




Snack bars enriched with *Spirulina* for schoolchildren nutrition

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Abstract

The aim of this study was to develop snack bars nutritionally enriched with *Spirulina* and evaluate if there was acceptance of the sensorial attributes by schoolchildren. Snack bars enriched with *Spirulina* (2% and 6%) and a control formulation (0% *Spirulina*) were prepared. The samples were evaluated regarding nutritional content, microstructure, and sensorial characteristics. Furthermore, the study of stability during storage (30 days) was carried out. The addition of 2% and 6% of *Spirulina* provided a protein increase of 11.7% and 29.9%, respectively. The physicochemical and microbiological parameters remained stable during the storage of 30 days. Sensory evaluation showed that snack bars enriched with 6% *Spirulina* presented no significant difference ($p > 0.05$) compared to the control samples. We concluded that *Spirulina* can be used as a nutritive ingredient in snack bars designed for infant feeding without sensorial characteristics change.

Keywords: cereal bars; microalga; ready-to-eat food; sensory evaluation; stability during storage.

Practical Application: *Spirulina* addition in snack bars results in protein increase without affecting sensorial quality.

1 Introduction

Diets with low concentrations of nutrients such as protein and minerals contribute to the increase of diseases (Müller & Krawinkel, 2005; World Health Organization, 2014), besides affecting the growth and development of children (United Nations Children's Fund, 2018). In 2014, during the Second International Conference on Nutrition, over 170 countries adopted the political Declaration to ensure that all people have access to more healthy and sustainable diets. Such countries should achieve concrete results by 2025 through firm policies and actions (World Health Organization, 2014). In this context, the microalga *Spirulina*, which has a high concentration of nutrients and biocompounds (Batista et al., 2017; Rodríguez De Marco et al., 2014), has been used to improve the nutritional health of the population.

The Food and Drug Administration (FDA) approved *Spirulina* as safe food without toxicological effects on human health and provide GRAS (*Generally Recognized as Safe*) certification (Food and Drug Administration, 2002a). The compounds present in *Spirulina* biomass can exhibit antiviral, antibacterial, anticancer, antioxidant and anti-inflammatory roles which could be useful in the treatment of diseases (Hosseini et al., 2013). Foods such as pasta (Rodríguez De Marco et al., 2014), cookies (Batista et al., 2017; Morais et al., 2006), and ready-to-eat (RTE) extruded snacks (Lucas et al., 2017) have already been enriched with *Spirulina*.

Snack bars are also considered ready-to-eat food and are increasingly present in the diet of children. These foods attract the consumers due to their versatility and convenience (Ramírez-Jiménez et al., 2018), besides the high sensorial quality (Aigster et al., 2011; Bialek et al., 2016; Prazeres et al., 2017). In this

context, studies have been carried out aiming for the introduction of bioactive compounds into formulations of snack bars in order to provide healthier food for the consumers (Pinto et al., 2017).

Several studies have reported the influence of functional ingredients such as bean flour (Ramírez-Jiménez et al., 2018), lentils (Ryland et al., 2010) and fruit pulp (Prazeres et al., 2017) in the nutritional enrichment of snack bars. However, there are only a limited number of studies that evaluate the influence of *Spirulina* in the characteristics of this food.

Furthermore, several works that evaluated the sensory acceptance of foods enriched with *Spirulina* are found in the literature (Batista et al., 2017; Lucas et al., 2018; Santos et al., 2016), however, there are no studies which evaluated the sensory acceptance among children. Therefore, the aim of this study was to develop snack bars nutritionally enriched with *Spirulina* and evaluate if there was acceptance of the sensorial attributes by schoolchildren.

2 Material and methods

2.1 Materials

The ingredients used for the development of the snack bars were: *Spirulina* sp. LEB 18 biomass, oat flakes, rice flakes, brown sugar, glucose syrup, water, vegetable fat, lecithin and maltodextrin. The microalga *Spirulina* sp. LEB 18, was isolated from Mangueira Lagoon (Morais et al., 2008) and their biomass produced at the Pilot Plant of the Laboratory of Biochemical Engineering located in Rio Grande do Sul, Brazil (33°30'13"S 53°08'59"W). The biomass was collected, dried and ground to a fine powder

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in a ball mill (Model Q298, Quimis, Brazil). Afterwards, the biomass was sieved through a 48 mesh (0.3 mm) and stored in metallized bags at 4 °C. The other ingredients were purchased in a local market.

2.2 Preparation of snack bars

Three formulations were developed: C (Control), SP2 and SP6 (Table 1). Samples SP2 and SP6 were prepared by addition of 2% and 6% *Spirulina* sp. LEB 18, respectively, replacing the same amount of oat flakes in the control formulation.

Firstly, the oat and rice flakes were weighed and mixed. Afterward, the other ingredients were heated under constant mixing in a stainless-steel pan until form the agglutination syrup (79 °Brix). Then, this syrup was added and homogenized with the cereals pre-mixed. After, the resultant mixture was molded on portions of 35 g into a rectangular aluminum tray to obtain a snack bar with dimensions of 112 mm × 43 mm × 13 mm and then baked in an oven at 130 °C, during 15 min (Sun-Waterhouse et al., 2010). Afterward, the samples were cooled at room temperature and stored (25 ± 2 °C) in metalized bags of polyethylene (internal layer) and polyester (external layer) under vacuum for further analyses.

2.3 Analyses of snack bars

Proximate composition

The Association of Official Analytical Chemists (AOAC) procedures were used to determine the proximate composition of the snack bars. Protein quantification was performed according to micro-Kjeldahl method (nº. 960.52), with conversion factor of 6.25. Lipids, ash and moisture were quantified according to methods 920.39, 923.03 and 925.10, respectively (Association of Official Analytical Chemists, 1995). Carbohydrates were calculated by difference.

Color

The color of the snack bars was measured instrumentally using a colorimeter (Chroma meter CR-400, Konica Minolta, Tokyo, Japan). The results were expressed as L* (black = 0 and white = 100), a* (green = -60 and red = +60) and b* (blue = -60 and yellow = +60).

Total color difference (ΔE) between the samples with and without *Spirulina* was calculated according to Equation 1, where, L^*_0 , a^*_0 , and b^*_0 were the values obtained in the control sample

(without *Spirulina*). Chroma C^* (saturation) and hue angle (h°) were determined following Equations 2 and 3, respectively (Konica Minolta, 2007).

$$\Delta E = \sqrt{(L^* - L^*_0)^2 + (a^* - a^*_0)^2 + (b^* - b^*_0)^2} \quad (1)$$

$$C^* = (a^{*2} + b^{*2})^{1/2} \quad (2)$$

$$h^\circ = \tan^{-1} \left(\frac{b^*}{a^*} \right) \quad (3)$$

Texture

The texture of the snack bars was measured with a texture analyzer TA-XTplus (Stable Microsystems, Surrey, UK) equipped with a 25 kg load cell, using a cutting test at a speed of 2 mm/s and distance of the sample of 10 mm. The hardness was calculated as the maximum force required (N) for the probe to penetrate 50% of the snack bars thickness.

Microstructure

Samples were cut to a thickness of 5 mm and placed in double-sided adhesive tape mounted on aluminum stub. The microstructures were observed in a scanning electron microscope (JSM-6610LV, JEOL Ltd., Japan) using low vacuum and 15 kV accelerating voltage.

Microbiological evaluation

The snack bars were analyzed microbiologically following the methods described by Bacteriological Analytical Manual (BAM) (Food and Drug Administration, 2002b, 2007). The analyses performed were coliforms at 45 °C and *Salmonella* (Lucas et al., 2018).

Stability during storage

The fresh snack bars were stored in metalized bags (polyethylene: internal layer and polyester: external layer) under vacuum and maintained at room temperature (25 ± 2 °C). The properties of color, texture, moisture and microbiological attributes (coliforms at 45 °C and *Salmonella*) were analyzed after 15 and 30 days of storage, according to the procedures described previously.

Sensory properties

The sensory evaluation was performed with fifty children aged between 8 and 13, students of the Center for Comprehensive Care for Children and Adolescents (CAIC) (Rio Grande, Brazil), after obtained the consent from children's parents and the head teacher. Children evaluated the freshly prepared snack bars in terms of their attributes (appearance, flavor, and taste). The analysis was performed using a 5-point facial scale with verbal anchors (1 = dislike a lot, 2 = dislike, 3 = neither like nor dislike, 4 = like, 5 = like a lot). The sensory analysis was approved by the Research Ethics Committee of the Federal University of Rio Grande (approval nº 49/2016, Process 23116.002572/2016-05).

Table 1. Formulations of snack bar control (C) and snack bars with 2% of *Spirulina* (SP2) and 6% of *Spirulina* (SP6).

Ingredients (g/100 g)	C	SP2	SP6
Oat flakes	35.3	33.3	29.3
Rice flakes	15.8	15.8	15.8
Brown sugar	13.2	13.2	13.2
Glucose syrup	16.0	16.0	16.0
Water	9.2	9.2	9.2
Vegetable fat	1.5	1.5	1.5
Lecithin	2.0	2.0	2.0
Maltodextrin	7.0	7.0	7.0
<i>Spirulina</i> powder	0.0	2.0	6.0

Statistical analysis

Data were analyzed using analysis of variance (ANOVA) and the Tukey test was used to determine the differences between the means at 95% ($p < 0.05$) (Montgomery, 2009).

3 Results and discussion

3.1 Proximate composition of freshly prepared snack bars

The proximate composition showed that snack bars enriched with *Spirulina* presented protein content significantly higher ($p < 0.05$) than the control formulation (Table 2). The nutritional increase obtained after the addition of 2% and 6% of microalga was 11.7% and 29.9% in protein content, respectively. This occurs due to the high protein concentration (59.5%) of *Spirulina* sp. LEB 18 biomass used in the present study (Lucas et al., 2017).

Several studies have used *Spirulina* as an ingredient for nutritional enrichment of food. Batista et al. (2017) developed cookies with 2% and 6% of *Spirulina* biomass and observed increases of 24% and 59% in protein content, respectively, when compared to the control cookie (0% *Spirulina*). Lucas et al. (2018) prepared snacks enriched with *Spirulina* sp. LEB 18 and reported a nutritional increase of protein content (22.6%). Furthermore, the authors revealed that the concentration of carotenoids increased from $1.57 \pm 0.02 \mu\text{g/g}$ in the control formulation to $14.68 \pm 0.03 \mu\text{g/g}$ in the formulation with *Spirulina*, without showing significant modifications on the physical parameters of the snacks as expansion index and hardness. Rodríguez De Marco et al. (2014) added *Spirulina* biomass in pasta and observed an improvement in protein concentration. In this work, authors obtained 23.74% of protein content for the cooked pasta prepared with 20% of *Spirulina* addition, while the control formulation (without microalgae) showed a protein concentration of 13.09%. Furthermore, the authors verified that there were no biological activity losses during the cooking process.

The replacement of oat flakes by *Spirulina* biomass did not influence lipid concentration ($p > 0.05$) due to the similarity between the lipid composition of oat flakes (Núcleo de Estudos e Pesquisas em Alimentação, 2011) and *Spirulina* biomass (Lucas et al., 2017). Regarding ash content, the addition of 2% and 6% of *Spirulina* resulted in a significant increase ($p < 0.05$) of that component, indicating an increase in mineral concentration. This is relevant from a nutritional aspect, as according to Henrikson (2009), among the minerals contained within the biomass of this microalgae are calcium, iron, and magnesium. These micronutrients are essential for the health and the development of children (United Nations Children's Fund, 2018).

Similar behavior was observed by Santos et al. (2016), who elaborated shake for the elderly enriched with *Spirulina* sp. LEB 18 biomass and obtained an increase in mineral content when compared to the control sample. The shake with *Spirulina* presented 3.48% of ash, a result significantly greater ($p < 0.05$) than in the shake without microalga (3.14%). In another study, researchers developed food supplement for athletes added with biomass of *Spirulina* sp. LEB 18 and observed an increase in mineral content. The authors obtained 3.65% of ash content in the electrolyte replenisher without the microalga, while in the product added with *Spirulina* sp. LEB 18, this content was 4.00% (Carvalho et al., 2017).

Moisture content is a relevant indicator of food shelf life (Oyeyinka et al., 2018). The high moisture content is directly related to food degradation and microbial growth (Mathlouthi, 2001). In the present study, snack bars presented moisture content according to the value established by FDA (*Food and Drug Administration*) for cereal flours and related products ($< 15\%$) (Food and Drug Administration, 2017). The same trend was observed by Gutkoski et al. (2007) who developed oat based snack bars and obtained moisture content ranging from 10.75 to 13.95% and also by Srebernich et al. (2016) who reported moisture content ranging from 13.36 to 13.50% in snack bars developed from rice and oat flakes added with acacia gum, inulin, and sorbitol.

3.2 Color characteristics of freshly prepared and stored snack bars

The snack bars with *Spirulina* biomass presented an appearance similar to the control, but with the green color from the microalga (Figure 1). Table 3 presented the color parameters of the snack bars. The addition of 2 and 6% *Spirulina* significantly affected ($p < 0.05$) the color parameters of the snack bars when compared to the control formulation. These results should be related to the presence of pigments from *Spirulina*, such as chlorophylls, carotenoids and phycocyanin (Barkallah et al., 2017; Lucas et al., 2018).

The addition of this microalga in the formulation led to lower values of L^* , a^* , b^* , and C^* parameters as well as to an increase of hue angle. The results showed that the sample enriched with 6% of *Spirulina* presented a more intense green color when compared to samples C and SP2. According to Fradique et al. (2010), this makes microalgae a sustainable alternative as coloring agents with nutritional relevance (Table 2) over synthetic coloring agents.

Regarding the hue angle, the enrichment with *Spirulina* resulted in values between yellow (90°) and green (180°), which

Table 2. Proximate composition of snack bar control (C) and snack bars with 2% of *Spirulina* (SP2) and 6% of *Spirulina* (SP6).

Parameters	C	SP2	SP6
Protein (g/100 g dry basis)	9.31 ^c ± 0.13	10.40 ^b ± 0.03	12.09 ^a ± 0.05
Lipids (g/100 g dry basis)	6.12 ^a ± 0.09	6.02 ^a ± 0.12	6.16 ^a ± 0.13
Ash (g/100 g dry basis)	1.24 ^c ± 0.01	1.46 ^b ± 0.02	2.00 ^a ± 0.03
Moisture (g/100 g)	10.73 ^b ± 0.38	10.75 ^b ± 0.10	12.68 ^a ± 0.66
Carbohydrates (g/100 g)	72.60	71.37	67.07

Mean ± standard deviation ($n = 3$). Different letters in the same line mean significant differences between samples ($p < 0.05$).

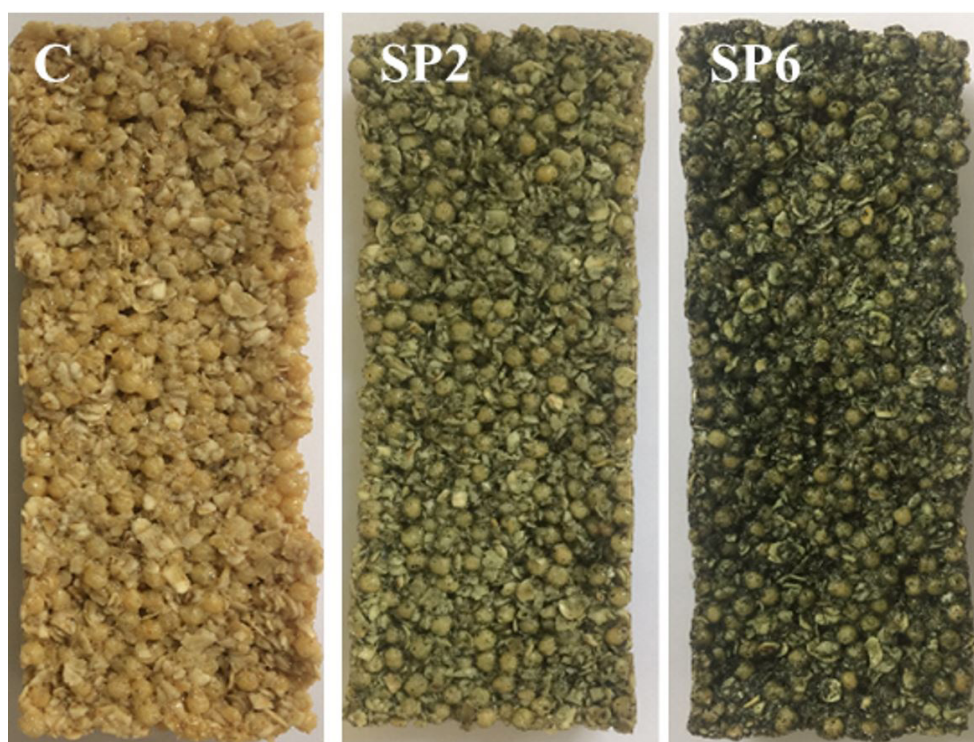


Figure 1. Snack bar control (C) and snack bars with 2% of *Spirulina* (SP2) and 6% of *Spirulina* (SP6).

Table 3. Color parameters of snack bar control (C) and snack bars with 2% of *Spirulina* (SP2) and 6% of *Spirulina* (SP6).

Storage	L*	a*	b*	C*	h	ΔE
0 days						
C	50.37 ^a ± 0.28	5.10 ^a ± 0.05	24.30 ^a ± 0.13	24.83 ^a ± 0.12	78.14 ^c ± 0.17	*
SP2	34.04 ^b ± 0.66	-1.34 ^b ± 0.01	14.76 ^b ± 0.19	14.82 ^b ± 0.19	95.17 ^b ± 0.02	19.98 ^b ± 0.49
SP6	27.41 ^c ± 1.00	-2.05 ^c ± 0.05	9.79 ^c ± 0.20	10.00 ^c ± 0.21	101.81 ^a ± 0.21	28.09 ^a ± 0.74
15 days						
C	50.25 ^a ± 0.31	5.07 ^a ± 0.03	24.48 ^a ± 0.23	25.00 ^a ± 0.23	78.30 ^c ± 0.09	*
SP2	34.30 ^b ± 0.87	-1.36 ^b ± 0.03	14.25 ^b ± 0.13	14.32 ^b ± 0.13	95.44 ^b ± 0.10	20.01 ^b ± 0.74
SP6	27.59 ^c ± 0.66	-2.06 ^c ± 0.07	9.50 ^c ± 0.20	9.72 ^c ± 0.19	102.24 ^a ± 0.56	28.09 ^a ± 0.63
30 days						
C	50.45 ^a ± 0.44	5.11 ^a ± 0.19	24.38 ^a ± 0.16	24.91 ^a ± 0.20	78.15 ^c ± 0.37	*
SP2	33.84 ^b ± 0.91	-1.35 ^b ± 0.08	14.37 ^b ± 0.26	14.43 ^b ± 0.26	95.37 ^b ± 0.28	20.44 ^b ± 0.86
SP6	27.35 ^c ± 0.85	-2.03 ^c ± 0.13	9.63 ^c ± 0.44	9.84 ^c ± 0.40	101.96 ^a ± 1.26	28.33 ^a ± 0.80

Means ± standard deviation ($n = 3$). Different letters in the same column mean significant differences between samples ($p < 0.05$). L*: Lightness; +a*: redness; -a*: greenness; +b*: yellowness; -b*: blueness; C*: Chroma; h: hue angle; ΔE: Total color difference; (*): Standard values (L^*_0 , a^*_0 ; b^*_0) used in the calculation of ΔE of SP2 and SP6.

could be due to the pigments present in the raw material, such as carotenoids and chlorophylls. The total color variation was directly related to the concentration of *Spirulina* added, being higher after larger microalga increments. Results of ΔE superior to 12 indicate that the colors of the samples are perceptible and different from the control (Limbo & Piergiovanni, 2006; You et al., 2018).

Lucas et al. (2018) also obtained a decrease in color parameters (L^* , b^* and, C^*) after addition of 2.6% *Spirulina* in the extruded snacks formulation as well as a total color difference (ΔE) of 30.50. Barkallah et al. (2017) developed yogurt and observed a reduction in L^* , a^* , and b^* parameters after addition of *Spirulina*.

Silva Figueira et al. (2011) prepared gluten-free bread enriched with *Spirulina* microalga and verified a reduction of L^* from 75.15 (control sample) to 28.83 (sample with 5% *Spirulina*).

Concerning the storage of 15 and 30 days, no significant changes ($p > 0.05$) were observed on colors parameters (L^* , a^* , b^* , C^* , hue, and ΔE), demonstrating the color stability of all the formulations (C, SP2, and SP6) along 30 days. Similar to our results, Barkallah et al. (2017) revealed that there was no significant change in a^* and b^* parameters of *Spirulina*-fortified yogurts during 28 days of storage. Thus, we can point out that the color obtained from *Spirulina* could remain stable during the storage of different food.

3.3 Physicochemical characteristics of freshly prepared and stored snack bars

Hardness is an important physical characteristic to be evaluated and is directly related with the food sensory acceptance. In the present study, the formulations C, SP2, and SP6 did not show changes in hardness or in moisture content during the storage of 30 days ($p > 0.05$). Furthermore, there was no significant difference ($p > 0.05$) between SP2 and C (Table 4), indicating that addition of 2% *Spirulina* does not alter the hardness and concentration of moisture of the snack bars. Batista et al. (2017) observed similar behavior in cookies enriched with microalgae. In the study, authors revealed that the addition of 2% microalgal biomass did not cause structural changes enough to modify the hardness of the cookies.

The moisture content of the SP6 sample was statistically higher ($p < 0.05$) than the content observed in samples C and SP2. The result obtained for SP6 may be due to the substitution of higher concentration of oats flakes which contains approximately 9% of moisture (Núcleo de Estudos e Pesquisas em Alimentação, 2011) by *Spirulina* biomass with 15.8% of moisture (Unpublished data). Moreover, the moisture content in the sample SP6 (Table 4) may also have influenced directly the texture.

Table 4. Physical properties of snack bar control (C) and snack bars with 2% of *Spirulina* (SP2) and 6% of *Spirulina* (SP6).

Storage	Hardness (N)	Moisture (g/100 g)
0 days		
C	81.35 ^a ± 3.74	10.73 ^b ± 0.38
SP2	82.45 ^a ± 5.42	10.75 ^b ± 0.10
SP6	52.36 ^b ± 3.37	12.68 ^a ± 0.66
15 days		
C	83.17 ^a ± 4.20	10.85 ^b ± 0.12
SP2	84.14 ^a ± 4.85	10.97 ^b ± 0.53
SP6	52.97 ^b ± 1.75	12.87 ^a ± 0.23
30 days		
C	86.76 ^a ± 5.83	10.61 ^b ± 0.30
SP2	89.37 ^a ± 5.74	10.81 ^b ± 0.41
SP6	56.91 ^b ± 0.98	12.18 ^a ± 0.24

Means ± standard deviation ($n = 3$). Different letters in the same column mean significant differences between samples ($p < 0.05$).

The SP6 (0, 15, and 30 d) showed hardness significantly lower ($p < 0.05$) than the observed in samples C and SP2 (Table 4). The micrographs (Figure 2) confirm that the SP6 sample presented a less compact structure when compared to C and SP2 structures. Despite that, the values obtained for all the samples are in agreement with previous studies that developed snack bars. Similar results were observed by Sun-Waterhouse et al. (2010) and by Prazeres et al. (2017) in snack bars added with fruit.

3.4 Microbiological characteristics of freshly prepared and stored snack bars

The results of the microbiological analyses (< 3 MPN g^{-1} for coliforms at 45 °C and absence in 25 g of *Salmonella*) indicated that the snack bars were processed under adequate hygienic sanitary conditions and therefore were considered suitable for consumption. Furthermore, the results confirm that the samples C, SP2, and SP6 were packaged and stored properly without risk of microbial contamination (by coliforms at 45 °C or *Salmonella*) during the storage of 30 days.

3.5 Sensory evaluation of freshly prepared snack bars

The sensory attributes are the factors that most influence the consumer preference (Pinto et al., 2017). According to Potter et al. (2013), children above 8 years are able to express the degree of liking of the sensory attributes using a 5-point facial scale with verbal anchors. The samples C and SP6 had no significant differences ($p > 0.05$) regarding the appearance, which demonstrates that the children visually accepted the addition of higher concentrations of *Spirulina* (6%) (Figure 3). This was probably due to the color of the SP6 sample remained greener (Table 3) than the SP2 sample, being more attractive for schoolchildren. Thus, *Spirulina* can be used as a coloring agent and additionally increase the nutritional content (Table 2) of snack bars.

Studies have already reported the high sensory acceptance of food enriched with *Spirulina* by the consumers. Santos et al. (2016) elaborated a shake for the elderly added with *Spirulina* sp. LEB 18 and verified higher sensory acceptance scores (7.68) when compared to the commercial shake (6.89). Lucas et al. (2018) reported that extruded snacks enriched with *Spirulina* sp. LEB 18 had a high acceptance rate (82%). In such study, the authors had average scores for flavor, texture, taste and overall acceptance between “like moderately” and “like very much”. Batista et al.

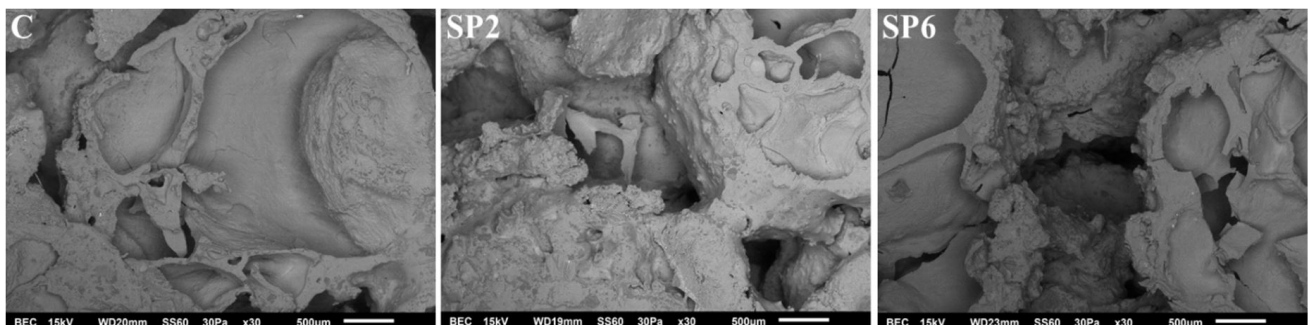


Figure 2. Microstructure of snack bar control (C) and snack bars with 2% of *Spirulina* (SP2) and 6% of *Spirulina* (SP6).

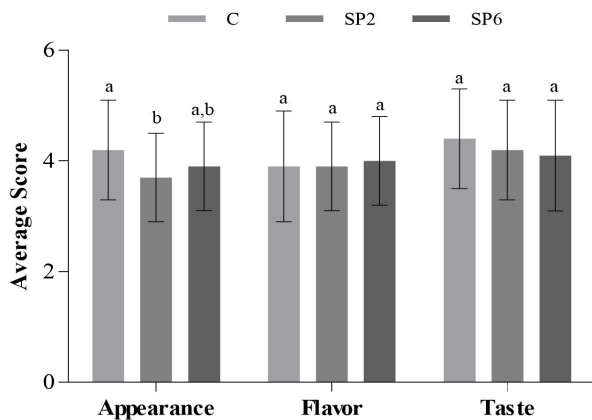


Figure 3. Sensory evaluation of snack bars control (C) and snack bars with 2% of *Spirulina* (SP2) and 6% of *Spirulina* (SP6). Means \pm standard deviation ($n = 50$). Different letters mean significant differences between samples ($p < 0.05$).

(2017) evaluated the sensorial characteristics of cookies added with 2 and 6% of the microalgae *Spirulina* and *Chlorella*. The authors revealed that the cookies enriched with 2% *Spirulina* were the preferred by consumers concerning sensory parameters of taste and global appreciation.

In the present study, the snack bars received scores between (3) neither like nor dislike and (4) like for the flavor, without showing a significant difference ($p > 0.05$) between the samples. Regarding the taste, the mean was considered high for all samples of snack bars, since it remained between (4) like and (5) like a lot (Figure 3), without significant differences between the samples ($p > 0.05$). These results indicate that although the hardness and moisture of the samples have shown significant differences ($p < 0.05$) (Table 4), the sensorial quality was not influenced. Thus, *Spirulina* may be considered relevant as an ingredient of snack bars intended for schoolchildren feeding.

4 Conclusion

We conclude that *Spirulina* can be used in the nutritional enrichment of snack bars. The addition of 2% and 6% of this microalga resulted in an increase of 11.7% and 29.9% in protein content, respectively, besides significantly affecting the color of the snack bars. The snack bars maintained their texture and color stable besides remaining microbiologically safe during the storage period evaluated (30 days).

Sensory evaluation showed that the snack bars enriched with 2% and 6% of *Spirulina* had the attributes appearance, flavor, and taste enjoyed by schoolchildren. Therefore, the use of *Spirulina* as an ingredient of snack bars designed for infant feeding is considered promising as an alternative to improve their nutritional health.

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