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Effect of *Spirulina* addition on the physicochemical and structural properties of extruded snacks

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Abstract

Nowadays the demand for practical food like snacks increases worldwide, however the nutritional value in most these formulations is reduced. Due to its chemical composition with high protein concentration, the microalga *Spirulina* has been used on the production of enriched foods. The present study aimed to evaluate the effects of *Spirulina* sp. LEB 18 addition on snacks formulations and extrusion conditions on the physicochemical and structural properties of snacks. Protein concentration and physical properties such as expansion index, bulk density, hardness, water absorption index, water solubility index and color were determined. The results showed that the addition of *Spirulina* sp. LEB 18, temperature in the last zone of the extruder and feed moisture influenced the product responses. The increase in feed moisture increased the hardness, bulk density and water absorption index of the snacks. Higher concentrations of microalga produced snacks with higher protein content, total color difference (ΔE) and compact structure. The addition of 2.6% *Spirulina* produced snacks with up to 11.3% protein and with adequate physical and structural properties for consumption. Thus, snacks containing *Spirulina* are an alternative to the demand for healthy food of practical consumption.

Keywords: extrusion; microalga; microstructure; nutrition; snack.

Practical Application: Snacks added of *Spirulina* can be developed with adequate physical and microstructural properties.

1 Introduction

Extruded snack food are growing worldwide due to the variety of shapes, textures and flavors, as well as the practicality of consumption. This class of food is highly accepted by children. However, most of the formulations have low concentrations of nutrients such as proteins, amino acids and vitamins (Anton et al., 2009; Basto et al., 2016). Furthermore, they are manufactured from transgenic cereals and have high levels of sodium.

Facing the challenges of attend the nutritional demands of a growing population and with high rates of malnutrition, it is essential to use new sources of nutrition, associating the development of agricultural products with biotechnology innovation. As an alternative to the supply of nutrients, *Spirulina* has a high concentration of digestible protein (Morais et al., 2008), besides being a source of essential fatty acids, vitamins, minerals and pigments.

Spirulina has possible effects that benefit health, such as improvement of the lipid profile (Parikh et al., 2001), suppression of hypertension (Ichimura et al., 2013), in addition to proving efficient in the treatment of child malnutrition (Li et al., 2012). *Spirulina* does not require arable land for its cultivation, and use a lower amount of water compared to the production of other foods such as soy, milk, eggs and meat (Henrikson, 2009).

Spirulina has been approved as safe food with no toxicological effects on human health and has received GRAS (Generally Recognized As Safe) certification (Food and Drug Administration, 2002). Studies conducted using this microalga have reported the increase of nutritional quality in foods (Marco et al., 2014; Morais et al., 2006).

Although the effect of different protein sources on extrudates has been extensively investigated to increase the nutritional value of these products (Goes et al., 2015; Jozinović et al., 2016; Lazou & Krokida, 2010; Onwulata et al., 2001b; Pastor-Cavada et al., 2011; Rathod & Annapure, 2016), little information is available on microalgae-added extruded snacks. Some studies using *Spirulina* on extrudates composition verified nutritional increase (Morsy et al., 2014; Joshi et al., 2014). Therefore, this study aimed to evaluate the effects of *Spirulina* sp. LEB 18 addition on snacks formulations and extrusion conditions on the physicochemical and structural properties of snacks.

2 Materials and methods

2.1 Raw material and centesimal composition

The microalga used was *Spirulina* sp. LEB 18, isolated from Mangueira Lagoon (Morais et al., 2008) and produced at the Pilot Plant of the Laboratory of Biochemical Engineering, located in Santa Vitória do Palmar, Rio Grande do Sul, Brazil (33°30'13"S and 53°08'59"W). The flours used were commercial organic corn flour (Indústria e Comércio de Erva Mate Salet, Coronel

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Bicaco, Brazil) and commercial organic rice flour (Volkmann Alimentos Ltda, Camaquã, Brazil).

The quantification of the total protein concentration was performed according to micro-Kjeldahl method nº 960.52 (Association of Official Analytical Chemists, 1995). The conversion factor of nitrogen used for *Spirulina* sp. LEB 18 was 5.95 (González López et al., 2010), 6.25 for corn flour and 5.95 for rice flour.

The total lipid content for *Spirulina* sp. LEB 18 was determined using the Folch gravimetric method (Folch et al., 1957), and the Soxhlet extraction method nº 920.39C was used for the flours (Association of Official Analytical Chemists, 1995). Ash and moisture determinations were performed according methods nº 923.03 and nº 925.10, respectively (Association of Official Analytical Chemists, 1995). The concentration of carbohydrates in *Spirulina* sp. LEB 18 biomass was determined from the phenol-sulfuric method (Dubois et al., 1956). For flours, carbohydrates were determined by difference.

2.2 Experimental design and statistical analysis

A Central Composite Design (2^3) was employed (Montgomery, 2009; Rathod & Annapure, 2016), with three independent variables (Table 1), six axial points and three repetitions of the central point. The independent variables were: concentration of *Spirulina* sp. LEB 18 in the formulation, feed moisture and temperature in the last zone of the extruder.

The dependent variables evaluated were protein concentration, expansion index, bulk density, hardness, water absorption index (WAI), water solubility index (WSI) and total color difference (ΔE). The responses were evaluated using linear and quadratic adjustment (Montgomery, 2009; Jeyakumari et al., 2016).

The regression models were obtained from significant terms ($p < 0.10$), where the adequacy of the fit model was evaluated from the coefficient of determination ($R^2 > 0.70$) (Borah et al., 2016; Jeyakumari et al., 2016; Sumargo et al., 2016). Only the best-fitted model was selected for each response in order to find the best process condition. Results were analyzed using Statistica software version 7.0 (Statsoft Inc, version 7.0, Tulsa, USA).

2.3 Sample preparation

The 2:1 ratio of rice and corn flour was used, defined after performing extrusion preliminary trials. All the ingredients were homogenized with filtered water using a planetary mixer (Kitchen Aid Professional, Model K45SS, Cameron Park, Australia).

Moisture content of each formulation was adjusted according to experimental design (Table 1). Corrections of deviations in moisture were performed from mass balance. Afterwards, the samples were packed in polypropylene bags and stored at 7 °C for 24 h, to ensure a uniform level of hydration.

2.4 Extrusion

The experiments were performed in a co-rotating twin-screw extruder (Werner Pfleiderer Co., Model ZSK-30, Ramsey, USA). The geometry of the screw was 872 mm in length and 30 mm in diameter (L/D ratio = 29.07).

Table 1. Variables and their levels employed in Central Composite Design (2^3), in coded and uncoded values.

Variables	Levels				
	-1.68	-1.0	0	+1.0	+1.68
Concentration of *Spirulina* sp. LEB 18 (%)	0.4	1.0	1.8	2.6	3.2
Feed moisture (%)	15	16.2	18	19.8	21
Temperature in the last zone (°C)	120	128	140	152	160

$\alpha = (2^n)^{1/4}$; n = number of independent variables; $\alpha = 1.68$.

A feed rate of 12.6 kg h^{-1} was used. The screw configuration starting from the feed extremity towards the die, considering n:a/b, KB c/d/a, a/b LH or KB c/d/a LH was: 2:60/30; 2:42/21; 1:28/14; 1:KB 90/5/28; 1:21/21; 1:28/14; 4:20/10; 1:KB 90/5/28; 1:21/21; 1:28/14; 5:20/10; 1:28/14; 1:14/14; 1:KB 45/5/14LH; 4:20/10; 1:28/14; 1:10/10LH; 3:20/10; 1: KB45/5/20; 3:20/10, where "n" is the number of elements; "a" is the element length (mm); "b" is the screw pitch of each element (mm); "KB" (kneading block) is a kneading element; "c" is the angle formed by adjacent crests, "d" is the number of crests of the kneading element and "LH" is the flow inverter which creates a blockage and generates a high pressure zone.

The screw rotation speed was set at 250 rpm and the temperatures of the 1st, 2nd and 3rd heating zones were fixed at 75 °C, 100 °C and 120 °C, respectively (Oliveira et al., 2015). Temperature in the 4th heating zone was varied from 120 to 160 °C, according to experimental design (Table 1). At the exit, a circular die with diameter of 3.8 mm was used.

After extrusion, the snacks were dried in a forced air oven at 85 °C (Tecnal, Model TE-394/2, Piracicaba, Brazil) until moisture less than 6%. Afterwards snacks were stored in laminated bi-oriented polypropylene bags at 18 °C for further analyses.

2.5 Physicochemical analyses of snacks

Protein

The protein concentration was determined by micro-Kjeldahl method of total nitrogen nº 960.52, with conversion factor of 6.25 (Association of Official Analytical Chemists, 1995).

Expansion index

The expansion index (EI) (Equation 1) was calculated according to Gujska & Khan (1990), where D_{ex} is the diameter of the extrudate (cm) and D_o is the diameter of the die (cm).

$$EI = \frac{D_{ex}}{D_o} \quad (1)$$

Bulk density

Bulk density (g cm^{-3}) was determined according to Alvarez-Martinez et al. (1988), from Equation 2, where m is the sample weight (g), D is the diameter of each snack (cm) and L is the length of the extrudate (cm).

$$BD = \frac{4 \times m}{\pi \times D^2 \times L} \quad (2)$$

Color analysis

Color of the extrudates was evaluated using a colorimeter (Konica Minolta, model Chroma meter CR-400, Tokyo, Japan) and expressed as L* (lightness/darkness), a* (redness+/greenness-) and b* (yellowness+/blueness-). The total color difference (ΔE) was calculated according to Equation 3, where the subscript '0' indicates the values of the extrudates developed without addition of *Spirulina* sp. LEB 18, from extrusion preliminary trials. Chroma (C*) and hue angle (h) were also calculated according to Equations 4 and 5, respectively.

$$\Delta E = \sqrt{\left(L - L_0\right)^2 + \left(a - a_0\right)^2 + \left(b - b_0\right)^2} \quad (3)$$

$$C^* = \left(a^{*2} + b^{*2}\right)^{1/2} \quad (4)$$

$$h^o = \tan^{-1}\left(\frac{b^*}{a^*}\right) \quad (5)$$

Hardness

Texture measurements was performed using a texture analyzer (Stable Micro Systems, model TA-XTplus, Surrey, UK), with a cylindrical probe of 20 mm. Speed of 1 mm s^{-1} and distance of 25 mm was used. Hardness was the maximum force (N) achieved during the run.

Water Absorption Index (WAI) and Water Solubility Index (WSI)

Water absorption index and water solubility index were determined according to the method developed by Anderson et al. (1969).

Scanning Electron Microscopy (SEM)

Samples cross sections were cut at a thickness of 4-5 mm, mounted on stubs and sputter coated with gold (Denton Vacuum, model Desk-V-Standard, Moorestown, USA). Microstructure of the snacks was examined with a scanning electron microscope (JEOL Ltd., model JSM-6610LV, Tokyo, Japan) using accelerating voltage of 15 kV.

3 Results and discussion

3.1 Composition of raw material

The proximate composition of organic rice flour were protein (8.6%), fat (1.6%), carbohydrate (89.2%) and ash (0.6%). Organic corn flour contained 9.7% protein, 3.8% fat, 85.7% carbohydrates and 0.8% ash. All results were expressed on a dry basis.

Spirulina sp. LEB 18 biomass presented 59.5% protein, 7.0% lipids, 17.2% carbohydrates and 16.5% ash, on a dry basis. The high protein concentration demonstrates the viability of the application of this source as nutritional enrichment of foods. This protein content is higher than that found in other commonly consumed sources of protein such as soy (35%), milk powder (35%) and eggs (12%) (Henrikson, 2009).

3.2 Physicochemical characteristics of snacks

Extrudates with different physical properties were obtained under different processing conditions (Table 2). Regression models were obtained after evaluating the influence of the independent variables on the responses (Table 3).

Protein

The protein concentration of the formulations ranged from 9.4 to 11.3%. Trials added with 2.6% and 3.2% of *Spirulina* sp. LEB 18 resulted in snacks with higher protein content. Other

Table 2. Matrix of the Central Composite Rotational Design (in coded and uncoded values) and the evaluated responses.

Trial	S (%)	U (%)	T (°C)	EI	BD	HD	WAI	WSI	ΔE
1	-1 (1.0)	-1 (16.2)	-1 (128)	4.30	0.072	22.34	8.24	28.20	27.72
2	+1 (2.6)	-1 (16.2)	-1 (128)	4.22	0.075	26.44	7.62	38.18	31.14
3	-1 (1.0)	+1 (19.8)	-1 (128)	3.90	0.098	28.46	9.14	31.78	26.15
4	+1 (2.6)	+1 (19.8)	-1 (128)	3.80	0.102	32.84	8.30	35.73	32.55
5	-1 (1.0)	-1 (16.2)	+1 (152)	4.50	0.080	21.87	9.06	28.81	26.83
6	+1 (2.6)	-1 (16.2)	+1 (152)	4.44	0.083	25.89	8.59	33.32	30.84
7	-1 (1.0)	+1 (19.8)	+1 (152)	3.70	0.089	22.55	9.08	33.20	28.33
8	+1 (2.6)	+1 (19.8)	+1 (152)	3.63	0.103	27.89	9.02	37.10	31.98
9	-1.68 (0.4)	0 (18)	0 (140)	4.36	0.071	19.35	8.99	23.00	26.72
10	+1.68 (3.2)	0 (18)	0 (140)	3.07	0.119	21.76	8.47	33.00	36.55
11	0 (1.8)	-1.68 (15)	0 (140)	3.90	0.088	14.13	8.40	25.09	29.96
12	0 (1.8)	+1.68 (21)	0 (140)	2.49	0.185	31.66	8.84	24.75	31.00
13	0 (1.8)	0 (18)	-1.68 (120)	2.77	0.124	23.18	8.66	26.11	29.73
14	0 (1.8)	0 (18)	+1.68 (160)	3.64	0.091	23.47	8.58	32.49	31.38
15	0 (1.8)	0 (18)	0 (140)	3.90	0.077	15.38	8.63	28.05	29.36
16	0 (1.8)	0 (18)	0 (140)	3.83	0.085	15.32	8.63	28.60	29.98
17	0 (1.8)	0 (18)	0 (140)	3.86	0.079	16.29	8.62	30.57	29.63

S = concentration of *Spirulina* sp. LEB 18 (%); U = feed moisture (%); T = temperature in the 4th extrusion zone (°C); EI = expansion index (cm cm^{-1}); BD = bulk density (g cm^{-3}); HD = Hardness (N); WAI = water absorption index (g gel.g^{-1}); WSI = water solubility index (%); ΔE = Total color difference.

Table 3. Regression equations of response variables.

Responses	Regression model	R^2	F_{test}
EI	EI = 4.01 – 0.30U	0.80	10.90
BD	BD = 0.09 + 0.01U	0.71	6.44
Hardness	Hardness = 15.44 + 2.48S^2 + 3.27U + 3.31U^2 + 3.46T^2	0.71	3.93
WAI	WAI = 8.63 - 0.25S + 0.25U + 0.31T + 0.12ST – 0.14UT	0.97	10.74
ΔE	ΔE = 29.50 + 2.19S	0.89	20.97

$F_{test} = F_{value} / F_{tabulated}$; EI = expansion index (cm cm^{-1}); BD = bulk density (g cm^{-3}); WAI = water absorption index (g gel.g^{-1}); ΔE = Total color difference; S = concentration of *Spirulina* sp. LEB 18 (%); U = feed moisture (%); T = temperature in the 4th extrusion zone (°C).

studies also showed an increase in protein concentration in foods added with *Spirulina*. After incorporating *Spirulina* biomass in pasta, Marco et al. (2014) observed increase in protein content, phenolic compounds and antioxidant activity. Morais et al. (2006) developed cookies added with *Spirulina* sp. LEB 18 and obtained 7% increase in protein content compared to the control sample without microalgae.

Expansion index

Expansion index of the extrudates ranged from 2.5 to 4.5. Snacks with a higher expansion index present a less dense structure, providing greater crispiness. The expansion obtained in this work presented results similar to those found by others studies. Sumargo et al. (2016) developed extruded of rice and beans and obtained values between 3.5 and 4.2. Ding et al. (2005) reported values between 2.02 and 3.87 for rice-based extrudates.

The moisture had a significant ($p < 0.001$) and negative effect on the expansion index of snacks, at all temperatures and concentrations of *Spirulina* sp. LEB 18. Previous studies also used rice as raw material and observed similar behavior of moisture on the expansion of extrudates (Borah et al., 2016; Sumargo et al., 2016). According to Ding et al. (2005) increasing feed moisture content during extrusion reduces the mechanical energy dissipation, resulting in the mass less viscous, reducing the degree of gelatinization of the starch and resulting in less expansion.

The *Spirulina* sp. LEB 18 addition had not significant probably due to the concentration used was not sufficient to influenced the viscous and elastic forces who governing the extrusion expansion (Sumargo et al., 2016). Elevated temperatures tend to affect and reduce the rate of expansion due to degradation of the starch granules (Borah et al., 2016). In the present study the temperature had no significant effect on the expansion, probably because of the degradation of the starch did not occur in high proportions inside the extruder.

Bulk density

Bulk density considers the expansion in all directions, which is very important evaluation in extruded snacks (Jeyakumari et al., 2016; Rathod & Annapure, 2016). Studies generally evaluate the influence of extrusion parameters and raw material composition on this property.

Ding et al. (2005) when developed extruded rice-based snacks, obtained bulk density values between 0.10 and 0.43 g cm^{-3}. Jeyakumari et al. (2016) produced snacks based on rice, corn and shrimp protein hydrolysates and obtained values between 0.045 and 0.082 g cm^{-3}. These results are in agreement with our study (0.071 to 0.185 g cm^{-3}).

The linear model was adjusted from the moisture effect ($p < 0.01$) with coefficient of determination (R^2) of 0.71 (Table 3). Higher feed moisture values led to an increase in the bulk density of the extrudate at all temperature and *Spirulina* sp. LEB 18 concentration levels. Feed moisture was also the main factor influencing this response of extrudates developed by Oliveira et al. (2017) and by Stojceska et al. (2009). This is probably due to the partial evaporation of the moisture content of the blend, resulting in less expanded and higher bulk density products.

The addition of *Spirulina* sp. LEB 18 did not have a significant effect on bulk density probably because the concentration used in substitution of starch was low. Onwulata et al. (2001a) observed a similar result when milk protein was added in extrudates, without significantly affecting the density of the product. Additionally, the temperature did not affect significantly the bulk density, probably because in the temperatures of 152 °C, the degradation of the starch was not occurring in high proportions.

Hardness

All variables were found to have significant effect on the hardness of the snacks (Figure 1A-C). The results showed that the best-fitted model was the quadratic, with coefficient of determination (R^2) of 0.71.

The increase in feed moisture resulted in a significant increase ($p < 0.01$) in the hardness of the extrudate. This effect is due to the fact that higher moisture content decreases the shear of the plasticized mass inside the extruder, reducing the gelatinized starch and prevent the growth of air bubbles of the snacks (Stojceska et al., 2009; Sumargo et al., 2016). The hardness of the snacks ranged from 14.1 to 32.8 N. Similar results (16.2 to 43.4 N) were reported by Joshi et al. (2014) on corn extrudates added with microalga.

Furthermore, the microalga also influenced the hardness, probably due to the high concentration of protein present in the composition of this microalga (59.5%). This fact is due to the protein competes with the starch for the water during extrusion, reducing gelatinization and resulting in lower expansion and higher hardness. In addition, the higher protein concentration may also result in differences in pressure and temperature of the mass inside the extruder, affecting the texture properties (Sumargo et al., 2016).

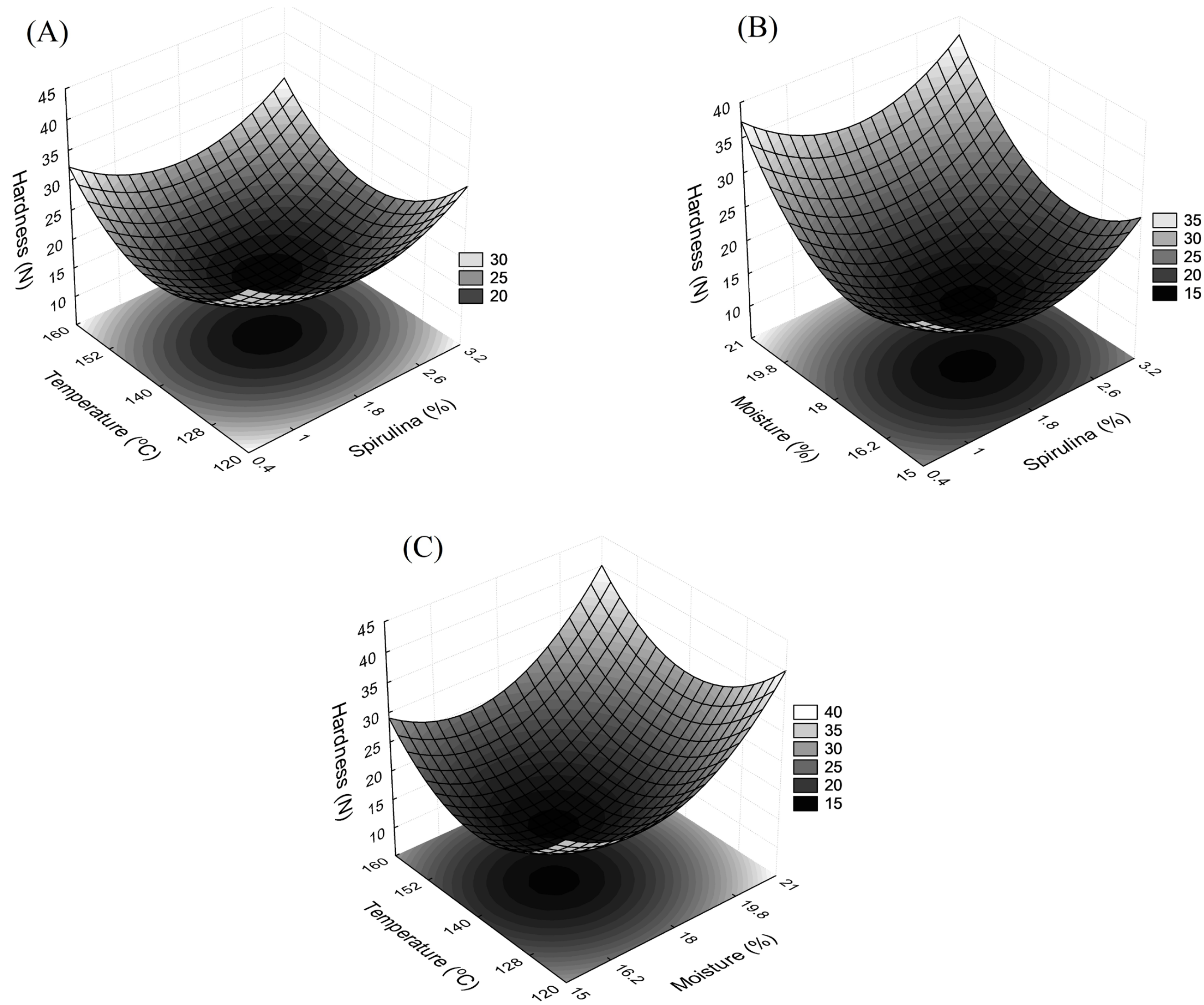

Figure 1. Effect of the independent variables temperature and *Spirulina* sp. LEB 18 (A), moisture and *Spirulina* sp. LEB 18 (B) and temperature and moisture (C) in the hardness of the extruded snacks.

This observation is in agreement with studies conducted by Lazou & Krokida (2010), Sumargo et al. (2016) and by Onwulata et al. (2001b). These studies obtained enhance in hardness when they increased the concentration of protein in their formulations.

In the trials 9 and 10, who had 0.4 and 3.2% of *Spirulina* sp. LEB 18 addition, the results of hardness result in similar region on the surface of response (Figure 1). This is probably due to the feed moisture (18%) and the temperature in the last zone (140 °C) used, which is intermediate (central points), and generally result in good properties in the extrudates (Ding et al., 2005).

In the present study, the highest hardness was obtained in the trial containing 2.6% *Spirulina* sp. LEB 18, 19.8% moisture and 128 °C in the last zone of the extruder. This result confirms that high levels of protein (*Spirulina* sp. LEB 18) and feed moisture should not be used in conjunction with lower temperatures, since they reduce the technological quality of the snacks.

Water absorption index and water solubility index

WAI is related to the absorption and retention of water by the constituents of the raw material, and can be used as a gelatinization index (Joshi et al., 2014). The increase in feed moisture positively influenced the WAI ($p < 0.01$), due to the increase in content of water available. Similar results were obtained in others studies (Ding et al., 2005; Stojceska et al., 2009).

Spirulina sp. LEB 18 also had a significant effect ($p < 0.01$), resulting in a decrease in the water absorption index of the extrudates. This decrease may be associated to the reduction of the flour concentration due to replacement by *Spirulina* sp. LEB 18, which resulted in lower gelatinization of the starch within the extruder.

Other studies found this behavior after include raw materials with high protein content in extrudates. Sumargo et al. (2016) reported this performance for beans during extrusion. However, the results obtained (7.6 to 9.1 g g^{-1}) were higher than those found in other studies that used rice flour (Ding et al., 2005), microalgae and corn flour as raw material (Joshi et al., 2014).

In addition, the temperature also had a significant and positive effect ($p < 0.01$). According to Rathod & Annapure (2016) with increasing temperature, the starch becomes more cooked and more able to expand, however, an excessive temperature may cause weakening of the structure and fragmentation of starch molecules. Thus, it is assumed that at the temperatures used in the extruder zones, the starch degradation process was not occurred in high proportions and, therefore, the WAI did not decrease.

WSI is a measure of the mechanical depolymerisation of starch, releasing free polysaccharides during extrusion (Seth et al., 2015; Sumargo et al., 2016). The results of the variable response WSI ranged from 23.0 to 38.2% and is comparable to other study which obtained values between 21.5 and 32.7% in the development of rice-based extrudates (Ding et al., 2005). However, no variables independent had a significant effect on this response ($p > 0.10$) and the coefficient of determination indicated a lack of fit ($R^2 = 0.46$), thus no surfaces and regression model were generated for this variable.

Color

Color is an important attribute in food acceptability, being an indicator of quality, conservation state, flavor expectation and commercial value (Fradique et al., 2010). The results of ΔE ranged from 26.2 to 36.5. Data obtained for the total color difference (ΔE) showed that the linear model was adjusted from the effect of concentration of *Spirulina* sp. LEB 18 (p <0001), with a coefficient of determination (R^2) of 0.89 (Table 3).

Although rice-corn based snacks exhibit yellow coloration (+ b*) and high luminosity (L*), the green color provided by the addition of *Spirulina* sp. LEB 18 can be appreciated in products developed specially for children.

Higher values of ΔE were obtained with the increase of *Spirulina* sp. LEB 18, at all temperature and moisture levels. This behavior is due to the major pigments in microalga biomass (chlorophyll, carotenoids, phycocyanin) (Fradique et al., 2010). The color parameters for snacks extruded with different concentrations of *Spirulina* sp. LEB 18 under the same extruder conditions can be observed in Table 4.

The addition of higher concentrations of *Spirulina* sp. LEB 18 in the extruded snacks caused the reduction of a*, b*, C* and luminosity (L*). Furthermore, the trials with higher microalgae content obtained an increase in the Hue angle (h), which was expected due to the green coloration of the microalga, leading to green hues. The results were similar to those obtained in other studies that evaluated the influence of the addition of *Spirulina* on the color of foods (Fradique et al., 2010; Joshi et al., 2014).

3.3 Microstructure

The effects caused by the extrusion conditions and *Spirulina* sp. LEB 18 were observed by SEM micrographs. Air cells with different sizes inside the snacks were observed (Figure 2). The extrudates presented cell walls with thickness between 30.3 μm and 114.6 μm.

Structures more compacted, with smaller bubble size and thicker cell walls (Figure 3) were associated with the increase of *Spirulina* sp. LEB 18, which also significantly influenced the hardness of the extruded. The cell walls of the trials containing 0.4, 1.8 and 3.2% *Spirulina* sp. LEB 18 presented in average 30.25 μm, 58.02 μm and 98.06 μm of thickness, respectively. Lazou & Krokida (2010), when increasing the amount of lentil flour observed a similar influence on the extruded microstructure. The authors also obtained the thickening of the extruded cell walls, as well as reduction in the number of air bubbles formed in the extrusion, resulting in more rigid structures.

Table 4. Color parameters for the trials (9, 15 and 10) containing 0.4, 1.8 and 3.2% *Spirulina* sp. LEB 18, respectively.

Parameters	Trials		
	9	15	10
*L**	61.5 ± 0.6^{a}	49.9 ± 0.4^{b}	41.7 ± 1.8^{c}
*a**	1.9 ± 0.2^{a}	1.8 ± 0.1^{b}	-0.8 ± 0.1^{c}
*b**	22.7 ± 0.7^{a}	22.3 ± 0.3^{a}	20.0 ± 1.1^{b}
*C**	22.8 ± 0.7^{a}	22.3 ± 0.3^{a}	20.0 ± 1.1^{b}
h	85.1 ± 0.5^{c}	85.5 ± 0.2^{b}	92.4 ± 0.3^{a}
ΔE	26.4 ± 0.8^{c}	29.7 ± 0.1^{b}	36.5 ± 1.4^{a}

Different letters in the same row indicate significant difference between samples ($p < 0.05$). *L** = luminosity; a* and b* = chromatic coordinates (+*a** = red and -*a** = green; + *b** = yellow and -*b** = blue); *C** = Chroma; *h* = Hue angle and ΔE = Total color difference; trial 9 = snacks with 0.4% *Spirulina* sp. LEB 18; trial 15 = snacks with 1.8% *Spirulina* sp. LEB 18; trial 10 = snacks with 3.2% *Spirulina* sp. LEB 18.

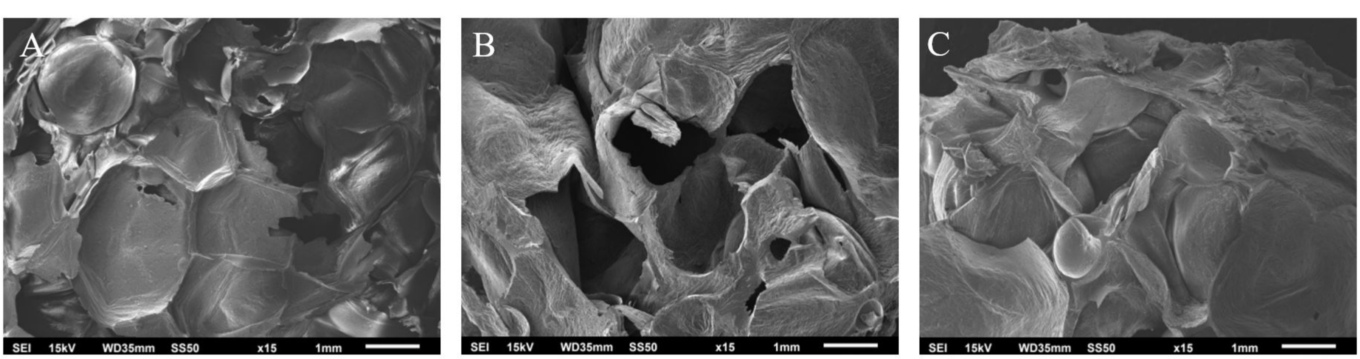

Figure 2. Micrographs of extruded snacks with (A) 0.4% of *Spirulina* sp. LEB 18 (trial 9); (B) 1.8% *Spirulina* sp. LEB 18 (trial 16); (C) 3.2% *Spirulina* sp. LEB 18 (trial 10).

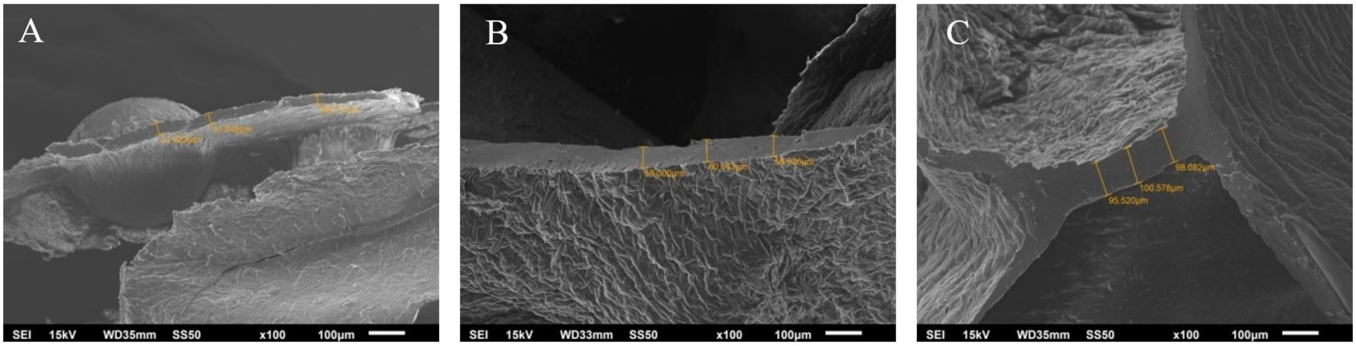

Figure 3. Micrographs of cell wall of the extruded snacks with (A) 0.4% *Spirulina* sp. LEB 18 (trial 9); (B) 1.8% *Spirulina* sp. LEB 18 (trial 16); (C) 3.2% *Spirulina* sp. LEB 18 (trial 10).

Despite the influence on the physical parameters, the use of higher concentrations of *Spirulina* sp. LEB 18 is interesting from nutritional enrichment of food, since this microalga has high contents of biocompounds. Several studies have been carried out with the addition of *Spirulina* in foods and have reported improvements in different nutritional and sensory parameters (Marco et al., 2014; Fradique et al., 2010; Morais et al., 2006).

The microstructure evaluation contributed for demonstrated the influence of the *Spirulina* sp. LEB 18 in the process, which is interesting for associate with the quantitative responses (for example: hardness, bulk density, expansion index). This type of analysis has been used in extruded snacks, providing understanding the structure of the product, avoiding the use of inadequate levels of protein and feed moisture during the extrusion and preventing loss on sensory quality.

3.4 Best process condition

After the interpretation of the all data and by overlapping response surfaces, the best process condition obtained was 2.6% *Spirulina* sp. LEB 18, 16.2% feed moisture and 143 °C in the 4^{th} zone of extruder. This condition was selected to increase protein concentration, expansion index, ΔE and to reduce the bulk density and hardness of the snacks.

4 Conclusions

The best process condition obtained for the development of the snacks was 2.6% *Spirulina* sp. LEB 18, 16.2% feed moisture and 143 °C in the last zone of the extruder. Increased in feed moisture make it difficult the process and the expansion in the die of the extruder, providing more rigid snacks, with compacted structures and higher bulk density.

The results showed that *Spirulina* sp. LEB 18 can be incorporated as nutritional enrichment in extruded snacks and still result in a product with physical and structural properties suitable for consumption. The addition of 2.6% of *Spirulina* sp. LEB 18 provided snacks with up to 11.3% protein. Thus, the snacks enriched with *Spirulina* sp. LEB 18 are an alternative to the demand for healthy food of practical consumption.

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