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Properties of wheat and rice breads added with chia (*Salvia hispanica* L.) protein hydrolyzate

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Abstract

Bioactive, technological and sensory properties of wheat and rice breads added of chia (*Salvia hispânica* L.) protein hydrolyzate were evaluated. The hydrolyzate was added to breads at concentrations of 1, 3 and 5 mg of hydrolyzate/g of flour. The specific volume, total score, bread crumb firmness, color, sensory analysis and antioxidant activity of breads were evaluated. The results showed that the protein content of the protein concentrate (79.56%) increased significantly compared with the defatted chia flour (32.52%). The results of antioxidant activity of chia protein hydrolyzate showed that at 0.50 and 1.0 mg of hydrolyzate/mL showed the highest values of activity by the DPPH method (66.09 and 65.43%). However, the chia protein hydrolyzate at 0.5 mg/mL presented the highest activity with a value of 83.74% by ABTS method. The reducing power results indicated that the hydrolyzate showed a dose-dependent relation since an increase from 0.50 to 2.00 mg/mL resulted in a significant increasing from 0.380 to 0.881 in absorbance. The addition of chia hydrolyzate resulted in good technological characteristics and antioxidant properties, in both breads. The breads with 3 mg of chia hydrolyzate/g flour had good sensory characteristics, which were not lower than those of the control breads.

Keywords: hydrolysis; antioxidant; gluten-free; concentrate; sensory characteristics.

Practical Application: Breads with chia hydrolyzate presented good technological and antioxidant properties.

1 Introduction

Bread is a food that has been consumed all over the world for thousands of years, and wheat has been used in bread (Rosell, 2011; Shah et al., 2018; Kouassi-Koffi et al., 2019). However, bread made from wheat flour contains gluten, which is not tolerated by celiac disease patients, who find it necessary to completely restrict this protein. In the last few decades, breads made from rice flour, which contains easily digestible carbohydrates and no gluten, have become increasingly popular, fueling a growing market and serving individuals with medical needs and millions who seek healthy food (Figueira et al., 2011; Coelho & Salas-Mellado, 2015; Torrelio Martos & López, 2018; Brites et al., 2019).

Chia (*Salvia hispanica* L.) is an annual herbaceous plant belonging to the family *Lamiaceae* and is a good source of protein, which ranges from 15-25% (Veggi et al., 2018); this content is greater than that in traditionally used grains such as wheat (14%), oat (15.3%), maize (14%) and rice (8.5%) (Orona-Tamayo et al., 2015). Due to its properties, some foods have already been developed with chia, such as bar (Iuliano et al., 2019), cookies (Brites et al., 2019) and milk sweet (Chaves et al., 2018). Coelho & Salas-Mellado (2018) reported that the process for obtaining proteins from chia can be studied to obtain products with high protein content, which can be used as raw material for the production of protein hydrolyzate. Chia proteins can be provided as biologically active peptides by enzymatic hydrolysis (Segura-Campos et al., 2013; Coelho & Salas-Mellado, 2018; Coelho et al., 2018).

Synthetic antioxidants are commonly used to prevent lipid oxidation and the formation of free radicals in food. However, their use is restricted in some countries due to potential health risks. In view of this, much attention has been paid to protein hydrolyzate as natural antioxidants due to beneficial health effects (Di Bernardini et al., 2011; Orona-Tamayo et al., 2015; Guijarro-Fuertes et al., 2019). Chia can provide antioxidant peptides; however, there have been few studies on the development of its protein hydrolyzate. As there are no studies on the application of chia hydrolyzate in rice bread and few in relation to the application in wheat bread, this study is interesting in order to obtain more nutritious foods that may, in addition, have some biotivity. Thus, the objective of this study was to evaluate the influence of the addition of chia protein hydrolyzate on the technological, sensory and antioxidant characteristics of wheat and rice breads.

2 Materials and methods

2.1 Raw materials and chemical characterization

Defatted chia flour was supplied by Giroil, Santo Ângelo/RS, Brazil. Rice flour containing carbohydrates (91.58%), moisture (10.03%), proteins (6.77%), lipids (0.51%) and ashes (0.37%) was supplied by Cerealle Indústria e Comércio de Cereais Ltda., Pelotas/RS, Brazil. Wheat flour containing carbohydrates (72.27%), moisture (13.49%), proteins (10.03%), ashes (0.68%) and lipids

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(0.53%) was supplied by Galópolis mill, Rio Grande/RS, Brazil. Ingredients such as yeast, sugar, salt, and oil were purchased locally. The enzyme transglutaminase was supplied by Ajinomoto Industry, São Paulo/SP, Brazil. Methylcellulose (Methocel A4M premium) was supplied by Colorcon, São Paulo/SP, Brazil. The enzyme Alcalase® 2.4 L FG was supplied by the Latin American LNF, Bento Gonçalves/RS, Brazil.

The contents of moisture (method nº. 935.29), ashes (method nº. 923.03), lipids (method nº. 920.85) and proteins (micro-Kjeldahl method, nº. 920.87) of the raw materials were determined according to Association of Official Analytical Chemists (2000). Carbohydrate content was obtained subtracting 100 from the summation of other chemical components.

2.2 Protein-rich fraction, protein concentrate and Chia protein hydrolyzate obtainment

The protein-rich fraction (PRF) was obtained according to the method described by Otto et al. (1997), with modifications. The protein concentrate of the protein-rich fraction was obtained by the *pH-shifting* method of Nolsøe & Undeland (2009). The protein-rich fraction was solubilized at pH 10 and 25 °C for 20 min. and then centrifuged (Hanil, Supra 22k, Japan) at 14308 × g and 25 °C for 20 min. The supernatant was precipitated at pH 3 and 25 °C for 20 min, followed by centrifugation at 14308 × g and 25 °C for 20 min. The precipitate was lyophilized (Liotop, L108, Brazil) for 48 h to obtain the dried chia protein concentrate.

The chia protein concentrate was hydrolyzate by the method described by Pedroche et al. (2002) using Alcalase as enzyme until a degree of hydrolysis of 30% was reached monitored by the pH-stat method of Adler-Nissen (1986). The following conditions were used: protein at a concentration of 2% (w/v, protein/water) and Alcalase at a concentration of 30 U/g (enzyme/substrate), taking into account the enzymatic activity of 7.9 U/mg. The obtained hydrolyzate was lyophilized (Liotop, L108, Brazil) and conditioned at -18 °C until use.

2.3 Evaluation of antioxidant activity

Antioxidant activity was evaluated for hydrolyzate at the concentrations of 0.5, 1 and 2 mg of chia hydrolyzate/mL of buffer (according to the antioxidant activity method) that were homogenized in a vortex agitator (Ionlab, Warmnest, Brazil) for 30 s; afterward, the antioxidant activity of the slurries was determined. The ability to sequester the DPPH radical was determined for the hydrolyzate and the wheat and rice breads according to the method described by Nicklisch & Waite (2014). The buffer used for samples dispersion is a citrate-phosphate buffer (0.1 M, pH 7.0) containing 0.3% Triton X-100. The results are expressed as percent (%) of inhibition. The determination of the reduction capacity of the chia hydrolyzate and the wheat and rice breads was performed according to the method described by Yen & Hsieh (1995). The buffer used for samples dispersion was sodium phosphate buffer (200 mM, pH 6.6). The ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid] assay was performed on chia hydrolyzate and on wheat and rice breads according to the method of Re et al. (1999). The buffer used was the phosphate saline solution (50 mM, pH 7.4). The results are expressed as percent (%) of inhibition.

2.4 Breads making

Rice or Wheat breads were made with the addition of chia protein hydrolyzate in the following proportions: 1, 3 and 5 mg of hydrolyzate/g of flour. Table 1 shows the wheat bread formulations and bread were prepared as follows: dry ingredients were added in a planetary mixer (KitchenAid, BEA30A, Brazil) and homogenized for 1 min at medium speed, followed by addition of water, vegetable fat and chia hydrolyzate solubilized in distilled water. Then, the mixture was homogenized for 9 min, and the same speed was maintained until the gluten was completely developed. The dough was allowed to stand for 10 min and then divided into 165 g portions, which were molded into the recipients and fermented at 30 °C for 90 min with 80% relative humidity. The doughs were then baked in an electric oven to 220 °C for 20 min. After 1 h of baking, the breads were sliced for further analysis.

Table 1. Wheat (W) and Rice (R) bread formulations.

Ingredients (g/100g)	Wheat				Rice			
	WC	W1	W3	W5	RC	R1	R3	R5
Flour	100	100	100	100	100	100	100	100
Salt	2	2	2	2	2	2	2	2
Sugar	5	5	5	5	5	5	5	5
Dry yeast	2	2	2	2	2	2	2	2
Vegetable fat Hydrogenated	3	3	3	3	-	-	-	-
Soy oil	-	-	-	-	2	2	2	2
Ascorbic acid	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009
Water	60	60	60	60	120	120	120	120
Transglutaminase enzyme	-	-	-	-	0.5	0.5	0.5	0.5
Methylcellulose	-	-	-	-	2	2	2	2
Chia hydrolysate protein	-	0.1	0.3	0.5	-	0.1	0.3	0.5

WC: Wheat bread control; W1, W3, and W5: Wheat breads added with 1, 3 and 5 mg of hydrolysate/g of flour.; RC: Rice bread control; R1,R3 and R5: Rice breads added with 1, 3 and 5 mg of hydrolysate/g of flour.

Gluten-free breads were prepared according to the method described by Figueira et al. (2011) and the formulations are presented in Table 1. The dry ingredients were homogenized in the planetary mixer for 2 min at medium speed. The oil, water, and hydrolyzate solubilized in distilled water were then added and homogenized for 10 min. The dough was first fermented at 30 °C for 60 min with an 80% relative humidity. Subsequently, 175 g of dough were added into the recipients, and the dough was again fermented at 30 °C for 55 min. The doughs were then baked in an electric oven (Fischer, Diplomata, Brazil) at 200 °C for 20 min. After 1 h of baking at room temperature, breads were sliced for further analysis.

2.5 Technological, physical-chemical and sensorial characterization of breads

Determination of the specific volume (SV) and the firmness of the bread crumb was performed according to the method described by (Fernandes & Salas-Mellado 2017).

The internal and external characteristics of the breads were evaluated by the method according to El-Dash (1978), which assigns scores to the products with a maximum value of 100 points distributed among the following parameters: volume (VE x 3.33), crust color, symmetry, crust characteristic, crumb color, crumb cell structure, crumb texture, aroma and flavor. The color analysis was performed in a colorimeter (Minolta, CR-400, Japan) following the color system in the L*, a* and b* space defined by CIE-L*a*b* (Commission International de l'Éclairage, 1986).

Bread samples were presented as 2 cm edge cubes and coded with three numbers. The wheat and rice breads with 3 mg added chia hydrolyzate/g flour were subjected to paired comparison. The analysis was performed with 60 panelists. An evaluation form was used for each panelist to choose the bread of his or her choice by comparing the control bread and the tested bread. Two evaluations were carried out separately: one for wheat bread and one for rice bread. The interpretation of the results was based on the number of total judgments versus the number of positive judgments. If the number of positive judgments was greater than or equal to the bilateral table value, it was concluded that there was a significant difference between the samples at the corresponding probability level (Queiroz et al., 2017).

2.6 Antioxidant capacity of wheat and rice breads with added chia hydrolyzate

The Antioxidant capacity of wheat and rice breads was evaluated with the same methods used for chia protein hydrolyzate. Thus, the bread samples were prepared based on the method of Franco-Miranda et al. (2017). For the DPPH method, 250, 500 and 750 mg of ground bread crumbs were homogenized in 10 mL of a citrate-phosphate buffer (0.1 M, pH 7.0) containing 0.3% Triton X-100. For the reducing power determination, the same amounts of ground bread were homogenized with sodium phosphate buffer (200 mM, pH 6.6). For ABTS, the same amounts of ground bread with phosphate saline solution (50 mM, pH 7.4) were used. The suspensions were homogenized in the vortex mixer (Ionlab, Warmnest, Brazil) for 30 s and then centrifuged at 14308 × g (Biosystems, MPW 350/350 R, Brazil) for 10 min. The supernatant from each sample was filtered with nº. 1 Whatman paper. The filtrates were used for the determination of antioxidant activity.

2.7 Statistical analysis

All analyses were performed in triplicate. The results of the analyses were treated statistically using analysis of variance (ANOVA) and compared using the Tukey test ($p < 0.05$) with Statistica 5.0 program. For the sensory evaluation was used Bilateral Preference test.

3. Results and discussion

3.1 Proximate composition and antioxidant capacity of the hydrolyzate

The results showed that the protein content of the protein-rich fraction (46.84%) and of the protein concentrate (79.56%) increased significantly ($p < 0.05$) compared with defatted chia flour (32.52%) as shown in Table 2. Furthermore, the ash and lipids content of the protein concentrate decreased significantly ($p < 0.05$). In the rich fraction obtainment, the retained fraction was composed mainly of fibers, and the passing fraction was composed mainly of proteins (Otto et al., 1997). Coelho & Salas-Mellado (2018) verified similar results for the protein-rich fraction and for the protein concentrate with values of 49.7% and 70.9% of protein content, respectively. These authors also reported a decreasing of 4% in ash content from the protein-rich fraction to the protein concentrate. In this study, it was verified an increase of 100% of protein content in protein concentrated compared to defatted chia flour.

The antioxidant activities of hydrolyzate are shown in Figure 1. The hydrolyzate with concentrations of 0.50 and 1.0 mg/mL had the highest values of antioxidant activity, as determined by the DPPH method (66.09 and 65.43%), respectively. Evangelho et al. (2017) verified that the antioxidant activity of 1.0 mg/mL of black bean (*Phaseolus vulgaris* L.) protein hydrolyzate by Alcalase showed a DPPH radical scavenging activity of 37.15%. The differences

Table 2. Proximate compositions of the defatted chia flour, protein-rich fraction and protein concentrate.

Components (%)	Defatted chia flour	Protein-rich fraction	Protein concentrate
Moisture	9.08 ± 0.10^{a}	8.97 ± 0.04^{a}	2.05 ± 0.39^{b}
Ash	6.29 ± 0.02^{a}	7.31 ± 0.04^{a}	1.26 ± 0.09^{b}
Lipids	1.55 ± 0.11^{b}	2.02 ± 0.15^{a}	0.46 ± 0.01^{c}
Protein	32.52 ± 0.54^{c}	46.84 ± 2.70^{b}	79.56 ± 4.25^{a}
Carbohydrates	50.46	34.86	16.66

Different lowercase letters on the same line indicate that there is a significant difference ($p<0.05$).

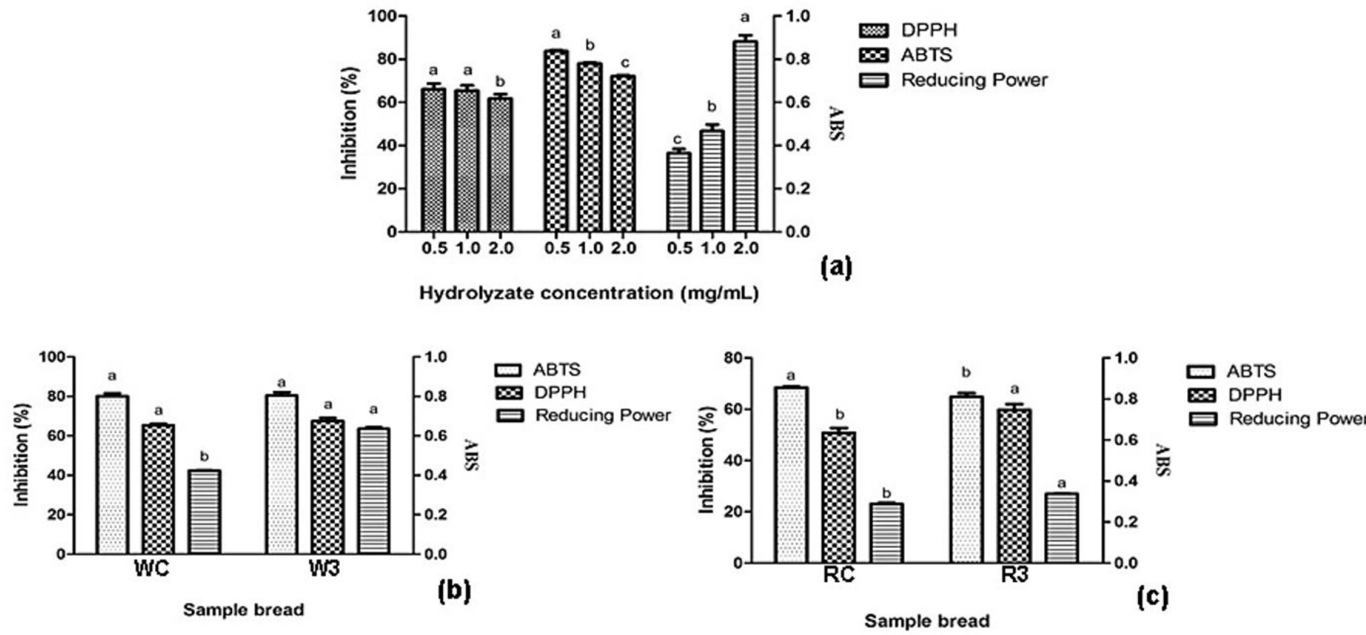

Figure 1. Antioxidant activity of chia protein hydrolysate (a) and (b) wheat and (c) rice breads determined by different methods. Equal lowercase letters indicate that there is no significant difference (p> 0.05) between the different concentrations of hydrolysate tested for the same method of determining antioxidant activity. ABS: absorbance; WC: wheat control bread; W3: wheat bread with 3 mg added hydrolysate/g flour; RC: rice control bread; R3: rice breads with 3 mg added hydrolysate/g flour. Different letters indicate a significant difference between the samples for the same method (p <0.05).

obtained in the different researches can be associated with the degree of hydrolysis reached in the hydrolyzate, the type of protease used and the different raw materials used, which had different protein compositions, respectively.

The chia protein hydrolyzate in a concentration of 0.5 mg/mL presented the highest antioxidant activity with a value of 83.74% by the ABTS method ($p< 0.05$). In this study, the chia protein hydrolyzate can be acted as electron donors and free radical thus providing antioxidant protection. According to Segura-Campos et al. (2013), enzymatic hydrolysis results in increased exposure of antioxidant amino acids in the chia proteins, consequently providing them greater antioxidant activity, which was verified in the present study. According to Re et al. (1999), antioxidant activity may be attributed to the binding ability of ABTS hydrophilic radicals with hydrophobic proteins.

The results of this study, suggest that chia protein hydrolyzate possess hydrophobic proteins. The reducing power results indicated that the hydrolyzate showed a dose-dependent relation since an increase from 0.50 to 2.00 mg/mL resulted in a significantly increasing from 0.380 to 0.881 in absorbance values ($p< 0.05$). Chang et al. (2007) reported values of 0.080-0.250 for reductive capacity at the highest concentration (5 mg/mL) of a hemoglobin hydrolyzate; these values are lower than those obtained in the present study, which showed reducing power in the range of 0.360-0.880. According to the results obtained in this study, by DPPH, ABTS and reducing power methods, the chia protein hydrolyzate showed potential for application as a natural antioxidant.

Table 3. Technological characteristics of breads with added chia protein hydrolysate.

Breads	Formulations	SV (mL/g)	Firmness (N)	Total score
Wheat	WC	4.31 ± 0.04^{ab}	2.44 ± 0.09^{b}	93.78 ± 0.74^{a}
	W1	4.35 ± 0.04^{ab}	2.69 ± 0.06^{b}	88.47 ± 0.24^{b}
	W3	4.63 ± 0.29^{a}	2.05 ± 0.13^{c}	86.98 ± 0.89^{b}
	W5	3.96 ± 0.14^{b}	4.65 ± 0.23^{a}	80.69 ± 0.89^{c}
Rice	RC	3.38 ± 0.16^{AB}	1.32 ± 0.18^{A}	88.75 ± 0.52^{A}
	R1	3.32 ± 0.13^{B}	1.56 ± 0.37^{A}	83.05 ± 0.44^{B}
	R3	3.63 ± 0.10^{A}	1.25 ± 0.07^{A}	84.61 ± 0.34^{B}
	R5	3.43 ± 0.05^{AB}	1.36 ± 0.14^{A}	80.57 ± 0.46^{C}

WC: Wheat bread control; W1, W3 and W5: Wheat breads added with 1, 3 and 5mg of hydrolysate/g of flour; RC: Rice bread control; R1, R3 and R5: Rice breads added with 1, 3 and 5 mg of hydrolysate/g of flour; SV: Specific volume. Average of three values with standard deviation, the same letter in the column indicates that there is no significant difference between the means by Tukey test (p<0.05).

3.2 Technological characteristics of wheat and rice breads

Table 3 shows the specific volume (SV), firmness and total score values of breads with added of chia hydrolyzate. The addition of chia hydrolyzate to wheat bread showed no influence on the SV value until the addition of 3 mg hydrolyzate/g flour (W3). Above this concentration, there was a significant reduction in SV. This may have occurred due to the inclusion of chia protein in the formulation that interfered with the formation of gluten.

The chia hydrolyzate may have influenced the formation of many smaller and denser alveoli in the bread crumb, thus reducing the SV value (Franco-Miranda et al., 2017). Coelho & Salas-Mellado (2015) evaluated the effect of the addition of dried and hydrated chia seeds and dried and moist chia flour to wheat bread and verified an increase in SV value when chia seed was added at 2%. These results suggest that chia, as well as their proteins, affects the development and growth of bread dough.

In relation to the SV value of the rice breads, it was found that only the formulation R1 (1 mg hydrolyzate/g flour) was significantly lower than the other formulations ($p< 0.05$). Selmo & Salas-Mellado (2014) found similar results, verifying an increasing of the SV value according to the amount of added *Spirulina*. In addition to the gas retention capacity, the specific volume is influenced by the elasticity of the dough, a characteristic of great importance in the formation of the alveoli and expansion of this product during the fermentation and filling stages during its manufacture (Steffolani et al., 2015).

The increase in the amount of hydrolyzate added in the wheat bread resulted in lower firmness with 1 and 3 mg of added chia protein hydrolyzate/g of flour as shown in Table 3. Above this value, there was a significant increase in firmness ($p< 0.05$). This behavior was expected since the highest concentration of hydrolyzate showed the lowest SV and these characteristics acted in an inverse way; that is, breads with lower SV presented higher crumb firmness values. Increasing the amount of hydrolyzate in wheat breads results in a greater change in the formation of the gluten network due to the type and nature of the present proteins (Franco-Miranda et al., 2017).

Franco-Miranda et al. (2017) studied the addition of lime bean hydrolyzate and cowpea beans at 1 and 3% to the formulation of Mexican type sweet bread. It was verified that the higher inclusion of the hydrolyzate resulted in higher firmness of the products. In the present study, the firmness was not significantly different in all formulations of rice breads, as presented in Table 3. The incorporation of protein ingredients can be improved with the addition of transglutaminase, an enzyme that catalyzes reactions between proteins and promotes the formation of protein networks, which contributes to the structure of gluten-free breads (Capriles et al., 2016). The combination of hydrocolloid methylcellulose, transglutaminase enzyme, and chia protein hydrolyzate contributed to the softness of breads made with rice flour. The gluten-free breads presented an inverse correlation between the SV and the firmness, so that the greater the compaction of the gas particles in the breads, the smaller the SV, causing an increase in the resistance to the deformation of these breads, and consequently resulting in high firmness values.

In this study, the total score ranged from 80.69 to 93.78 and wheat breads with 5 mg of hydrolyzate/g of flour (W5) presenting the lowest score as shown in Table 3. The highest score was obtained by the control breads. According to Dutcosky (1996), the W1 and W3 formulations were considered as good bread and the W5 sample was considered a regular bread. Oliveira et al. (2017) examined bread enriched with insect (*Nauphoeta cinerea*) flour at concentrations of 5, 10 and 15% and theses authors observed that the score of the breads decreased as the concentration of insect flour increased. These results are similar to those obtained in the present study. Thus, the addition of unconventional ingredients in bread decreases the overall evaluation score of the products.

Considering all bread technological parameters, the chia hydrolyzate can be added to breads in concentrations up to 3 mg hydrolyzate/g of flour, so that it does not affect the technological characteristics, since above this concentration, the chia hydrolyzate can produce the interruption of the protein network.

Table 4 shows the color parameters of the crust and crumb of the wheat and rice added chia protein hydrolyzate. The luminosity (L*) of the crust and the crumb of the rice bread were lighter than that of the wheat bread because rice flour presented higher L* than wheat flour. In wheat breads, the L* of the crust was higher in the formulation W3, while in rice breads did not differ statistically, showing that the addition of chia hydrolyzate influenced only the wheat breads crust color. Regarding the L* breads crumb values, there was no significant difference between the wheat and gluten-free formulations, except for the R5 formulation, which presented lower L*.

The chroma a* values of the crust of the wheat bread indicated a tendency for red coloration, while the chroma an* of the crust of the rice breads and of the crumb of both types of breads tested presented a slight tendency to green coloration. In relation to chroma b*, both in the crust and in the crumb, it could be observed a tendency to yellow and did not occur significant difference between the samples in the crust of the rice breads and in the crumb of the wheat breads. Normally the color of bread crumb tends to yellow (near 90°). In all formulations were obtained

Table 4. Color parameters of the crust and crumb of wheat and rice breads with added chia protein hydrolysate.

Breads	F	Crust				Crumb			
		L*	a*	b*	Hue (°)	L*	a*	b*	Hue (°)
Wheat	**WC**	50.34±4.17^{b}	14.37±1.72^{b}	31.47±2.07^{a}	65.45	64.87±3.02^{a}	-0.74±0.03^{a}	15.14±1.22^{a}	87.20
	W1	34.88±0.76^{d}	14.85±0.13^{b}	19.10±1.14^{c}	52.13	64.96±1.38^{a}	-0.65±0.10^{b}	15.57±0.11^{a}	87.61
	W3	61.05±2.46^{a}	13.39±1.51^{b}	33.84±0.38^{a}	68.41	64.29±0.97^{a}	-0.52±0.07^{c}	15.60±0.11^{a}	88.09
	W5	46.58±0.94^{c}	17.65±1.22^{a}	29.63±0.77^{b}	59.21	64.19±2.43^{a}	-0.79±0.09^{a}	15.37±0.28^{a}	87.05
Rice	**RC**	77.50±2.06^{A}	-0.33±0.14^{A}	13.42±2.25^{A}	88.59	69.06±2.43^{A}	-1.07±0.15^{B}	6.09±0.56^{C}	80.03
	R1	76.91±3.04^{A}	-0.23±0.47^{A}	15.00±1.54^{A}	89.12	70.73±0.16^{A}	-1.34±0.09^{A}	7.81±0.36^{B}	80.26
	R3	73.03±3.80^{A}	-0.08±0.10^{B}	16.60±2.20^{A}	89.72	69.60±1.41^{A}	-0.87±0.09^{C}	9.76±0.52^{A}	84.90
	R5	77.33±0.69^{A}	-0.40±0.12^{A}	17.10±2.27^{A}	88.66	68.01±0.57^{B}	-0.90±0.05^{C}	9.12^{a}±0.58^{A}	84.36

F: Formulation; WC: Wheat bread control; W1, W3 and W5: Wheat breads added with 1, 3 and 5 mg of hydrolysate/g of flour; RC: Rice bread control; R1, R3 and R5: Rice breads added with 1, 3 and 5 mg of hydrolysate/g of flour. Average of three values with standard deviation, the same letter in the column indicates that there is no significant difference between the means by Tukey test ($p<0.05$).

values near 90°, being more pronounced in wheat breads, with the Hue angle (h) ranged from 87.05 to 88.09. In this work, it was verified that there were alterations in some parameters of color, evidencing that chia hydrolyzate can influence the color of wheat and rice breads, but maintaining the color characteristics desired by the consumers.

3.3 Antioxidant capacity of wheat and rice breads with added chia hydrolyzate

Figure 1 presents the antioxidant capacities determined by the DPPH, ABTS and reducing power assays of wheat (Figure 1b) and rice (Figure 1c) breads with the addition of 3 mg chia hydrolyzate/g flour. W3 (Figure 1b) showed a higher antioxidant activity by the reduction power method (0.600) than that of the WC (0.420) sample without incorporation of chia protein hydrolyzate ($p < 0.05$). However, in DPPH and ABTS assays the antioxidant activity of the control (WC) and wheat breads added with hydrolyzate showed the same antioxidant capacities ($p > 0.05$). Segura-Campos et al. (2013) also found that the antioxidant activity by ABTS method in wheat bread with added chia hydrolyzate at concentrations of 1 and 3 mg/g of flour was not different from that of control wheat bread. According to those authors, the fermentation and high temperatures during the baking process can be results in lower bioactive properties potential. Some carotenoids present in many types of wheat flours have antioxidant compounds. These compounds are present in the outer part of the grain (i.e. husk, pericarp, aleurone, germ) and, although during milling processes many bioactive components located in the peripheral parts of the grain are lost, bioactive compounds still present in mill streams, can play an important role in determining the nutritional quality of end products.

On the other hand, the bread sample R3 presented higher antioxidant activity than that of the control bread, as determined by the DPPH and reducing power methods, suggesting that chia hydrolyzate incorporation had a greater influence in these breads than in wheat bread. Franco-Miranda et al. (2017) added hydrolyzate of lima beans and cowpea at concentrations of 1% and 3% in sweet bread of Mexican type and verified that the breads with the two added hydrolyzate presented the highest antioxidant activity, as determined by the ABTS method. This result is different from that obtained in the present study and may be due to the relation of the antioxidant activity of the peptides with the specific proteases used to produce the hydrolyzate, the concentration of the enzyme used, the DH achieved, the nature of the peptides released, which have different molecular weights, compositions and amino acid sequences, and the type of raw material used (Segura-Campos et al. 2013).

3.4 Sensory analysis of wheat and rice breads with added chia hydrolyzate

Figure 2 shows the results of the sensory analysis performed on the wheat and rice breads.

The control sample had 23 positive responses, and the bread with 3% of hydrolysate showed 37 positive responses. For a total number of 60 judges, there must be 39 positive responses for one of the samples, to indicate that there is a significant difference ($p < 0.05$). Consequently, it can be inferred that the use of chia hydrolyzate flour did not affect the preference of both pieces of bread.

Silva et al. (2014) evaluated the nutritional value, preference and intention to buy for salt bread produced with mixed flour and composed of wheat and ora-pro-nobis (*Pereskia aculeata*) at concentrations of 5 and 10% and verified that bread with the lower concentration of ora-pro-nobis was preferred. These authors commented that this preference may be due to the texture and flavor conferred by the fiber in the product with the greater amount of *P. aculeata*. These results are different from those obtained in this study.

Figure 2b shows the results of the sensory analysis performed on the rice bread. The control sample had 24 positive responses, and the rice bread with 3% of hydrolyzate showed 36 positive responses. As in the case of wheat bread, there must be 39 positive responses for one of the samples to indicate that there was a significant difference between them. The matched comparison between the two samples tested indicated that neither of them was preferred, demonstrating that the addition of chia protein hydrolyzate in rice bread had a positive influence since the bread with added chia hydrolyzate did not present lower sensory characteristics than the control bread.

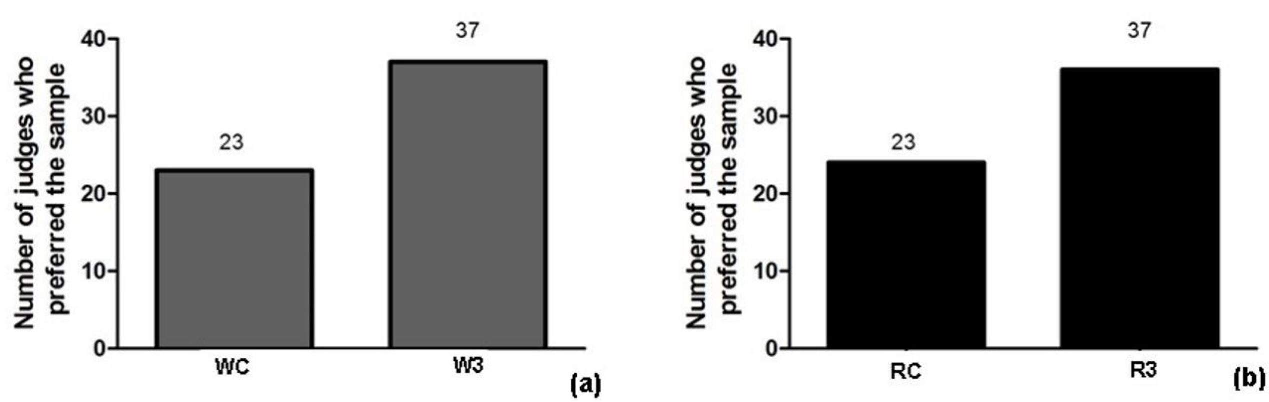

Figure 2. Sensory evaluation of (a) wheat and (b) rice breads. WC: Wheat control bread; W3: Wheat bread with 3 mg added hydrolysate/g flour; RC: Rice control bread; R3: Rice breads with 3 mg added hydrolysate/g flour.

If there were interest to study the characteristics of the wheat and rice breads, it could be applied another sensorial test like descriptive and projective methods that can provide a greater depth of understanding of what people truly think and feel about the products.

4. Conclusion

Wheat and bread rice presented good properties because the addition of chia hydrolyzate resulted in good technological characteristics such as bulkiness, softness and good antioxidant activity too. In addition, the sensory characteristics of the wheat and rice bread with 3 mg of chia hydrolyzate/g flour were not inferior to those of the control bread, showing that the addition bread had adequate sensory characteristics.

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