

# Sourdough and Bread Properties as Affected by Soybean Protein Addition

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## 1. Introduction

Soybean benefits in human health have been the target of numerous studies during the last decades. This interest has been the result of the findings about soybean consumption, where this legume or some of its components, mainly proteins and isoflavones, have been related to the reduction of some human diseases, such as cardiovascular diseases, diabetes, obesity, hypertension, dyslipidemia or even cancer (Piñeiro, 2006; Martin, 2001; Barnes, 1995).

Soybean is an economically important crop, which usually has served as a source of energy and good-quality protein for animals and humans, as it presents a high content of protein (36-48%), lipids (18-21%) and carbohydrates (33.5%), besides the amount of crude fibre and non saturated lipids which make them to be considered as healthy foods (Chávez et al., 1992). Moreover, a number of nutrients and micronutrients with nutraceutical properties have been identified in soybean, including isoflavones, phytosterols, inositol hexaphosphate, saponins, protease inhibitors, and bioactive peptides (Barnes, 1995; Hawrylewicz et al., 1995; Kennedy, 1995; Rao & Sung, 1995; Badger et al., 2005; Gálvez et al 2001; Vucenik et al., 2003; Badui, 1993).

Nowadays, different kinds of foods containing soybean flour or some of its products can be found in the market, being bread one of these items. Adding soybean to bread has been proposed to improve its nutritional quality, however, the level of soybean addition has been restricted to less than 10%-15% (wheat flour basis) as detrimental effects on bread quality (lower bread volume, coarser crumb structure, and a stronger flavour) have been reported (Sabani & Tzia, 2009; Olaoye et al., 2003; Dhingra & Jood, 2004; Shogren et al., 2003; Halle et al, 2004). This effect makes necessary to keep looking for other alternatives that allow higher levels of addition of this legume in bread making.

Sourdough breads are very popular foods worldwide. They are a mixture of flour (rye and wheat) and water that is fermented with lactic acid bacteria (LAB), mainly heterofermentative strains, which generate, as fermentation by-products, lactic and acetic acids and hence resulting in a sour taste of the end product. At the same time, yeast fermentation takes place, resulting in the dough expansion. The action of both microorganisms determines the sourdough bread characteristics in terms of acid production, aroma and leavening, resulting in an improvement of the volume, texture, flavour, nutritional value and shelf life of bread. These

characteristics make sourdough bread a very good alternative for being added with soybean flours or isolates, as the soybean off flavours could be less noticeable because of the stronger taste and aroma of this kind of bread. The aim of this work was to evaluate the effect of the addition of soybean isolate (0, 12.5 and 25%) as well as the effect of varying the starter fermentation time (0, 24 and 48h) on dough extensographic and farinographic properties and sourdough bread quality.

## 2. Materials and methods

### Materials

A commercial soybean isolate (Cenit, Mex, 90 g protein/100 g sample (db)) and commercial wheat flour (11.0 g protein/100 g sample (db), N×5.7,) with a farinographic water absorption value of 58.1 g water per 100 g flour were employed. This type of wheat flour is suitable for traditional sourdough bread products. All other ingredients like table salt (La Fina, Sales del Itsmo, Mex), sugar (Great Value, Wal-Mart, Mex), white rye flour (ConAgra Foods, Inc, Omaha, NE), skim milk powder (Svelty, Nestle), dry instant yeast (Nevada Oro, Safmex), caraway seeds (*Carum carvi*, Terana, Mex ) and extra virgin olive oil (Borges, Tarrega, Spain) were also commercial grade.

### Wheat-soy isolate flour samples

Soybean isolate was added to complete 100 g of wheat flour at three different concentrations (0, 12, 25g).

### Farinographic measurements

The farinograms of wheat-flour-soy isolate mixtures added or not with rye flour (18.5%), as well as the full sourdough bread formulations were acquired in a 300g Brabender Farinograph (Brabender OHG, Duisburg, Germany), obtaining, for all these samples, the farinographic water absorption, the maximum consistency time and the dough stability.

### Extensographic measurements

The extensographic properties of the wheat flour- soybean mixtures with or without rye flour were determined in a Brabender extensograph (Brabender Duisburgh, T150, Germany) obtaining the maximum resistance to extension ( $R_{max}$ , UB) and extensibility ( $L$ , mm) at three different resting time (45, 90, 135 min) as reported by the manufacturer.

### Bread making procedure

Sourdough bread was prepared following a traditional three steps method which includes obtaining the starter, the sourdough and finally the bread dough. Three different starters were obtained by fermenting (Precision Scientific, USA) a mixture of wheat flour (48.01g), active dry yeast (2.81 g), skim milk powder (2.95g) and tap water (46.2g) for different times (0, 24, 48h) at constant temperature ( $28\pm 1^\circ\text{C}$ ). Once the selected fermentation time was completed a portion of the starter (31.4 g) was mixed with rye flour (38.1g) and tap water (30.5g) resulting in the sourdough after fermenting it for 24h at  $30^\circ\text{C}$ . The bread dough was prepared by mixing (Minor-pin mixer, Henry Simon Limited, Cheshire UK) all dry ingredients for 1 min: wheat flour-soy isolates mixtures (42.69g), caraway seeds (0.27g), dry active yeast (1.04g), table salt (1.09g) and sugar (1.64g). Then the olive oil (1.09g) and the sour dough (28.79 g) were added to the other ingredients and mixed for another minute. Finally tap water ( $30^\circ\text{C}$ ) was added and all ingredients were mixed until optimum dough

development, as obtained from the farinographic evaluation of full sour bread formulations. The quantity of water added to obtain the bread dough was based on the farinographic water absorption percentage at 500 Brabender Units (BU) of the wheat flour-soy isolates mixtures (Calderón-Domínguez et al., 2003). After mixing, dough was weighed (50 g), rounded by hand, and placed into an adapted fermentation chamber (Precision Scientific, USA) at constant temperature (30 °C) and humidity (85%) for 120 min. After this fermentation period, the dough was punched, rounded, moulded, placed into a baking pan (aluminium pudding mould), and left to rest for 60 min. Bread was baked at 210 °C for 20 min in a rotary oven (Henry Simon). Bread quality was analyzed considering loaf specific volume, colour, pH, titratable acidity, texture, crumb-grain structure and consumer acceptance.

### **Loaf specific volume**

Loaf weight was measured immediately after baking, and loaf volume was evaluated 120 min later, using the rapeseed displacement method (AACC 2000, 10-05).

### **Colour**

Crust and crumb colour were evaluated using a colorimeter (CR-400 Chroma metre, Konica Minolta, USA) with a D65 illumination source and at a 0° viewing angle. Luminosity (L\*),  $\pm a^*$  and  $\pm b^*$  values were obtained (Rosales-Juárez et al., 2008). Colour was measured on five different points on each sample, taking them in the central and outward parts of the loaf, avoiding its edges.

### **pH and titratable acidity**

pH was measured by dispersing 10 g of sample in 100 mL of cool, recently boiled water (25°C) and employing a potentiometer equipment (Hanna Instruments, Rumanía). Titratable acidity was evaluated following the 02-31 AACC method (2000).

### **Texture evaluation**

Texture analysis was carried out in a Texture Analyzer-TX2 (Stable Micro Systems Ltd., UK), applying a double compression test and employing a 1.5" (3.81 cm) diameter acrylic cylinder probe (TA-11) as cited by Rosales-Juárez et al., (2008). Cross head's speed was set at 5 mm/s, and the analysis was carried out 18 h after baking. Samples were prepared by cutting the loaf crust off to obtain a 3-cm height sample, allowing only crumb texture measurements. Bread slices were compressed to 50% of their original height. The type of probe was chosen to avoid the effect of loaf edges. Reported textural parameters were: compression force (g), firmness (g), and resilience.

### **Crumb grain evaluation**

Crumb grain characteristics were evaluated by an image analysis technique following the methodology reported by López-Guel et al., (2009). All samples were allowed to cool for at least 2 hours at room temperature (20°C) and kept inside polyethylene hermetic bags to avoid moisture loss until they were analyzed for crumb structure which was performed 24 hours after baking. Breads were cut in halves with an electric knife (Moulinex Classic 012, France). Bread crumb images were obtained by scanning the samples at a resolution of 550 dpi in a BenQ 5000 colour scanner (colour depth 48 bit, optical resolution 1,200 dpi×2,400 dpi). Images were saved as bitmap files and they were cropped using the ImageJ software (National Institutes Health, Bethesda, MD, USA) to obtain a field of view (FOV)

representing a 65% of the total crumb bread area. Cropped colour images were converted into an 8-bit greyscale images. Thresholding was carried out by means of the Otsu algorithm (Gonzales- Barron & Butler 2006), using the ImageJ software. Count and measurements of cells were done using the Sigma Scan Pro 5.0 software to get the total cells number of the cropped image, pore size (overall mean cell area), number of pores per square centimetre (cell density), and circularity of pores (shape factor). The final data were processed in Excel 2003 software (Microsoft Office Corporation, USA) where a subdivision based on the pore size area (A) for all the parameters measured was established: all pores,  $A > 0.002 \text{ mm}^2$ ,  $0.002 < A < 4 \text{ mm}^2$  and  $0.004 < A < 4 \text{ mm}^2$ .

### Sensory analysis

A multi-sample likeability rating test, as reported by Rosales-Juárez et al., (2008), was carried out on two selected samples, where a hedonic scale (seven levels of acceptance) was used, asking to grade the samples with values from 7 for the "like very much" level of acceptance down to 1 for the "dislike very much" level of acceptance. The evaluation was performed with a panel that consisted of 55 non-trained adults ( $30 \pm 13$  years old) as the product was intended for adults that want to consume healthy products. Loaf samples were sliced with an electric slicing knife (Moulinex Classic 012, France), discarding crusts. Both samples were labelled with a three-digit random code.

### Experimental design

A  $3^2$  factorial design, with three repetitions of each sample, was used. Two factors were analyzed (soy isolate level and fermentation time) at three different levels (Table 1). Selected responses were farinographic and extensographic parameters, bread quality (specific volume, colour, texture, crumb structure), and dough titratable acidity and pH. These responses were expressed individually as a function of independent variables. The experimental sequences, the data analysis and the response surface plots were obtained from the Design Expert Program software, version 6.0 (Stat-Ease Inc., Minneapolis, USA). Sensory analysis and biological evaluation data were analysed using SigmaStat 3.5 for Windows (Systat Software, Inc. USA), using ANOVA and the Holm-Sidak method or Tukey test as obtained from the software.  $P < 0.05$  was considered to be significant.

Factor	Code	Levels		
WA (%)		-1	0	+1
Soybean isolate (%)	SI	0	12.5	25
Fermentation Time (h)	FT	0	24	48

Table 1. Response surface experimental design

### SDS-PAGE electrophoresis

The effect of mixing, fermentation and baking on the protein structure of the two best sourdough samples was studied by SDS-PAGE electrophoresis. Proteins were extracted following the methodology proposed by Tuukkanen et al., (2005) with some modifications. Prolamins were obtained by mixing (30 min,  $52^\circ\text{C}$ ) 0.1g of the dry defatted sample with 1mL of 70% ethanol solution (v/v) and keeping to analyze the soluble fraction as obtained after centrifugation ( $11000 \times g$ ,  $4^\circ\text{C}$ , 10 min). Albumins and globulins were extracted by mixing (10 min, vortex) 0.1g of sample (dry and defatted) with 1 mL of a 0.5M NaCl solution (w/v) and keeping the liquid phase after centrifugation ( $11000 \times g$ , 10 min,  $4^\circ\text{C}$ ). Glutelins were

obtained by mixing (10 min, vortex) 0.1 g of sample (dry and defatted) with 1 mL of 0.05M acetic acid solution (w/v) and analyzing after centrifugation (11000 x g, 10 min, 4°C) the supernatant. SDS-PAGE electrophoresis was conducted in a 250 Mighty Small II electrophoresis unit (Hoeffer, USA) using resolving gels with different concentration of total acrylamide (%T). An 11%T resolving gel was used to study the prolamin and glutelin fractions, while a 16%T resolving gel was employed to separate NaCl soluble fraction. The analysis was carried out at non reducing conditions and at 110 V (25 mA) and loading 20 µL each protein sample. A low molecular weight protein marker (5 µL) and BSA (5 µL) were included as protein markers.

### Biological analysis

Male and female 21 days old albino Wistar rats, weighting an average of 68±10g were used for evaluating the protein quality of the two selected breads. All animals were allowed a 5-day adaptation period after receipt. After this 5-day period, rats were weighted and fed (ad libitum) with water and with a diet containing 10% protein and 90% of a mix which was composed of 1% of vitamins, 10% corn oil, 5% non nutritive cellulose, 4% minerals and corn starch to complete the mix. The laboratory conditions were kept at controlled temperature and moisture under 12h light and 12h dark. Diets were weighted everyday during the experiment and the rats were weighted on each seven days. The feed intakes were calculated weekly over the 28-day experimental period, except the protein free groups that lasted 14 days. Faeces were collected from the 7<sup>th</sup> to the 14<sup>th</sup> days of experiment. The biological indexes were determined from the results of the weight gain, the total amount of protein consumed, the total amount of diet consumed, and the total nitrogen excreted in the faeces. The biological indexes calculated were: protein efficiency ratio (PER), net protein ratio (NPR), apparent digestibility (AD) and true digestibility (TD).

## 3. Results and discussion

### Farinographic and extensographic parameters of flours.

Table 2 shows the results of the farinographic and extensographic evaluation of the flours used in the bread making process (wheat, wheat-rye, and wheat- soybean isolate- rye).

Farinographic & Extensographic parameters	Flours			
	Wheat	Wheat-Rye	Wheat-Soy 12.5%- Rye	Wheat-Soy 25%-Rye
WA (%)	58.1	61.0	68.0	71.0
MCT (min)	10.3 ± 0.1	15.1 ± 0.5	11.3 ± 0.4	10.5 ± 0.7
S (min)	19 ± 0.8	16.4 ± 0.8	6.5 ± 0.4	6.3 ± 0.4
L <sub>135 min</sub> (mm)	140 ± 11	140 ± 7	101 ± 6	82 ± 5
R <sub>max 135 min</sub> (BU)	>1000	745 ± 41	653 ± 59	540 ± 14

L and Rmax were evaluated after 135 min of resting time.

Table 2. Farinographic and extensographic results. (WA: water absorption. MCT: maximum consistency time. S: stability. L: extensibility. Rmax: maximum resistance to extension).

Water absorption (WA), which represents the amount of water required to centre the farinogram curve on the 500 BU line, increased when adding rye flour or the soybean isolate as a result of the larger level of protein. This information is in agreement with the reports of Rosales-Juárez et al., (2008) and López-Guel et al., (2009). WA data was used to calculate the quantity of water to add in the full bread formulations. Table 2 also presents the results of dough maximum consistency time (MCT) where it can be seen that the addition of rye flour increased the time required to obtain an optimum developed dough, while the addition of the soybean isolate decreased this parameter, maybe as a consequence of the less gluten proportion in the formulation. The dough stability (S) showed a decrement in their values as soybean isolate or rye flour was increased. On the other hand, extensographic information showed that the addition of rye flour or soybean isolate decreased both the extensibility ( $L_{135}$ ) and the maximum resistance to extension ( $R_{max}$ ). Hegazy & Faheid (1990) reported that adding soybean flour promotes a decrement in wheat dough extensibility, which is in accordance with our results. However it has been reported in other works that the addition of soybean flour increased this parameter (Rosales-Juárez et al., 2008). These differences could be explained as a result of the larger levels of soybean isolate used in our bread formulations as compared to the lower levels of the other studies. The addition of soybean isolate or rye flour also resulted in a decrement in the maximum resistance to extension ( $R_{max}$ ). Similar results have been published by Indrani et al., (1991) and Maforimbo et al., (2008).

#### Farinographic maximum consistency time and stability of full bread formulations

The time required to obtain an optimum developed bread dough was determined by farinographic evaluation. Dough stability was also evaluated. Figure 1 shows these results.

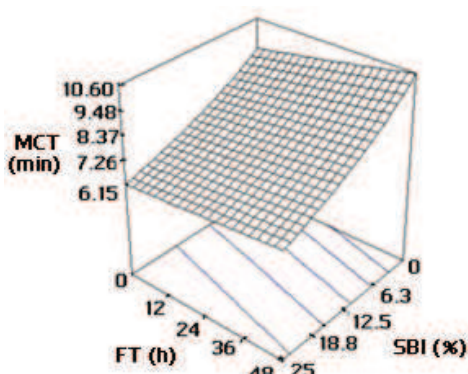


Fig. 1-A

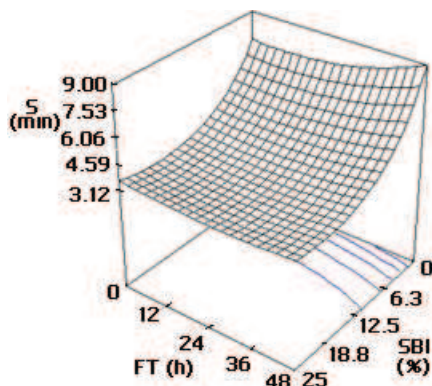


Fig. 1-B

Fig. 1. Effect of fermentation time (FT) and soybean isolate concentration (SBI) on maximum consistency time (MTC)(A) and dough stability (S) (B) of the sour bread dough.

It can be seen from the figure that both variables had an effect on the sourdough bread maximum consistency time, being the soybean isolate the component which produced the largest changes by decreasing the response (MCT) as SBI increases. Statistical analysis showed that data could be fitted, to lineal model ( $P < 0.0001$ ), with determination coefficient ( $R^2$ ) of 0.9383 (Table 3). Figure 1 also shows the effect of fermentation time and soybean isolate concentration on farinographic stability (Fig 1B). It can be observed from this graph

that this parameter decreased from 7.2 min to 3.4 min as soybean isolate increased, while fermentation time had a lesser effect. Stability data was fitted to a logarithmic model (Table 3) with a determination coefficient of 0.9575, having the proportion of soybean isolate a larger effect ( $P < 0.0001$ ). When comparing the maximum consistency time or the farinographic stability of the full sourdough formulations (Figure 1A, 1B) against flours (Table 2), it was noticeable that the first one presented smaller values in both parameters for all formulations. The decreasing effect of soybean isolate in sourdough full bread formulations is contradictory to the information reported by other authors (López-Guel et al., 2009). This difference could be ascribed to very different proportion of soybean protein used in both works, being the effect over the wheat gluten network very different.

#### Effect on soybean isolate addition and fermentation time on pH and titratable acidity

Figure 2 shows the change of titratable acidity (Fig 2A) and pH (Fig 2B) as affected by soybean isolate addition and fermentation time.

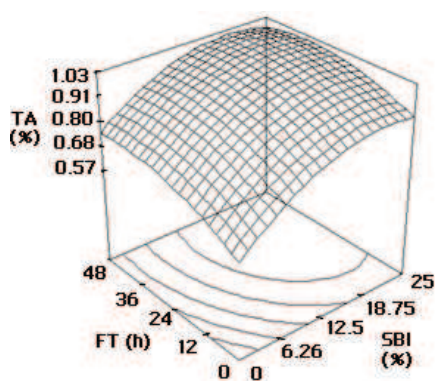


Fig. 2-A

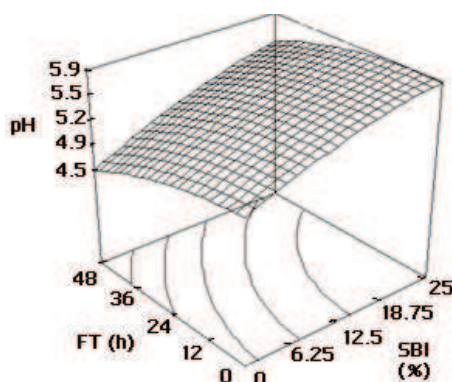


Fig. 2-B

Fig. 2. Changes on titratable acidity (TA) (A) and pH (B) of sourdough samples added with soybean isolate (SBI) and fermented for different times (FT).

It can be observed from Fig. 2 that both variables affected the responses ( $P < 0.0001$ ). The statistical analysis showed that titratable acidity data can be fitted to a quadratic model ( $P < 0.0001$ , Table 3), with a determination coefficient of 0.9646, and increasing its value as the fermentation time and soybean isolate levels were risen. The sourdough bread pH follows a different trend, decreasing the pH values as fermentation time increased, but presenting larger values at higher soybean isolate proportions. These data were adjusted to a quadratic model too (Table 3), with a determination coefficient of 0.9983. It has been reported that a sourdough fermentation promotes the production of organic acids, and that these substances, along with the action of enzymes and microorganisms, could generate a proteolytic effect on the proteins of the soybean added, resulting in the formation of positively charged free amino acids, which could increase the pH value (Leon et al., 2006).

#### Bread specific volume

The effect of soybean isolate levels and fermentation duration on sourdough bread specific volume is shown in Fig. 3, where it can be noticed that the higher the soybean isolate concentration the less the bread loaf volume is ( $P < 0.0001$ ). Statistical analysis showed that the

effect of fermentation time was non significant on this response ( $P>0.05$ ). Similar results have been reported by different authors (López-Guel et al., 2009; Indrani et al., 1997; Hallen et al., 2004), who cited that the smaller loaf specific volumes of soybean breads could be the result of a decrement in the gas retention capacity of the gluten network. Specific volume data was fitted to a quadratic model ( $P<0.0001$ ) with a determination coefficient of 0.9649 (Table 3).

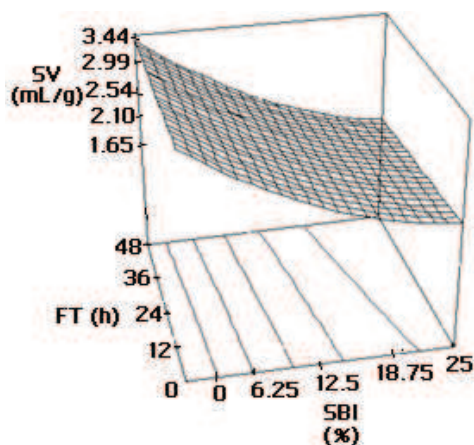


Fig. 3. Change on bread specific volume (SV) as a result of soybean isolate (SBI) addition and fermentation time (FT).

### Bread crumb and crust quality

Sourdough bread quality was evaluated by the changes in the crumb and crust colour (luminosity,  $a^*$  and  $b^*$ ), crumb textural parameters (compression force, firmness and resilience) and crumb grain structure (mean cell area, cell density and shape factor).

#### Crust and crumb colour.

Crumb and crust luminosity (L) were not affected ( $P>0.05$ ) by the changes in soybean isolate concentration or fermentation time. Crust luminosity showed a mean value of 53, while crumb luminosity stood in a value of 60. Similar results were reported by Rosales-Juárez et al., (2008), however other studies have shown that bread becomes darker when adding soybean products at increasing levels (López-Guel et al., 2009; Marco & Rosell, 2008; Hellen et al., 2004). The differences could be the result of the kind of soybean product added and the proportion used as compared to other studies.

CIE Lab chromaticity parameters ( $a^*$ ,  $b^*$ ) were also evaluated at the crust and crumb of the sourdough bread. Figure 4 shows that the addition of soybean isolate increased ( $P<0.0001$ ) the red colour of the crumb ( $a^*$ ), while the crust colour was not affected by this variable ( $P>0.05$ ). In both cases, fermentation time did not have significant effect ( $P>0.05$ ). The red colour data ( $a^*$ ) of the crumb was fitted to a lineal model ( $P<0.0001$ , Table 3) with a determination coefficient of 0.9113. These results do not accord with the ones reported by Rosales-Juárez et al., (2008) who described that soybean addition decreased the red colour of the crumb. The differences could be ascribed to the type and quantity of the soybean product added.



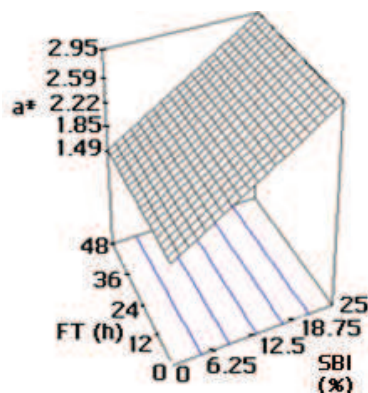


Fig. 4. Effect of soybean isolate (SBI) proportion and fermentation time (FT) on crumb red colour ( $a^*$ )

The changes in the CIE Lab chromaticity yellow-green parameter ( $b^*$ ) followed a quadratic or lineal tendency ( $P < 0.0001$ , Table 3) when evaluating the crumb and crust of the sourdough breads respectively (Fig. 5A, 5B). In both cases, the yellow colour increased as the soybean isolate was risen in the formulation. Fermentation time presented a significant but smaller effect on crumb yellow colour. López-Guel et al., (2009) and Halle et al., (2004) reported similar results.

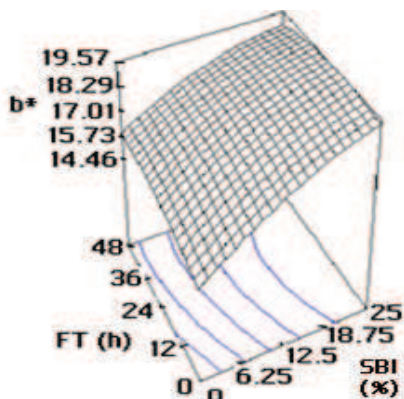


Fig. 5-A

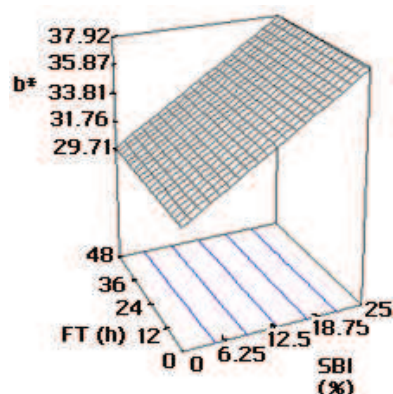


Fig. 5-B

Fig. 5. Effect of soybean isolate (SBI) and fermentation time (FT) on CIE Lab  $b^*$  parameter. A: crumb, B: crust.

### Crumb texture

The changes in crumb texture were evaluated by measuring the compression force, firmness and resilience. These results are presented in Fig. 6 where it is possible to see that as the concentration of soybean isolate was increased in the formulation the compression force (Fig. 6A) also increased. Fermentation time showed a smaller but significant ( $P < 0.05$ ) effect on this parameter. Data was fitted to a quadratic model (Table 3) with a determination

coefficient of 0.9097. Similar results were reported by Stauffer (2002) who showed that the bread added with soybean flour (3-12 g/ g flour) required a larger compression force. However other researchers found the opposite tendency (Leon et al., 2004). These discrepancies could be the result of the different materials and concentrations used in the experiments. Fig. 6B shows the changes in the crumb firmness, where the higher the concentration of soybean isolate the larger firmness of the product is. Fermentation time also had a significant effect on this response ( $P < 0.0001$ ), being more notorious when adding more soybean isolate. Firmness data was fitted to a first degree polynomial model ( $P < 0.0001$ , Table 3) with a determination coefficient of 0.8474. Rosales-Juárez et al., (2008) reported a similar effect when adding soybean flour to white bread. On the other hand, resilience did not change with soybean isolate addition or fermentation time ( $P > 0.05$ ), remaining almost constant (0.16) for all the conditions tested. Marco & Rosell (2008) reported similar results. Table 3 shows the response surface mathematical models of the evaluated parameters.

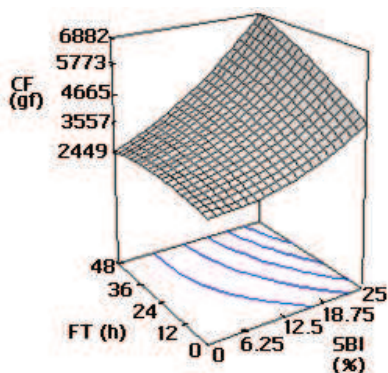


Fig. 6-A

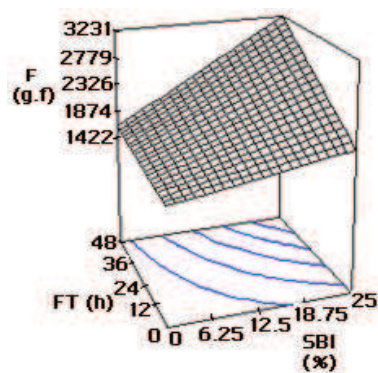


Fig. 6-B

Fig. 6. Changes in the compression force (CF) (A) and firmness (F) (B) of sourdough crumb bread as affected by soybean isolate (SBI) addition levels and fermentation time (FT).

### Crumb grain structure

Crumb grain structure was evaluated by image analysis, reporting mean cell area ( $\text{mm}^2$ ), cell density (pores/ $\text{cm}^2$ ) and shape factor (Figure 7). Results showed that the addition of soybean isolate or the fermentation time did not affect the size of the crumb bread pores (0.23-0.29  $\text{mm}^2$ ). Cell density and shape factor were not either affect by soy isolate addition or fermentation time, showing average values of 42 pores/ $\text{cm}^2$  and 0.75 respectively. Rosales-Juárez et al., (2008) reported similar results. On the other hand, Lagrain et al., (2006) and Zghal et al., (1999) reported mean cell areas ranging from 77 to 112 pores/ $\text{cm}^2$  for white bread, which means that the soybean isolate addition promotes a more open structure. Statistical analysis showed that any of the variables has a significant effect ( $P > 0.05$ ) on any of the responses.

Response surface model	Equation
$MCT^{-1} = 0.1089 + 2.1515 \times 10^{-3} SBI - 3.0247 \times 10^{-4} FT$	1
$\ln S = 1.99 - 0.084 SBI - 2.7 \times 10^{-3} FT + 2.3 \times 10^{-3} SBI^2 + 1.5 \times 10^{-4} FT^2 - 1.9 \times 10^{-4} SBI FT$	2
$TA = 0.57 + 0.03 SBI + 9.3 \times 10^{-3} FT - 7.4 \times 10^{-4} SBI^2 - 1.2 \times 10^{-4} FT^2 - 6.7 \times 10^{-5} SBI FT$	3
$pH = 5.1 + 0.05 SBI - 2.3 \times 10^{-3} FT - 7.1 \times 10^{-4} SBI^2 - 2.1 \times 10^{-4} FT^2 + 1.9 SBI FT$	4
$SV = 3.4 - 0.11 SBI - 8.3 \times 10^{-4} FT + 1.9 \times 10^{-3} SBI^2 - 2.1 \times 10^{-5} FT^2 - 1.1 SBI FT$	5
$A = 1.5 + 0.06 SBI + 3.5 \times 10^{-4} FT$	6
$B = 14.5 + 0.3 SBI + 0.07 FT - 3.9 \times 10^{-3} SBI^2 - 7.8 \times 10^{-4} FT^2 - 1.5 \times 10^{-3} SBI FT$	7
$BC = 29.7 + 0.3 SBI + 3.3 \times 10^{-3} FT$	8
$CF = 2457 - 10.6 SBI + 20.6 FT + 3.4 SBI^2 - 0.4 FT^2 + 2.1 SBI FT$	9
$F = 1421 + 18.5 SBI + 4.3 FT + 0.95 SBI FT$	10

Table 3. Response surface models of the evaluated parameters. MCT: maximum consistency time (min); S: stability (min); TA: titratable acidity (%); SV: specific volume (cm<sup>3</sup>/g); A: crumb chromaticity "a" value; B: crumb chromaticity "b" value; BC: crust chromaticity "b" value; CF: compression force (g); F: firmness (g). SBI: Soybean isolate concentration (g/100 g flour); FT: fermentation time (min).

### Sensory analysis

As the effect of fermentation time was negligible for many of the parameters evaluated, the breads fermented for 48 h and at both levels of soybean isolate addition were selected. Results show that both samples were accepted without having significant differences between them ( $P < 0.05$ ). Figure 8 shows this information.

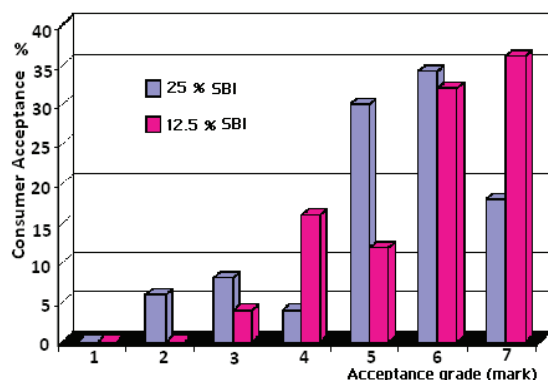


Fig. 8. Sensory analysis evaluation of soybean isolate added bread.

It can also be seen from this information that as the amount of soybean isolate added to the formulation increased the level of acceptance by the panellists decreased, which could be related to the off flavours generated by these high levels of soybean (Rosales-Juárez et al., 2008; Olaoye et al., 2006; McWatters et al., 2003). Even though it is possible to consider that the process of fermentation helps to hide the off flavours of soybean as the level of substitution was higher as compared to other published reports and both samples were well accepted.

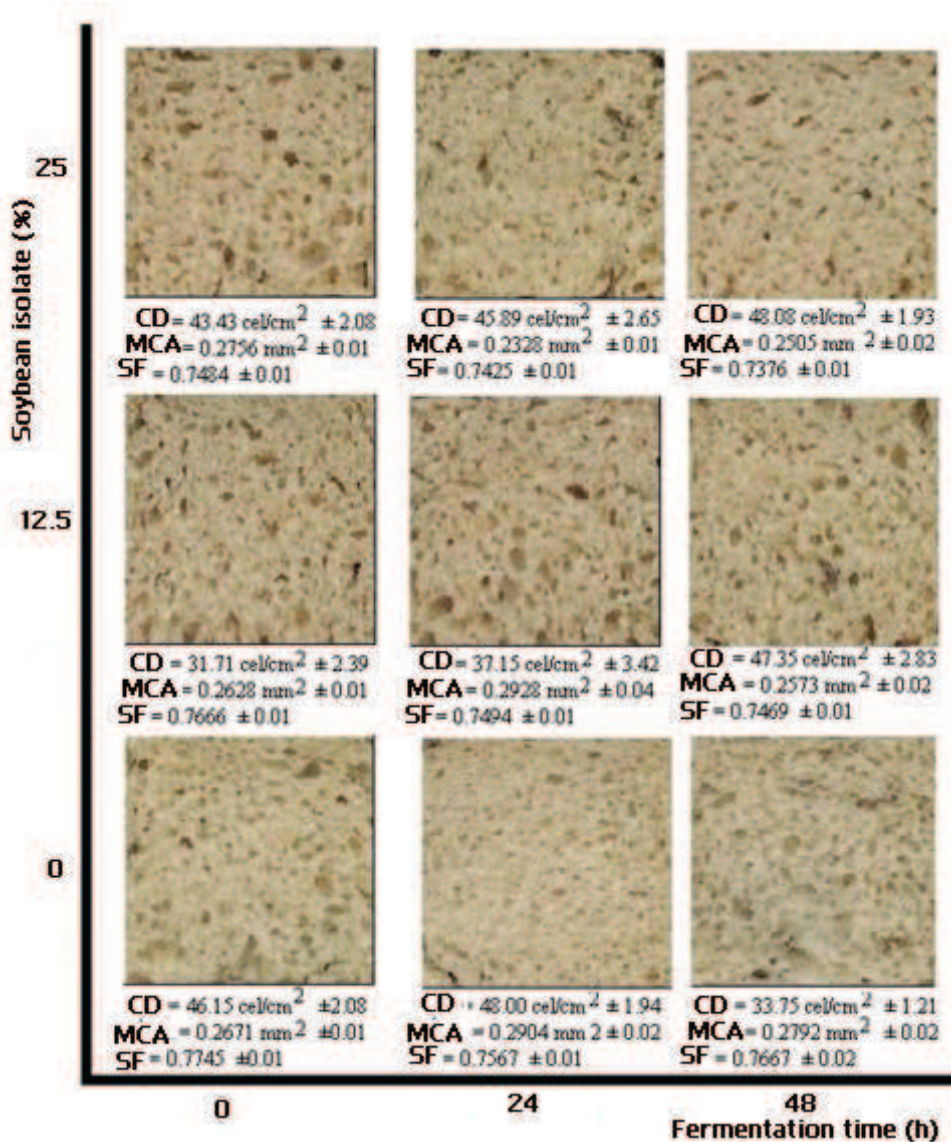


Fig. 7. Crumb grain structural analysis of sourdough breads added with different levels of soybean isolate (SBI) and fermented for different times (FT). CD: cell density, MCA: mean cell area; SF: shape factor. Results are the mean value of three repetitions  $\pm$  standard deviation.

#### Biological protein quality analysis

Table 4 shows the protein biological indexes calculated: protein efficiency ratio (PER), net protein ratio (NPR), apparent digestibility (AD) and true digestibility (TD).

	Control	12.5 % SI-sourdough bread	25% SI-sourdough bread
PER	1.8 ± 0.3	2.2 ± 0.4	2.4 ± 0.3
NPR	2.4 ± 0.3	2.7 ± 0.4	2.9 ± 0.3
D (%)	80.7 ± 1.9	83.1 ± 3.2	84.4 ± 1.8
TD (%)	85.6 ± 2.1	87.7 ± 2.9	88.2 ± 1.8

Table 4. Protein quality biological indexes of three diets made of soybean isolate sourdough bread. SI: soybean isolate. PER: protein efficiency ratio; NPR: net protein ratio; D: apparent digestibility; TD: true digestibility.

It can be observed from these results that the addition of soybean isolate (as expected) significantly increased ( $P < 0.05$ ) the PER and the NPR indexes of the sourdough bread as compared to the control sample. However there were no differences between the two samples added with soybean isolate and in the digestibility values (D, TD) either. This information shows that depending on the purpose of the product (better protein quality or nutraceutical bread), the addition of soybean isolate can be carried out at both substitution levels.

#### Changes on protein fractions during the bread making process of sourdough

Figure 9 shows the change in the electrophoretical pattern of NaCl soluble proteins. These results showed that the globulin fraction was mainly affected by the baking stage, while the fermentation process had an effect on the higher molecular weight subunits, as well as in the lowest molecular weight subunits. The other fractions (prolamins and glutelins) were mainly affected during baking, without an appreciable change in conformation.

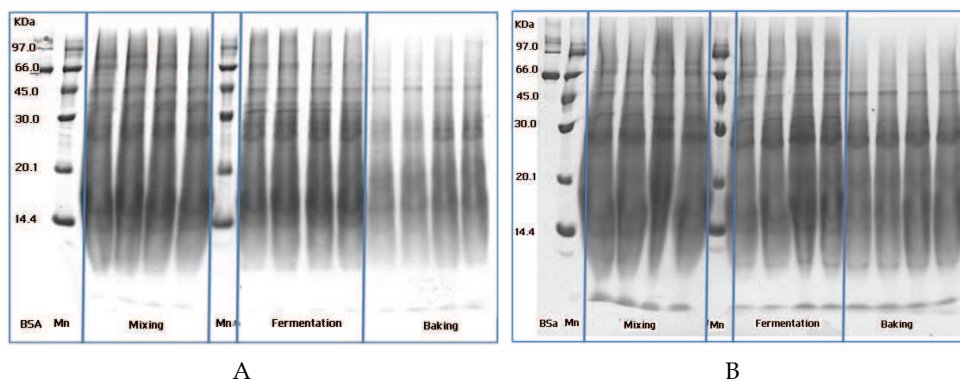


Fig. 9. Changes in the electrophoretical pattern of NaCl soluble proteins of sourdough added with different soybean isolate (SBI) concentrations. A:12.5% SBI; B: 25% SBI.

#### 4. Conclusions

Adding soybean isolate to produce sourdough bread resulted in a product with lower volume than then control but with good acceptance by consumers. The off flavour of

soybean was hidden by the sour flavour of the bread, besides that the nutritional quality was highly improved. Rheological properties were affected by the addition, as well as the texture of the product.

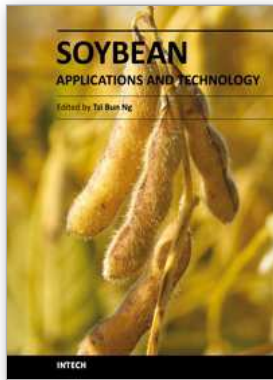
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