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Research Article

Soybean Sourdough as Bio-ingredients to Enhances Physical and Functional Properties of Wheat Bakery Products

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Abstract: The aim of this study was to evaluate the strain *Lactobacillus plantarum* C8 as starter culture for soybean sourdough fermentation, in terms of rheological properties, phenolic content and antioxidant activity. After this, wheat breads were prepared with fermented (sourdough) and unfermented soybean flour (control) at three different ratios (4.0:1.0, 3.5:1.0 and 1.5:1.0). Soybean sourdough showed less consistency and increased viscosity compared with unfermented dough. The content of phenolic compounds and the antioxidant activity significantly ($p<0.05$) increased after lactic fermentation. The overall characteristics of breads depended on the amount of soybean sourdough or flour addition while the best results were obtained with the lower substitution level. In fact, higher specific loaf volume, less crumb hardness and chewiness and intensification in lightness with respect to control breads, were obtained. Besides, sourdough breads showed high phenolic content and antioxidant activity with respect to breads made with unfermented soybean flour. In addition, sourdough breads were widely accepted by the consumers. This study put in evidence that sourdough addition improved the physical and functional properties of traditional wheat bread.

Keywords: Antioxidant activity, *Lactobacillus plantarum*, physical properties, sourdough, soybean flour

INTRODUCTION

Sourdough is a mixture of flour (traditionally rye and wheat flours) and water that is fermented with Lactic Acid Bacteria (LAB) and yeasts. Fermentation is a complex process not yet fully understood and depends on numerous factors that determine the subsequent bread quality. In the traditional production of sourdough, spontaneous fermentation by the indigenous flour microbiota determines the properties of the dough (De Vuyst and Vancanneyt, 2007). However, for a better control of the fermentation process, the inoculation of the sourdough with starter cultures is recommended.

Numerous studies reported the positive effects of wheat and rye sourdough addition on bread quality (flavour, crumb structure, dough volume), shelf life and production (Clarke *et al*., 2004; Gerez *et al*., 2010; Mamhoud *et al*., 2016). LAB fermentation may improve the macromolecule hydrolysis, thus enhancing digestibility and nutritional quality of breads (Bartkiene *et al*., 2012; Rizzello *et al*., 2016). Nevertheless, specific factors, such as the type of flour and the lactic starter culture, have to be controlled to reach an optimal fermentation process (Rizzello *et al*., 2016).

Sourdough of alternative flours such as soybean may offer nutritional benefits to human health. Among legumes, soybean is the only one containing the nine essential amino acids in a proper proportion, being also an important source of fibre, lecithin, vitamins, minerals and isoflavones (natural antioxidant) (He and Chen, 2013).

Some studies have already focused on fermentation as a potential tool to improve the technological and mainly nutritional characteristics of soybean flour. Bartkiene *et al*. (2012) reported that fermentation of soybean dough with a *Pediococcus acidilactici* strain improved its nutritional profile; nevertheless, a decrease in the specific volume and acceptability of breads was observed. As was recently reported a soybean-wheat dough fermented by *Lactobacillus* (*L.*) *brevis*, *L. plantarum* and *Saccharomyces* (*S.*) *cerevisiae* yielded

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less extensible dough and consequently, a harder bread crumb was obtained (Yezbick *et al*., 2013). Nevertheless, to our knowledge the available information about lactic fermentation of soybean dough is still poor. Therefore, the aims of the present work were to:

- Characterize a soybean flour sourdough fermented by the selected starter *L. plantarum* C8
- Study the rheological behaviour, phenolic content and antioxidant activity of soybean flour sourdough
- Evaluate the effect of soybean sourdough addition to Wheat Bread (WB) at three different levels. Subsequently, an integrated characterization (including texture, antioxidant and sensory features) of breads was carried out to select the best formulation and compare the advantages of the sourdough fermentation process with respect to the use of unfermented soybean flour.

MATERIALS AND METHODS

Materials: Commercial full-fat soybean and wheat flour retailed on the local market were used in the study. 1, 1-Diphenyl-2-Picryl-Hydrazyl (DPPH), 2, 4, 6-tris(2-pyridyl)-s-triazine (TPTZ), 6-hydroxy-2, 5, 7, 8-tetramethyl-2-carboxylic acid (Trolox), Gallic Acid (GA) and Ascorbic Acid (AA) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Proximate analysis of flour: The moisture, ash, protein and lipid contents of commercial flour samples were determined according to the Association of Official Analytical Chemists (AOAC) (AOAC, 1990) standard methods (925.10, 923.03, 920.87 and 922.06, respectively). Protein was calculated from total nitrogen using a factor of 5.7 for both wheat flour and soybean flour.

Genotypic identification of lactic acid bacteria used as starter: The strain *L. plantarum* C8 used in this study was previously isolated from chia sourdough and selected on the basis of acidification rate and proteolytic activity (unpublished data). The genotypic identification was determined on the basis of sequencing of the variable (V1) region of the 16S rDNA, as previously described (Hébert *et al*., 2000). The oligonucleotides for PCR reactions were: PLB16, 5′-AGAGTTTGATCCTGGCTCAG-3′; and MLB16, 5′-GGCTGCTGGCACGTAGTTAG-3′. PCR amplification consisted of 30 cycles of 30 s/94°C, 30 s/50°C and 1 min/72°C. All PCR runs included a blank control consisting of PCR-grade water and a non-template control. Final PCR products were purified using a commercial kit (AccuPrep® PCR Purification Kit; Bioneer Corporation, Genbiotech, Buenos Aires, Argentina) and subjected to sequencing (Servicio de Secuenciación, Centro Científico Tecnológico CONICET Tucumán, Argentina). The resulting sequences were analysed on line using the NCBI BLAST algorithms (National Center of Biotechnology Information, http://blast.ncbi.nlm.nih.gov/Blast.cgi) and the Ribosomal Database Project (http://rdp. cme. msu. edu/ seqmatch/ seqmatch_intro.jsp).

Preparation of soybean flour sourdoughs: *L. plantarum* C8 was grown in MRS broth (Sigma-Aldrich) at 37°C for 16 h. Cells were harvested by centrifugation (4000 *g* for 10 min) and washed twice with sterile saline solution. The mixture of soybean flour with tap water containing the cell suspension was prepared at 1% (w/v) to have a final cell density of 7 cfu/g dough and a dough yield [DY = (mass of dough/mass of flour) ×100] of 200. Mixing was done manually for 5 min. Doughs were fermented at 37°C for 24 h, the best conditions for growth and proteolytic activity of the starter culture. Cell growth was determined by the plate dilution method using MRS agar; the plates were incubated at 37°C for 48 h and results were expressed as log cfu/g dough. The sourdough pH was measured with a pH meter (pH 209; Hanna Instruments, Amorim-Povoa de Varzim, Portugal) and the organics were determined by high performance liquid chromatography (ISCO 2350 model) according to Gerez *et al*. (2009) using an Aminex HPX-87H ion-exclusion column (300 mm×7.8 mm, Bio-Rad, Hercules, CA). The fermentation quotient (FQ) was determined as the molar ratio between lactic acid and acetic acid.

Rheological measurements: Rheological determinations were carried out using a controlled stress rheometer (AR 2000, TA Instruments, New Castle, DE) with computer control software (Rheology Advantage Data Analysis Program) and according to Banu and Aprodu (2012) with modifications. The sourdough samples taken at zero time and after 24 h fermentation were fixed between the plates (d = 40 mm with a gap of 1500 μm), the overlaying dough was softly removed and the measurements started after a relaxation time of 10 min. Tests were conducted at constant temperature (25°C) using a Peltier plate system attached to a water circulation unit. Frequency sweeps were performed using a strain value in the linear viscoelastic region and a frequency range of 0.1-10 Hz. The rheological parameters measured were storage modulus (G’), loss modulus (G’’), complex modulus (G*) and tangent of loss angle (tan δ).

Wheat bread manufacture using soybean sourdough: WB were prepared with the addition of either unfermented Soybean Flour (SF-WB) or Soybean Sourdough (SS-WB) according to the formulations presented in Table 1. WB was used as reference. All preparations contained equal amounts of tap water,

Table 1: Formulations of different doughs

		SF-WD			SS-WD		
Ingredients	WD	A*	B	C	A	B	C
Wheat flour (g)	100	80	70	60	80	70	60
Soybean flour (g)	-	20	30	40	-	-	-
NaCl (g)	8	8	8	8	8	8	8
S. cerevisiae (g)	3	3	3	3	3	3	3
Soybean sourdough (g)	-	-	-	-	40	60	80
Water (mL)	55	55	55	55	35	25	15

WD: wheat dough; SF-WD: wheat dough with unfermented soybean flour; SS-WD: Wheat Dough with soybean sourdough, *A, B and C indicate the different wheat: soybean flour ratios, A = 4.0:1.0, B = 3.5:1.0 and C = 1.5:1.0

since the amount of this ingredient in the formulations containing sourdough was corrected. In all cases, commercial yeast *S. cerevisiae* (Calsa, Yeast Company Argentina S.A.) was used as leavening agent (7 log cfu/g dough). The following baking schedule was adopted: ingredient mixing for 10 min (first 2 min at slow speed and 8 min at increased speed), fermentation for 60 min at 30±1°C and 85% of relative humidity (rh), degassing, loaves hand-moulded into 200 g pieces, proofing for 60 min at 30±1°C and 85% rh and baking for 20 min at 180±1°C.

The bread loaves were cooled down at room temperature for 2 h and then sealed in polyethylene bags.

Dough hardness: Hardness of the doughs was determined using the universal testing machine TA.XT.Plus Texture Analyser (Stable Microsystems, Surrey, UK) according to Rizzello *et al*. (2016) with modifications. Dough samples were prepared as described in Table 1 without addition of yeast and were formed into balls of 10 g of dough and left to rest covered for 15 min before the analysis. A compression test was performed using a 20 mm diameter cylinder probe (TA-11) to compress the dough samples to 50 % of their original height.

Physical properties of breads: Loaf volumes were measured by the rapeseed displacement method (Mashayekh *et al*., 2008). Specific loaf volumes were calculated by dividing the loaf volume by the loaf weight and expressing the results as mL/g. The texture profile was determined using the universal testing machine TA.XT.Plus Texture Analyser (Stable Microsystems, Surrey, UK) following the procedure described above. Samples were prepared by cutting a 2-cm height sample, allowing only crumb texture measurements. The textural parameters were: hardness (g), chewiness (g) and springiness. Five replicates were used for colour measurement using a Minolta Chroma Meter CR400 colorimeter (Minolta CR-400, Osaka, Japan) calibrated against a standard light white reference tile; the measurements were performed under standard illuminant D65. Results were expressed in CIE (Commission Internationale d' Eclairage) system, where L* (lightness, L* = 0, black; L* = 100, white), a* (redness-greenness), b* (yellowness-blueness) colour space (Jambrec *et al*., 2011).

Total phenols and antioxidant activity of soybean sourdoughs and breads:

Extract preparation: Ten g of doughs as well as slices of bread were dried at 50°C for 12 h and then manually crumbed, grounded in a traditional stone mortar to obtain dust samples. For extraction, 1 g of dust sample was blended with 10 mL of 80 % (v/v) aqueous methanol solution for 2 h at room temperature. Samples were afterwards centrifuged (9000 *g* for 15 min). The supernatant (extracts) were stored at -20°C until use.

Determination of total phenols: Total phenolic contents were determined by the Folin-Ciocalteu method, as previously described (Ezekiel *et al*., 2013). Absorbance of the mixture was determined at 725 nm using the spectrophotometer Jasco V-630 (Medson, Paczkowo, Poland). Calibration plot was performed using GA as the reference compound in the concentration range of 15 to 200 ppm. Results were expressed as g GA/kg dry weight (dw).

Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity: Antiradical activity was measured according to Banu and Aprodu (2012) with modifications. A 1:10 dilution of the extract in methanolic DPPH radical solution (12 mg DPPH in 100 mL methanol; absorbance ~ 1.1) was vigorously shaken and allowed to stand at room temperature in the dark for 20 min. Then, the decrease in absorbance of the resulting solution was determined at 515 nm. A DPPH solution in methanol was used as blank, the absorbance (A) was measured at t = 0 (A control t = 0). Additionally, an Ascorbic Acid (AA) standard solution (concentration ranging from 10 to 60 µM) was measured under the same conditions. The antioxidant activity was expressed as µM of AA per gram of dry weight (µM AA/g dw) and in terms of percentage of discoloration calculated according to the following formula:

% DPPH radical discoloration: $(1\text{-Asample}_{t20}/\text{Acontrol}_{t0})\times 100$

Ferric Reducing Antioxidant Power (FRAP) assays: The FRAP assay was carried out according to Benzie and Strain (1996). Briefly, the oxidant in the FRAP assay (reagent mixture) consisted of ferric chloride solution (20 mmol/L), TPTZ solution (10 mmol

TPTZ/L in 40 mmol HCl/L) and acetate buffer (pH = 3.5) in a proportion of 1:1:10, respectively and was freshly prepared. To each reaction 0.05 mL of the methanolic extract was added to 0.95 mL of the reagent mixture, shaken vigorously and allowed to stand at room temperature in the dark for 20 min. The absorbance was afterwards measured and the final results were expressed as µg Trolox g/dw.

Sensory evaluation of breads: Sensory attributes of breads were evaluated by an untrained panel (45 tasters, mean age: 26 years, range: 18-35 years) using a qualitative 5-point hedonic scale: 1 ''very less'', 2 ''less'', 3 ''indifferent'', 4 ''much'' and 5 ''very much'' with respect to wheat bread used as control (Wronkowska and Soral-Śmietana, 2008). They assessed for colour, flavour and taste and crumb texture. The overall acceptability rating was calculated as the mean score of the organoleptic characteristics. The breads were sliced into 2-cm long portions. The panellists tasted approximately two slices of each sample wrapped in aluminium paper and identified with three-digit codes. Between samples, panellists rinsed their mouths with mineral water.

Data analysis: All measurements were carried out in at least three independent assays and the results were reported as mean values with the standard deviations. The Analysis of Variance (ANOVA) and LSD Fisher post tests were performed using InfoStat 2014, InfoStat, FCA, Universidad Nacional de Córdoba, Argentina; URL http://www.infostat.com.ar. All statistical analyses were performed at a significance level of $p<0.05$.

RESULTS AND DISCUSSION

Sourdough fermentation: The proximate composition of the soybean and wheat flours used in this study was determined. Protein (355±11 g/kg), ash (63±2 g/kg) and fat (209±10 g/kg) contents in soybean flour were higher than in wheat flour (146±11; 7±0.5 and 16±1 g/kg, respectively). These values are in line with those reported by other authors (He and Chen, 2013) and indicate that the fortification of WB with soybean flour could dramatically improve its protein content.

The soybean flour was fermented by *L. plantarum* C8 to obtain a sourdough. After 24 h of fermentation, the soybean sourdough reached a cell count of 9.34 cfu/g and final pH of 4.25±0.3. The pH decrease was associated with the production of organic acids by a lactic strain. The soybean sourdoughs showed high content in lactic (3.66 g/kg dough) and acetic (0.23 g/kg dough) acids. The value of FQ, given by the lactic acid/acetic acid ratio, was 15.9. This value is in the range of those reported by Rizzello *et al*. (2016) and Banu and Aprodu (2012) in sourdough made with quinoa and rye, respectively. In addition, phenyl lactic acid (73 µg/kg dough) was detected. This latter organic acid is produced by some strains of LAB and is a novel antimicrobial agent able to prevent fungal spoilage (Gerez *et al*., 2010).

The acidification observed in the soybean flour sourdough could alter the enzymatic activity of soybean flour and consequently change the physical properties of dough. Therefore, small deformation and oscillatory rheological measurements were performed in order to obtain information about the impact of lactic fermentation on the viscoelasticity of the soybean sourdoughs. Figure 1 shows the frequency sweeps for the soybean sourdough at the beginning and at 24-h fermentation. The oscillatory frequency sweep tests in the linear viscoelastic domain indicate that storage, loss and complex moduli had an increasing trend for the entire frequency domain and also that a solid-like behaviour was predominant (G' > G'') in both studied samples. The G* of the samples was also different: unfermented soybean dough showed a higher value (58300±4340 Pa at 1 Hz) than that of the 24 h-fermented sample (37375±375 Pa at 1 Hz). The spectrum analysis revealed a loss of consistency and an increase in the viscous character of sourdough with respect to the unfermented sourdough. Additionally, tan δ values confirmed the reduction of soybean sourdough elasticity due to the effect of the lactic fermentation process (tan δ 0.14±0.0013 and 0.20±0.0008 of soybean

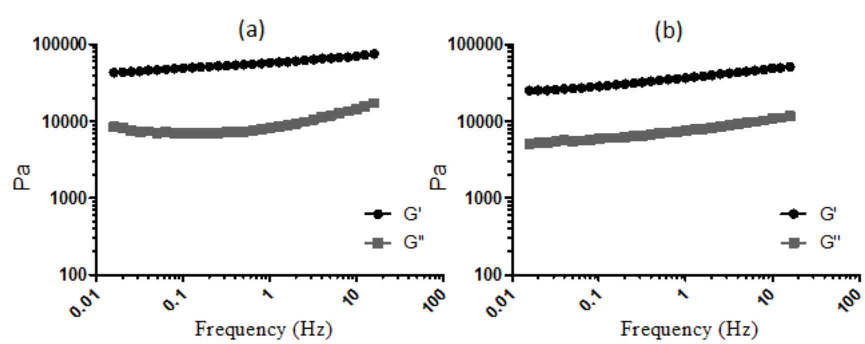

Fig. 1: Storage modulus (G') and loss modulus (G'') versus frequency; (a): Soybean sourdough at the beginning of fermentation; (b): soybean sourdough after fermentation

Table 2: Effect of soybean flour sourdough addition on dough hardness and specific volume of breads

Formulations	Dough hardness (g)	Bread specific volume (mL/g)
WD	930±77[b]	3.3±0.23[a]
SF-WD		
A	1080.5±45[c]	2.65±0.31[b]
B	1320.0±85[d]	2.21±0.23[c,d]
C	1650.0±76[e]	1.83±0.42[d,e]
SS-WD		
A	830.0±66[a]	3.28±0.20[a]
B	953.0±95[b,c]	2.44±0.22[b,c]
C	1260.0±66[d]	1.73±0.25[e]

WD: Wheat Dough; SF-WD: wheat dough with unfermented soybean flour; SS-WD: wheat dough with soybean sourdough, *A, B and C indicate the different wheat: soybean flour ratios, A = 4.0:1.0, B = 3.5:1.0 and C = 1.5:1. Variables with the same superscript letter in the same column show no significant differences between them (p<0.05)

dough and sourdough, respectively). Houben *et al*. (2010) and Moroni *et al*. (2011) reported a similar decrease in the complex shear modulus and elasticity for amaranth sourdoughs made with *L. plantarum* AL30 or *L. paralimentarius* AL28 and buckwheat sourdoughs prepared with *L. plantarum* AB 26, *L. brevis* AB 27 and *L. paralimentarius* AB 28, respectively. Enzymatic activity (particularly proteolytic) and the formation of organic acids are the main causes for the rheological changes observed in sourdough (Clarke *et al*., 2004). However, chemically acidified doughs (wheat, amaranth, among others) showed the opposite behaviour, resulting in more elastic dough probably because the acidification is instantaneous and not progressive as in the sourdough process and in this way, microbial and flour enzymes are not activated (Katina, 2005; Houben *et al*., 2010).

Technological characterization of doughs and breads:

Hardness of raw doughs: Soybean flour sourdough was added as an ingredient in baking formulations, observing different effects on the properties of wheat doughs and breads according to the addition level. In order to estimate bread quality, the hardness of raw doughs was measured. Wheat dough hardness showed a significant (p<0.05) increase with each addition level of unfermented Soybean Flour (SF-WD) (Table 2). The hardness of Wheat Dough with Soybean Sourdough (SS-WD) was lower than that of the SF-WD. Considering the high elasticity of the unfermented soybean sourdough, the hardness found in the SF-WD samples can be reasonably expected. Regarding the soybean sourdough systems, the lower hardness is related to their rheological behaviour and could be explained by gluten and protein denaturation caused by acidification. It was reported that in an acidic environment there is a sizeable positive net charge and protein solubility increases. The greater intramolecular electrostatic repulsion leads to an unfolding of the gluten proteins and prevents the formation of new bonds, the net effect of these events being a weakening of the structure and thus a softening of the dough (Arendt *et al*., 2007).

Specific volume of breads: In addition, the specific volume of breads was evaluated (Table 2). In control breads, the specific volume significantly (p<0.05) decreased as the percentage of soybean flour (SF-WB type A, B and C) in the formulation increased. Some authors also reported smaller loaf specific volumes of breads prepared with increasing concentrations of soybean and other flours and related these findings to a decrement in the gas retention capacity of the gluten network (Dhingra and Jood, 2004; Mashayekh *et al*., 2008; Taghdir *et al*., 2016). On the other hand, the effect of soybean sourdough on volume was also dependent on the substitution level. In fact, the specific loaf volume of the SS-WB type A was the highest and close to that of the WB used as reference, while the type C showed the lowest volume with a clear structure collapse at the highest substitution level.

Enzymatic reactions taking place during soybean sourdough fermentation could modify dough components, resulting in a softer and less elastic texture of SS-WB. The weaker structure allows the higher expansion of dough and as a consequence, an improved specific volume (Clarke *et al*., 2004). However, if the pH of sourdough is too low or the addition level is high, its addition could inhibit the CO_2 produced by yeast, resulting in less volume of bread (Katina, 2005; Moroni *et al*., 2011). In this sense, recent studies reported that the use of 20 % (w/w) of quinoa and wheat sourdough improved the specific volume of WB compared to the use of unfermented flours (Mamhoud *et al*., 2016; Rizzello *et al*., 2016). On the contrary, Yezbick *et al*. (2013) informed no differences in the specific volume of bread with the incorporation of 21.2 % (w/w) of fermented and unfermentd soybean-wheat dough, while Bartkiene *et al*. (2012) observed a decrease in the bread volume when soybean sourdough (86.5:13.5 wheat: soybean ratio) instead of raw soybean flour was added to WB.

Texture analysis: As shown in Fig. 2, the instrumental texture variables studied were affected by both the increase in soybean flour substitution level and sourdough addition. The hardness of the crumb increases with each addition level in all breads. However, the crumb of SS-WB was softer than that of SF-WB except at the highest sourdough concentration (type C) where the hardest crumb of all formulations was observed. As was mentioned earlier, as the level of substitution increases, changes in the structure of the dough occur, affecting the bread quality probably due to a decrease in the amount of gluten and an increase in fibre concentration. In this sense, Ribotta *et al*. (2005) showed that the breads with increased concentration of soybean flour required a larger compression force in

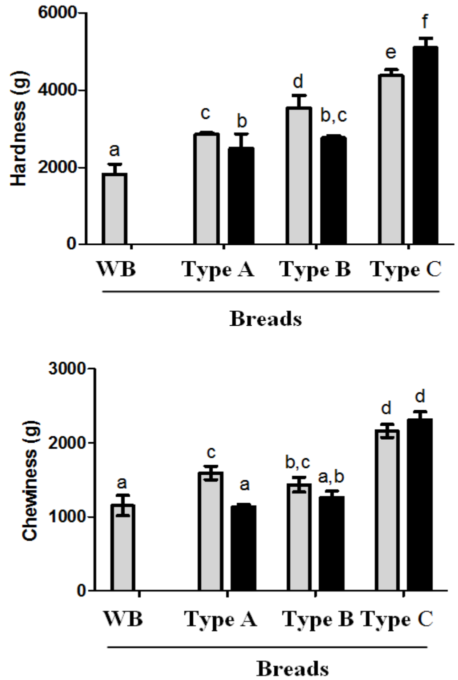

Fig. 2: Effect of soybean flour and sourdough addition on the textural parameters of breads; WB: wheat bread; Grey bars: wheat bread with unfermented soybean flour (SF-WB); black bars: wheat dough with soybean sourdough (SS-WB). * A, B and C indicate the different wheat: soybean flour ratios, A = 4.0:1.0, B = 3.5:1.0 and C = 1.5:1.0. Variables with the same superscript letter show no significant differences between them (p<0.05)

textural tests. Regarding the sourdough systems, the results could be explained by the textural and rheological behaviour of the doughs. In fact, the lower hardness and elasticity moduli (G') found in these systems (Table 2 and Fig. 1) could decrease bread hardness. On the other hand, the highest hardness of the SS-WB type C could be related to the lowest specific volume, probably showing a collapsed structure. In this sense, other authors mentioned a negative correlation between specific volume and hardness in wheat sourdough breads (Katina, 2005).

Chewiness, which can be interpreted as the energy required to chew solid food, presented a similar behaviour to that of hardness. Interestingly, between the SF-WB type A and B no significant (p<0.05) differences in chewiness values were observed. Since the chewiness parameter is the product of elasticity, cohesiveness and hardness, even though the hardness increased, the marked decrease in the cohesiveness and elasticity obtained in this sample caused the observed effect (data not shown). In SS-WB, as was observed in hardness, the chewiness was lower than that of SF-WB except at high addition level (type C) where the chewiness value was the highest. In this sense, Moroni *et al*. (2011) observed a significant (p<0.05) increase in chewiness and hardness at higher sourdough addition levels in buckwheat sourdough breads.

Finally, crumb springiness is a desirable and expected feature in fresh bread, which means that when it is subjected to compression, it could return to its original position. In evaluating this parameter, no significant (p<0.05) differences were found in all the systems studied (data not shown).

Colour of breads: Bread colour together with texture and flavour can determine the consumer's preference for the product. In this study, crust and crumb colour were affected by the substitution level of soybean flour as well as by sourdough addition (Table 3). In fact, a decrease in lightness (L*) as well as a significant (p<0.05) increase in a* and b* values were observed with each level of soybean flour or sourdough addition due to the characteristic colour of soybean flour. Also, other studies have shown that breads become darker and redder when soybean flour was added at increasing levels (López-Guel *et al*., 2012).

SS-WB type A showed an increase in lightness (L*) and a significant (p<0.05) decrease of redness (a*) values with respect to SF-WB type A. The higher volume reached in this formulation (SS-WB type A) could dilute the pigments present in the crust and would be responsible for these findings.

The crumb was less brown than the crust because the crust is formed during baking and the Maillard

Table 3: Crust and crumb colour bread analysis

	Crust			Crumb		
Samples	L (lightness)	a (green-red)	b (yellow-blue)	L (lightness)	a (green-red)	b (yellow-blue)
WB	63.06±4.50^{a}	9.13±2.54^{a}	30.96±1.83^{e}	72.61±1.50^{a,b}	-0.23±0.74^{a}	16.82±0.65^{a}
SF-WB						
A	57.22±3.82^{b}	12.09±1.11^{b}	16.45±3.45^{a}	71.50±1.45^{b}	0.47±0.15^{b}	17.87±1.47^{b}
B	51.87±2.26^{c}	13.16±0.64^{b,c}	19.30±1.74^{b}	69.09±1.29^{c}	0.96±0.11^{c}	20.08±0.75^{c}
C	46.53±2.54^{c,d}	14.38±0.60^{c}	24.16±2.21^{c}	67.21±1.28^{d}	1.40±0.19^{d}	22.29±0.75^{d}
SS-WB						
A	63.40±2.86^{a}	10.16±1.68^{a}	24.30±2.62^{c}	73.90±1.54^{a}	0.39±0.10^{c}	20.20±1.25^{a}
B	56.43±1.06^{b}	14.26±0.49^{c}	27.88±0.95^{d}	72.01±1.43^{b}	0.80±0.14^{d}	22.48±1.30^{b}
C	44.12±2.78^{d}	15.82±1.76^{d}	31.73±0.60^{d}	67.96±1.42^{c,d}	1.43±0.15^{e}	25.03±1.19^{c,d}

WB: Wheat Bread; SF-WB: wheat bread with soybean flour; SS-WB: wheat bread with soybean sourdough. *A, B and C indicate the different wheat: soybean flour ratios, A = 4.0:1.0, B = 3.5:1.0 and C = 1.5:1.0. Variables with the same superscript letter in the same column show no significant differences between them (p<0.05)

Table 4: Total phenolic content and antioxidant activity of sourdoughs and breads

Samples	Total phenolic content (g GA/g dw)	Antioxidant activity DPPH (% inhibition)	DPPH (µM AA/g dw)	FRAP (µg Trolox/g dw)
Soybean sourdough 0 h	1.55±0.08[a]	43.33±3.70[a]	2365.24±48[a]	6432.55±182[a]
24 h	2.38±0.05[b]	60.30±5.50[b]	3513.33±124[b]	8260.55±330[b]
WB	nd	4.02 ±0.25[a]	590.69±48[a]	450±15 [a]
SF-WB Type A*	0.71±0.04[c]	13.55±1.50[b]	1866.07±72[b]	2045.35±88[b]
SS-WB Type A	1.05±0.08[d]	22.02±1.70[b]	2914.33±98	2610.88±210[c]

WB: Wheat Bread; SF-WB: wheat bread with soybean flour; SS-WB: wheat bread with soybean sourdough; GA: Gallic Acid; dw: dry weight; AA: ascorbic acid; nd: not detected. *A indicates the wheat: soybean flour ratios of 4.0:1.0. Variables with the same superscript letter in the same group show no significant differences between them ($p<0.05$)

reaction occurs (Weenen, 1998). As was observed in the crust, a decrease in lightness (L*) as well as an increase in a* and b* values were observed with each soybean flour and sourdough addition level.

Phenolic compounds and total antioxidant activities of doughs and breads: Breads containing soybean sourdough type A showed the highest specific loaf volume, less hardness and crumb chewiness and an increase in lightness, as well as a decrease in red crust colour with respect to the control breads, displaying a behaviour close to that of WB. Based on these results, SS-WB type A was used for further phenolic content and antioxidant evaluations as well as for sensory analysis. The SF-WB type A (with unfermented soybean flour) was included as control.

Soybean flour has been shown to be a basic source of antioxidant compounds such as polyphenols, phospholipids, tocopherols, amino acids and peptides. The presence of Phenolic Compounds (PC) in the diet is beneficial to health due to their probable role in the prevention of various diseases associated with oxidative stress, such as cancer and cardiovascular diseases, mainly due to their antioxidant activity (Hole *et al*., 2012). In this study, the total PC in sourdough and breads was determined (Table 4). The PC content showed a significant ($p<0.05$) increase from 154.98±8.50 to 238.17±19.20 g GA/g dw after 24 h of sourdough fermentation. Antioxidant activity was evaluated by two complementary methods (FRAP assay and DPPH radical activity). Both methods indicate higher (1.3 to 1.5 times) antioxidant activity in the soybean flour sourdoughs at 24-h fermentation. The antioxidant activity of the sourdough is highly influenced by the metabolic activity of LAB used for fermentation. Other authors also reported an increase in the total PC and antioxidant activity in sourdoughs prepared from different flours such as rye, whole wheat, spelt, among others and fermented by LAB (Banu and Aprodu, 2012; Coda *et al*., 2012). As previously described, during sourdough fermentation, the esterase activities of LAB are able to hydrolyse the complex PC and their glycosylated forms and release the corresponding phenolic acids (Nionelli *et al*., 2014; Rizzello *et al*., 2012). The increased solubilisation of phenolic compounds might be related to the highest antioxidant activity found in soybean sourdough. In addition, the β-glucosidase activity of lactic strains leads to an increase of bioactive isoflavones and consequently, to high antioxidant activity of a fermented soy beverage (Marazza *et al*., 2012).

A significant ($p<0.05$) increase of PC and antioxidant activity was found in WB when soybean sourdough instead of the unfermented soybean flour was added (Table 4). In fact, the antioxidant activity of SS-WB was 1.3 to 1.6 and 6 times higher than that of SF-WB (with unfermented soybean flour) and WB, respectively. These results are consistent with the high activity observed in the soybean sourdough.

Consumer acceptance test: The consumer acceptance test was done by hedonic evaluation of the samples with a 5-point category scale. By analysing flavour and taste, a significantly ($p<0.05$) higher score (4.05 vs. 3.25) was reached by SF-WB type A compared with SS-WB type A, while the opposite behaviour was observed in crumb texture (3.45 vs. 3.65). Finally, no significant ($p<0.05$) differences were observed in colour score (3.60 vs. 3.50). In consequence, the overall acceptability of SF-WB was significantly ($p<0.05$) higher than that of SS-WB (3.70 vs. 3.47); however, this product was widely accepted by the consumers. Even though in our country there is no tradition in the consumption of breads with acid taste, the formulation of SS-WB type A was well accepted by consumers, probably due to the good technological profile observed in these breads.

CONCLUSION

In recent years, there has been an increasing interest in the so-called functional foods that provide physiological benefits, improving health or reducing the risk of disease beyond the basic nutritional requirements. In this sense, soybean is an excellent source of proteins, dietary fibres and a variety of micronutrients and phytochemicals, such as natural phenol antioxidants. In this study, a soybean flour sourdough fermented by a selected *L. plantarum* strain was characterized. This sourdough was used, at three different substitution levels, as an ingredient for the manufacture of WB and its characteristics were compared to those of bread made with or without unfermented soybean flour. According to our results, the best formulation was obtained by the use of soybean sourdough in the formulation of bread type A (4.0:1.0, wheat: soybean flour ratio). In fact, SS-WB type A showed the highest specific loaf volume, less hardness and crumb chewiness and an increase in lightness, as well as a significant decrease in red crust colour with respect to bread with unfermented soybean flour (SF-WB type A). Furthermore, sourdough incorporation increased 1.4 times the phenolic content with respect to that of SF-WB, 1.3 to 1.6 times the antioxidant activity compared to that of the control bread and 6 times with respect to traditional WB. In addition, SS-WB type A was widely accepted by the consumers. This study evidenced that sourdough fermentation modified the overall characteristics of soybean flour and its use could improve the physical and antioxidant properties of traditional wheat breads.

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