

# Functional bread with n-3 alpha linolenic acid from whole chia (*Salvia hispanica* L.) flour

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**Abstract** This work proposed to study the effects of the addition of whole chia flour (WCF) on the technological, nutritional and sensory qualities of bread. Different WCF contents (0 and 20 %) and vital gluten (VG) (0 and 4 %) were added to bread according to a  $2^2$  central composite rotational design. WCF decreased the specific volume, lightness and hue angle of the bread loaves, but did not affect the chroma values. WCF and VG contributed to maintenance of the moisture content of the loaves during the storage period. The increased firmness found with the addition of high levels of WCF (more than 10 %) was countered by larger amounts of VG (more than 2 %). The optimum loaf (10 % WCF and 2 % VG) showed 26 % more lipids, 19 % more protein and 11 % more ash than the standard loaf (0 % WCF and 0 % VG). A better lipid profile was also found (higher omega-3 fatty acid content and a better omega-6/omega-3 ratio). Both breads were positively rated in the sensory profile analysis.

**Keywords** Polyunsaturated fatty acids · Response surface methodology · Vital gluten · Bread quality · Sensory analysis

## Introduction

Chia (*Salvia hispanica* L.) is an ancient seed that has been revived in recent years due to its nutritional value (Jiménez et al. 2010). For centuries it was consumed by the Aztecs who

considered it a basic dietary component. They milled raw, cooked or toasted seeds to produce a flour that they incorporated into tortillas and tamales (Sahagun 1982). The cultivation of chia originated in Central America, and it is an herbaceous plant of the *Lamiaceae* family. The plant is sensitive to daylight and produces small, white and dark seeds with an ellipsoid shape. The characteristic length, width and thickness dimensions are 2.11, 1.32 and 0.81 mm, respectively, for dark seeds and 2.15, 1.40 and 0.83 mm, respectively, for white seeds. The bulk density of chia seeds ranges from 0.667 to 0.722 g cm<sup>-3</sup>, and the porosity from 22.9 to 35.9 %. The volume of a single chia seed can range from 1.19 to 1.42 mm<sup>3</sup> and the mass of 1,000 chia seeds is approximately 1.3 g. Similar to many crops, the productivity of chia is sensitive to the weather and the planting date (Ixtaina et al. 2008). The evolution of the quality of chia is closely related to ageing of the plant. The chia plant provides forage with good nutritive value when harvested at a stage before the shooting period. After this stage, the nutritional quality of the plant decreases considerably with an increase in fibrous fractions and decrease in crude protein content (Peiretti and Gai 2009).

The fatty acid composition of chia seed oil may be of interest for use in healthy food and cosmetic applications, because it contains a large amount of polyunsaturated fatty acids (PUFAs). Chia seed lipids are composed of 30 % oil. In general, alpha-linolenic (C18:3 n-3, ALA) and linoleic acids (C18:2 n-6, LA) account for 60 and 20 %, respectively, of the total oil content, with small amounts of palmitic and stearic acids (Jiménez et al. 2010; Ixtaina et al. 2010). Chia seeds are an important source of dietary fibre (approximately 40 %) and they have nutritional and technological potential. The approximate ratio between insoluble and soluble fibre in chia seeds is 5. Thus insoluble dietary fibre is the predominant fraction with lignin as the main component, which accounts for 39–41 % of the total dietary fibre. Lignin contains important antioxidant compounds and in addition, due to its capacity to absorb bile

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acids, lignin is responsible for the hypocholesterolemic effect associated with fibre intake. Cellulose and hemicellulose are also present as components of dietary fibre (Reyes-Caudillo et al. 2008). Chia seeds also have a high protein content, which justifies the use of chia seed flour as a source of protein for animals and humans. The major protein fractions of chia seeds are alkali-soluble glutelins and prolamins. Chia seeds also have large amounts of glutamic acid (123 g kg<sup>-1</sup> crude protein), arginine (80.6 g kg<sup>-1</sup> crude protein) and aspartic acid (61.3 g kg<sup>-1</sup> crude protein). However, chia seeds are deficient in the essential amino acid lysine (Olivos-Lugo et al. 2010).

The nutritional benefits resulting from a combination of cereals with other seeds in the alimentary diet have been known for years. Due to their functional properties, seeds are a good option for balanced feeding, and nutritional specialists highlight the importance of incorporating seeds into meals. An example of this is the consumption of bread prepared with whole seeds (Aguilar et al. 2004). Bread is one of the most consumed food products known to humans, and for some it is the principal source of nutrition. Bread is an inexpensive source of energy containing carbohydrates, lipids and proteins, and is important as a source of essential vitamins of the B complex and of vitamin E, minerals and trace elements (Collado-Fernández 2003). Several kinds of flour are mixed with wheat flour in the baking industry, and these mixtures are called mixed or composite flours (El-Dash and Germani 1994). These mixtures are commonly used to increase the nutritional value of the bread but technological problems can exist. The gluten content is reduced when these mixtures are used, and new components, such as fibre and lipids, are introduced into the system. Thus, the objective of this study was to verify possible changes in the technological quality of bread due to the addition of different amounts of whole chia flour and vital gluten, according to a central composite rotational design. The best result was evaluated in relation to its nutritional and sensory qualities. The study aimed to obtain a product with good technological quality and sensory and nutritional profiles.

## Material and methods

### Material

The following raw materials were used: chia seeds (A. Sturla, Buenos Aires, Argentina), wheat flour NITA (Moinho Paulista Ltda., Santos, Brazil) and vital gluten (Meelunie, Amsterdam, Holland). Whole chia flour was obtained by milling chia seeds in a laboratory scale mill Quadrumat Senior (Brabender GmbH & Co. KG, Duisburg, Germany). The physicochemical characteristics of the wheat and chia flours (proximate composition and particle size range) were presented in Luna Pizarro et al. (2013).

### Methods

#### *Loaf preparation*

The following formulation was used to prepare the loaves: combination of wheat flour, whole chia flour and vital gluten (100 %), water (50–60 %), instant baker's yeast (2 %), salt (2 %), sugar (5 %), hydrogenated vegetable fat (6 %), egg (1 %), powdered milk (4 %) and bread improver (1 %). The amounts of whole chia flour (WCF) and vital gluten (VG) were established according to a complete 2<sup>2</sup> factorial experimental design (Table 1) with a total of 11 trials (Rodrigues and Iemma 2005). The amount of WCF added ranged between 0 and 20 % and that of VG between 0 and 4 %. The loaves were produced using the straight dough method. The bread formulation was mixed in an HAE 10 spiral mixer (Hypo, Ferraz de Vasconcelos, Brazil) for 15 min, the dough shaped into balls (400 g), moulded in a HM2 Hp moulder (Hypo, Ferraz de Vasconcelos, Brazil), put into baking pans and left to proof in the proofing chamber 20B (Klimaquip, Pouso Alegre, Brazil) at 32°C and 80 % relative humidity for 2 h. After proofing, the loaves were baked in an HF 4B open hearth oven (Hypo, Ferraz de Vasconcelos, Brazil) at 160°C for 35 min.

#### Bread analysis

The bread loaves were examined 24 h after baking. Three separate loaves were examined for each batch, and the results were averaged.

The apparent loaf volume was determined by rapeseed displacement using AACC Method n° 10–05.01 (AACC 2010), and loaf mass was determined using a semi-analytical scale. The specific volume was determined from the volume/mass ratio and expressed in mL/g.

The crumb colour of the loaves was evaluated by the tristimulus method followed by the CIE-L\*a\*b\* colour space method, which determined the lightness (L\*), chroma (C\*) and hue angle (h) values using a Colour Quest II HUNTERLAB (Minolta, Reston, USA) spectrophotometer. The test conditions were as follows: illuminant D65, visual angle of 10° and calibration with reflectance specular included (RSIN). This determination was made in the centre of the bread by extracting three central slices from each sample, and the evaluation was carried out in triplicate.

For the shelf life evaluation of the loaves, they were packed into polyethylene plastic bags and stored at room temperature (24 °C). The moisture content and firmness values of the loaves were evaluated after 1, 4 and 7 storage days. The moisture content of the bread loaves was determined in triplicate by AACC Method n° 44–15.02 (AACC 2010) and crumb firmness by AACC Method n° 74–09.01 (AACC 2010) using a TA-XT2i texture analyser (Stable Micro Systems, Surrey, UK) with a 25 kg capacity and XTRA

**Table 1** Specific volume and instrumental crumb colour of the loaves in relation to the additions of whole chia flour and vital gluten

Trials	WCF	VG	Specific volume (mL/g)	Crumb instrumental color		
				L*	C*	h
1	−1 (3)	−1 (0.6)	4.53±0.77 b	70.59±1.09	15.49±0.37	88.00±0.50
2	+1 (17)	−1 (0.6)	3.47±0.10 e	57.96±0.68	15.16±0.16	80.45±0.27
3	−1 (3)	+1 (3.4)	3.90±0.05 d	72.40±1.25	16.54±0.27	87.26±0.22
4	+1 (17)	+1 (3.4)	3.12±0.15 f	60.01±0.97	15.05±0.32	80.99±0.78
5	−1.41 (0)	0 (2)	5.25±0.01 a	72.93±1.13	15.50±0.62	90.34±0.39
6	+1.41 (20)	0 (2)	2.78±0.03 g	57.55±1.40	15.63±0.19	79.27±0.20
7	0 (10)	−1.41 (0)	3.61±0.07 e	66.71±0.45	14.46±0.29	83.86±0.34
8	0 (10)	+1.41 (4)	4.21±0.03 c	67.17±1.32	14.61±0.33	84.80±0.38
9	0 (10)	0 (2)	4.43±0.03 cb	68.05±1.27	14.70±0.16	84.14±0.34
10	0 (10)	0 (2)	4.41±0.15 cb	65.71±1.34	14.39±0.75	85.72±0.70
11	0 (10)	0 (2)	4.50±0.02 b	65.84±1.03	15.53±0.48	83.97±0.42

mean±standard deviation,  $n=3$ .

WCF=whole chia flour;

VG=vital gluten; L\*=lightness;

C\*=chroma and h=hue angle.

The values in brackets correspond to the amounts of whole chia flour and vital gluten incorporated

(in percentages). Means follow-

ed by the same superscript letter

in the same column are not sig-

nificantly different according to

Tukey's test ( $p<0.05$ )

Dimension program equipped with a P/35 mm aluminium cylindrical probe, which compressed the central area of two slices taken from the centre of each loaf. The bread loaves were sliced transversely using a slice regulator to obtain uniform slices with a thickness of 12.5 mm. The following parameters were used: pre-test speed of 4.0 mm/s, test speed of 1.0 mm/s, post-test speed of 5.0 mm/s and auto trigger of 20 g. Ten measurements were made per trial.

#### Nutritional and sensory characteristics of the loaves

The loaf with the best technological parameters was selected according to the results obtained in the technological analyses, and both the selected loaf (optimum chia loaf) and the standard loaf (without WCF and VG) were evaluated for their nutritional and sensory qualities. Since the objective of this study was to develop bread with the incorporation of WCF, the optimum chia loaf was selected according to the WCF content, colour values and the lowest firmness values. After analyzing the results of the experimental trials, the following formulation was selected: that produced with 10 % WCF and 2 % VG. A standard loaf was also produced with 0 % WCF and 0 % VG as described in the loaf preparation section.

The nutritional characterization of the loaves was determined from the proximate composition (protein, lipids and ash contents) according to AACC methods n° 46–13.01, 30–10.01 and 08–12.01 (AACC 2010), respectively. The carbohydrate content was calculated by difference. The fatty acid profile of the lipids extracted from the loaves was obtained according to AACC Method n° 44–15.02 (AACC 2010). The central slices were dried, milled and the lipids extracted as described in AOAC Method n° 922.06 (AOAC 2000). The fatty acid methyl esters (FAMES) were obtained according to Method UNE n° 55-037-73 (AENOR 1991) and their composition determined by capillary gas chromatography (CGC 6890 System Plus, Agilent Technologies, Mississauga,

Canada) connected to a flame ionisation detector (FID). A DB–225 J&W 122-2232- 50 % Cyanopropylphenyl-dimethylpolysiloxane capillary column (Agilent Technologies, Mississauga, Canada) with the following dimensions was used: 30 m long, 0.25 mm inner diameter and 0.25  $\mu$ m film. The analytical conditions were: injector temperature 220°C; detector temperature 220°C; oven temperature 60°C (1 min) programmed to increase to 210°C at a rate of 6°C/min and maintained at this temperature for a further 20 min; carrier gas: N<sub>2</sub>-UAP; and make-up gas: N<sub>2</sub>-UAP. Individual FAMES were identified using Sigma 189–1 Lipid Standards (Sigma Chemical Co. Ltd., Poole, UK) and the Supelco FAME-Mix C4-C24 18919–1 (Supelco Inc., Madrid, Spain). The results were expressed as the total fatty acid (TFA) content.

With respect to the sensory evaluation of the loaves, acceptance tests and purchase intention were carried out 1 day after baking. The samples were evaluated by 40 untrained panellists in isolated cabins under white light. The attributes of colour, flavour and texture were evaluated using a 9-point hedonic scale (Stone and Sidel 1993) where 1=dislike extremely and 9=like extremely. The purchase intention was measured on a five point scale where 5=“certainly would buy” and 1=“certainly would not buy”. The samples were served monadically in a randomized order. A positive purchase intention was calculated according to the percentage of panellists who attributed scores from 4 to 5.

#### Statistical analysis

The Statistica 7.0 statistical program (Statsoft Inc., Haslemere, USA) was used for the analysis of variance (ANOVA), and to calculate the regression coefficients ( $R^2$ ) to obtain the mathematical models and build the response surfaces. Whole chia flour (WCF) and vital gluten (VG) were used as the independent variables, and the specific volume, crumb colour,

moisture content and firmness during shelf life periods of 1, 4 and 7 storage days used as the dependent variables or responses. The differences between the average values for specific volume, moisture content and firmness during the storage period and the nutritional and sensory results of the loaves, were assessed by ANOVA and Tukey's test ( $p < 0.05$ ) using the same statistical program.

## Results and discussion

### Specific volume

The specific volumes obtained for the loaves from the experimental design varied from 2.78 to 5.65 mL/g (Table 1). The highest volume corresponded to trial 5 without the addition of chia, and the lowest value was attributed to trial 6 with the highest incorporation of whole chia flour (20 %). For this variable, a mathematical model could not be established as a function of the WCF and VG, since no linear or quadratic effect or effect of the interaction between the variables presented significance ( $p < 0.05$ ).

An analysis of the variation in specific volume between trials (1 vs. 2; 3 vs. 4; and 5 vs. 6) where the WCF content increased but the VG content remained constant, demonstrated that the specific volume decreased with increased incorporation of WCF. In this study, WCF contributed to a reduction in specific volume when the vital gluten content was fixed. The results obtained for specific volume in this study suggest that the chemical composition of WCF may interfere with the formation and aggregation of protein structures around the air bubbles inside the dough, thus contributing to the reduction in bread volume.

An analysis of the variation in specific volume between trials (1 vs. 3; 2 vs. 4; and 7 vs. 8) where the VG content increased but the WCF content remained constant showed that no conclusion could be obtained regarding the effect of vital

gluten. A comparison between trials 1 and 3 and between trials 2 and 4 showed that an increased vital gluten content (2.4 %) provided a reduction in specific volume. However, a comparison between trials 7 and 8, where the increase in vital gluten was greater (4 %), showed that this increase in vital gluten content provided an increase in specific volume. Brites et al. (2008) demonstrated that the gluten quantity was important in bread loaves prepared with large amounts of gluten-free cereals.

### Instrumental crumb colour

Table 1 shows the values obtained for the colour parameters for all the trials in the design: the lightness values ( $L^*$ ) ranged between 57.55 and 72.93; the chroma values ( $C^*$ ) between 14.39 and 16.54; and the hue angle values ( $h$ ) ranged between 79.27 and 90.34°. For the  $L^*$  and  $h$  parameters, mathematical models could be established as a function of the WCF and VG contents, but this was not possible for the  $C^*$  parameter. The chroma values were not significantly affected by the addition of WCF and VG, indicating that these ingredients did not interfere with the chroma values. Thus, independent of the amounts of WCF and VG added, the chroma values were within the interval of the mean value and its standard error.

The equations obtained for the parameters  $L^*$  and  $h$  (Table 2) show that they were not affected by the variation in VG. These colour parameters only depended on WCF, which contributed to a reduction in the two parameters. Moreover, the lowest and highest values for  $L^*$  and  $h$  were found in trials 6 and 5, respectively, where the WCF contents were the maximum and minimum ones, respectively (axial points of the experimental design).

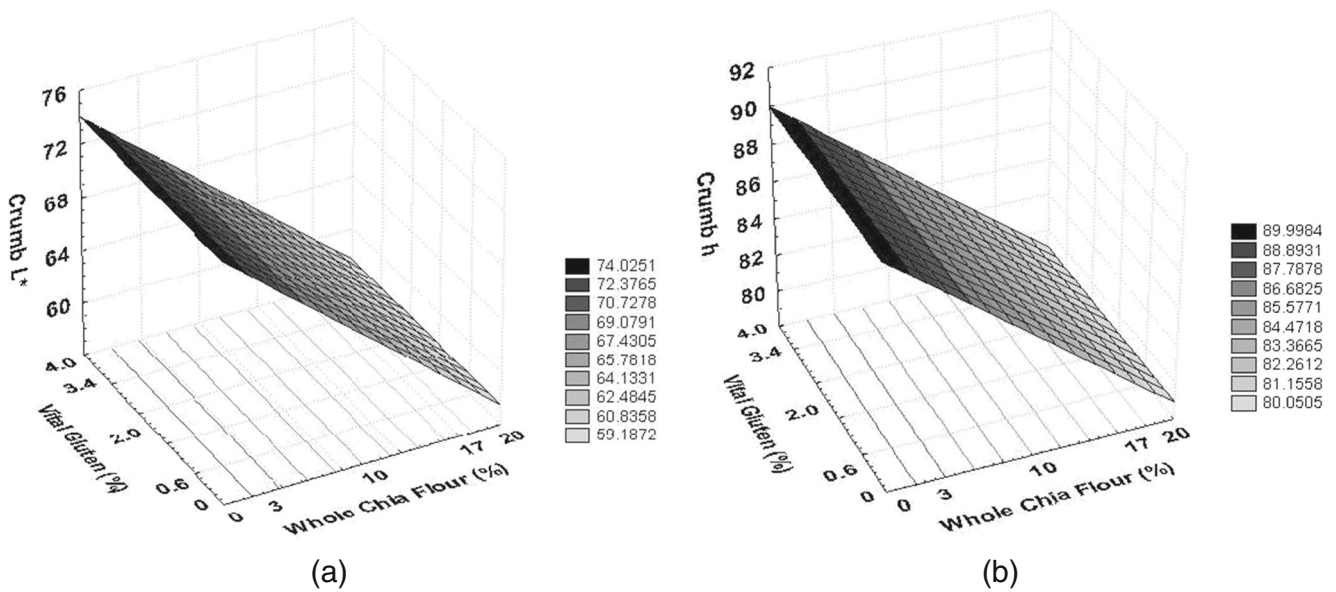
An increase in the incorporation of WCF from 0 to 20 % resulted in a decrease in  $L^*$  from 72.93 to 57.55 (Fig. 1). Higher values for  $L^*$  indicate greater light reflectance, which signifies a light coloured bread. The reduction of  $L^*$  resulted from the incorporation of WCF due to the colour of this raw material, which provided the bread with a different colour. An

**Table 2** Coded models for the quality parameters of the loaves as a function of the amounts of whole chia flour and vital gluten (coded values of the independent variables must be used)

Parameters	Coded model
Crumb $L^*$ =	$65.85 - 5.85 \text{ WCF}$ ( $r^2=0.9435$ ; $F_{\text{calc}}/F_{\text{tab}}=29.36$ )
Crumb $h$ =	$84.47 - 3.68 \text{ WCF}$ ( $r^2=0.9681$ ; $F_{\text{calc}}/F_{\text{tab}}=53.32$ )
Firmness on day 1 of storage =	$5.27 + 1.49 \text{ WCF} - 0.97 \text{ VG} - 0.91 \text{ WCF VG}$ ( $r^2=0.9023$ ; $F_{\text{calc}}/F_{\text{tab}}=4.94$ )
Firmness on day 4 of storage =	$7.03 + 2.05 \text{ WCF} + 0.62 \text{ WCF}^2 - 1.42 \text{ VG} + 0.82 \text{ VG}^2 - 1.80 \text{ WCF VG}$ ( $r^2=0.9774$ ; $F_{\text{calc}}/F_{\text{tab}}=8.58$ )
Firmness on day 7 of storage =	$9.77 + 2.41 \text{ WCF} + 1.09 \text{ WCF}^2 - 2.02 \text{ VG} - 2.31 \text{ WCF VG}$ ( $r^2=0.9178$ ; $F_{\text{calc}}/F_{\text{tab}}=3.69$ )

WCF=coded value ( $-\alpha$  to  $+\alpha$ ) of the amount of whole chia flour added; VG=coded value ( $-\alpha$  to  $+\alpha$ ) of the amount of vital gluten added;  $F_{\text{calc}}$ =calculated F;  $F_{\text{tab}}$ =tabled F





**Fig 1** Response surfaces for (a) crumb  $L^*$ , (b) crumb  $h$  of the loaves as a function of the additions of whole chia flour and vital gluten

increase in incorporation of WCF decreased the hue angle. With no WCF incorporation, the hue angle values were proximate to the axis+b/yellow ( $90.34^\circ$ ), but with higher WCF levels (20 %), the hue angle values were proximate to the axis+a/red ( $79.27^\circ$ ). Thus, the reduction in  $h$  demonstrated that the addition of WCF resulted in breads with a redder colour.

#### Moisture content

A predictable mathematical model for the moisture content on storage days 1, 4 and 7 could not be established. Table 3 shows

that, for the same storage day, the moisture content values were similar, indicating that neither WCF nor VG interfered with moisture content. Thus, the moisture contents were within the range of the mean value and its standard deviation, independent of the amounts of added WCF and VG.

During the period evaluated, there was no significant variation in the moisture content values in the formulations studied. Thus the bread loaves had a high moisture retention capacity and, consequently, no water loss. Sciarini et al. (2008) and Vasconcelos et al. (2006) reported that the moisture content did not vary during storage when the flours were mixed with soy, due to its fibre content and capacity to fix

**Table 3** Moisture and firmness of the loaves in relation to the amounts of whole chia flour and vital gluten added

Trials	WCF	VG	Moisture (%)			Firmness (N)		
			Day 1	Day 4	Day 7	Day 1	Day 4	Day 7
1	−1 (3)	−1 (0.6)	26.56±0.17 a	26.41±0.43 a	26.38±0.17 a	4.68±0.15 c	6.60±0.18 b	9.93±0.48 a
2	+1 (17)	−1 (0.6)	28.15±0.39 a	27.52±0.15 a	27.44±0.31 a	9.23±0.16 c	14.19±0.28 b	18.83±0.82 a
3	−1 (3)	+1 (3.4)	30.32±0.20 a	29.64±0.59 a	29.14±0.99 a	4.17±0.09 c	7.20±0.10 b	8.69±0.61 a
4	+1 (17)	+1 (3.4)	30.75±0.52 a	30.18±0.86 a	30.11±0.19 a	5.07±0.08 c	7.61±0.24 b	8.36±0.50 a
5	−1.41 (0)	0 (2)	26.94±0.43 a	26.48±0.06 a	26.37±0.10 a	2.98±0.10 c	4.88±0.20 b	7.57±0.52 a
6	+1.41 (20)	0 (2)	30.20±0.15 a	30.12±0.12 a	29.46±0.51 a	7.53±0.27 c	10.80±0.33 b	15.15±0.61 a
7	0 (10)	−1.41 (0)	28.53±0.23 a	28.48±0.11 a	27.32±0.25 b	6.30±0.12 c	10.14±0.38 b	11.17±0.89 a
8	0 (10)	+1.41 (4)	28.63±0.47 a	27.19±0.18 b	26.86±0.14 b	4.11±0.13 c	6.35±0.10 b	8.05±0.20 a
9	0 (10)	0 (2)	28.98±0.89 a	28.86±0.21 a	27.95±0.08 a	4.19±0.09 c	7.11±0.31 b	9.91±0.33 a
10	0 (10)	0 (2)	28.77±0.07 a	28.56±0.23 ba	28.22±0.11 b	4.65±0.19 c	6.93±0.20 b	9.39±0.27 a
11	0 (10)	0 (2)	28.05±0.09 a	27.38±0.59 a	27.47±0.16 a	4.72±0.06 c	7.05±0.28 b	9.48±0.36 a

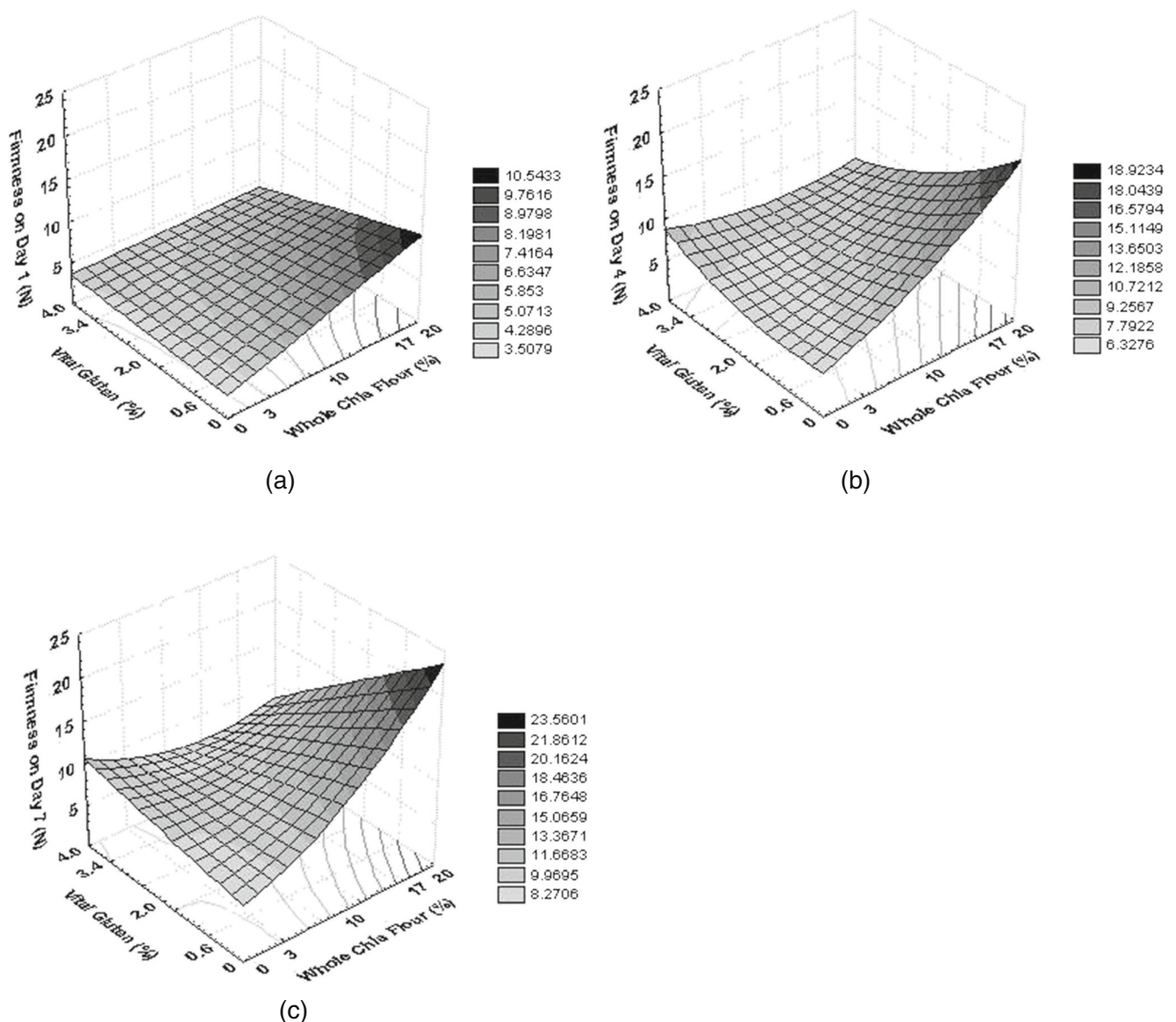
mean±standard deviation,  $n=3$  to 10. WCF=whole chia flour; VG=vital gluten. The values in brackets correspond to the amounts of whole chia flour and vital gluten incorporated (in percentages). Means followed by the same superscript letter in the same line for the same parameter evaluated are not significantly different according to Tukey's test ( $p<0.05$ )

water. In the present study, there was no water loss when WCF was added, due to its high level of fibre (31.51 %) (Luna Pizarro et al. 2013), but VG also resulted in no water loss, because in trial 5, which did not contain WCF, the moisture content was also maintained during storage.

### Firmness

Texture is an important indicator of bread quality, and its analysis is the parameter most used to evaluate the shelf life of bread during storage. The values obtained for crumb firmness are shown in Table 3. The mathematical models obtained (Table 2) demonstrated an interaction between the independent variables (WCF and VG). Moreover, the firmness was influenced by WCF and VG throughout the storage period

evaluated. From the models obtained (Table 2), it was possible to construct the response surfaces for the variable of firmness on storage days 1, 4 and 7 (Fig. 2). Of all the storage periods evaluated, the highest firmness values were obtained with the higher WCF contents (greater than 10 %) and lower VG contents (less than 2 %). Similar results were observed for mixed wheat, rice, maize and soy flours, which resulted in increases in crumb texture when the percentage of mixed flours increased, as a consequence of dilution of the gluten content by the flours (Sabanis and Tzia 2009). The lowest gluten content in the dough resulting from to the largest addition of WCF, weakened the structure that entraps the gas produced by the yeast, resulted in bread with a lower specific volume. Consequently, a more compact crumb was obtained, which was reflected by a greater firmness values. Trials 2 and



**Fig 2** Response surfaces for firmness on (a) day 1, (b) day 4 and (c) day 7 of loaf storage as a function of the additions of whole chia flour and vital gluten

6, which contained the highest levels of WCF and lowest levels of VG, had the firmest crumbs throughout the storage period. Trial 5, which did not contain WCF, was the softest bread.

Greater firmness resulting from higher levels of WCF (greater than 10 %) was countered by a greater addition of VG (greater than 2 %). A higher level of VG countered dilution of the wheat flour gluten by the WCF. The bread structure, as related to the amount of gluten, was the main factor determining the values obtained for bread firmness. Since WCF contains a high lipid content (30.97 %) (Luna Pizarro et al. 2013), the addition of WCF may provide several benefits to the bread texture which cannot be observed. The high fat content of WCF may promote a volume increase acting as a gas retainer, which softens the texture as the fat layers and gluten network are intermingled. Thus, the high fat content may improve product conservation by a better interaction of fat and starch, hampering recrystallisation between the chains and granting an age-diminishing effect to the product.

To compare the different storage periods, the average values were analysed by Tukey's test. Table 3 shows that crumb firmness increased with increase in storage time. The firmness values showed the following variations during storage: from 2.98 to 9.23 N on the first day; from 4.88 to 14.19 N on the fourth day; and from 7.57 to 18.83 N on the seventh day. Since the moisture content can influence texture, this result was not expected, because the moisture content of the breads did not vary during the storage period evaluated. Bread staling is related to starch retrogradation, gluten proteins, gluten-starch interactions and water loss (Lai and Lin 2006), but the latter was not observed in the present study. An increase in firmness was found without a water loss.

#### Nutritional and sensory characteristics

The incorporation of WCF in the bread formulation improved the nutritional value of the product (Table 4). The optimum chia loaf presented significant increases in the protein (19 %), lipids (26 %) and ash (11 %) contents as compared to the standard loaf. This increase may be due to the high contents of these nutrients in the WCF (Luna Pizarro et al. 2013).

In relation to the lipids, it is important to emphasize the improvement in the fatty acid profile of the optimum chia loaf formulation (Table 4), which presented a decrease in total saturated fatty acids (14 %) and mono unsaturated acids (16 %) and an increase in polyunsaturated fatty acid (60 %). The decrease of saturated and mono unsaturated fatty acids was due to the decrease in the fatty acids C14:0; C18:0; C22:0; C16:1; C18:1 and C20:1. The increase in polyunsaturated fatty acids was mainly due to the increase in the alpha-linolenic acid content (2776 %), which makes the optimum chia loaf a source of  $\omega$ -3 fatty acid. The alpha-linoleic acid

**Table 4** Nutritional and sensory characterization of standard loaf and optimum chia loaf

	Standard loaf	Optimum chia loaf
Centesimal composition (%)		
Moisture	29.72±0.23 a	28.95±0.16 b
Protein	11.24±0.09 b	13.32±0.17 a
Lipids	6.74±0.06 b	8.48±0.04 a
Ash	2.48±0.04 b	2.76±0.04 a
Carbohydrates	49.82±0.26 a	46.49±0.34 b
Fatty acid profile (%)		
C 6:0	0.03±0.01 a	0.03±0.01 a
C 8:0	0.38±0.02 b	0.42±0.01 a
C 10:0	0.09±0.01 b	0.12±0.01 a
C 12:0	1.13±0.02 a	1.22±0.06 a
C 14:0	1.00±0.02 a	0.89±0.01 b
C 16:0	22.52±0.09 a	19.08±0.08 b
C 18:0	10.45±0.02 a	9.00±0.09 b
C 20:0	0.37±0.04 a	0.35±0.05 a
C 22:0	0.28±0.01 a	0.09±0.01 b
Total SFA	36.26±0.01 a	31.22±0.01 b
C 16:1	0.39±0.06 a	0.12±0.01 b
C 18:1	43.36±0.06 a	36.89±0.12 b
C 20:1	0.29±0.01 a	0.12±0.06 b
Total MUFA	44.03±0.01 a	37.12±0.19 b
C 18:2 $\omega$ -6	19.26±0.02 a	18.72±0.07 b
C 18:3 $\omega$ -3	0.45±0.03 b	12.94±0.13 a
Total PUFA	19.71±0.01 b	31.66±0.19 a
SFA : MUFA : PUFA Ratio	1 : 1.21 : 0.54	1 : 1.19 : 1.01
PUFA/SFA	0.54±0.01 b	1.01±0.01 a
$\omega$ -6/ $\omega$ -3	42.68±0.41 a	1.45±0.01 b
Sensory acceptance <sup>1</sup>		
Colour	8.0±0.7 a	6.5±1.4 b
Flavour	7.7±1.2 a	7.0±1.4 a
Taste	7.9±0.8 a	7.5±1.2 a
Texture	7.8±1.2 a	7.7±1.0 a
Purchase intention <sup>2</sup>		
Positive purchase intention (%) <sup>3</sup>	97	83

mean±standard deviation. Centesimal composition and fatty acid profile ( $n=3$ ); sensory evaluation ( $n=40$ ). Means followed by the same letter in the same line, did not differ according to Tukey's test ( $p<0.05$ ). SFA=saturated fatty acids, MUFA=mono unsaturated fatty acids, PUFA=polyunsaturated fatty acids

<sup>1</sup> Hedonic scale ranging from 1="disliked extremely" to 9="liked extremely"

<sup>2</sup> Hedonic scale ranging from 1="would certainly not buy" to 5="would certainly buy"

<sup>3</sup> Panelist who attributed scores from 4 to 5 (in a scale from 1="would certainly not buy" to 5="would certainly buy") were considered

content decreased and hence an excellent omega-6/omega-3 ratio was observed in the optimum chia loaf formulation (1.45/1), which was not found in the standard loaf.

Simopoulos and Cleland (2003) recommended an omega-6/omega-3 ratio of 4:1 or less. On the other hand, a high ratio of omega-6/omega-3 is detrimental to health and may lead to the development of chronic diseases. Improving the dietary ratio by increasing the omega-3 fatty acids is essential for brain function and for the management of cardiovascular disease, arthritis and cancer.

The loaf produced with the addition of WCF showed good sensory acceptance. Although it presented lower scores for the attribute of colour acceptance in relation to the standard loaf, the scores for flavour, taste and texture were similar for both samples. In general, the loaves were well accepted by consumers, with scores between 6 and 8 (“liked slightly” to “liked a lot”) for the sensory attributes studied. The results for purchase intention varied between “certainly buy” to “maybe not buy” for the chia loaf and between “certainly buy” to “maybe buy, maybe not buy” for the standard loaf. Both formulations showed no rejection of purchasing (“certainly not buy”). More than 83 % of the panellists would possibly or certainly buy the chia loaf, representing a positive purchase intention. No statistical difference was observed in positive purchase intention between the formulations.

## Conclusion

This study showed that it is possible to produce bread with better nutritional characteristics by the addition of whole chia flour (WCF) to the formulation. Higher lipid, protein and ash contents and better lipid profiles were found in the bread produced with 10 % WCF, when compared to the standard loaf. An incredible amount of n-3 alpha linolenic was observed as well a better omega-6/omega-3 ratio. The use of WCF contributed to a reduction in specific volume, lightness and hue angle of the bread crumb. Moreover, incorporation of WCF weakened the bread dough protein network, resulting in a denser product. However, potential technological problems could be circumvented by the addition of VG (more than 2 %).

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