after the second fermentation and baking. In Figure 3, the appearance of doughs and breadsticks studied can be seen. Previously, colour changes by Chlorella and Spirulina addition were studied by Uribe-Wandurraga et al. (2019). Visible differences among samples can be seen in pictures (Figure 3), and between the doughs and breadsticks. The largest visual change in the last comparison is in Spirulina, when dough colour is compared to the breadstick colour. Moreover, this is like the high loss of studied pigments in Spirulina breadsticks and its dough.

To explain the influence of the quantified pigments on the colour coordinates of the samples in this study, correlation statistical analyses were performed (Table 2). All Pearson correlation coefficient were significant ($p < 0.05$). The trend observed in the relationship between studied pigments and colour coordinates was similar in TC and chlorophyll a. When higher studied pigment content in samples were found, lower values of a* (TC: -0.90, Ca: -0.90), b* (TC: -0.90, Ca: -0.88), L* (TC: -0.88, Ca: -0.76), and C* (TC: -0.89, Ca: -0.86) and higher h*value (TC: -0.81, Ca: 0.94) was observed. The highest Pearson correlation with colour coordinates were shown by in TC content, except for h*. Doughs and breadsticks with higher TC and chlorophyll a content showed tonalities of bluish-green and darker.

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4. CONCLUSIONS

Microalgae addition in breadstick formulations is a good alternative, because they present a greater content of carotenoids and chlorophyll a than control breadsticks. Consequently, microalgae enriched breadsticks can provide health benefits available to consumers. Here, Chlorella breadsticks showed the highest studied pigments content. However, despite the microalgae powder contained vitamin C, doughs and breadsticks did not.

Conflict of interest

The authors declare no conflict of interest.

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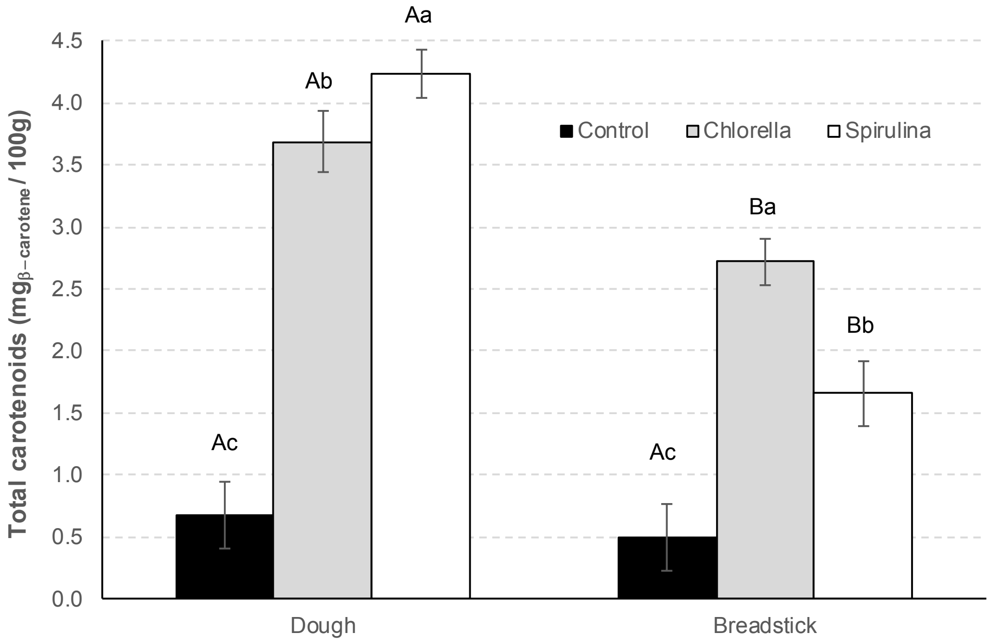

Figure 1. Mean values and standard deviation of total carotenoids of dough and breadstick. Letters indicate homogeneous groups established by the ANOVA (p < 0.05) for sample state (A,B) and for kind of samples (a-c).

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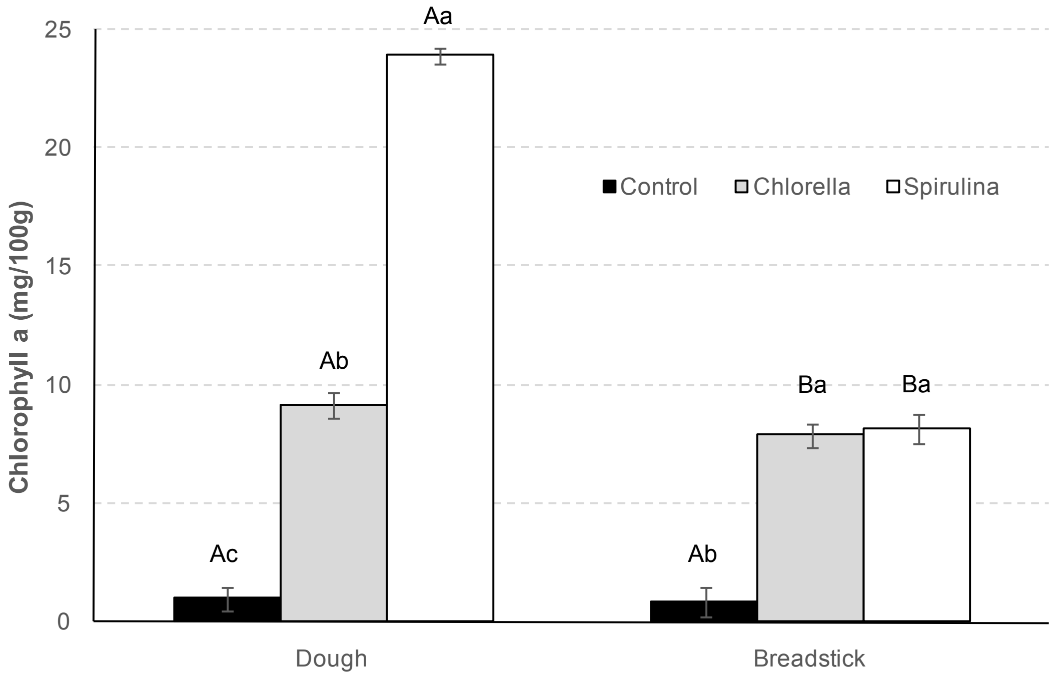

Figure 2. Mean values and standard deviation of chlorophyll a of dough and breadstick. Letters indicate homogeneous groups established by the ANOVA (p < 0.05) for sample state (A,B) and for kind of samples (a-c)

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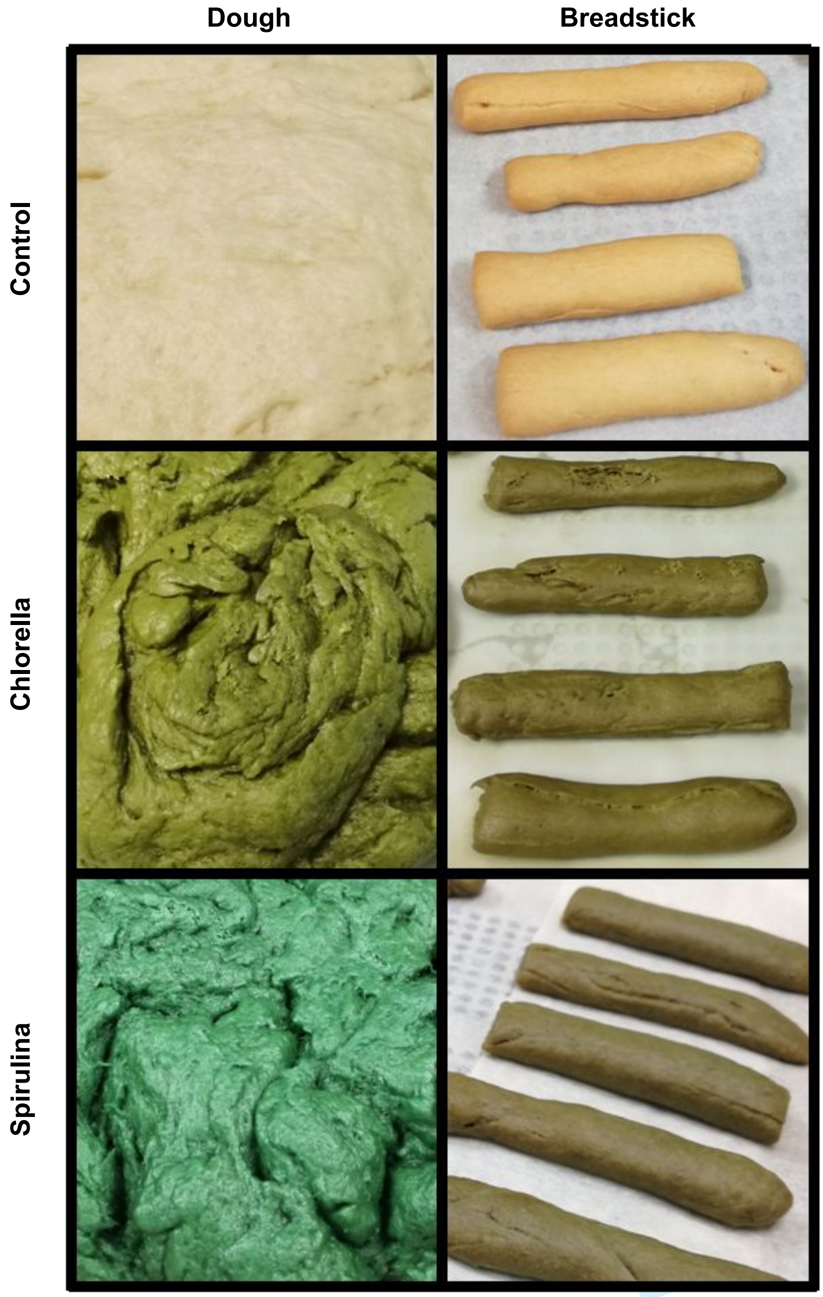

Figure 3. Pictures of dough and breadstick of studied samples

Table 1. Compound's mean values (mg/100 g) and standard deviation of microalgae powder.

Compounds	**Chlorella**	**Spirulina**
Vitamin C	170 (15)^{b}	204 (32)^{a}
Total carotenoids	370 (23)^{a}	385 (10)^{a}
Chlorophyll a	999 (46)^{b}	2312 (183)^{a}

Letters indicate homogeneous groups within rows established by the ANOVA ($p < 0.05$).

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Table 2. Pearson correlation coefficients among total carotenoids (TC), chlorophyll a (Ca), and colour coordinates.

	Ca	L*	a*	b*	h*	C*
TC	0,8666*	-0,8793*	-0,9004*	-0,8992*	0,8104*	-0,8934*
Ca		-0,7631*	-0,8972*	-0,8794*	0,9473*	-0,8557*
L*			0,7927*	0,9189*	-0,6013*	0,9249*
a*				0,9401*	-0,9059*	0,9328*
b*					-0,7930*	0,9987*
h*						-0,7654*

* Correlation is significant at the 0.05 level.

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