Verdú Amat, S.; Vasquez, F.; Ivorra Martínez, E.; Sánchez Salmerón, AJ.; Barat Baviera, JM.; Grau Meló, R. (2015). Physicochemical effects of chia (Salvia Hispanica) seed flour on each wheat bread-making process phase and product storage. Journal of Cereal Science. 65:67-73. doi:10.1016/j.jcs.2015.05.011.

Physicochemical effects of chia (Salvia Hispanica) seed flour on each wheat bread-making process phase and product storage.

Samuel Verdú[1]*, Francisco Vásquez [3], Eugenio Ivorra[2], Antonio J. Sánchez[2], Jose M. Barat[1], Raúl Grau[1]

[1]Departamento de Tecnología de Alimentos. Universidad Politècnica de València, Spain.

[2]Departamento de Ingeniería de Sistemas y Automática, Universidad Politècnica de València, Spain

[3]Departamento de Tecnología de Alimentos de Origen Vegetal. Centro de Investigación en Alimentación y Desarrollo A,C, Hermosillo, Sonora, Mexico.

*Author for correspondence: Samuel Verdú
Address: Edificio 8G - Acceso F - Planta0
Ciudad Politécnica de la Innovación
Universidad Politécnica de Valencia
Camino de Vera, s/n
46022 VALENCIA – SPAIN
E-mail: saveram@upvnet.upv.es
Phone: +34 646264839

Abstract

Some chia seed flour effects relating to different bread-making process phases and variables were studied by distinct image analysis and physicochemical techniques. Wheat flours with three different degrees of substitution (5%, 10% and 15%) were tested. In technological terms, the aim was to study the influence and properties of chia flour on several relevant parameters, such as pasting properties, growth kinetics and internal crumb structure during dough fermentation; and baking process, mass loss, water activity and texture profile of the end product during its storage. Some changes in pasting properties were observed. The effects obtained by image analysis techniques proved that addition of chia improved gas retention in dough and cut the time required to reach maximum dough development. A delay in hardness and water loss during storage of breads was also observed. Bread presented reduced water activity, and contained the same amount of moisture compared with the control. The mucilage provided by chia has properties that can explain these observed effects given the influence on water-holding capacity and its interactions with gluten proteins throughout the gluten matrix-forming process.

Keywords: Chia, bread-making process, image analysis, wheat flour

1. Introduction

Some relevant factors affect productivity in the cereal by-product industry due to changes between the properties and the behavior of the flour sort and other ingredients used in production phases. Flour features, such as flour origin and quality, cultivation and milling method, and cereal variety are some of the most influential factors. Variability conferred by raw materials directly affects the process and product variables (Cocchi et al. 2005), which are necessarily controlled to obtain high yields and homogeneous characteristics in both the production chain and product quality. Some of the most affected properties derive from the rheology of dough and batters, such as gas retention capacity, water interactions and flow behaviors. How different strategies act with these factors has been studied to solve the problems that appear in some phases of industrial processes (Ahlborn et al. 2005). Work has been done mainly in those cases in which flours present defects or low technological quality to reach the properties of reference products, such as wheat flour with a small amount of gluten, flours without gluten for gluten-free products, modifying properties during storage, etc. (Lazaridou et al. 2007). In relation to the above objectives, one of the most studied areas is the development of new ingredients, among which, several of the most important ones are compounds that are situated in any plant part, like grains, which improve gas retention in the dough matrix and water activity modification, among others (Houben et al. 2012). The nature of those compounds is diverse, but the most influential ones can form hydrocolloid structures. Hydrocolloids can induce some structural changes in the main flour components from the bread-making process to product storage. So they are usually added to dough to improve its viscoelastic properties, as well as the structure, mouthfeel, acceptability and shelf life of bakery products (Ahlborn, et al. 2005). Hydrocolloids are used either alone or combined to achieve specific synergies between their respective functional properties. Numerous types of hydrocolloids are obtained from the flour of whole grains and have been analyzed in this approach to take advantage of the other active compounds and nutrients in these products (Del Rio et al. 2013). One grain with interesting properties for this area is chia seeds (*Salvia hispanica*). Chia is an annual herb of the *Labiatae* family and was one of the basic nourishments of Central American civilizations in pre-Columbian times (Ayerza and Coates, 2005). Some studies have shown the potential uses of chia based on its compositional profile (defatted chia seeds have 22% of fiber and 17% of protein). These contents are similar to those of other oilseeds currently used in the food industry (Cumbyet al. 2008; Vázquez-Ovando et al., 2010). The consumption of chia provides numerous health benefits, such as a high content of oil, protein and bio-active peptides, antioxidants, minerals and dietary fiber (Ixtaina et al. 2008). Some works have been published with information about dough behavior and organoleptic acceptance, with degrees of substitution between 4-5% with both chia by-products and whole seed flour (Iglesias & Haros, 2013; Moreira et al., 2013). This percentage of substitution is based on the daily bread intake recommended by the World Health Organization, which would result in considerably improved nutrient contribution if all bread consumed contained 5% of chia. Those results report interesting technological properties apart from nutritional improvement. The effect of high substitution degrees could be interesting from a technological application point of view for the development of new formulas based on gluten-free flours, and for possible improvements to different bread-making process phases. From this technological viewpoint, the most important component of these seeds is fiber content, which includes a polysaccharide gum with high-molecular-weight mucilage. It has been proposed that the structure of the basic mucilage unit is a tetrasaccharide with 4-O-methyl-a-D-glucoronopyranosyl residues which occurs in branches of β-D-xylopyranosyl in the main chain (Lin et al. 1994). This compound presents a high water-holding capacity and forms an active hydrocolloid. The work of Iglesias and Haros, 2013 observed how this compound could improve the dough volume rate due to the formation of hydrophilic complexes between their ionic groups and proteins as gluten, which favors gluten matrix formation. For all these reasons, chia seeds present interesting features for testing some aspects of the bread-making process, and to evaluate their plausible applications in order to improve some of these aspects. Therefore, the purpose of the present study was to report further information about how substituting wheat flour for chia seed flour at the 5%, 10% and 15% degrees affects some important technological features; e.g., pasting properties of flour mixtures; dough fermentation process; baking loss; physicochemical properties (texture profile, mass loss and water activity) during storage of the end product for 0, 1, 3 and 7 days.

2. Material and Methods

2.1. Raw materials and dough preparation

The commercial wheat flour used was obtained from a local producer (Molí del Picó-Harinas Segura S.L. Valencia, Spain) whose chemical composition was: 14.7±0.6% of proteins, 1.1±0.03% of fat, 14.5±0.5% of water, and 0.32±0.1 of ash (w.b). The alveographic parameters were also facilitated by the company, which were P = 94±2 (maximum pressure (mm)), L = 128±5 (extensibility (mm)), W = 392±11 (strength (J^{-4})) and 0.73 of P/L. Chia flour was obtained from a commercial black chia seed format (BIOCESTA S.L., Valencia Spain) by milling in a stainless steel grinder (Retsch GmbH, ZM 200, Haan, Germany) to obtain a particle size distribution as close to the used wheat flour as possible. The particle size of flours was measured 6 times by laser scattering in a Mastersizer 2000 (Malvern, Instruments, UK) equipped with a Scirocco dry powder unit. The results are expressed as a maximum size in µm at 10%, 50% and 90% (*d (0.1)*, *d (0.5)* and *d (0.9)*, respectively) of the total volume of the analyzed particles as their averages (*D [4, 3]*). The wheat flour results were *d (0.1)* = 25.5±1.1, *d (0.5)* = 92.0±0.6, *d (0.9)* = 180.6±0.8 and D *[4, 3]* = 99.4±1.2. The chia flour results were *d (0.1)* = 23.9±1.9, *d (0.5)* = 95.3±1.2, *d (0.9)* = 181.9±1.3 and *D [4, 3]* = 100.1±1.1. Chia flour contained 2±1% of proteins, 30.4±0.9% of fat, 8±0.3% of moisture and 4.1±0.8% of ash (w.b). Four flour mixtures, which presented 0%, 5%, 10% and 15% wheat flour substitution degrees for chia flour, were prepared to carry out the study.

The control formulation used to prepare dough was the following: 56% flour, 2% refined sunflower oil (maximum acidity 0.2º Koipesol Semillas, S.L., Spain), 2% commercial pressed yeast (*Saccharomyces cerevisiae*, Lesafre Ibérica, S. A., Spain), 4% white sugar (≥ 99.8% of saccharose, Azucarera Ebro, S.L., Spain), 1.5% salt (refined marine salt ≥ 97% NaCl, Salinera Española. S.A., Spain) and water 34.5%. This formulation slightly increased the amount of water, following degree of substitution, to 1% in the case of 15% mixture, due to the low moisture of chia to maintain a constant ratio between moisture (provided by wheat, chia and the added water) and dry matter (provided by wheat and chia), observed in the control sample (0.89 g water/g dry matter). Bread dough was made according to a closed process with no modifications between mixes to observe the effect on a possible continuous industrial process. The process was carried out by mixing all the ingredients in a food mixer (Thermomix® TM31, Vorwerk, Germany) according to the following method: in the first phase, liquid components (water and oil), sugar and NaCl were mixed for 4 minutes at 37 ºC. Pressed yeast was added in the next phase to be mixed at the same temperature for 30 seconds. Finally, flour was added and mixed with the other ingredients according to a default bread dough mixing program, which provides homogeneous dough. The program system centers on mixing ingredients with random turns of the mixer helix in both directions (550 revolutions/minute) to obtain homogeneous dough. This process was applied for 4.5 minutes at 37 ºC. Then 450 g of dough were placed in the metal mold (8x8x30cm) for fermentation. Height was approximately 1 cm.

2.2. Characterization of flour mixing dough by a viscometer Rapid Viscosity Analyser (RVA)

The pasting properties of the flour mixtures were analysed using the viscosity profile obtained by the viscometer RVA (Rapid Visco Analyser Super 4, Newport Scientific). For this purpose, the method approved by AACC (America Association of Cereal Chemists), whose reference is "General Pasting Method for Wheat or Rye Flour of Starch Using the Rapid Visco Analyser. AACC 2000, number: 76-21"), was used. Samples of 3 g±0.01g were weighed and the amount of water incorporated was 25 g±0.01g. The test started at 50 °C and 960 RPM, and was slowed down to 160 RPM at 10 s. Temperature was maintained during the first minute. The temperature from 50 °C to 95 °C was increase during the next 4 minutes to reach 95 °C at minute 5 in a second step. The third step involved maintaining a temperature of 95 °C until minute 7.5. The fourth step was to lower the temperature to 50 °C, which was reached at minute 11. The last step was to maintain a temperature of 50 °C until minute 13. Measurements were taken in triplicate.

2.3. Fermentation process and its continuous monitoring

The fermentation process was carried out in a chamber with controlled humidity and temperature (KBF720 Binder Tuttlingen, Germany). The conditions were 37 °C and 90% of Relative Humidity (RH). Dough growth evolution was monitored by a device installed and calibrated inside the chamber. This device was based on an image analysis of Structured Light (SL), following the method described previously by Ivorra et al. (2014). Only the points between mold edges were analyzed by this method. During the process, one picture per second was captured until the required analysis time according to the phase study. The 3D information calculated from each image during the process focused on maximum height (*H*: the maximum Z edge value) and transversal area (*A*: the integration of the Z-values along the X direction of the sample). Data acquisition and data processing were carried out using an own code developed in the Matlab computational environment (The Mathworks. Natick. Massachusetts, USA). The behavior of the dataset average during fermentation was modeled by the SL method, obtained from dough samples per mixture and adapted to the Gompertz prediction model. The Gompertz function is a nonlinear sigmoid growth function which was developed by Gompertz (1825) to calculate the mortality rate of microorganisms. The equation is as follows:

$$t = A \cdot \exp((-\exp\,(\frac{V}{A} \cdot (Lt - t) + 1\,)) \qquad (1)$$

where t is time, A is area during the process, V is the maximum growth rate, and Lt represents the latency time before dough development begins. Model parameters were determined by a nonlinear regression procedure and were obtained by minimizing the sum of squares from prediction errors.

2.4. Study of internal dough structure in the fermentation phase

In order to study the chia effect on crumb evolution during the process, dough fermentation was interrupted at 25%, 50%, 75% and 100% compared to its maximum developing time (*Ft*). Thus the study of maximum fermentation development was firstly analyzed (until dough depletion). In each sample, dough was baked and sliced to be studied by 2D image segmentation. The baking process took place in an oven (Rotisserie, DeLonghi, Italy) at 180ºC for 30 minutes. Once baking finished, six 1 cm-thick slices were obtained from the center of the bread. In order to study crumbs, both sides of each slice were captured in a scanner (Aficio™ MP C300-Ricoh. Tokyo, Japan) to be then analyzed by 2D image segmentation (Verdú et al., 2014; Datta et al. 2007; Esteller et al. 2006). Three bread products of each mixture and fermentation time were examined, which meant that 36 crumb images were obtained. Images were acquired at a resolution of 300 dpi. A black background was used in all the captures to improve contrast and to enhance both cell wall structure and porosity measurements.

2.5. Study of the end product

To study the effect of chia on several of the most relevant bread properties, various assays were carried out at different times before and after packing to test their evolution. Baking loss (ΔM_b) was first determined. In this phase, mass loss during the baking process was concluded by the difference between the pre-baking dough weight and the finished bread weight (both bread products were cooled at room temperature for 1 h). Then samples were packed in a low-density polyethylene bag, similar to that used in commercial presentations, and were stored under environmental conditions (23 ºC and 72% R.H. approximately). The assay was carried out for 1, 3 and 7 days of storage. Mass loss of whole breads, the texture profile analysis (*TPA*) and water activity (a_w) of crumbs were performed for each time.

Mass loss (ΔM_t) was analyzed by paying attention to the weight at the beginning and at each sampling time. *TPA* was performed following the method used by Miñarro et al. (2012), where two 12.5 mm-thick cross-sectional slices were obtained from the center of each bread product. The texture profile analysis was carried out in a TA-TX2 texture analyzer (Stable Micro Systems, Surrey, UK). A 25kg load cell (35 mm diameter) was used. The assay speed was set at 1.7 mm/s to compress the bread crumb center to 50% of its previous height. The time between compressions was 5 s. The studied parameters were: hardness (*D*), springiness (*S*), cohesiveness (*C*), gumminess (*G*), chewiness (*Ch*) and resilience (*R*). Water activity of crumbs (a_w) was determined in an Aqualab® dew point hygrometer (DECAGÓN Aqualab CX-2, Pullman, WA, USA).

2.6 Statistical analysis

The results of pasting properties, fermentation parameters (*A, V, Ft*), bubble size and baking loss were studied by a one-way variance study (ANOVA). A multifactor ANOVA was used to study the main effects and interactions on the evolution of the parameters studied during storage (texture, mass loss and water activity). In those cases where the effect was significant (P-value < 0.05), the average was compared by Fisher's least significant difference (LSD).

3. Results and Discussion

3.1. Characterization of flour mixtures by Rapid Viscosity Analysis

The results of the pasting properties obtained by RVA and the profiles of the different mixtures samples are shown in Fig. 1. The corresponding pasting parameters are summarized in Table 1. The pasting times of the samples lowered due to degree of chia substitution, and significant differences were observed for the 10% and 15% substitution mixtures. Peak viscosity presented an inverse behavior and became higher with increasing chia substitution, where the 10% and 15% mixtures continued to show significant differences compared to the control and 5% mixture despite the peak time presenting this inverse behavior. Trough, breakdown and setback also showed a clear increment with degree of substitution.

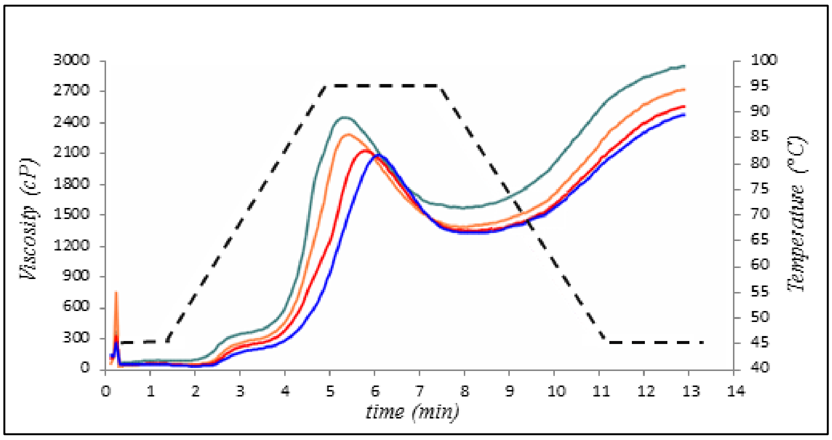

Figure 1. Pasting profile of control wheat flour —; 5% —; 10% —; 15% —, temperature — —

Initially, reduced water availability due to the presence of chia compounds should make starch gelatinization difficult. Thus the pasting temperature should present the opposite behavior to that observed in the results. One possible explanation is that chia mucilage, in combination with water and heat, produces increased viscosity at a lower temperature compared to starch. Therefore, the increase in viscosity at the beginning of the assay, at the 10% and 15% degrees of substitution could be attributed more to mucilage hydration than to starch gelatinization.

Table 1. Pasting characteristics of different mixtures

Pasting time	*Peak viscosity*	*Trough*	*Breakdown*	*Final visc*	*Setback*	*Peak time*
68.5 ± 0.6 c	2245 ± 15 a	1421 ± 49 a	823 ± 10 a	2660 ± 56 a	1238 ± 10 a	6.1 ± 0.1 c
67.7 ± 0.1 c	2289 ± 98 a	1430 ± 14 a	859 ± 12 a	2738 ± 12 a	1308 ± 21 b	5.8 ± 0.1 b
66.1 ± 0.1 b	2472 ± 26 b	1530 ± 65 b	942 ± 4 b	2965 ± 58 b	1435 ± 70 c	5.5 ± 0.2 a
63.9 ± 0.6 a	2639 ± 91 c	1673 ± 10 c	966 ± 19 b	3173 ± 49 c	1500 ± 15 d	5.3 ± 0.1 a

t letter within columns means significant differences at $p \leq 0.05$

The peak time results were also affected for the same reason, which lowered. This indicates a higher degree of substitution. Yet despite the reduction in peak time, peak viscosity increased with degree of substitution. These behaviors proved that chia components mainly affected the viscosity of mixtures, independently of wheat, because the degree of substitution incremented the viscosity level. Similarly, final viscosity was another parameter that showed considerable changes. This implies that the changes in pasting properties observed in the behaviors of the mixtures could be produced by chia seed mucilage. This component has a high water-holding capacity and hydration features (Inglett et al. 2014) and this phenomenon had no major influence on starch granule gelatinization. However, the rapid formation of hydrocolloids, when they came into contact with water, was the main factor responsible for the variations in the viscosity parameters observed.

3.2. Fermentation process

Figure 2A shows the results obtained by the Gompertz fitting model of fermentation curves generated by a 3D device. The parameters obtained from each mixture are presented in Table 2. Maximum dough growth, represented by parameter *A*, did not present significant differences among samples, except for the 15% mixture, which was significantly higher than the rest.

Table 2. Results of fermentation process characterization

		Control	***5%***	***10%***	***15%***
Curve parameters	A ($m^2.10^{-4}$)	12.9 ± 1.1 a	13.1 ± 1.2 a	13.5 ± 1.5 a	15.8 ± 0.9 b
	V ($m^2.10^{-4}$/min)	32.9 ± 3.5 a	45.4 ± 4.1 b	47.5 ± 2.4 b	50.9 ± 1.3 c
	Lt (min)	0.55 ± 0.1 a	1.62 ± 0.1 c	1.70 ± 0.2 c	1.10 ± 0.1 b
	Ft (min)	81 ± 3.2 b	77 ± 2.1 b	68 ± 3.1 a	65 ± 2.9 a
Bubble size ($m^2.10^{-6}$)	T1 (25%)	1.1 ± 1.0 b	1.2 ± 1.3 b	1.8 ± 1.9 b	0.8 ± 0.9 a
	T2 (50%)	3.7 ± 1.2 b	3.4 ± 1.4 b	5.0 ± 1.9 b	3.4 ± 1.0 a
	T3 (75%)	4.1 ± 2.3 a	4.2 ± 2.0 a	7.1 ± 2.2 b	5.3 ± 1.4 a
	T4 (100%)	4.9 ± 1.9 a	5.2 ± 2.0 a	7.7 ± 2.5 a	7.2 ± 1.7 a
Nº of bubbles/$m^2.10^{-4}$	T1 (25%)	13.4 ± 1.0 a	13.2 ± 1.3 a	14.4 ± 1.9 b	13.0 ± 0.9 a
	T2 (50%)	5.9 ± 1.2 a	8.2 ± 1.4 b	7.4 ± 1.9 b	10.5 ± 1.0 c
	T3 (75%)	6.4 ± 2.3 b	6.9 ± 2.0 b	5.1 ± 2.2 a	7.5 ± 1.4 b
	T4 (100%)	7.4 ± 1.9 a	7.5 ± 2.0 a	6.7 ± 2.5 a	7.5 ± 1.7 a
Time parameters comparing to control	tA_c (min)	79.2	60.8	52.8	44.1
	ΔtA_c (%)	-	23.2	33.3	44.3

Different letters within rows mean significant differences at $p \leq 0.05$

Velocity features (*V*) were similar in the 5% and 10% mixtures. The control sample gave the lowest value, while the 15% mixture obtained the highest one, and both showed significant differences with the 5% and 10% mixtures. *Lt* (time latency) also presented significant differences between the control and mixtures, with values up to 3-fold lower than the chia mixtures. The control obtained the highest *Ft* (maximum development time) value, followed by the 5%, 10% and 15% mixtures in ascending order, and the differences among them were significant. The highest values in the 10% and 15% mixtures were for the average bubble size data (Table 2), where these mixtures gave significant differences compared to the other samples at *Ft*.

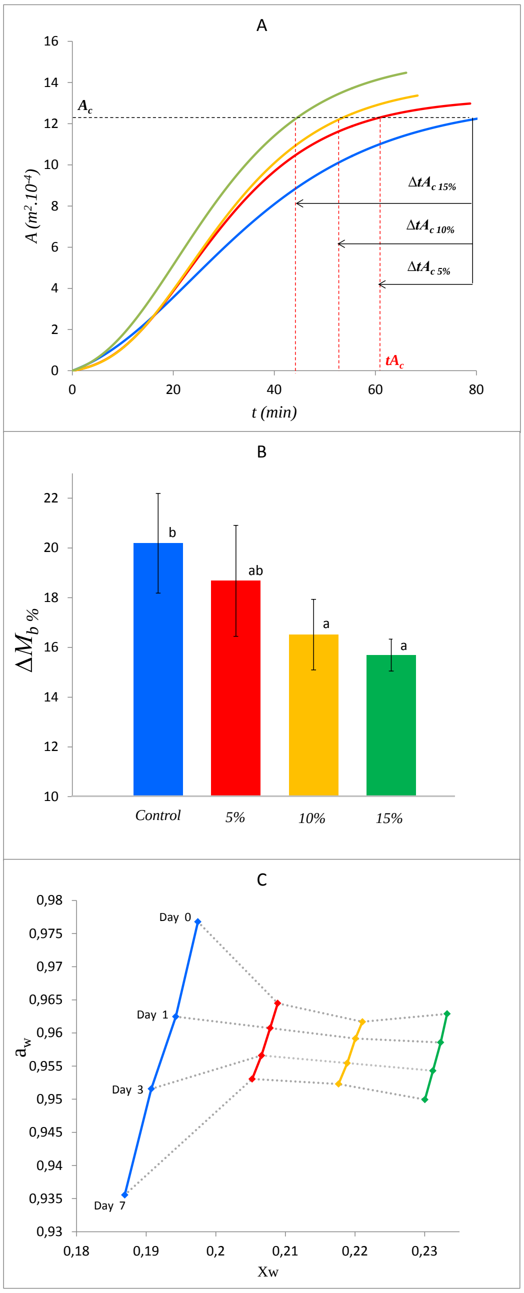

Figure 2.

A: Fermentation curves resulted from Gompertz curve-fitting procedure.

B: Percentage of mass-loss during baking process (ΔM_b). Bars express standard deviation and letters on the columns mean significant differences at $p \leq 0.05$.

C: Evolution of X_w and a_w during the storage of breads

Colors mixtures correspond as follows: Control —; 5% —; 10% —; 15% —; control area (*Ac*) – –; time to reach control area (*tAc*) – –.

In order to evaluate the influence of chia in the fermentation phase, the resultant data of the fermentation curves, which took the control curve as a reference, were studied. Thus the time when each mixture reached the same *A* value of the control was determined (*Ac*). This time factor was called *tAc*. Once each mixture *tAc* was obtained, it was calculated how long it took to reduce in each mixture to reach *Ac*. This parameter was called Δ*tAc*. The results showed that *tAc* reduced according to the increase in the degree of substitution. After analyzing the Δ*tAc* results, the 5% mixture reached 23.2% *Ac* faster than the control, 33.3% for the 10% mixture, while the 15% mixture obtained the highest value and reduced the time to 44.3%.

Several authors have reported studies about different chia flour properties, which have proven the improvement of gas retention in the dough matrix through variations in their pasting properties; for example, in products prepared with gluten-free flours (Moreira et al., 2013). Some changes in the rheological properties of dough resulted mainly from the polymeric structure of chia mucilage, whose capacity to form hydrocolloids increases water retention, and thus the surface tension of the matrix. This property could improve the volume of dough through the formation of hydrophilic complexes between their ionic groups and gluten proteins to favor the gluten matrix formation and, therefore, to help avoid gas from leaking (Iglesias & Haros, 2013).

Therefore, the improvement of gas retention could lead to increased growth velocity, and reaching *Ac* required less time. The 15% of degree of substitution, and probably higher degrees, could keep enough gas to overcome *Ac* for the same initial dough mass. Likewise, the effect of this property on bubble size was increased mechanical resistance to the matrix walls, which confers more porous matrix to the gluten network, and permits greater stability and further expansion during the fermentation process (Bárcenas & Rosell, 2005). Consequently, the structure coalescence delay took place, followed by collapsing dough.

The number of bubbles in the different mixtures was similar to that in the control. However for the highest substitution mixtures, 10% and 15%, bubble size was significantly larger at T4 (100% of dough growth). Those results are in agreement with Iglesias and Haros (2013), whose study did not report differences in bubble size between the wheat flour control and the 5% mixture. However, the significantly larger bubble size in the 10% and 15% mixtures, and the fact that, at the same time, no differences were found in their number of bubbles /$m^2 \cdot 10^{-4}$, could explain their high *A* at *Ft* values in the other samples due to a reduced gas leak. Therefore some changes in crumb structure were observed from degrees of substitution above 5%, which also had a direct effect on *A*.

3.3. End product

3.3.1. Baking process phase

Figure 2B represents the middle increment of mass (ΔM_b) in % in relation to the baking process phase of each mixture at *tAc*. This baking-loss was due mainly to the water loss which resulted from heat treatment. The results show reduced baking loss when incrementing the degree of substitution. The 10% and 15% mixtures presented significant differences to the control, but not to the 5% mixture, and significant differences were found among them. The chia compounds property on water retention, as previously postulated by some authors, was also observed in this phase of the process. In line with this, the studies into chia mucilage by Muñoz et al. (2012) reported that it is able to hydrate 27 times its own weight. Likewise, the results obtained by Vázquez-Ovando et al. (2009), but on a fiber-rich fraction of defatted chia, confirmed its good water-holding capacity, among other aspects.

In order to study the observed effect and the results based on the literature in detail, diverse parameters were calculated for the different compositional fractions of each mixture and bread product, and how the process affected them. Table 3 summarizes the calculated parameters and ratios. Firstly, although fat influenced several dough properties, the final dry matter without fat (*DMf)* of each mixture was calculated following the assays on matter balances by Vázquez-Ovando et al. (2009) as proteins, starch and other carbohydrates, such as fiber, are the main influential components of flour in terms of the interactions of dough in water (Wilhelm et al. 2005). In order to obtain an overview of the variations in this parameter with degree of substitution, the decrease in *%DMf* was calculated compared to the control flour.

Table 3. Parameters of dry matter of flour mixtures and their effect on baking process.

bstitution	***DMf***	***Xw of bread***	***g Water of bread / g DMf***	***%DMf reduced***	***%Xw increment***	***%gWater / g DMf increment***
Control	0.84	0.20 ± 0.01 a	0.10 ± 0.01 a	-	-	-
5%	0.83	0.21 ± 0.02 ab	0.12 ± 0.02 b	1.3	5.8	26.4
10%	0.82	0.22 ± 0.01 bc	0.16 ± 0.01 cd	2.5	13.5	64.7
15%	0.81	0.24 ± 0.00 c	0.18 ± 0.01 d	3.8	18.1	80.9

DMf : Fraction of dry matter without fat of each flour mixture.

Xw of bread: moisture of bread after baking process.

g Water of bread / g DMf : ratio between grams of water in bread per each gram of dry matter without fat of flour mixture.

%DMf reduced: Reduction of dry matter without fat by the increase of % substitution compared to control flour.

%Xw increment: Increment of moisture in bread after baking in each flour mixture compared to control flour.

% g Water / g DMf increment: Increment of grams of water in bread per each gram of dry matter without fat of flour mixture compared to control flour.

-Different letters within columns mean significant differences at $p \leq 0.05$.

Thus the results showed that *DMf* lowered when degree of substitution increased and, therefore, the decrease in *%DMf* varied from 1.3% for the 5% mixture to 3.8% in the 15% mixture. Those variations were produced because chia flour has more than 10 times the amount of lipids compared to wheat flour and, consequently, due to a lower proportion of the remaining dry matter. Secondly, moisture of bread products was calculated (*Xw)*. It was observed how bread moisture augmented with an increasing degree of substitution. To link these two parameters, the grams of water contained in each bread product per gram of *DMf* (*g Water of bread / g DMf*) were calculated. The results indicated that although there was less dry matter in highly substituted flour, water retention per gram rose when the degree of substitution was higher. Thus this last parameter was estimated as a % in relation to the control *(%gWater / g DMf increment).* The 15% mixture contained 80.9% ore water per gram of dry matter of flour mixture than the control flour. It was, followed by the 10% mixture with 64.7% and the 5% mixture with 26.4%. The contribution of chia to water retention in the matrix allowed baking loss to lower with the same g of water/ g of dry matter ratio of the control sample.

3.3.2 Texture, mass loss and water activity of bread

End product package evolution over time was analyzed. All bread products were firstly fermented until *tAc* to evaluate differences with the control at the same degree of dough development. Table 4 shows the results of the mass loss and texture parameters of *TPA* at four different time points: days 0, 1, 3 and 7.

On day 0, hardness did not present any important difference between the control and the chia mixtures, nor on day 1. On day 3, high levels of hardness were observed for them all (more than 1400 g), where the highest value went to the control compared to the chia mixtures. After seven days, the hardness of the control increased to reach values of around 2600g, while the values of the chia mixtures were maintained from day 3 with no statistical differences. Significant differences were found for springiness between the 15% mixture and the other mixtures on day 3, between the 5% mixture and the other mixtures on day 0, and for the 5% and 15% mixtures on day 1. Cohesiveness presented significant differences for the 10% and 15% mixtures, principally on day 0. The chewiness of the 5% mixture obtained significantly lower values compared to the control for days 3 and 7, and the 10% and 15% mixtures presented a significantly high value on day 1 compared to the control. However, significant differences with lower values compared to the control on days 5 and 7 were observed. Obviously the fact that all the samples presented textural properties on day 3 is unfavorable for consumption from an organoleptic point of view. However, the observation made until day 7 allowed us to note the effect of chia under extreme conditions.

Mass loss also differed between mixtures and control. From day 1 onward, the control lost the largest amount of water than the chia mixtures. Thus the control presented a mass loss of 2% on day 7 compared to the mass on day 0, while the chia 5%, 10% and 15% mixtures lost 0.7%, 0.6% and 0.5% respectively. A significant effect for the "storage day" main effect was observed for all the variables, except springiness. The "degree of substitution" main effect also had a significant effect, but only for hardness and a_w. The interactions between the main effects with a significant effect were observed in hardness, cohesiveness and a_w, although with a lower F-ratio compared to the main effects.

This effect could also derive from water-retention capacity of chia mucilage. Water in the bread matrix was better maintained in the mucilage structures, which made its outward leak difficult (Capitani et al. 2012). Furthermore, these high levels of moisture could allow to make water available to delay starch retrogadation and could, therefore, influence texture features. This could explain the differences among hardness, gumminess and chewiness throughout storage, and would agree with Bárcenas et al. (2005), whose results concluded that the incorporation of hydrocolloids into bread dough improves the crumb texture profile by reducing crumb hardness.

The moisture retention property could also be counterproductive from the standpoint of conservation features. So a decision was made to study this water availability with a water activity analysis. This parameter was studied because the sole determination of moisture content does not inform about the nature of water; that is, whether it is bound, free, inert or occluded (Mathlouthi, 2001). This is a critical factor that affects a product's shelf life, which controls food behavior during processing and storage (Anese et al. 1996; Yang & Paulson. 2000). Figure 2C shows the tendencies of the evolution of a_w and X_w during the storage time of mixtures.

From day 0 to 7, the a_w values showed marked differences between the control and chia mixtures. On day 0, the control had a higher a_w than the rest of the samples, which had between 5.8% and 13.1% less Xw (Table 3). The reduction of a_w for the control between the study time (days) was also more intense. The chia mixtures underwent some considerably slighter changes in this parameter throughout storage time. Finally, moisture reduction in the control brought about a sharper drop in the a_w values than the chia mixtures.

The most interesting phenomenon noted during storage was the property of chia to maintain the a_w levels close to an initial value despite the large amount of water they retained in the matrix. The results agree with previously reported studies by Muñoz et al. (2012), who observed the chia mucilage properties of increasing the linked water in matrix to further reduce the availability of it being used by other reactions, which could prolong storage time and, therefore, the shelf life.

4. Conclusions

The parameters of the different bread-making phases studied herein were modified by substituting wheat flour with chia seed flour. Those modifications can be associated with the effect of the hydrophilic compounds of chia, which has good water-holding capacity, and can develop stable hydrocolloids and improve the gluten net. These properties imply increased viscosity, mainly for 10% and 15% mixtures, and can amend the other pasting properties of flours. In the same way, the kinetics of dough fermentation was modified to cut the time to reach the control's maximum volume to 40%. Bread properties also were modified and less water was lost during both the baking process and storage. Chia also had effect on degree of water activity, which remained the same despite containing a larger amount of crumb moisture. Texture presented the least influenced properties, and retardation of hardness at prolonged times was mainly observed. The results concluded that the degrees of substitution, up to 15%, could be used to improve not only nutritional provision, but also the technological properties of wheat flours. More studies are needed to evaluate consumers' acceptance of these degrees of substitution and the properties of each fraction of chia seed components.

5. Acknowledgements

We wish to thank the Polytechnic University of Valencia and the Generalitat Valenciana for the financial support they provided through the Projects PAID-05-011-2870 and GVPRE/2008/170, respectively.

6. References

Ahlborn, G. J., Pike, O. A., Hendrix, S. B., Hess, W. M., & Huber, C. S. (2005). Sensory, mechanical, and microscopic evaluation of staling in low-protein and gluten-free breads. Cereal Chemistry, 82, 328-335.

Anese, M., Shtylla, I., Torreggiani, D., Maltini, E. (1996). Water activity and viscosity-relations with glass transition temperatures in model systems. *Thermochimica Acta*, 275, pp. 131–137.

Ayerza, R., Coates, W., (2005). Chia: Rediscovering a Forgotten Crop of the Aztecs. The University of Arizona Press, Tucson, AZ, USA.

Barcenas M. E, Rosell CA (2005) Effect of HPMC addition on the microstructure, quality and aging of wheat bread. *Food Hydrocolloids*. 19:1037–1043

Capitani, M.I., Spotorno, V., Nolasco, S.M.,. Tomás M.C. Physicochemical and functional characterization of by-products from chia (Salvia hispanica L.) seeds of Argentina. *LWT - Food Science and Technology,* 45 (2012) 94-102

Cocchi, M., , Corbellinib M, Focaa,G., Lucisanoc, M., Paganic, M. A., Lorenzo T. & Alessandro, U. (2005). Classification of bread wheat flours in different quality categories by awavelet-based feature selection/classification algorithm on NIR spectra. *Analytica Chimica Acta*, *544*, 100–107.

Cumby, N., Zhong, Y., Naczk, M., & Shahidi, F. (2008). Antioxidant activity and waterholding capacity of canola protein hydrolysates. *Food Chemistry*, 109, 144-148.

Datta, A. K., Sahin, S., Sumnu, G., & Keskin, O. (2007). Porous media characterization of breads baked using novel heating modes. *Journal of Food Engineering*, 79, 106–116.

Del Rio, D., Rodriguez-Mateos, A., Spencer, J. P. E., Tognolini, M., Borges, G., & Crozier, A. (2013). Dietary (poly)phenolics in human health: Structures, bioavability, and evidence of protective effects against chronic diseases. Antioxidant & Redox Signaling, 18, 1818–1892.

Esteller, M. S., Zancanaro, O., Jr., Santos-Palmeira, C. N., & da Silva-Lannes, S. C. (2006).The effect of kefir addition on microstructure parameters on physical properties of porous white bread. *European Food Research Technology*, 222, 26–31.

Houben, A., Höchstötter, A., & Becker, T. (2012). Possibilities to increase the quality in gluten-free bread production: An overview. *European Food Research Technology*, 235, 195–208.

Iglesias-Puig, E, Haros, M. Evaluation of performance of dough and bread incorporating chia (Salvia hispanica L.). *European Food Research Technology* (2013) 237:865–874

Inglett G.E, Chen, D., Liu, S., Lee, S. Pasting and rheological properties of oat products dry-blended with ground chia seeds. *LWT - Food Science and Technology* 55 (2014) 148-156.

Ivorra, E., Verdú, S, Sánchez A., Barat, J.M., Grau., R. (2014). Continuous monitoring of bread dough fermentation using a 3D vision Structured Light technique. *Journal of Food Engineering*, 130 8–13

Ixtaina VY, Nolasco SM, Tomas MC (2008) Physical properties of chia (Salvia hispanica L.) seeds. *Ind Crop Prod* 28:286–293.

Lazaridou, A., Duta, D., Papageorgiou, M., Belc, N., & Biliaderis, C. G. (2007). Effects of hydrocolloids on dough rheology and bread quality parameters in gluten-free formulations. *Journal of Food Engineering*, 79, 1033-1047.

Lin KY, Daniel JR, Whistler RL (1994) Structure of chia seed polysaccharide exudate. *Carbohyd Polym* 23(1):13–18

Mathlouthi, M. (2001) Water content, water activity, water structure and stability of foodstuffs. *Food Control*, 12, pp. 409–417.

Miñarro B., Albanell , E., Aguilar , N., Guamis B., Capellas , M., (2013). Effect of legume flours on baking characteristics of gluten-free bread. *Journal of Cereal Science* ,56 (2012) 476-481.

Moreira, R., Chenlo, F., Torres, M.D. Effect of chia (Sativa hispanica L.) and hydrocolloids on the rheology of gluten-free doughs based on chestnut flour. LWT - *Food Science and Technology* 50 (2013) 160e166

Muñoz, L. A , Cobos, A., Diaz, O., Aguilera, J.M. Chia seeds: Microstructure, mucilage extraction and hydration. *Journal of Food Engineering* 108 (2012) 216–224.

Vázquez-Ovando, A., Rosado-Rubio, G., Chel-Guerrero, L., Betancur-Ancona, D., 2009. Physicochemical properties of a fibrous fraction from chia (Salvia hispanica L.). *LWT – Food Science and Technology,* 42 (1), 168–173.

Vázquez-Ovando, J. A., Rosado-Rubio, J. G., Chel-Guerrero, L. A., & Betancur-Ancona, D. A. (2010). Dry processing of chia (Salvia hispanica L.) flour: chemical and characterization of fiber and protein. *CYTA Journal of Food*, 8, 117-127.

Verdú, S., Ivorra, E., Sánchez, A.J., Barat, J.M., Grau, R. (2014). Relationship between fermentation behaviour, measured with a 3D vision Structured Light technique, and the internal structure of bread. *Journal of Food Engineering*, 146 (2015) 227–233.

Wilhelm, L.R., Suter, D.A, Brusewitz, G.H., 2005. Physical properties of food materials. Food and Process Engineering Technology. American Society of Agricultural, St. Joseph, Michigan, pp. 23–52.

Yang, L., Paulson, A.T. (2000). Effects of lipids on mechanical and moisture barriers properties of edible gellan film. *Food Research International*, 33, pp. 571–578.