



## Development of gluten-free premixes with buckwheat and chia flours: Application in a bread product

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### ABSTRACT

The objective of the present work was to develop novel alternative gluten-free premixes for their application in the production of a bread product. Eight formulations of premixes based on light buckwheat flour (LBF) or whole buckwheat flour (WBF) supplemented with chia flour (CF) in a 90:10 ratio (w/w), with and without the addition of xanthan gum (XG 2 g/100 g) were evaluated. A commercial gluten-free premix was used as control. Breads were baked using each formulation and the control premix. The loaves prepared with buckwheat flour and supplemented with CF and XG exhibited a significantly lower mass loss during baking than the control. All the loaves made with LBF presented a higher air fraction, alveolar area and lightness than those prepared with WBF. The joint addition of XG:CF decreased the hardness of the LBF-based loaves. The selected premix (LBF:XG:CF) and the bread exhibited a higher protein, ash, crude fiber and polyunsaturated fatty acid content and antioxidant activity than the commercial premix and its bread (control). The development of premixes based on buckwheat and chia flour represents an alternative with high nutritional value that can be applied to the formulation of gluten-free breads, with adequate technological properties.

### 1. Introduction

Gluten-free products represent an interesting sector in the food industry mainly due to the increase in the incidence of the celiac disease over the past several decades, and to a current trend toward the consumption of non-allergenic ingredients. Making gluten-free breads of high quality and with a healthier nutritional profile presents a technological challenge given the absence of gluten and the deficiency in certain nutrients. Thus, it is necessary to use ingredients with viscoelastic properties similar to gluten in order to obtain products with good characteristics and that are nutritionally balanced (Jnawali, Kumar, & Tanwar, 2016). Among hydrocolloids, xanthan gum is characterized by achieving a good imitation of the viscoelastic properties of gluten, being one of the most commonly used ingredients in this type of product, giving the dough a softer texture, greater volume, reduced crumbling

and better elastic properties, probably due to its high water retention capacity and viscosity (Moreira, Chenlo, & Torres, 2011).

Various works on the formulation of gluten-free foods combined buckwheat with rice flours (Torbica, Hadnadev, & Dapcevic, 2010), and with different hydrocolloids (Kaur, Sandhu, Arora, & Sharma, 2015). Buckwheat (*Fagopyrum esculentum*) is rich in proteins of good quality (good balance of essential amino acids, high lysine content), dietary fiber and resistant starch (Tömösközi & Langó, 2017). Buckwheat flour is an important source of polyphenolic compounds with high antioxidant activity, vitamins and minerals. It has a total, insoluble and soluble dietary fiber content of 6.5, 5.33 and 1.20 g/100 g, respectively (Costantini et al., 2014).

Chia and the by-product of the cold-pressed chia oil extraction process (partially-deoiled chia flour) are a good source of  $\omega$ -3 fatty acids, antioxidants, proteins and dietary fiber, which makes them a novel

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ingredient for functional foods (Capitani, Spotorno, Nolasco, & Tomás, 2012; Ixtaina et al., 2011). The European Commission (2013, p. 34) gave marketing authorization for whole and ground chia seeds as a novel food ingredient to be used in bread products (maximum 10 g/100 g flour). Partially-deoiled chia flour is mainly used as animal feed and is sold for human consumption at health food stores. Recent studies show that this by-product has been applied in the production of bioactive peptides and in the formulation of bakery products (muffins, bread) containing gluten (Aranibar, Aguirre, & Borneo, 2019; Cotabarren et al., 2019; Guiotto, Tomás, & Haros, 2020). As it is a by-product of high nutritional value, it could be used in the formulation of gluten-free bakery products. The high total dietary fiber content of partially-deoiled chia flour (44.11 g/100 g dry basis - d.b.), consisting mostly of insoluble dietary fiber (40.30 g/100 g d.b.) and a small proportion (3.81% mucilage) of soluble dietary fiber, makes it an interesting ingredient for developing gluten-free breads. In addition, the presence of mucilage confers a high water holding capacity, which helps improve the stability of gluten-free doughs (Capitani et al., 2012; Guiotto et al., 2020). Studies have been conducted on the addition of whole or ground chia seeds to gluten-free breads (Costantini et al., 2014; Huerta, Alves, da Silva, Kubota, & da Rosa, 2016; Pal & Kumari, 2017; and others); however, there is no literature on the formulation of gluten-free breads with chia flour from cold-pressed oil extraction and their combination with buckwheat flour. Therefore, the objective of this work was to develop a gluten-free premix based on buckwheat flour supplemented with chia flour obtained as a by-product of the cold-pressed oil extraction, with and without the addition of xanthan gum, for the production of a baked product (gluten-free bread).

## 2. Materials and methods

### 2.1. Materials

Buckwheat flours (light and wholegrain, LBF and WBF, respectively) were supplied by Centro INTI Cereales y Oleaginosas (9 de Julio, Buenos Aires, Argentina). Chia flour (CF) was obtained by the procedure (cold press) proposed by Capitani et al. (2012). Dry yeast (Levex®), white sugar (Ledesma S.A.A.I.), refined salt (Dos Anclas S.A.), powdered milk (La Serenísima S.A.) and xanthan gum (XG) (Onza de Oro) were purchased at a local market in Olavarría.

### 2.2. Analysis of the flour samples

#### 2.2.1. Gluten determination

The gluten content was measured by the sequential competitive ELISA method developed by Chirido, Añón, and Fossati (1995) (detection limit 1 mg/kg).

#### 2.2.2. Particle size distribution

All the samples were submitted to granulometry determination according to Capitani et al. (2012), using a Zonytest agitator (Buenos Aires, Argentina) equipped with a series of ASTM meshes between number 10 and 325, corresponding to particle sizes between 44 and 2000 µm. Twenty grams of flour were weighed and placed onto the top sieve, and then agitated for 60 min. The material retained in each sieve was weighed and the percentage of each fraction was calculated.

#### 2.2.3. Proximate composition and color of the flour

The water, crude fiber and ash contents were determined according to AOCS (1998) procedures. The oil and protein content were determined following IUPAC (1992) and AOAC (2016), respectively. The carbohydrate content was calculated by difference (Capitani et al., 2012).

Color measurements were carried out using a Minolta Chroma Meter CR-400 (Sensing Inc., Japan) colorimeter. The results were expressed in terms of  $L^*$  (lightness),  $a^*$ , and  $b^*$  values, in accordance with the CIELab

system with reference to illuminant D65 and an observation angle of 10°.

### 2.2.4. Functional properties

Water-holding capacity (WHC), oil-holding capacity (OHC), water absorption capacity ( $WA_bC$ ), water adsorption capacity ( $WA_dC$ ), organic molecule absorption capacity (OMAC), emulsifying activity (EA), and emulsion stability (ES) were determined according to Capitani et al. (2012).

### 2.3. Formulation of the gluten-free premixes

Mixtures of LBF and CF and of WBF and CF were prepared, with and without XG (Table 1), in order to offer an alternative to premixes available in the market that allows obtaining a bread with similar characteristics to that produced with a commercial premix. The different premixes were prepared just before making the loaves. A commercial gluten-free premix purchased at a local health food store was used as control (CP). The composition of the commercial premix is rice flour, cassava starch, corn starch, potato starch, sugar, vegetable oil, milk, egg, salt, emulsifiers (sterol, sodium lactate, soybean lecithin) and stabilizers (carboxymethylcellulose, xanthan gum, guar gum).

### 2.4. Gluten-free bread making procedure

Eight gluten-free bread formulations were tested. The concentrations of water (110 g), dry yeast (2 g), salt (1.5 g) and sugar (4 g) (all based on 100 g of mixture) were kept constant for all the gluten-free bread formulations. The control bread was made from a commercial gluten-free premix.

The ingredients were weighed and added to a planetary mixer (MP-7, Santini, Italy), mixing the dry ingredients first, and then adding the water at  $30 \pm 1$  °C. Kneading was performed for 1 min at 145 rpm and then, 7 min at 210 rpm in the same mixer. The bread dough was divided into 165 g portions and placed on teflon-coated baking pans (13.5 × 7.0 × 4.5 cm), which in turn were placed in an oven at 30 °C and 85% RH, 60 min for fermentation (modified drying oven San Jor SL60CDB, Argentina). Subsequently, the loaves were baked in an electric oven (Moretti, Argentina) for 25 min, 180 °C, removed immediately from the pans and cooled at  $25 \pm 1$  °C, 2 h before the analyses. Each bread formulation was performed in duplicate.

### 2.5. Physical and technological evaluation of the bread samples

#### 2.5.1. Moisture content measurement

The moisture content of the loaves was determined in a forced convection oven for 2 h at 130 °C (drying oven DHG-9123A, China). For each measurement, approximately 3 g of crumb were taken from 3 slices of each bread, previously ground in a coffee grinder (Moulinex, Argentina).

**Table 1**

Gluten-free flour mixture formulations (g/100 g of total mixture).

Formulation	Ingredients	LBF	CF	WBF	XG
1	LBF	100.0			
2	LBF: XG	98.0			2.0
3	LBF: CF	90.0	10.0		
4	LBF: CF: XG	88.2	9.8		2.0
5	WBF			100.0	
6	WBF: XG			98.0	2.0
7	WBF: CF		10.0	90.0	
8	WBF: CF: XG		9.8	88.2	2.0

LBF: light buckwheat flour; WBF: wholegrain buckwheat flour; CF: chia flour; XG: Xanthan gum.

### 2.5.2. Specific volume (SV)

The SV measurement was calculated from the ratio between the loaf apparent volume (mL), using the rapeseed displacement procedure (AACC method 2003) and the weight (g) after baking, using a semi-analytical balance PA1502 (Ohaus, USA).

### 2.5.3. Baking loss (BL)

The amount of loss that occurred during baking, was determined according to [Silveira Coelho and Salas-Mellado \(2015\)](#).

### 2.5.4. Color of the gluten-free bread crumb and crust

The color of the crumb and crust was determined using the procedure described in 2.2.3. The determination of crumb color was made on the central portion of three slices of each bread, whereas crust color was determined on six pre-selected locations of the crust of each bread.

### 2.5.5. Digital image analysis of the bread crumb structure

Three slices (thickness 20 mm) were cut from each bread loaf, and images were acquired with a scanner (Epson Stylus TX135, China), with a resolution of 400 pixels per inch. Digital images of the crumbs were analyzed using ImageJ 1.51 v software (National Institutes of Health, Bethesda, MD, USA). The center of each slice image was cropped to a square field of view of 20 × 20 mm and converted to an 8 bit-grey level image. The images were then binarized (converted from greyscale to black and white) using an automated fuzzy thresholding method to differentiate gas cells from non-cells. The mean alveolar area (MAA), circularity and air fraction (percentage of the total area occupied by alveoli over the total area of the image) were then determined.

### 2.5.6. Texture profile analysis (TPA)

The TPA was performed with a TA. XT2i texture analyzer (Stable Micro System, Surrey, UK) following the method described by [Arp, Correa, and Ferrero \(2018\)](#). Five slices (thickness 15 mm) were compressed in the center of the texture analyzer platform using a cylindrical stainless steel probe of 75 mm diameter (SMS probe P/75). The selected settings were: test speed 0.5 mm/s, 40% deformation of the sample, and two compression cycles. The Texture Expert software for Windows (v.1.2) was used to evaluate the following textural parameters: hardness, springiness, cohesiveness, fracturability, and resilience.

Based on the physical and technological analysis of the baked loaves, the premix formulation that allowed to obtain the bread with similar or better characteristics than the control (higher moisture content, lower baking loss, larger specific volume, higher lightness, higher circularity and air fraction, less hardness and more homogeneous crust) was selected. This premix formulation was prepared in a planetary mixer (MP-7, Santini, Italy), then placed in hermetic plastic containers and stored at 5 ± 1 °C until use.

## 2.6. Characterization of the selected gluten-free premix and bread

The proximate composition and color of the selected gluten-free premix and bread were determined following the procedures described in 2.2.3. The caloric value was calculated using the Atwater system according to [Silveira Coelho and Salas-Mellado \(2015\)](#).

The different functional properties of the premix were determined following the procedures described in section 2.2.4.

### 2.6.1. Antioxidant activity (AA)

The extraction of the phenolic compounds was carried out following the procedures described by [Capitani et al. \(2012\)](#). Ten mL of ethanol were added to 1 g of sample; then it was homogenized in Vortex for 2 min, decanted and filtered (0.25 mm nylon paper). The supernatant was then transferred into a flask and evaporated using a rotavapor apparatus (BUCHI R-3000, Germany) to concentrate the sample, which was then dissolved in 1 mL ethanol. The AA of the extracts was screened by measuring the DPPH radical scavenging activity according to [Carciochi,](#)

[Manrique, and Dimitrov \(2014\)](#). Aliquots (50 µL) of extracts were added to 1950 µL of a methanolic solution (100 l M) of DPPH radical. After agitation, the mixture was incubated in the dark for 30 min, and the absorbance was measured at 517 nm. AA was calculated on the basis of the percentage of DPPH radical scavenging activity.

### 2.6.2. Fatty acid profile

This parameter was determined as described by [Capitani, Mateo, and Nolasco \(2011\)](#). Methyl esters were obtained using acetyl chloride in methanol and chloroform as solvent. A Shimadzu GC-2014 chromatograph (Japan) equipped with a capillary column (DB-23 × 60 m x 0.25 mm) and a flame ionization detector (FID) with a split ratio of 95/1 was used. Furnace, injector and detector temperatures were 210 °C, 240 °C and 300 °C, respectively. N<sub>2</sub> was used as carrier gas. The identification of the peaks in the chromatogram was carried out considering retention times and using the patterns of methyl esters. The results were expressed as g of fatty acids per 100 g of total fatty acids.

## 2.7. Statistical analysis

Determinations were performed in triplicate. Results were analyzed using ANOVA (Tukey's test  $p \leq 0.05$ ) with the Infostat software ([Di Rienzo et al., 2019](#)).

## 3. Results and discussion

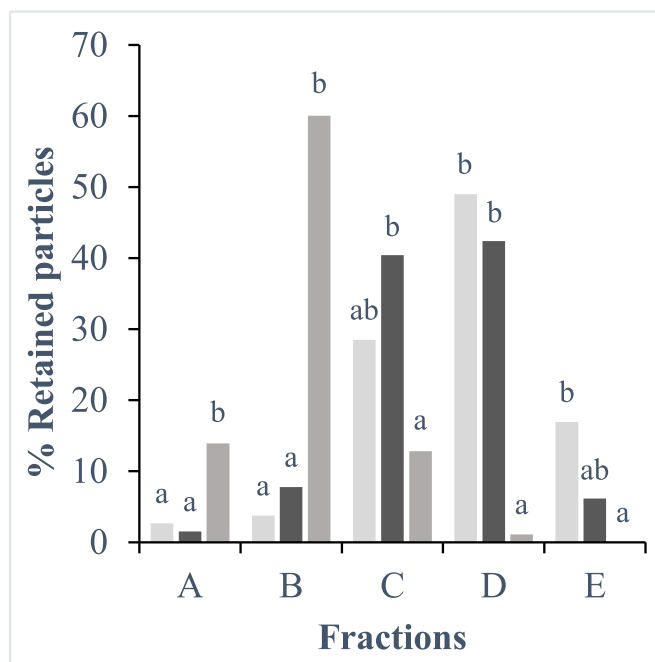
### 3.1. Physicochemical characteristics and functional properties of the flour samples

#### 3.1.1. Gluten determination

Gliadin content was lower than 1 mg/kg in LBF, WBF and CF, which makes them suitable raw materials for the preparation of gluten-free products.

#### 3.1.2. Particle size distribution

LBF and WBF presented a larger percentage of smaller sized particles (105–149 µm) (49 and 42 g/100 g for LBF and WBF, respectively) than



**Fig. 1.** Particle size distribution of buckwheat and chia flours. Each value is an average of three determinations ( $n = 3$ ). A (420–840 µm), B (250–420 µm), C (149–250 µm), D (105–149 µm), E (74–105 µm). ■ = LBF; ■ = WBF; ■ = CF.

CF that exhibited 60 g/100 g of larger-sized particles (250 and 420  $\mu\text{m}$ ) (Fig. 1). These results are in agreement with the visual observations (see supplementary material), where the buckwheat flours showed a finer granulometry. While the percentage of large size particles was higher for WBF than LWF, no significant differences ( $p > 0.05$ ) were observed between the various fractions of these samples. The larger granulometry of CF could be a result of the agglomeration of particles due to the significant presence of residual lipids from the cold pressing extraction, as explained by Capitani et al. (2012).

### 3.1.3. Proximate composition, color and functional properties

The buckwheat flours exhibited significantly higher ( $p \leq 0.05$ ) carbohydrate content than CF, with WBF showing a significantly higher crude fiber level than LBF (Table 2), associated with the presence of hull. CF showed a significantly higher ( $p \leq 0.05$ ) protein, ash, crude fiber and fat content than the buckwheat flours, with a similar composition to that reported by Capitani et al. (2012). However, it should be noted that studies by Ayerza and Coates (2011) showed a significant difference in the protein content of chia grown commercially in different ecosystems.

The three flour samples were different in terms of color (Table 2). The  $L^*$  parameter (lightness) was significantly higher for LBF, indicating a whiter appearance of this flour with respect to WBF and CF, with CF showing the significantly lowest  $L^*$  value, indicating a darker flour. This behavior is consistent with the visual observation of the three flour samples (see supplementary material). The  $a^*$  and  $b^*$  parameters were positive for all the samples, showing a greater tendency to redness and yellowness. The coordinate  $a^*$  was significantly higher ( $p \leq 0.05$ ) for CF, expressing a more reddish shade for this flour, while the coordinate  $b^*$  was significantly higher for LBF indicating a more yellowish hue, related to its greater lightness. The darker color of CF shows less intensity of the yellow color (significantly lower  $b^*$  value), which could be attributed to the significant presence of chia oil soluble pigments ( $\beta$ -carotene) (Ixtaina et al., 2011).

LBF and WBF had significantly higher ( $p \leq 0.05$ ) emulsifying properties (EA and ES) than CF, with LBF exhibiting the significantly highest values (Table 2). The high ES could be related to the differences in carbohydrate composition between the flours. The buckwheat flour has a high starch content in its carbohydrate composition (59%–70%, d.b.), characteristic makes this kind of flour an ingredient with interesting

stabilizing and thickening properties (Tömösközi & Langó, 2017). As for OMAC, LBF presented a significantly higher value than WBF and CF, which can be attributed to its lower lipid content compared to CF and to its smaller particle size with respect to WBF.

CF was characterized by exhibiting significantly higher ( $p \leq 0.05$ ) WHC and  $WA_bC$  values than the buckwheat flours. The larger WHC of CF could be associated with its higher fiber content compared to the buckwheat flours. In addition, the mucilage present in the chia acts as soluble dietary fiber (SDF), increasing WHC.

### 3.2. Technological characteristics of the bread samples

The breads formulated only with LBF and WBF, as well as the control, exhibited a significantly lower moisture level than the rest of the formulations (Table 3). When XG and CF were added, jointly or separately, moisture increased significantly. This could be explained by the higher WHC and  $WA_bC$  of CF with respect to buckwheat flours (see Table 2) associated with the mucilages in the chia seeds that can act as SDF. The inclusion of hydrocolloids favors the fresh quality of bread products because of their excellent water-holding properties (Kaur et al., 2015).

On the other hand, the loaves made with only LBF and WBF showed a significantly higher SV than the rest of the samples, except for the control bread (CB) that presented the highest SV (Table 3). This could be due to the presence of stabilizing agents (xanthan gum, guar gum and carboxymethyl cellulose) and emulsifiers (soybean lecithin) declared in the CP, which contribute to better retain gas during fermentation and baking.

The incorporation of XG and CF to the gluten-free bread formulations produced a significant decrease ( $p \leq 0.05$ ) in the SV of the loaves. This can be attributed to the amount of added water, which remained constant in all formulations, favoring the increase in viscosity of the dough, its development and gas retention. The addition of various hydrocolloids to gluten-free doughs in general increases the SV of the bread; however, the materials used, the interaction between them, the concentrations used, and the amount of added water can affect the results (Lazaridou, Duta, Papageorgiou, Belc, & Biliaderis, 2007; Sciriani, Ribotta, León, & Pérez, 2010). As for CF (fiber rich matrix), it should be noted that even though the addition of fiber to bread products is beneficial in terms of moisture retention, on the other hand it can cause poor bread quality, for example a smaller SV (Katina, Salmenkallio-Marttila, Partanen, Forsell, & Autio, 2006). Similar technological limitations of CF were observed by Huerta et al. (2016) when adding different concentrations of chia flour to gluten-free bread formulations. However, the bread based on LBF with the joint addition of XG and CF (formulation 4) presented a significantly higher SV than when they were added separately (formulations 2 and 3), which could be indicating a synergistic effect of the two ingredients.

The loaves prepared with only LBF and WBF (formulations 1 and 5, respectively) and the control bread (CB) showed a significantly higher ( $p \leq 0.05$ ) mass loss during baking (BL) than the rest of the formulations (Table 3). This behavior could be due to their larger volume, which produces a greater surface for the water exchange during baking (Steffolani, de la Hera, Pérez, & Gómez, 2014), and in the case of formulations 1 and 5, it could also be associated with the absence of hydrocolloids (XG, and CF with mucilage), which provide a higher WHC thus helping to maintain the moisture of the dough during baking (Steffolani et al., 2014). Thus, the inclusion of CF and XG in the premixes produced a significantly lower BL during baking ( $p \leq 0.05$ ) compared to the loaves prepared with only LBF and WBF. In the breads based on LBF, the joint addition of XG and CF produced significantly lower BL than when the ingredients were added separately.

The CB showed a whiter crumb than the rest of the loaves, which presented parameters  $L^*$ ,  $a^*$ , and  $b^*$  consistent with the color characteristics observed for the flours (see Tables 2 and 3). Fig. 2 shows the colors of the nine formulations of gluten-free bread.

The crust color of the LBF-based loaves and the control was

**Table 2**  
Physicochemical and functional properties of buckwheat and chia flours.

	LBF	WBF	CF
Proximate composition (g/100 g d.b.)			
Moisture	16.25 $\pm$ 0.02 <sup>c</sup>	14.64 $\pm$ 0.25 <sup>b</sup>	10.18 $\pm$ 0.13 <sup>a</sup>
Protein (Factor = 6.25)	12.52 $\pm$ 0.36 <sup>a</sup>	11.67 $\pm$ 0.04 <sup>a</sup>	31.02 $\pm$ 0.62 <sup>b</sup>
Crude fiber	0.56 $\pm$ 0.02 <sup>a</sup>	10.86 $\pm$ 0.21 <sup>b</sup>	24.62 $\pm$ 0.22 <sup>c</sup>
Oil	2.71 $\pm$ 0.01 <sup>a</sup>	2.59 $\pm$ 0.04 <sup>a</sup>	10.45 $\pm$ 0.61 <sup>b</sup>
Ash	2.07 $\pm$ 0.02 <sup>a</sup>	2.13 $\pm$ 0.002 <sup>a</sup>	7.52 $\pm$ 0.04 <sup>b</sup>
Carbohydrate	82.14 $\pm$ 0.36 <sup>c</sup>	72.75 $\pm$ 0.21 <sup>b</sup>	26.39 $\pm$ 0.20 <sup>a</sup>
Color			
$L^*$	83.69 $\pm$ 0.07 <sup>c</sup>	72.17 $\pm$ 0.36 <sup>b</sup>	41.41 $\pm$ 0.20 <sup>a</sup>
$a^*$	1.31 $\pm$ 0.03 <sup>a</sup>	2.20 $\pm$ 0.02 <sup>b</sup>	4.27 $\pm$ 0.03 <sup>c</sup>
$b^*$	8.32 $\pm$ 0.04 <sup>c</sup>	8.00 $\pm$ 0.04 <sup>b</sup>	6.83 $\pm$ 0.16 <sup>a</sup>
Functional property			
WHC (g/g)	1.73 $\pm$ 0.04 <sup>a</sup>	1.87 $\pm$ 0.02 <sup>b</sup>	7.72 $\pm$ 0.02 <sup>c</sup>
OHC (g/g)	1.16 $\pm$ 0.05 <sup>a</sup>	1.19 $\pm$ 0.03 <sup>a</sup>	1.20 $\pm$ 0.06 <sup>a</sup>
$WA_bC$ (g/g)	1.17 $\pm$ 0.002 <sup>a</sup>	1.16 $\pm$ 0.002 <sup>a</sup>	7.72 $\pm$ 0.09 <sup>b</sup>
$WA_dC$ (g/g)	0.43 $\pm$ 0.02 <sup>ab</sup>	0.40 $\pm$ 0.02 <sup>a</sup>	0.48 $\pm$ 0.01 <sup>b</sup>
OMAC (g/g)	1.17 $\pm$ 0.003 <sup>b</sup>	1.01 $\pm$ 0.003 <sup>a</sup>	1.00 $\pm$ 0.01 <sup>a</sup>
EA (mL/100 mL)	42.54 $\pm$ 0.53 <sup>c</sup>	41.29 $\pm$ 0.88 <sup>b</sup>	6.21 $\pm$ 0.37 <sup>a</sup>
ES (mL/100 mL)	46.29 $\pm$ 0.55 <sup>c</sup>	44.38 $\pm$ 0.25 <sup>b</sup>	10.04 $\pm$ 0.42 <sup>a</sup>

Mean values  $\pm$  standard deviation ( $n = 3$ ).

Different letters in the same row indicate significant differences ( $p \leq 0.05$ ).

LBF: light buckwheat flour; WBF: wholegrain buckwheat flour; CF: chia flour. WHC: water-holding capacity; OHC: oil-holding capacity;  $WA_bC$ : water absorption capacity;  $WA_dC$ : water adsorption capacity; OMAC: organic molecule absorption capacity; EA: emulsifying activity, and ES: emulsion stability.

**Table 3**

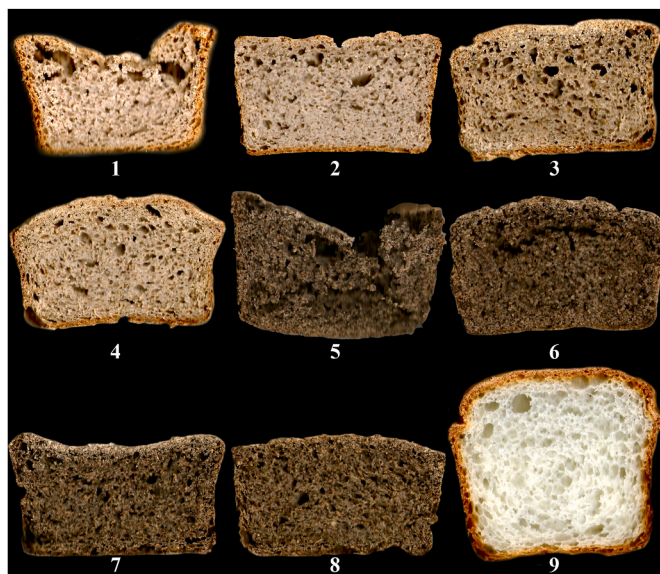
Moisture, physical characteristics, crumb structure (digital image analysis) and texture parameters of the gluten-free breads.

Properties	Formulation									
	LBF	LBF: XG	LBF: CF	LBF: CF: XG	WBF	WBF: XG	WBF: CF	WBF: CF: XG	CP	
Moisture (g/100 g)	40.79 ± 0.51 <sup>a</sup>	43.98 ± 0.81 <sup>b</sup>	46.75 ± 0.86 <sup>c</sup>	45.76 ± 0.76 <sup>bc</sup>	39.18 ± 0.58 <sup>a</sup>	44.51 ± 0.48 <sup>bc</sup>	45.34 ± 0.93 <sup>bc</sup>	44.49 ± 0.39 <sup>bc</sup>	39.20 ± 0.51 <sup>a</sup>	
Specific volume (mL/g)	2.41 ± 0.07 <sup>c</sup>	1.64 ± 0.08 <sup>a</sup>	1.73 ± 0.02 <sup>a</sup>	2.04 ± 0.05 <sup>b</sup>	2.40 ± 0.04 <sup>c</sup>	1.84 ± 0.07 <sup>ab</sup>	1.99 ± 0.06 <sup>b</sup>	1.90 ± 0.02 <sup>ab</sup>	3.97 ± 0.19 <sup>d</sup>	
Bake loss (g/100 g)	26.07 ± 0.11 <sup>e</sup>	19.96 ± 0.65 <sup>bcd</sup>	19.79 ± 0.26 <sup>bcd</sup>	17.00 ± 1.00 <sup>a</sup>	25.02 ± 0.27 <sup>e</sup>	18.51 ± 0.06 <sup>abc</sup>	20.56 ± 0.87 <sup>cd</sup>	17.85 ± 0.67 <sup>ab</sup>	21.58 ± 0.50 <sup>d</sup>	
Crumb color	<i>L</i> <sup>*</sup>	55.58 ± 1.07 <sup>c</sup>	56.09 ± 0.12 <sup>c</sup>	46.74 ± 0.52 <sup>b</sup>	50.62 ± 0.47 <sup>bc</sup>	31.41 ± 0.44 <sup>a</sup>	31.91 ± 0.37 <sup>a</sup>	30.07 ± 0.22 <sup>a</sup>	33.31 ± 1.22 <sup>a</sup>	78.83 ± 1.18 <sup>d</sup>
	<i>a</i> <sup>*</sup>	4.91 ± 0.12 <sup>c</sup>	4.89 ± 0.06 <sup>c</sup>	4.38 ± 0.02 <sup>bc</sup>	4.21 ± 0.002 <sup>b</sup>	4.97 ± 0.21 <sup>c</sup>	4.71 ± 0.27 <sup>bc</sup>	4.38 ± 0.06 <sup>bc</sup>	4.53 ± 0.23 <sup>bc</sup>	-3.17 ± 0.21 <sup>a</sup>
	<i>b</i> <sup>*</sup>	12.35 ± 0.19 <sup>c</sup>	12.37 ± 0.11 <sup>c</sup>	12.38 ± 0.48 <sup>c</sup>	12.84 ± 0.12 <sup>c</sup>	10.58 ± 0.09 <sup>ab</sup>	9.72 ± 0.22 <sup>a</sup>	9.80 ± 0.05 <sup>a</sup>	10.63 ± 0.06 <sup>ab</sup>	11.93 ± 1.03 <sup>bc</sup>
Crust color	<i>L</i> <sup>*</sup>	55.31 ± 1.63 <sup>cd</sup>	58.37 ± 0.25 <sup>d</sup>	52.91 ± 1.80 <sup>b</sup>	51.75 ± 1.07 <sup>b</sup>	41.87 ± 0.99 <sup>a</sup>	39.83 ± 0.44 <sup>a</sup>	41.26 ± 1.73 <sup>a</sup>	37.47 ± 0.19 <sup>a</sup>	47.90 ± 1.57 <sup>b</sup>
	<i>a</i> <sup>*</sup>	11.69 ± 1.24 <sup>de</sup>	8.92 ± 0.01 <sup>bc</sup>	9.97 ± 0.29 <sup>cd</sup>	10.01 ± 0.46 <sup>cd</sup>	9.58 ± 0.17 <sup>c</sup>	6.71 ± 0.28 <sup>a</sup>	7.40 ± 0.21 <sup>ab</sup>	7.50 ± 0.07 <sup>ab</sup>	13.11 ± 0.29 <sup>e</sup>
	<i>b</i> <sup>*</sup>	31.14 ± 1.25 <sup>d</sup>	24.54 ± 0.24 <sup>bc</sup>	27.33 ± 1.42 <sup>c</sup>	24.86 ± 0.32 <sup>bc</sup>	23.32 ± 0.49 <sup>b</sup>	16.37 ± 0.69 <sup>a</sup>	18.37 ± 0.16 <sup>a</sup>	16.47 ± 0.32 <sup>a</sup>	30.90 ± 0.29 <sup>d</sup>
Crumb structure	MAA (cm <sup>2</sup> )	0.050 ± 0.01 <sup>ab</sup>	0.040 ± 0.001 <sup>ab</sup>	0.040 ± 0.003 <sup>ab</sup>	0.030 ± 0.001 <sup>ab</sup>	0.010 ± 0.002 <sup>a</sup>	0.020 ± 0.002 <sup>a</sup>	0.020 ± 0.002 <sup>a</sup>	0.020 ± 0.001 <sup>a</sup>	0.060 ± 0.03 <sup>b</sup>
	Circularity	0.30 ± 0.04 <sup>a</sup>	0.34 ± 0.06 <sup>ab</sup>	0.44 ± 0.01 <sup>bc</sup>	0.35 ± 0.02 <sup>ab</sup>	0.29 ± 0.01 <sup>a</sup>	0.31 ± 0.01 <sup>a</sup>	0.28 ± 0.06 <sup>a</sup>	0.30 ± 0.003 <sup>a</sup>	0.49 ± 0.01 <sup>c</sup>
	Air fraction (%)	14.34 ± 1.40 <sup>b</sup>	15.15 ± 0.18 <sup>b</sup>	17.25 ± 0.51 <sup>b</sup>	18.29 ± 0.22 <sup>b</sup>	2.63 ± 0.50 <sup>a</sup>	2.24 ± 0.11 <sup>a</sup>	4.56 ± 1.15 <sup>a</sup>	4.68 ± 0.53 <sup>a</sup>	20.14 ± 1.03 <sup>b</sup>
Texture profile analysis	Hardness (N)	5.06 ± 0.19 <sup>b</sup>	6.54 ± 0.32 <sup>cd</sup>	10.54 ± 0.01 <sup>f</sup>	8.54 ± 0.29 <sup>e</sup>	5.35 ± 0.13 <sup>bc</sup>	6.96 ± 0.01 <sup>d</sup>	11.11 ± 0.01 <sup>f</sup>	12.65 ± 0.47 <sup>g</sup>	0.88 ± 0.63 <sup>a</sup>
	Springiness (mm)	0.87 ± 0.01 <sup>c</sup>	0.73 ± 0.02 <sup>b</sup>	0.88 ± 0.03 <sup>c</sup>	0.83 ± 0.01 <sup>c</sup>	0.75 ± 0.04 <sup>b</sup>	0.59 ± 0.02 <sup>a</sup>	0.74 ± 0.02 <sup>b</sup>	0.73 ± 0.003 <sup>b</sup>	0.97 ± 0.01 <sup>d</sup>
	Cohesiveness	0.39 ± 0.02 <sup>ab</sup>	0.42 ± 0.03 <sup>ab</sup>	0.45 ± 0.02 <sup>bc</sup>	0.50 ± 0.01 <sup>c</sup>	0.37 ± 0.01 <sup>a</sup>	0.39 ± 0.01 <sup>ab</sup>	0.40 ± 0.02 <sup>ab</sup>	0.44 ± 0.01 <sup>bc</sup>	0.57 ± 0.01 <sup>d</sup>
	Chewiness (Nxmm)	1.70 ± 0.02 <sup>b</sup>	1.97 ± 0.11 <sup>b</sup>	4.17 ± 0.01 <sup>e</sup>	3.50 ± 0.24 <sup>cd</sup>	1.48 ± 0.08 <sup>b</sup>	1.60 ± 0.11 <sup>b</sup>	3.26 ± 0.08 <sup>c</sup>	3.98 ± 0.27 <sup>de</sup>	0.49 ± 0.03 <sup>a</sup>
	Resilience (Nxmm)	0.28 ± 0.01 <sup>b</sup>	0.24 ± 0.03 <sup>ab</sup>	0.33 ± 0.02 <sup>c</sup>	0.34 ± 0.02 <sup>c</sup>	0.21 ± 0.01 <sup>ab</sup>	0.20 ± 0.02 <sup>a</sup>	0.22 ± 0.02 <sup>ab</sup>	0.24 ± 0.01 <sup>ab</sup>	0.46 ± 0.02 <sup>d</sup>
	Gumminess (N)	1.96 ± 0.002 <sup>b</sup>	2.73 ± 0.07 <sup>c</sup>	4.75 ± 0.17 <sup>d</sup>	4.25 ± 0.25 <sup>d</sup>	1.97 ± 0.11 <sup>b</sup>	2.75 ± 0.09 <sup>c</sup>	4.41 ± 0.01 <sup>d</sup>	5.50 ± 0.36 <sup>e</sup>	0.50 ± 0.03 <sup>a</sup>

Mean values ± standard deviation (n = 3). Values followed by different letters in the same row differ significantly (p ≤ 0.05).

MAA: Mean alveolar area.

LBF: light buckwheat flour; XG: Xanthan gum; CF: chia flour; WBF: wholegrain buckwheat flour; CP: control premix.



**Fig. 2.** Gluten-free bread slices and structure of the crumbs. Formulation: (1) LBF, (2) LBF:XG, (3) LBF:CF, (4) LBF:XG:CF, (5) WBF, (6) WBF:XG, (7) WBF:CF, (8) WBF:XG:CF, (9) control.

significantly lighter than that of the WBF-based loaves. The inclusion of XG did not cause a significant change to the lightness of the bread crust, whereas the addition of CF produced a darker color in the crust of the LBF-based breads. Parameter *a*<sup>\*</sup> was significantly higher for the control and most LBF-based formulations. The addition of XG and CF (individually or jointly) significantly decreased the *b*<sup>\*</sup> value of the crust, indicating a lower intensity of the yellow color.

No significant difference was observed in the alveolar area of the different gluten-free breads, with the alveolar area of the LBF-based breads being similar to that of the control (Table 3). The incorporation of 10 g of CF/100 g of LBF allowed obtaining loaves with circularity similar to the control. The LBF-based breads and the control also presented a significantly higher (p ≤ 0.05) gas percentage than those obtained with WBF, which could be related to the bran particles present in WBF that penetrate the gas cells causing leaks and breaking the gas bubbles, affecting the amount of air incorporated into the dough (Seyur & Gelinas, 2009). The greater air fraction present in the CB could also be related to its significantly higher SV, because a larger volume is associated with a higher gas percentage in the bread crumb (Arp et al., 2018). The combination of CF and XG produced more spongy and less brittle breads, with a better alveolar distribution. The alveoli of the WBF-based loaves were smaller, producing a more compact and drier crumb (Fig. 2).

The breads based on LBF and WBF exhibited significantly higher (p ≤ 0.05) hardness than the control. The incorporation of XG and CF increased significantly the hardness of the loaves, with this effect being

significantly lower when both ingredients were added jointly to the LBF-based breads. This behavior could be due to the lower SV of the bread formulated with added XG and CF, as there is a negative correlation between the SV of a loaf and its hardness (Steffolani et al., 2014). Also, the contribution of the CF fiber, could hinder the expansion of the gas cells. However, the bread formulations without XG presented a more friable texture, with sunken top, and they crumbled when sliced (Fig. 2); thus, the addition of this ingredient helps to reduce those effects, improving the overall appearance of the loaf.

On the other hand, the addition of CF, as well as the combination XG:CF, produced a significantly greater elasticity ( $p \leq 0.05$ ) than the addition of XG alone, both in LBF and WBF formulations. Elasticity was in general higher for the LBF-based breads, but lower than the control. The latter behavior was also observed for resilience. The addition of CF, and the combination XG:CF, also significantly increased chewiness, even higher than that of the control. This was probably due to the greater hardness of those samples. As for gumminess, a significant increase was observed when adding XG and CF, both for the LBF and WBF formulations, with the effect being the highest for the addition of CF and the combination XG:CF. This behavior could be attributed to the fact that the presence of hydrocolloids increases the WHC, thus affecting the gumminess of the bread products. As for cohesiveness, when XG and CF were added jointly this property increased significantly for both buckwheat flours, in all cases with the level being lower than that of the control, which could be associated with the declared ingredients of the CP favoring the inner strengths of the dough.

### 3.3. Selection of the premix formulation

Based on the properties determined for the breads prepared with different gluten-free premix formulations, the formulation 4 was selected (LBF:XG:CF) because it gave the best characteristics to the final product. LBF allowed to obtain loaves with higher lightness, greater tendency to yellowness, larger gas percentage, and a smaller alveolar area. The joint addition of XG and CF increased the moisture of the loaves and reduced the mass loss during baking, with a larger SV. The combination (XG:CF) also presented greater elasticity, gumminess and chewiness to the loaves, highlighting that the inclusion of XG improved the appearance of the breads, producing a more spongy crumb and a more homogeneous crust without cracks on the surface.

#### 3.3.1. Characterization of the selected gluten-free premix and bread

The selected premix (SP) exhibited significantly higher ( $p \leq 0.05$ ) moisture, protein, ash and crude fiber values than CP (Table 4), which can be associated with the significant protein, fiber and ash content of the flours of SP. However, the lipid and carbohydrate content were significantly higher for CP. Taking into account the first-mentioned component, this fact could be attributed to the presence of vegetable oils declared in its composition. The energy level of SP was also significantly lower with respect to CP. The caloric value of the proteins was 68.4 kcal/100g for SP, representing 17.35% of the total energy value, which is satisfactory according to the acceptable macronutrient distribution range (AMDR, 10–35%) (Vitali, Amidžić Klarić, & Dragojević, 2010). However, the percentage of contribution of the proteins of CP was lower than recommended. The contribution of carbohydrates to the total energy value was 292.2 Kcal/100g for SP, representing 74.11% of the total energy value, with this percentage being higher than the recommended AMDR (45–65%) but lower than that of CP (83.79%). In contrast, the contribution of fat to total energy was 33.66 and 49.95 Kcal/100g (SP and CP, respectively), which represent 8.54 and 11.90% of the total caloric value, with these values being lower than the recommended AMDR (25–35%). The latter behavior could be associated with the low oil content of the premixes.

Even though the lipid content of SP was low, its fatty acid composition is worth noting, which included as major components, in descending order, oleic, linoleic ( $\omega$ -6), linolenic ( $\omega$ -3), palmitic, and

**Table 4**

Physicochemical parameters and fatty acid composition of the selected gluten-free premix and bread.

	Premix		Bread	
	SP	CP	SB	CB
Proximate composition (g/100 g)				
Moisture	13.89 ± 0.04 <sup>b</sup>	12.71 ± 0.16 <sup>a</sup>	43.11 ± 1.13 <sup>b</sup>	37.93 ± 0.72 <sup>a</sup>
Protein (Factor = 6.25)	17.10 ± 0.16 <sup>b</sup>	4.52 ± 0.04 <sup>a</sup>	9.02 ± 0.02 <sup>b</sup>	5.33 ± 0.22 <sup>a</sup>
Crude fiber	3.35 ± 0.30 <sup>b</sup>	n.d. <sup>a</sup>	1.28 ± 0.004 <sup>b</sup>	n.d. <sup>a</sup>
Oil	3.74 ± 0.05 <sup>a</sup>	5.55 ± 0.04 <sup>b</sup>	1.08 ± 0.04 <sup>a</sup>	2.09 ± 0.01 <sup>b</sup>
Ash	2.76 ± 0.06 <sup>b</sup>	2.03 ± 0.03 <sup>a</sup>	2.13 ± 0.01 <sup>b</sup>	1.33 ± 0.01 <sup>a</sup>
Carbohydrate	73.05 ± 0.12 <sup>a</sup>	87.90 ± 0.04 <sup>b</sup>	86.49 ± 0.01 <sup>a</sup>	91.25 ± 0.23 <sup>b</sup>
Caloric value (kcal/100 g)	394.26 ± 0.19 <sup>a</sup>	419.63 ± 0.26 <sup>b</sup>	391.76 ± 0.20 <sup>a</sup>	405.13 ± 0.08 <sup>b</sup>
Color	Crumb			
$L^*$	80.32 ± 0.27 <sup>a</sup>	94.03 ± 0.13 <sup>b</sup>	50.62 ± 0.47 <sup>a</sup>	78.83 ± 1.18 <sup>b</sup>
$a^*$	1.35 ± 0.04 <sup>b</sup>	-0.15 ± 0.02 <sup>a</sup>	4.21 ± 0.002 <sup>b</sup>	-3.17 ± 0.21 <sup>a</sup>
$b^*$	7.89 ± 0.19 <sup>b</sup>	7.26 ± 0.03 <sup>a</sup>	12.84 ± 0.12 <sup>a</sup>	11.93 ± 1.03 <sup>a</sup>
Total antioxidant capacity	DPPH scavenging capacity (%)			
	69.43 ± 0.71 <sup>b</sup>	13.38 ± 0.69 <sup>a</sup>	21.04 ± 0.56 <sup>b</sup>	12.00 ± 0.18 <sup>a</sup>
Fatty acids (g/100 g of total fatty acids)	Palmitic acid (C16:0)			
	14.17 ± 0.18 <sup>a</sup>	19.22 ± 0.25 <sup>b</sup>	13.55 ± 0.13 <sup>a</sup>	20.16 ± 0.42 <sup>b</sup>
	Stearic acid (C18:0)			
	2.18 ± 0.08 <sup>a</sup>	15.06 ± 0.56 <sup>b</sup>	2.21 ± 0.03 <sup>a</sup>	15.13 ± 0.14 <sup>b</sup>
	Oleic acid (C18:1)			
	32.25 ± 0.04 <sup>a</sup>	56.31 ± 0.15 <sup>b</sup>	32.59 ± 0.14 <sup>a</sup>	56.62 ± 0.42 <sup>b</sup>
	Linoleic acid (C18:2n6)			
	32.16 ± 0.08 <sup>b</sup>	7.12 ± 0.12 <sup>a</sup>	32.83 ± 0.05 <sup>b</sup>	7.65 ± 0.18 <sup>a</sup>
	Linolenic acid (C18:3n3)			
	19.23 ± 0.15 <sup>b</sup>	0.52 ± 0.41 <sup>a</sup>	18.83 ± 0.35 <sup>b</sup>	0.44 ± 0.04 <sup>a</sup>

Mean values ± standard deviation (n = 3). Values followed by different letters in each row differ significantly ( $p \leq 0.05$ ) by premix and bread, respectively. SP: selected premix; CP: control premix; SB: selected bread; CB: control bread. n.d.: not detected.

stearic acids (Table 4). It should also be highlighted that approximately 51% of the fatty acids present in SP correspond to essential polyunsaturated fatty acids that provide many health benefits. This behavior is related to the large proportion of  $\omega$ -3 and  $\omega$ -6 fatty acids present in chia and buckwheat (Ixtaina et al., 2011; Tömösközi & Langó, 2017).

The AA of SP was significantly higher than that of CP, which could be related to the antioxidant compounds present in the flours of the premix (chlorogenic and caffeic acids, flavonols, myricetin, quercetin, kaempferol, rutin) in chia and buckwheat flours (Capitani et al., 2012; Tömösközi & Langó, 2017).

As for color, SP exhibited significantly lower lightness than CP showing positive values for coordinate  $a^*$  indicating a trend toward redness, while CP had negative  $a^*$  values, indicating a tendency toward greenness. On the other hand, coordinate  $b^*$  presented positive values for both premixes, indicating a tendency to yellow. The differences in lightness and the tendency to redness of SP could be attributed to the color of the CF present in this premix.

SP exhibited significantly higher ( $p \leq 0.05$ ) values for WHC, OHC, OMAC, WA<sub>b</sub>C, EA and ES than CP (Table 5). The higher OHC and OMAC could be related to the lower lipid content of SP, while the emulsifying properties could have been affected by its protein content, as well as by the presence of XG and CF (mucilage), for being stabilizing agents. The emulsifying properties of the proteins could have been affected by

**Table 5**

Functional properties of the selected gluten-free premix.

	SP	CP
Functional Property		
WHC (g/g)	2.59 ± 0.06 <sup>a</sup>	2.49 ± 0.05 <sup>a</sup>
OHC (g/g)	1.27 ± 0.03 <sup>b</sup>	0.98 ± 0.03 <sup>a</sup>
WA <sub>b</sub> C (g/g)	2.69 ± 0.01 <sup>b</sup>	1.89 ± 0.01 <sup>a</sup>
WA <sub>d</sub> C (g/g)	0.39 ± 0.02 <sup>a</sup>	0.47 ± 0.01 <sup>b</sup>
OMAC (g/g)	1.23 ± 0.01 <sup>b</sup>	0.64 ± 0.02 <sup>a</sup>
EA (mL/100 mL)	51.33 ± 0.54 <sup>b</sup>	29.89 ± 0.38 <sup>a</sup>
ES (mL/100 mL)	53.00 ± 0.38 <sup>b</sup>	16.71 ± 0.42 <sup>a</sup>

Mean values ± standard deviation (n = 3). Values followed by different letters in the same row differ significantly (p ≤ 0.05).

SP: selected premix; CP: control premix.

WHC: water-holding capacity; OHC: oil-holding capacity; WA<sub>b</sub>C: water absorption capacity; WA<sub>d</sub>C: water adsorption capacity; OMAC: organic molecule absorption capacity; EA: emulsifying activity, and ES: emulsion stability.

increasing temperature during the pressing of the seeds (Östbring, Malmqvist, Nilsson, Rosenlind, & Rayne, 2020). However, in this study chia flour was obtained by cold pressing.

The bread made with the selected premix (SB) presented a significantly higher (p ≤ 0.05) moisture, protein, ash and crude fiber content with respect to the control bread (CB), whereas its lipid and carbohydrate content was significantly lower (Table 4), which is consistent with the obtained results for the SP and CP. The moisture level of SB was similar to that reported by Pal and Kumari (2017) and Costantini et al. (2014), while the protein and carbohydrate contribution was higher than that observed by said authors. The caloric value of SB was significantly lower than CB, which is consistent with the values observed for the premixes (SP and CP, respectively). As SB was prepared with a premix consisting of raw materials (chia and buckwheat flours) rich in dietary fiber (Capitani et al., 2012; Costantini et al., 2014), and considering that the inclusion of dietary fiber in gluten-free breads presents different technological functions (increased water retention and viscosity) and health properties (increased feeling of satiety, helps reduce constipation) (El Khoury, Balfour-Ducharme, & Joye, 2018), it would be interesting to determine the dietary fiber of SB.

As for fatty acid profile, both SB and CB presented the same behavior of their premixes, with SB exhibiting a notable content of linoleic and linolenic acids. Also, SB presented higher AA than CB, which this property being lower in both loaves (SB and CB) than in the corresponding premixes (SP and CP), representing a loss of 30 and 12% for SB and CB, respectively. This could be associated with the baking process of the breads, due to the high temperatures reached during baking.

#### 4. Conclusions

Premixes based on buckwheat flour (light or whole grain) with the incorporation of chia flour (CF), xanthan gum (XG) or a combination of both were developed for the production of gluten-free bread. The combination of ingredients of the selected premix (light buckwheat flour with the addition of chia flour and xanthan gum) produced gluten-free breads with better nutritional characteristics (higher protein, and crude fiber content), higher antioxidant activity and a significant content of essential polyunsaturated fatty acids (linoleic and linolenic acids) compared to the control bread prepared with a commercial premix. This represents a promising use of a by-product of the cold-pressed chia oil extraction process (partially-deoiled flour) as a functional ingredient in the manufacture of gluten-free bread. In the light of these results, further studies are needed regarding the composition of the dietary fiber and the sensory acceptability of the selected bread.

#### CRedit authorship contribution statement

Estefania Belén Coronel: Investigation, Methodology, Data

curation, Formal analysis, Software, Writing - original draft. **Estefania Nancy Guioetto**: Methodology, Software, Formal analysis, Visualization. **María Cristina Aspiroz**: Methodology. **Mabel Cristina Tomás**: Writing - original draft, Visualization, Writing - review & editing, Funding acquisition, Project administration. **Susana María Nolasco**: Writing - original draft, Funding acquisition, Project administration, Writing - review & editing, Visualization. **Marianela Ivana Capitani**: Conceptualization, Supervision, Methodology, Data curation, Formal analysis, Software, Writing - original draft, Visualization, Writing - review & editing.

#### Declaration of competing interest

The authors declare no conflict of interest.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.lwt.2021.110916>.

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