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# Effects of substituting chia (*Salvia hispanica* L.) flour or seeds for wheat flour on the quality of the bread



# CrossMark

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#### ABSTRACT

The hydrogenated vegetable fat content was reduced and chia seeds or flour were added to the formulations of wheat flour bread based on a  $2^2$  CCRD, generating the two products: bread with 7.8 g/100 g chia flour and 0.9 g/100 g fat and bread with 11.0 g/100 g chia seeds and 1.0 g/100 g fat, resulting in a reduction of 27 and 24%, respectively, in the level of saturated fat compared to that of bread prepared with wheat flour. The ratio of polyunsaturated and saturated fats (PUFA:SAT), which was 1.01 in the control bread, was increased to 3.1 and 3.9, respectively, using chia flour and seeds. The content of fiber and  $\omega$ -3 fatty acid was increased in the final products. These new formulations might be used on an industrial scale to prepare products that could contribute to reducing the intake of saturated fatty acids and increasing that of essential fatty acids, such as  $\omega$ -3 fatty acid.

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#### 1. Introduction

The risk of cardiovascular disease, type-2 diabetes and colorectal cancer increases with obesity. Diet and lifestyle can be modified to prevent and reduce the risks of these diseases. There is epidemiological evidence that diets that promote health are rich in dietary fiber and omega-3 fatty acid and low in saturated fat, trans fat and cholesterol (Hu, 2002). A strategy to reduce the consumption of fat is to develop products with low fat content. Fat is one of the substances that is used more frequently in bakery products (Quaglia, 1991). Fat salter the sensory characteristics of bread and fermented products, providing a shorter and smoother bite and increasing the shelf life and duration of their softness. These effects are intensified as increasing amounts of fat are added. According to Brien, Mueller, Scannell, and Arendt (2003), the production of breads with a lower fat content that possess the quality characteristics derived from the functional properties of fats, such as a

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greater volume, smoother texture and delayed aging, is a technical challenge for food technologists. Based on concerns for health and for foods with specific features that improve health, there has been an increasing interest in so-called functional foods, which are consumed as part of the usual diet and which either provide physiological benefits or reduce the risk of chronic disease beyond the basic nutritional requirements (Shahidi, 2009).

Bread is one of the major components of the human diet. For thousands of years, wheat has been used to produce bread. In recent decades, researchers have worked on fortifying bread with natural compounds due to the demands for healthier food. Thus, whole grains and other seeds are commonly used in the production of bread. Chia (Salvia hispanica L.) is an annual plant of the family Lamiaceae. In pre-Columbian times, its seeds were one of the staple foods of Central American civilizations. Chia seeds are composed of proteins (15–20 g/100 g), lipids (30–33 g/100 g), ash (4–5 g/100 g) and carbohydrates (26-41 g/100 g) and have a high fiber content (18-30 g/100 g). These seeds contain a large amount of antioxidants, minerals and vitamins (Ixtaina, Nolasco, & Tomás, 2008). Puig and Haros (2011) reported that because of the nutritional properties of chia, its consumption can promote proper intestinal functioning, decrease blood cholesterol and glucose levels and decrease the incidence of diseases related to metabolic syndrome. Chia seeds are rich in polyunsaturated fatty acids, particularly the omega-3 (50-57 g/100 g) and omega-6 (17-26 g/100 g) fatty acids (Reves-Caudillo, Tecante, & Valdivia-López, 2008). These fatty acids are found in the tissues that constitute the central nervous system,

Abbreviations: CCRD, central composite rotational design; LM, loss of mass upon baking; SV, specific volume;  $L^*$ , lightness;  $a^*$  and  $b^*$ , chromaticity coordinates values;  $C^*$ , chroma;  $h_{ab}$ , hue angle; IA, index of acceptability; Control, wheat flour bread; F7.8, bread containing 7.8 g/100 g chia flour and 0.9 g/100 g hydrogenated vegetable fat; S11, bread containing 11.0 g/100 g chia seeds and 1.0 g/100 g hydrogenated vegetable fat.

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play a role in the proper functioning of vision, as well as in the prevention of cardiovascular diseases, cancer, and autoimmune and inflammatory diseases. In addition to being nutritionally important to good health, these fatty acids are beneficial for individuals suffering from heart disease, diabetes and immune response disorders (Djordjevic, McClements, & Decker, 2004; Simopoulos, 1991).

Considering these factors and the increased incidence and prevalence of obesity, the objectives of the present study were to study the effect of incorporating different amounts of chia seeds or flour and hydrogenated vegetable fat on the technological quality of wheat breads and to evaluate the nutritional and sensory characteristics of the products that presented the best technological properties.

#### 2. Materials and Methods

#### 2.1. Raw materials and ingredients

The S. hispanica L. chia seeds (34.6 g/100 g lipids, 4.6 g/100 g ash, 19.6 g/100 g protein, 23.7 g/100 g dietary fiber, 17.5 g/100 g other carbohydrates) used in the formulations were obtained from Chá e Cia – Ervas Medicinais para chá (São Paulo, Brazil). The raw material, wheat flour (3.6 g/100 g lipids, 0.6 g/100 g ash, 10.7 g/100 g protein, 0.1 g/100 g dietary fiber, 85.1 g/100 g other carbohydrates), used for the preparation of bread from Moinho Galópolis S.A. (Rio Grande do Sul, Brazil). Some of the ingredients that were used for the production of the breads, such as hydrogenated vegetable fat, sodium chloride, sucrose and dried yeast, were purchased in local shops. Ascorbic acid P.A. (Synth, Brazil) was also used an additive. To obtain the chia flour, chia seeds were ground (Arno, PL pic-liq model) and sieved through a 16-mesh screen, packed in plastic containers and maintained at 4 °C for later use.

#### 2.2. Formulation and preparation of breads

The basic formulation of the bread was 100 g wheat flour, 2 g sodium chloride, 5 g sucrose, 3 g fresh yeast, 3 g hydrogenated vegetable fat, 0.009 g ascorbic acid and 57–60 g water. The levels of chia flour and chia seeds in relation to the wheat flour and that of the hydrogenated vegetable fat varied depending on the experimental design. This formulation was that used by El-Dash (1978), with modifications. Fresh yeast was replaced proportionally by dry yeast. The quantities of chia flour or seed and fat were used in a 2<sup>2</sup> central composite rotational design (CCRD), with three replications at the center point and four axial points (Box & Draper, 1987), for a total of 11 trials for each experiment. Two experiments were conducted to verify the effects of the addition of chia in the forms of flour and seed and of decreasing the amount of hydrogenated vegetable fat on the final quality of the loaves.

Using the direct mass method, the dry ingredients (flour, sodium chloride, sucrose and ascorbic acid) were initially mixed using a planetary mixer (KitchenAid) at low speed for 3 min. For the formulations containing chia flour or seeds, these items were hydrated with 15% of the water in the formulation for 10 min before being stirred into the mixture at low speed for 1 min. Afterward, the rest of water was added and the fat and yeast were mixed for 6 min until the gluten had completely developed. The dough was left to rest for 10 min before being cut into pieces weighing 165 g, which were subsequently filleted and molded with a wood rolling pininto tubes. The shaped masses were taken to a greenhouse for fermentation, where they remained at a temperature of 30 °C for 90 min. Then, the items were baked in an electric oven to 220 °C for 20 min. After 1 h of baking, the loaves were sliced (electric knife, Moulinex) for further analysis. The breads storage conditions was room temperature (24  $^{\circ}C \pm 2 ^{\circ}C$ ).

The technological parameters of the loaves that were evaluated based on the experimental design were the following: the loss of mass upon baking, the specific volume (El-Dash, 1978), the color of the crumb (lightness, chroma and hue angle) (Minolta, 1993), the hardness of the bread 1 and 24 h after baking (AACC, 2000) and the total score (Pizzinatto, Magno, Campagnollli, Vitti, & Leitao, 1993).

# 2.3. Nutritional evaluation of the bread added chia (S. hispanica L.) flour or seeds

#### 2.3.1. Proximal composition and calorific value

The moisture (method number 935.29), ash (method number 923.03), protein (micro-Kjeldahl method, number 920.87) and total dietary fiber (method number 985.29) contents of the breads were determined according to the methods of the Association of Official Analytical Chemists International – AOAC (2005). The lipid content (method number 954.02) was determined by acid hydrolysis according to the AOAC method (2005). The carbohydrate content was obtained by the difference.

The caloric value of the loaves was calculated using the coefficients of Atwater (Watt & Merrill, 1963), based on the caloric coefficients corresponding to the protein, carbohydrate and lipid contents, according to Equation (1), as follows.

Caloric 
$$(\text{kcal} \cdot 100 \text{ g}^{-1}) = (\text{g of protein} * 4) + (\text{g of lipids} * 9)$$
  
+  $(\text{g of carbohydrates} * 4)$   
(1)

#### 2.3.2. Profile of the fatty acids

The lipids were extracted from the loaves using the acid hydrolysis method (AOAC, 2005). The extent of transformation into methyl esters and the fatty acid composition were determined according to the AOAC (2005) methods using a Thermo model Focus GC gas chromatograph with a FID detector.

The chromatographic conditions that were used were as follows: an initial column temperature of 100 °C for 4 min, increasing to a final column temperature of 240 °C at a rate of 3 °C min<sup>-1</sup>. The injector temperature was 225 °C, and the detector temperature was 285 °C. Helium was used as the carrier gas, and an SP2560 100 m  $\times$  0.25 mm capillary column was used. The results were expressed in g of fatty acids per 100 g of total lipids.

2.4. Physical and technological evaluation of the bread added chia (S. hispanica L.) flour or seeds

#### 2.4.1. Loss of mass upon baking

The physical property of the masses of the uncooked dough and the bread were determined. The loss of mass upon baking (% LM) was calculated according to Equation (2), as follows.

$$LM (\%) = \frac{M_{dough} - M_{bread}}{M_{dough}} \times 100$$
<sup>(2)</sup>

where  $M_{\text{dough}}$  corresponds to the mass of the dough and  $M_{\text{bread}}$  corresponds to the mass of the bread.

#### 2.4.2. Specific volume and total score

The technological evaluation of the loaves included the specific volume (SV) and the total scores assigned using the second

worksheet of El-Dash (1978), with a maximum value of 100 points. The SV  $(cm^3/g)$  was obtained from the ratio between the apparent volume  $(cm^3)$ , using the millet seeds offset according to Pizzinatto et al. (1993) and the mass (g) after baking.

#### 2.4.3. Crumb firmness

The firmness of the crumb of the breads was measured in the fresh bread and at 24 h after baking using a TA-XT2 texturometer (Stable Micro Systems, UK). For this analysis, the loaves were sliced using an electric knife (Moulinex). The tests were conducted according to the AACC (2000) (74-09.01) method, which consisted of compressing two 25-mm thick slices in the center of the Texture Analyzer platform using a cylindrical probe of 36 mm in diameter under the following working conditions: speed of 1.0 mm/s for the pre-test; speed of 1.7 mm/s for the test; speed of 10.0 mm/s for the post-test; 40% compression and 5-g trigger force. The firmness was expressed as g-force.

#### 2.4.4. Color of crumb and crust

The color analysis of the crumb and crust of the loaves were conducted using a Minolta<sup>®</sup> model CR400 colorimeter. The analysis were based on the  $L^*a^*b^*$  or CIE  $L^*a^*b^*$  system for breads that were defined by the CIE (International Commission on Illumination) in 1976 for determining the  $L^*$  (lightness),  $a^*$  and  $b^*$  (chromaticity coordinates) values. The value of chroma or  $C^*$  and the hue angle or  $h_{ab}$ , referred to as the CIELCh color system according to Minolta (1993) were also calculated using Equations (3) and (4), as follows.

Chroma 
$$(C^*) = \left( (a^*)^2 + (b^*)^2 \right)^{1/2}$$
 (3)

$$h_{\rm ab} = \tan^{-1}(b^*|a^*)$$
 (4)

# 2.5. Sensory evaluation of the bread added chia (S. hispanica L.) flour or seeds

The samples were evaluated by 58 non-trained judges, where the acceptability tests were applied and the purchase intent of the samples of breads made with chia flour and chia seeds obtained in CCRD model validation.

The bread samples were slices with a thickness of 1 cm. The purchase intent tests utilized a 5-point scale (1-certainly wouldn't buy to the 5-certainly buy). A nine-point hedonic scale was used in the test of acceptability, with one extreme being the qualification of "disliked very much", "indifferent" in the center and the other end being "extremely like", according to NBR 12994 (ABNT, 1993) for

evaluating the appearance attributes, color, aroma, texture of the crumb, flavor and overall quality. The index of acceptability (IA) was calculated using Equation (5), as follows.

$$IA(\%) = \frac{\text{Score} \times 100}{9} \tag{5}$$

#### 2.6. Data treatment

Response surface methodology was used to analyze the technological characteristics of the loaves with chia flour and chia seeds substituted for a portion of the wheat flour and with reduced amounts of hydrogenated vegetable fat. The Statistica 5.0 program was used to perform an analysis of variance (ANOVA), to obtain mathematical models and to build contour curves (p < 0.05). The data were compared using an analysis of variance (ANOVA) and the average values obtained were compared using Tukey's test, with statistical significance ( $\alpha$ ) set at p < 0.05.

#### 3. Results and discussion

#### 3.1. Experiment 1

Table 1 presents the values that were determined for the breads added chia flour and analyzed as responses in the experimental design, showing that the models were predictive of the SV, hue angle and total score values. This finding meant that only these responses were significantly different due to the addition of chia flour and the reduction of the hydrogenated vegetable fat content.

Appendix presents the values for the parameters used to validate the mathematical models obtained in Experiment 1. Based on the equations that corresponded to the significant and predictive mathematical models, it was found that the added fat had a positive effect on the specific volume and the total score for the technological features of the loaves. Adding chia flour had a negative effect on the specific volume, the hue angle and the total score. Fig. 1 shows the contour curves of predictive models of the parameters evaluated in bread obtained in Experiment 1.

The SV of the loaves ranged from 2.59 to 3.45 cm<sup>3</sup>/g. In Experiment 1, according to Fig. 1A, regardless of the fat content (0-3 g/100 g of flour) and with the lower concentrations of chia flour (approximately 2–8 g/100 g), the loaves attained a high SV. This result reflects the use of the mixed flours in the formulation. Pizarro, Almeida, Sammán, and Chang (2013) studied the effect of incorporating different amounts of chia flour and hydrogenated vegetable fat on the technological quality of cakes and found that

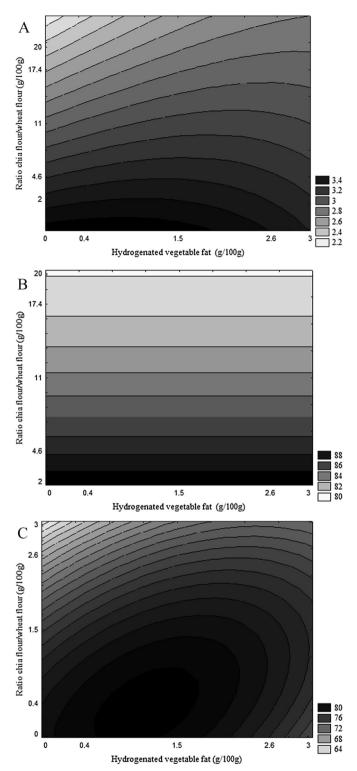
Table 1

Values for technological characteristics of the loaves made with different concentrations of hydrated chia flour in relation to wheat flour and of hydrogenated vegetable fat (Experiment 1).

Trial	Fat $(x_1)$	Ratio <sup>a</sup> ( $x_2$ )	Loss upon baking (%)	SV <sup>a</sup> (cm <sup>3</sup> /g)	Firmness (g)	Firmness 24 h (g)	L <sup>*a</sup>	$h_{ab}{}^{a}(^{\circ})$	C <sup>*a</sup>	Total score
1	0.4 (-1)	4.6 (-1)	6.78 ± 2.03	3.15 ± 0.06	50.60 ± 3.50	92.02 ± 8.15	65.12 ± 1.74	87.64 ± 1.09	15.86 ± 0.42	78.13 ± 0.22
2	2.6(1)	4.6 (-1)	$7.71 \pm 0.04$	$3.10 \pm 0.09$	$59.35 \pm 5.06$	$106.49 \pm 8.10$	63.75 ± 1.17	$86.57 \pm 0.94$	$15.78 \pm 0.47$	$76.82 \pm 0.26$
3	0.4 (-1)	17.4 (1)	$6.85 \pm 0.48$	$2.59 \pm 0.10$	70.29 ± 3.91	$94.94 \pm 6.14$	56.78 ± 1.79	$80.39 \pm 1.02$	$15.82 \pm 0.76$	$68.29 \pm 0.90$
4	2.6(1)	17.4 (1)	$8.60 \pm 1.67$	$2.84 \pm 0.20$	$65.24 \pm 5.43$	97.68 ± 10.48	$55.88 \pm 2.06$	$81.47 \pm 0.81$	$15.92 \pm 0.85$	74.12 ± 3.09
5	0 (-1.41)	11 (0)	$7.61 \pm 0.30$	$2.89 \pm 0.05$	$59.02 \pm 5.48$	114.60 ± 12.72	59.27 ± 1.27	$82.65 \pm 0.42$	15.53 ± 0.28	77.11 ± 1.43
6	3 (1.41)	11 (0)	$6.86 \pm 0.13$	$3.06 \pm 0.10$	38.29 ± 2.77	$75.00 \pm 7.74$	$61.40 \pm 1.86$	$83.40 \pm 0.60$	$15.40 \pm 0.38$	77.71 ± 1.07
7	1.5 (0)	2 (-1.41)	$6.83 \pm 0.33$	$3.45 \pm 0.14$	34.23 ± 2.34	75.58 ± 4.17	67.79 ± 3.12	$88.35 \pm 0.84$	$15.92 \pm 0.75$	82.99 ± 1.88
8	1.5 (0)	20 (1.41)	$6.81 \pm 0.15$	$2.61 \pm 0.08$	$61.89 \pm 10.49$	95.70 ± 10.19	53.73 ± 3.18	79.69 ± 1.12	$16.23 \pm 0.53$	$71.19 \pm 0.77$
9	1.5 (0)	11 (0)	$7.36 \pm 0.77$	$3.10 \pm 0.09$	$51.17 \pm 2.94$	89.54 ± 5.51	59.39 ± 1.20	83.06 ± 1.01	$15.77 \pm 0.47$	79.33 ± 0.66
10	1.5 (0)	11 (0)	$7.32 \pm 0.30$	$3.09 \pm 0.16$	54.73 ± 2.17	91.03 ± 2.39	$57.58 \pm 1.44$	$82.45 \pm 1.09$	15.37 ± 0.59	$79.46 \pm 0.46$
11	1.5 (0)	11 (0)	$7.90 \pm 0.23$	$3.05\pm0.08$	$50.52 \pm 1.43$	$78.35 \pm 2.78$	$60.80 \pm 2.38$	$83.86 \pm 0.82$	$14.96\pm0.52$	$80.14 \pm 1.23$

The values correspond to the mean values  $\pm$  standard deviation.

<sup>a</sup> Ratio: chia flour/wheat flour; SV: specific volume; L<sup>\*</sup>, h<sub>ab</sub> and C<sup>\*</sup> correspond to the color of the crumb. L<sup>\*</sup>: brightness, h<sub>ab</sub>: hue angle; C<sup>\*</sup>: chroma.



**Fig. 1.** Contour curves for the specific volume (A), the hue angle (B) and the total score (C) of the models that were obtained for the loaves developed in Experiment 1.

with the increase in the concentration of chia flour from 0 to 30 g/ 100 g in the flour mixture, them SV of the cakes decreased. These authors believed that this result was because adding chia flour decreased the amount of gluten present in the formulation. This result also suggested that incorporating chia flour in cakes may interfere in the formation of air bubbles in the dough and the aggregation of fat around them. In the experiment, the hue angle  $(h_{ab})$  ranged from 79.69 to 88.35. To obtain a product similar to wheat bread, the color should tend toward yellow (near 90°), which was obtained using the lower concentrations of chia flour (approximately 2–7 g/100 g) and regardless of the fat content, as shown in Fig. 1B.

The values for the total scores ranged from 68.29 to 82.99. According to the contour curve of the total point responses (Fig. 1C), regardless of the level of fat in the bread and with lower concentrations of chia flour (up to approximately 17.4 g/100 g), the highest total scores that were obtained ranged from 75 to 80 points. This result is because with the increased amount of chia flour, there was a decrease in the total score value because this value reflected the external features, particularly the SV, as well as the internal features, such as the aroma and taste of the bread. According to Dutcosky (1996), bread that has a score of 61–80 can be classified as regular quality bread.

From analysis of the contour curves, it was possible to determine the processing conditions under which the specific volume was increased (to more than 3 cm<sup>3</sup>/g), the hue angle was near 90° (closer to yellow) and the total score was increased (to greater than 75 and less than 100 (maximum)). The formulation containing 7.8 g/100 g chia flour and 0.9 g/100 g of hydrogenated vegetable fat was suitable for breads made with the addition of chia flour and the reduction of the fat content.

#### 3.2. Experiment 2

Table 2 presents the values that were determined for the breads with chia seeds added and analyzed as responses in the experimental design, showing that the models were predictive for the chroma, SV and total score values.

Appendix presents the values for the parameters used to validate the mathematical models obtained. Based on the equations that corresponded to the significant and predictive mathematical models, it was found that fat had a positive effect on the specific volume. The fat improved the volume and the softness of the loaves due to its ability to formation and aggregate around the CO<sub>2</sub> bubbles that form during fermentation, as seen in the proportional relationship between the hydrogenated vegetable fat content and the specific volume of the loaves with chia seeds added. The addition of chia seeds had a negative effect on the specific volume, the chroma values and the total score. These results occurred because chia seeds have no gluten and, moreover, the interactions between the proteins (gliadins and glutenins) of wheat flour and the chia fibers can prevent the expansion of bread during the fermentation process.

Fig. 2 shows the contour curves for the parameters that were evaluated in the bread that was obtained in Experiment 2. The value for  $C^*$  ranged from 13.10 to 16.46. According to Fig. 2A, in this experiment, regardless of the fat content (0–3 g/100 g of flour) of these breads, adding smaller amounts of chia seeds (approximately 2–11 g/100 g), resulted in a chroma value of greater than 15 and, thus, a more intense color.

The SV values ranged from 2.54 to 3.32 cm<sup>3</sup>/g. Good quality breads must have high SV values. According to the outline curve for the SV response shown in Fig. 2B, between 0.6 and 2.8 g/100 g fat content and up to 11 g/100 g chia seeds resulted in a high SV value. Therefore, it was possible to reduce the fat content of the breads by 50% without affecting their specific volume, even with the addition of up to 11 g/100 g chia seeds.

The total score results ranged from 74.81 to 84.97 points, according to the contour curve of the total score response (Fig. 2C). Bread with fat contents of between 0.4 and 2.6 g/100 g and lower concentrations of chia seeds (approximately 11 g/100 g) received higher total scores, ranging from 80 to 84 points, classifying them as good quality loaves (Dutcosky, 1996).

 Table 2

 Values for the technological characteristics of the loaves made with different concentrations of hydrated chia seed in relation to wheat flour and of hydrogenated vegetable fat (Experiment 2).

Trial	Fat $(x_1)$	Ratio <sup>a</sup> ( $x_2$ )	Losses on cooking (%)	SV <sup>a</sup> (cm <sup>3</sup> /g)	Firmness (g)	Firmness 24 h (g)	L <sup>*a</sup>	$h_{\mathrm{ab}}{}^{\mathrm{a}}(^{\circ})$	<i>C</i> <sup>*a</sup>	Total score
1	0.4 (-1)	4.6 (-1)	$7.62 \pm 0.44$	2.91 ± 0.02	$60.32 \pm 6.02$	120.73 ± 10.37	69.43 ± 2.96	88.76 ± 0.95	16.28 ± 1.07	80.87 ± 0.23
2	2.6(1)	4.6 (-1)	$6.91 \pm 0.17$	$3.20\pm0.06$	$58.58 \pm 3.46$	129.13 ± 13.15	70.19 ± 1.33	$88.70 \pm 0.72$	$16.38 \pm 0.81$	$80.81 \pm 0.62$
3	0.4 (-1)	17.4 (1)	$7.87 \pm 0.24$	$2.57\pm0.06$	$76.19 \pm 6.67$	118.49 ± 12.07	$64.67 \pm 3.45$	$88.83 \pm 0.78$	$13.56 \pm 1.02$	$74.81 \pm 0.42$
4	2.6(1)	17.4 (1)	$6.99 \pm 0.13$	$2.57\pm0.02$	$72.34 \pm 4.68$	$109.92 \pm 9.64$	$66.76 \pm 2.97$	$89.02 \pm 0.76$	$14.29 \pm 0.94$	$75.23 \pm 0.83$
5	0 (-1.41)	11 (0)	$6.57 \pm 0.20$	$2.54\pm0.12$	$80.63 \pm 8.87$	127.42 ± 13.23	66.96 ± 1.17	89.53 ± 0.35	$14.86\pm0.94$	$77.15 \pm 0.90$
6	3 (1.41)	11 (0)	$7.79 \pm 0.23$	$2.93 \pm 0.02$	$55.67 \pm 3.14$	97.79 ± 5.58	67.39 ± 1.44	89.11 ± 0.75	$14.62 \pm 0.75$	$78.91 \pm 0.71$
7	1.5 (0)	2 (-1.41)	$7.39 \pm 0.62$	$3.32 \pm 0.04$	$42.94 \pm 2.90$	84.54 ± 6.71	71.17 ± 1.95	$88.73 \pm 0.46$	$16.46 \pm 1.31$	$84.87 \pm 0.37$
8	1.5 (0)	20 (1.41)	$7.66 \pm 0.40$	$2.80\pm0.08$	$69.94 \pm 4.62$	106.55 ± 11.32	$61.22 \pm 2.69$	86.81 ± 1.53	$13.10 \pm 0.78$	$76.00 \pm 0.78$
9	1.5 (0)	11 (0)	$7.49 \pm 0.17$	$3.20 \pm 0.07$	$45.46 \pm 1.40$	76.85 ± 1.63	$68.14 \pm 1.94$	$88.60 \pm 0.57$	$14.45 \pm 0.77$	83.31 ± 0.76
10	1.5 (0)	11 (0)	$7.81 \pm 0.27$	$3.02\pm0.01$	$55.56 \pm 4.96$	$75.05 \pm 6.25$	$66.81 \pm 3.38$	$88.06 \pm 1.02$	$14.19 \pm 0.97$	$80.56 \pm 0.55$
11	1.5 (0)	11 (0)	$7.65 \pm 0.57$	$3.01 \pm 0.03$	$48.53 \pm 2.91$	$68.24 \pm 11.28$	$69.50\pm0.75$	$89.00 \pm 0.87$	$14.51\pm0.40$	$80.37 \pm 1.17$

The values correspond to the mean values  $\pm$  standard deviation.

<sup>a</sup> Ratio: chia seeds/wheat flour; SV: specific volume; L<sup>\*</sup>, h<sub>ab</sub> and C<sup>\*</sup> correspond to the color of the crumb. L<sup>\*</sup>: brightness, h<sub>ab</sub>: hue angle; C<sup>\*</sup>: chroma.

From analysis of the contour curves, it was possible to determine the processing conditions under which the specific volume was increased (to more than 3 cm<sup>3</sup>/g), the hue angle was near 90° (closer to yellow) and the total score was increased (to greater than 75 and less than 100 (maximum)). The bread formulation containing 11.0 g/100 g chia seeds and 1.0 g/100 g hydrogenated vegetable fat was suitable for obtaining the desirable characteristics of the final product.

#### 3.3. Nutritional evaluation of the breads

The breads developed with the addition of chia material, for which the proximal composition is presented in Table 3, had an increased nutritional value. Incorporating chia materials in the formulations increased (p < 0.05) the levels of ash, fiber and lipids and decreased (p < 0.05) the carbohydrate content compared to those of the control bread. The increase in the moisture content of the S11 formulation may have been caused by the integrated grains absorbing water and releasing it during baking.

In general, white bread has a low mineral content and must be supplemented to meet the daily requirements for the various elements (Škrbić & Filipcev, 2008). The ash content was increased significantly by the addition of and chia flour or seeds.

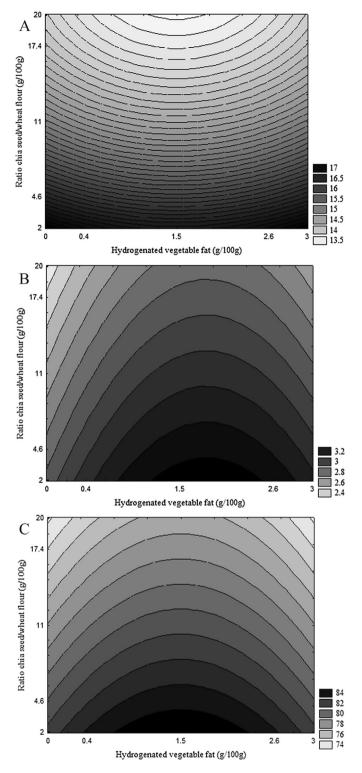
The incorporation of chia flour in the F7.8 bread formulation and chia seeds in the S11 bread formulation increased the content of total dietary fiber by 6.67 and 19 times, respectively, compared to that of the control bread. These results are superior to those obtained by Justo et al. (2007) for bread prepared using mixed flours of wheat:soybean:chia:flaxseed in the proportions of 80:10:05:05, which increased the fiber content by 1.78 times compared with whole-wheat flour bread. The chia fiber-rich fraction (Vázquez-Ovando, Rosado-Rubio, Chel-Guerrero, & Betancur-Ancona, 2009) and chia byproducts (Capitani, Spotorno, Nolasco, & Tomás, 2012) were indicated to be a potential ingredient in health and diet food products such as powders, nutrition bars, desserts, breads, cookies and among others. In Brazil, a food is allowed a functional property claim of assisting intestinal function when a portion of the product that is ready for consumption provides at least 3 g of fiber per 100 g if the food is solid (Anonymous, 1998). In this study, only the S11 formulation could be considered a functional food due to its elevated dietary fiber content. In the United States, the "Nutrition Labelling and Education Act (1990)" exercises control over the use of claims for functional foods, but provides no maximum or minimum values.

The calorific value was slightly greater for the S11 formulation. Similar results were obtained by Justo et al. (2007) for breads containing soybeans:chia (10:5) and soy:chia:flaxseed (10:5:5), with values of 422.3 and 419.1 kcal/100 g, respectively. Evaluating the characterized lipid contents (Table 3) revealed that there was an increase in the lipid content and also an improvement in the nutritional value of these.

The replacement of saturated fats by unsaturated fats in the diet or the addition of unsaturated fats reduces the risk of developing cardiovascular diseases. Compared to control bread, the breads developed in this study had reduced saturated and monounsaturated fat contents of 27 and 24% and 47 and 49%, respectively, in the F7.8 and S11 formulations. Borneo, Aguirre, and León (2010) studied the effect of replacing 75 g/100 g of the eggs or the oil in cake formulations by chia gel and observed a reduction the levels of saturated fat of 17.6 and 32.3%, respectively. The ratio of the polyunsaturated and saturated fat (PUFA:SAT) in the F7.8 and S11 formulations was increased compared to that of the control bread by 206.9 and 288.1%, respectively. These are high values compared with those of Maruyama et al. (2013), who reported that the addition of chia seeds and carrot leaves to white bread lead to a maximum increase of the PUFA:SAT ratio of 18.88% compared to that of control bread. To indicate whether a particular food is healthy, the Health Department of the United Kingdom recommends that the value of the PUFA:SAT ratio must be greater than 0.45. In this study, the values of this ratio in the F7.8 and S11 formulations were 3.10 and 3.92, respectively.

Trans fats can harm human beings because they increase the LDL-c value and reduce the HDL-c value, resulting in an increased risk of coronary artery disease and having adverse effects on the metabolism of essential fatty acids and the balance of prostaglandins; they can also lead to thrombosis (Marques, Valente, & Rosa, 2009). One of the satisfactory results of this study was that the trans fat content was practically null in the formulations in which the fat content was reduced and either chia flour or chia seed was added, causing a decrease in the trans-fat content of 69.2 and 73.1%, respectively, compared to that of the control bread. In 1999, the FDA proposed that in addition to the amount of saturated fatty acids, the amount of trans-fatty acids be listed on the labels of food products when there was more than 0.5 g in a specified portion. In Brazil, SVS ordinance number 27/98 (Anonymous, 1998) stipulated that for a claim of "free of trans fats" on a food label, the food as ready for consumption meet the following conditions: a maximum of 0.2 g of trans fat and a maximum of 2 g of saturated fat per serving (Anonymous, 2003). Therefore, the claim of "free of trans fat" can be applied to the products that were developed using chia material.

The polyunsaturated fat content was increased by approximately 125 and 195% in the F7.8 and S11 formulations, respectively, compared to that of the control bread. In particular, the level of  $\omega$ -3 fatty acid in the F7.8 and S11 formulations was increased by 40.3 and 61.7 times, respectively, and that of  $\omega$ -6, was increased by 17.0 and 26.1%, respectively. These values are high compared to those



**Fig. 2.** Contour curves for the chroma (A), specific volume (B) and total score values (C) of the models that were obtained for the loaves developed in Experiment 2.

obtained by Maruyama et al. (2013), who observed obtained an increase of 3.1 times in the  $\omega$ -3 fatty acid level and a decreased level of  $\omega$ -9 fatty acid in breads with chia seeds and carrot leaves added compared to those of the control bread. However, contrary to the results of this study, these same authors reported a reduction in the level of  $\omega$ -6 fatty acid. Justo et al. (2007) observed that wheat flour loaves with soybeans, flaxseed and chia added in the proportions of

#### Table 3

Nutritional, physical, technology and sensory characterization of the control bread, the bread with chia flour added (F7.8) and the bread with chia seeds added (S11).

nie breud with end nour ddded (r	Formulation	au with this sec	
	Control	F7.8	S11
D 1 1 1/1 / ///00			
Proximal composition (g/100 g Moisture	() and caloric va $37.2^{b} \pm 0.26$	lue (kcal/100 g $37.2^{b} \pm 0.04$	) 38.0 <sup>a</sup> ± 0.00
Ash	$2.5^{\rm b} \pm 0.05$	$3.0^{a} \pm 0.10^{4}$	$3.1^{a} \pm 0.04$
Proteins	$12.1^{a} \pm 0.09$	$13.7^{a} \pm 1.28$	$13.8^{a} \pm 1.13$
Dietary fiber	$0.3^{\circ} \pm 0.14$	$2.0^{\rm b} \pm 0.07$	$5.7^{a} \pm 0.54$
Lipids	$2.9^{a} \pm 0.89$	$3.2^{a} \pm 0.64$	$3.9^{b} \pm 0.03$
Others carbohydrates	$82.1^{a} \pm 0.09$	$78.1^{a,b} \pm 1.25$	$73.5^{b} \pm 1.50$
Caloric value	$404.1^{b} \pm 0.59$	$404.0^{\rm b} \pm 0.40$	$407.1^{a} \pm 0.17$
Lipids	$2.88 \pm 0.02$	$3.21 \pm 0.02$	$3.95 \pm 0.02$
Profile of fatty acids (g/100 g)	2.00 ± 0.02	5.21 ± 0.02	5.55 ± 0.02
Saturated fat <sup>a</sup>	$1.00 \pm 0.02$	$0.73 \pm 0.02$	$0.76 \pm 0.02$
Caproic acid <sup>a</sup> (C6:0)	-	-	$0.01 \pm 0.02$
Lauric acid <sup>a</sup> (C12)	$0.01 \pm 0.02$	_	-
Myristic acid <sup>a</sup> (C14:0)	$0.01 \pm 0.02$ $0.01 \pm 0.02$	$0.01 \pm 0.02$	$0.01 \pm 0.02$
Palmitic acid <sup>a</sup> (C16:0)	$0.60 \pm 0.02$	$0.51 \pm 0.02$	$0.51 \pm 0.02$ $0.53 \pm 0.02$
Margaric acid <sup>a</sup> (C17:0)	-	-	$0.01 \pm 0.02$
Stearic acid <sup>a</sup> (C18:0)	0.33 ± 0.02	$0.18 \pm 0.02$	$0.19 \pm 0.02$
Arachidic acid <sup>a</sup> (C20:0)	$0.01 \pm 0.02$	$0.01 \pm 0.02$	$0.01 \pm 0.02$
Behênico acid <sup>a</sup> (C22:0)	$0.02 \pm 0.02$	$0.01 \pm 0.02$	$0.01 \pm 0.02$ $0.01 \pm 0.02$
Lignocérico acid <sup>a</sup> (C22:0)	$0.02 \pm 0.02$ $0.01 \pm 0.02$	-	-
Monounsaturated fat <sup>a</sup>	$0.83 \pm 0.02$	$0.44 \pm 0.02$	$0.42 \pm 0.02$
Palmitoleic acid <sup>a</sup> (C16:1)	$0.01 \pm 0.02$	$0.02 \pm 0.02$	$0.02 \pm 0.02$
Oleic acid <sup>a</sup> (C18:1 – $\omega$ -9)	$0.65 \pm 0.02$	$0.37 \pm 0.02$	$0.35 \pm 0.02$
Cis-eicosenóico acid <sup>a</sup> (C20:1)	$0.01 \pm 0.02$	$0.01 \pm 0.02$	$0.01 \pm 0.02$
Polyunsaturated fat <sup>a</sup>	$1.01 \pm 0.02$	$2.27 \pm 0.02$	$2.98 \pm 0.02$
Linoleic acid <sup>a</sup> (C18:2 – $\omega$ -6)	$0.86 \pm 0.02$	$1.02 \pm 0.02$	$1.10 \pm 0.02$
Gamma linolenic acid <sup>a</sup>	$0.02 \pm 0.02$	$0.01 \pm 0.02$	$0.01 \pm 0.02$
$(C18:3 - \omega - 6)$	0102 - 0102	0101 - 0102	0101 ± 0102
Linolenic acid <sup>a</sup> (C18:3 – $\omega$ -3)	$0.03 \pm 0.02$	$1.21 \pm 0.02$	$1.85 \pm 0.02$
Trans fat <sup>a</sup>	$0.26 \pm 0.02$	$0.08 \pm 0.02$	$0.07 \pm 0.02$
Elaidic acid <sup>a</sup> (C18:1n9t)	$0.16 \pm 0.02$	$0.05 \pm 0.02$	$0.04 \pm 0.02$
Linolelaidic acid <sup>a</sup> (C18:2n6t)	$0.10 \pm 0.02$	$0.03 \pm 0.02$	$0.03 \pm 0.02$
Unsaturated fat <sup>a</sup>	$1.84 \pm 0.02$	$2.71 \pm 0.02$	$3.40 \pm 0.02$
Ratio PUFA:SAT	1.01	3.10	3.92
Physical and technological cha	racteristics		
Loss upon baking (%)	$7.8^{A,B} \pm 0.19$	$8.4^{A} \pm 0.55$	$6.9^{B} \pm 0.34$
SV (cm <sup>3</sup> /mL)	$3.2^{A} \pm 0.03$	$3.1^{A} \pm 0.03$	$2.9^{B} \pm 0.03$
Firmness after 1 h (g)	36.2 <sup>C</sup> ± 1.69	56.9 <sup>A</sup> ± 2.25	$48.9^{B} \pm 1.69$
Firmness after 24 h (g)	75.9 <sup>c</sup> ± 1.46	$108.0^{A} \pm 2.73$	$82.8^{B} \pm 2.28$
Color of the crumb			
$L^*$	$71.9^{A} \pm 1.04$	$63.8^{B} \pm 1.32$	$66.4^{B} \pm 2.46$
С*	$15.8^{A} \pm 1.06$	$15.7^{A} \pm 0.49$	$14.1^{A} \pm 0.73$
$h_{\rm ab}$ (°)	$85.9^{B} \pm 0.50$	$85.0^{B} \pm 0.60$	$89.0^{A} \pm 0.87$
Color of the crust			
$L^*$	$61.2^{B} \pm 0.92$	57.9 <sup>C</sup> ± 3.36	65.3 <sup>A</sup> ± 2.83
С*	36.5 <sup>A</sup> ± 1.03	33.7 <sup>A</sup> ± 2.09	$33.4^{A} \pm 2.18$
$h_{\rm ab}$ (°)	66.3 <sup>A</sup> ± 4.21	$68.9^{A} \pm 2.98$	$72.9^{A} \pm 2.38$
Total score	100 <sup>A</sup>	$80.1^{B} \pm 0.21$	$80.1^{B} \pm 1.12$
Score sensory analysis			
Appearance	_	$8.09^{A} \pm 0.960$	7.95 <sup>A</sup> ± 1.234
Crust color	_	7.81 <sup>A</sup> ± 1.051	$7.90^{A} \pm 1.255$
Color of the crumb	_	$8.12^{A} + 0.974$	$8.03^{A} \pm 1.108$
Odor	_	$7.72^{A} \pm 1.460$ $7.91^{A} \pm 1.328$	$7.97^{A} \pm 1.186$
Texture	_	7.91 <sup>A</sup> ± 1.328	$7.29^{A} \pm 1.777$
Flavor	_	8.29 <sup>A</sup> ± 0.858	7.91 <sup>A</sup> ± 1.442
Overall quality	_	$8.17^{\text{A}} \pm 0.825$	$8.11^{A} \pm 0.951$
Overall quality (IA) (%)	-	92.1	90,1
Purchase intent			
Certainly would buy	-	58.62	50.00
Probably would buy	-	34.48	32.75
Maybe buy/maybe	-	6.89	17.24
wouldn't buy			

The values correspond to the mean values  $\pm$  standard deviation.

<sup>a</sup> In relation to the total lipids; SV: specific volume;  $L^*$ : brightness,  $h_{ab}$ : hue angle;  $C^*$ : chroma; IA: index of acceptability; Control: wheat flour bread F7.8: bread containing 7.8 g/100 g chia flour and 0.9 g/100 g hydrogenated vegetable fat; S11: bread containing 11 g/100 g chia seeds and 1 g/100 g hydrogenated vegetable fat. Values denoted with the same letter in a column are not significantly different at the 5% probability level according to Tukey's test.

80:10:5:5 had 4.6 times the control content of  $\omega$ -3 fatty acid. According to the Brazilian legislation (Anonymous, 2008), a food may be alleged to have functional properties when it contains at least 0.1 g of  $\omega$ -3 fatty acid, which assists in maintaining healthy levels of triglycerides, in 100 g of product that is ready for consumption. Therefore, the products developed in this study possess functional properties.

#### 3.4. Physical and technological evaluation of the breads

A new product on the market must meet the level of quality that consumers demand. The effect of including chia materials on some of the parameters that describe the quality of bread is presented in Table 3.

The results demonstrate that there was a minor loss of mass upon baking (p < 0.05) in the S11 formulation compared to the control, which is because the fiber of chia seeds contains mucilage that absorbs a large quantity of water (Escudero-Álvarez & González-Sánchez, 2006), thus reducing the content of free water that can evaporate during the baking process.

The S11 formulation had a lower SV compared to the control bread, where as there were no differences between the F7.8 and control formulations. Puig and Haros (2011) developed breads containing 5 g/100 g chia seeds and 5 g/100 g chia flour, that were formulated using water, flour, yeast and salt, and obtained greater values for the SV due to the amount of mucilage in the chia and the absence of hydrogenated vegetable fat in the formulations and lower values for the hardness of these breads. The dilution of the gluten of the wheat flour of the mixed formulations by other flours that are free of this protein was responsible for the low retention of CO<sub>2</sub> during fermentation, the main consequence of which was reducing the volume (Sharma & Chauhan, 2000).

The firmness after 1 h and after 24 h following baking was lower for the S11 formulation than for the F7.8 formulation and both were significantly different (p < 0.05) from that of the control bread. These results demonstrated that the texture of the loaves was affected by adding chia to the formulation, mainly due to features related to the formation of the gluten dough.

The color analysis revealed significant differences in the lightness ( $L^*$ ) of the crumb and the crust and the hue angle ( $h_{ab}$ ) of the crumb. The value for brightness parameter of the crumb was significantly decreased by the addition of chia materials, tending toward dark and being more pronounced in the chia flour formulation (F7.8), an effect that was also observed by Puig and Haros (2011), who observed a decrease in the brightness with addition of 5 g/100 g chia flour and 5 g/100 g chia seeds compared to that of the control. The hue angle was greater for the S11 formulation containing chia seeds, with an increased tendency toward yellow (an angle closer to 90°). As for the crust, its brightness was decreased in the F7.8 formulation and increased in the S11 formulation compared to that of the control.

#### 3.5. Sensory evaluation of the breads

Most of the consumers were female (68.9%). Their ages were between 18 and 25 years (54.3%), between 26 and 35 years (37.1%), between 36 and 45 years (0.9%), between 46 and 50 years (1.7%) and greater than 50 years (6.0%). Table 3 presents the results of the sensory analysis of the loaves that were developed using chia materials.

The scores for all of the attributes were greater than 7, as shown in Table 3, the average acceptance of the judges ranged from "moderately liked" to "liked a lot" and the index of acceptability was greater than 90%. According to Spehar and Santos (2002), for a product to be considered acceptable in terms of its sensory properties, it must obtain a minimum score of 70%. Puig and Haros (2011) obtained a high acceptability rate (97.8%) for bread formulated with 5 g/100 g chia seeds. In evaluating the overall data, it appears that the chia flour bread attained better acceptability than did the bread formulated with chia seeds, which can be related to the higher SV value of the former.

Regarding the purchase intent (Table 3), samples of the breads containing added chia materials received positive responses, with 50% of the judges stating that they would certainly buy the breads and 33% stating that they most likely would buy them, for a total of 83% of the consumers. Approximately 60% of the consumers certainly would buy the product formulated with chia flour (F7.8) and 35% most likely would buy it, for a total of 95% of the consumers, proving that the bread added chia flour was better received by the judges than was the chia seed bread. This result may be related to the important technological feature of the breads, the specific volume, which was greater for the F7.8 formulation than for the S11 formulation.

#### 4. Conclusions

By reducing the content of hydrogenated vegetable fat and including chia (*S. hispanica* L.) seeds or flour in breads as determined by the experimental design, it was possible to reduce the levels of saturated fat and increase the levels of polyunsaturated fat, mainly  $\omega$ -3 fatty acid, in addition to increasing the level of fiber, yielding products with the features of functional foods. The technological quality of the loaves was affected by adding chia materials in the formulations, leading to a decrease in the specific volume and the total score values. In sensory evaluation, the breads with chia flour or seeds added obtained high levels of acceptability and purchase plans, demonstrating the commercial viability of these products, with the chia flour bread assigned a higher index of purchase intent than the chia seed bread.

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#### Appendix

Parameters used for validation of the model obtained in Experiment 1, where  $x_1$ : Concentration of fat;  $x_2$ : Concentration of chia flour/wheat flour:

SV = 
$$3.03 + 0.11 * x_1 - 0.12 * x_1^2 - 0.503 * x_2 + 0.144 x_1 \cdot x_2$$
  
 $R^2 = 0.9278 \quad F_{calc} / F_{tab} = 3.33 \quad p > 0.05$ 

Hue angle = 
$$83.194 - 6.150 * x_2 + 1.098 * x_2^2$$
  
 $R^2 = 0.9631 \quad F_{calc} / F_{tab} = 23.98 \quad p > 0.20$ 

Score Total = 
$$79.645 + 1.342 * x_1 - 3.688 * x_1^2$$
  
-  $7.307 * x_2 - 4.011 * x_2^2 + 3.57 * x_1 * x_2$   
 $R^2 = 0.8805 \quad F_{calc} / F_{tab} = 3.32 \quad p > 0.20$ 

Parameters used for validation of the models obtained in Experiment 2, where  $x_1$ : Concentration of fat;  $x_2$ : Concentration of chia flour/wheat flour:

$$SV = 3.05 + 0.21x_1 - 0.37x_1^2 - 0.43x_2$$
$$R^2 = 0.8934 \quad F_{calc} / F_{tab} = 3.82 \quad p > 0.10$$

$$C^* = 14.38 + 0.54x_1^2 - 2.39x_2 + 0.58x_2^2 \quad R^2 = 0.8934$$
$$F_{calc} / F_{tab} = 11.38 \quad p > 0.10$$

Score Total =  $80.65 - 3.56x_1^2 - 6.05x_2$   $R^2 = 0.8663$  $F_{calc}/F_{tab} = 2.63$  p > 0.05

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