LWT - Food Science and Technology 65 (2016) 1008–1016

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Fibre enrichment of wheat flour with mesquite (*Prosopis* spp.): Effect on breadmaking performance and staling

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ARTICLE INFO

Article history:
Received 9 June 2015
Received in revised form
15 August 2015
Accepted 16 September 2015
Available online 24 September 2015

Keywords:
Mesquite flour
Prosopis alba
Wheat flour
Breadmaking
Texture

ABSTRACT

Leguminous flours provide both nutritional and functional benefits when they are incorporated in a bread formula. Mesquite tree, (genus *Prosopis*) is widespread in semiarid regions of America and its fruit contains a high proportion of sugars, minerals and fibre. The objective of the present study was to analyse the effect of wheat replacement with different levels (15–35 g/100g) of mesquite flour (from *Prosopis alba* pods) on the leavening performance of dough, the nutritional and technological quality of bread and bread staling.

Farinograph assays were applied to obtain optimum water amounts and development times. The addition of mesquite led to less stable dough with respect to control wheat dough. Fermentation assays showed higher fermentation times and lower maximum volumes. High levels of mesquite led to lower specific volumes of breads and a more compact and darker crumb, with smaller alveolus size, higher firmness and lesser resilience and cohesiveness. Breads were considerably improved in fibre content (6–9 g/100g). During storage, hardness and chewiness increased while cohesiveness and resilience decreased in all formulations. Nevertheless, textural changes were attenuated in composite breads in comparison with wheat bread. X-ray difractometry and differential scanning calorimetry (DSC) also indicated less retrogradation in these products.

1. Introduction

Functional foods are those that have a potential benefit on health beyond the contribution of nutrients. These additional effects can reduce the risk of diseases or promote health maintenance (Roberfroid, 1999). Products enriched with dietary fibre are included within these foods. Among them, functional breads and other cereal-based products are promissory options since they may be incorporated in the diet of a wide range of consumers.

Leguminous flours can provide both nutritional and functional benefits when added to a bread formula. They are usually a good source of dietary fibre and leguminous proteins are complementary to cereal proteins. When combined in composite dough, a protein mixture with higher nutritional value is obtained (Fenn, Lukow, Humphreys, Fields, & Boye, 2010).

The leguminous family includes more than 19,000 species (Magallón & Sanderson, 2001). Mesquite tree, known in several countries as “algarrobo”, belongs to this family; the genus *Prosopis* is widespread in semiarid regions of America (from southern United States to Argentina and Chile). The ripe fruit of mesquite tree is a yellowish to black pod (depending on the species) whose mesocarp (pulp) contains a high proportion of fibre. Prokopiuk, Cruz, Grados, Garro, and Chiralt (2000) reported 27 g/100g of total dietary fibre in *Prosopis alba* pulp. Besides, in *Prosopis* seeds, a galactomannan was found (Ibañez & Ferrero, 2003).

Mesquite flour is the product obtained by grinding the whole pods. Besides fibre, it also has proteins, lipids and provides calcium and iron, among other minerals. The level of protein is variable (7–11 g/100g) (Bigne, Puppo, & Ferrero, 2015; Prokopiuk et al., 2000). The amino acidic composition of mesquite proteins reveals the presence of important amounts of essential amino acids (except for methionine, isoleucine and threonine, depending on the species) (Felker & Bandurski, 1977). Because of the relatively high content of lysine, mesquite protein is a potential good complement for cereal proteins, which are deficient in this amino acid (Marangoni & Alli, 1988).

Wheat dough is characterized by its viscoelastic behaviour.

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http://dx.doi.org/10.1016/j.lwt.2015.09.028
0023-6438/

Extensibility and elasticity properties are due to the gluten network formed upon hydration of gliadins and glutenins during kneading. The addition of flours other than wheat can interfere with the formation of this network, leading to a disrupted structure and so, to a weakened dough with less stability and poor breadmaking performance (Doxastakis, Zafiriadis, Irakli, Marlani, & Tananaki, 2002; Giménez et al., 2012). Several authors (Bigne et al., 2015; Mohammed, Ahmed, & Senge, 2012; Roccia, Ribotta, Ferrero, Pérez, & León, 2010) found that the replacement of wheat flour with different amounts of leguminous flours or protein isolates obtained from them (chickpea flour, soy flour, mesquite flour) affected the rheological properties of dough due to network weakening and consequently, the quality of the final product such as volume, internal structure and texture of the breads (Mohammed, Ahmed, & Senge, 2014). When dough quality diminishes, a poor product is obtained since proper leavening and performance in the oven are related to good gluten development.

In a previous work, Bigne et al. (2015) reported that the high level of fibre in mesquite flour led to changes in the textural properties of dough, as a consequence of an inferior development of the gluten network. Higher levels of added mesquite flour led to dough with increased consistency and less cohesiveness, where a disruption of the gluten matrix was observed.

The objective of the present study was to analyse the effect of wheat replacement with different levels of mesquite flour (up to 35 g/100g) on the leavening performance of dough, the nutritional and technological quality of the final product, and bread staling.

2. Materials and methods

2.1. Materials

Wheat flour 000 type (according to the Argentinean Food Code), provided by Molino Campodónico SA (La Plata, Argentina), has the following composition (mean ± SD): proteins, 12.11 ± 0.03 g/100g (AACC 46-12); lipids, 1.32 ± 0.04 g/100g (by using a Soxhlet device); moisture, 13.74 ± 0.11 g/100g (AACC 44-19); ash, 0.07 ± 0.1 g/100g (AACC 8-1). Mesquite flour from white mesquite species was provided by local producers (Baguala) from Santiago del Estero (Argentina). The composition determined in our laboratory was: proteins, 11.13 ± 0.04 g/g (AACC 46-12); lipids, 1.13 ± 0.04 g/100g (by using a Soxhlet extractor device); total dietary fibre, 26.77 ± 0.95 g/100g (AACC 32-07); moisture, 6.01 ± 0.03 g/100g (AACC 44-19); ash, 3.59 ± 0.04 g/100g (AACC 8-1); and carbohydrates other than fibre (calculated by difference), 52.21 g/100g. Other ingredients used in dough formulation were: margarine (DÁNICA, Argentina), fresh yeast (CALSA, Argentina) and food grade sodium chloride (CELUSAL, Argentina), all of them obtained from the local market.

2.2. Dough formulation and breadmaking

Wheat flour was mixed with different amounts of mesquite flour at the following replacement levels: 0 (control sample), 15, 25 and 35 g/100g composite flour (0, 15, 25 and 35% of replacement, respectively). The upper limit for replacement was fixed according to preliminary assays. Replacement levels higher than 35 g/100g led to dough with poor handling properties and deficient bread volume development.

Formulations for doughs, per 100 g of flour or mixture of flours, were composed of: water according to farinographic absorption, 2 g salt, 3 g compressed fresh yeast and 4 g margarine. A planetary kneader (KENWOOD, Italy) was used for dough preparation. Dry ingredients were premixed, yeast was dispersed in water before addition, and margarine was melted and then added. Kneading time was fixed according to the farinographic development time for each formulation: 18.0, 14.5, 17.4 and 23.0 min for the control sample and mixtures with 15, 25 and 35 g/100g of mesquite flour, respectively (Bigne et al., 2015). After kneading, the dough was covered with a plastic film and left to rest for 10 min at room temperature. Later on, the dough was laminated to 1 cm thick and then left to rest for another 10 min.

Dough pieces of 90 g were made up and left to rest for 10 min and then, they were shaped in MPZ equipment (Argentina). These loaves were leavened at 30 °C applying the optimum fermentation time for each formulation, determined as indicated below. Then, the pieces were baked at 210 °C for 25 min in an electric oven (ARISTON, Argentina). Breads were left at room temperature for 1 h before carrying out the tests for bread quality assessment.

2.3. Optimum fermentation times

Pieces of 50 g of dough prepared as described above were introduced in graduated cylinders and left in a fermentation chamber at 30 °C. The increase in volume of the dough was recorded. Data were plotted with OriginPro 8 (Origin Lab Corporation, USA) software and fitted with Boltzmann sigmoidal function (equation (1)). The selection of the best model to fit the data was empirical.

$$y = A_2 + (A_1 - A_2)/(1 + exp((x - x_0)/dx)) \tag{1}$$

Where the dependent variable y corresponds to volume increase (ΔV, cm^3), the independent variable x is the time (min), and parameters A_1, A_2, x_0 and dx are constants.

A_2 is related to the maximum increase in volume. Optimal fermentation time was calculated as the time to reach three quarters of the maximum volume.

2.4. Bread quality tests

2.4.1. Specific volume

It was calculated as the ratio between piece volume (obtained by displacement of rapeseeds) and piece weight. Eight replicates were tested for each formulation.

2.4.2. Crust and crumb color

Color was measured using a colorimeter (Minolta CR-400, Minolta, Japan). CIELAB parameters were determined: lightness ($L^* = 100$ for white and $L^* = 0$ for black), red–green (a^*, positive values indicate red and negative values indicate green) and blue–yellow (b^*, positive values indicate yellow and negative values indicate blue).

2.4.3. Crumb structure

Slices from the middle part of breads (eight per formulation) were scanned in an HP Scanjet 4070 Photosmart scanner (Hewlett–Packard, USA) and pictures were analysed with ImageJ 1.48q software (National Institutes of Health, USA) to obtain the number, mean size, area occupied and circularity of alveoli. For these measurements, an inferior limit was fixed for alveolus size (0.2 mm^2). Alveoli with sizes greater than 30 mm^2 were also disregarded.

2.4.4. Crumb moisture

Samples were dried in an oven at 135 °C for two hours according to the official method (AACC 44-19 method).

2.4.5. Crumb texture

From the middle part of each bread piece, two slices 2 cm thick and 3 cm in diameter were obtained. A texture analyser TA.XT2i

(Stable Micro Systems, Surrey, U.K.) provided with a 25-kg load cell was used to perform TPA of crumb. Slices were subjected to a double compression cycle up to 40% of the original height using a flat probe (SMSP/75) of 75 mm diameter and 1 mm s^{-1} crosshead speed. Eight slices for each formulation were tested. The parameters determined were: hardness (maximum force of the first cycle), cohesiveness (area under the curve of the second peak with respect to the area of the first peak), springiness (ratio between the distance corresponding to the maximum of the second cycle and the original compression distance) and chewiness (calculated as the product of hardness, cohesiveness and springiness).

2.5. *Storage tests*

Breads were stored at 20 °C in polyethylene bags for 6 days. After 1, 3 and 6 days, samples were taken to analyse crumb texture and moisture loss, as described above.

2.5.1. *Crystallinity of samples*

The crystalline structure of wheat flour, mesquite flour, mixture of flours and lyophilized bread crumbs (obtained with and without 35% of wheat flour replacement with mesquite flour) was determined by polarized light microscopy in an Olympus BX60 microscope (Olympus, Japan) under polarized light after dispersion in mineral oil and mounting on a slide.

2.5.2. *Starch retrogradation of crumbs*

Crumb samples from control bread and formulation with 35% of flour replacement, stored for 0 and 6 days, were taken to analyse starch retrogradation by X-ray diffractometry. Crumb samples were freeze–dried and pulverized. Pulverized samples were analysed in duplicate in a PW 1032 diffractometer (Philips, Netherlands) set to 40 kV and 20 mA. Diffraction patterns were obtained using Cu–K α radiation, scanning 2θ angles from 4° to 40° at a rate of 1°/min, and a step size of 0.02°. The diffraction patterns were analysed with PeakFit Version 4.12 software (SeaSolve Software Inc., USA) and relative crystallinity was calculated as the ratio between crystalline area (sum of all peak areas detected above the amorphous area) and the total area (crystalline plus amorphous area).

2.5.3. *Amylopectin retrogradation by DSC*

Gelatinization and amylopectin crystallization during storage were evaluated through differential scanning calorimetry (DSC). Dough samples were heated at 10 °C/min up to 140 °C in differential scanning calorimeter equipment (Q100 TA Instruments, USA). As a reference, an empty pan was used. Onset (T_0), peak (T_{pI} and T_{pII}) and end (T_f) temperatures were calculated, as well as the enthalpy value (ΔH_g). The peak temperature for amylose–lipid complex dissociation was also determined.

Amylopectin retrogradation during storage was studied. Dough samples were heated from 5 to 105 °C at 10 °C/min (in order to simulate the baking process), and then stored at 20 °C. At each storage time (0, 1, 3 and 6 days) a sample was heated from 5 to 140 °C at 5 °C/min. Onset, peak and final temperatures, and enthalpies of amylopectin retrogradation were measured. The retrogradation index (RI) was calculated as the ratio between ΔH (after storage) and ΔH_g, and expressed as percentage.

2.6. *Composition analysis of breads*

Moisture (AACC 44-19), ash (AACC 08-01), lipids (AACC 30-10), proteins by Kjeldahl method (AACC 46-12) and total dietary fibre by enzymatic method (AACC 32-05) were determined. Assays were performed at least by duplicates.

2.7. *Statistical analysis*

ANOVA and ANCOVA were performed to determine significant effects of treatments when necessary; LSD test was applied to compare mean values. Software Statgraphics Centurion XV version 2.15.06 (USA) was used to perform the analysis.

3. Results and discussion

3.1. *Dough performance during leavening*

Typical fermentation curves for doughs and their modelling curves are shown in Fig. 1. Adjusted R^2 was higher than 0.99 in all cases. For control dough, the maximum volume increase (A_2) was 109.7 ± 6.5 cm^3, while the optimum fermentation time was 62 ± 3 min. The addition of mesquite flour led to a reduction in maximum volume and increased fermentation times. When wheat flour was replaced with 15, 25 and 35 g/100g of mesquite flour, maximum volume values diminished to 95.1 ± 0.2, 93.8 ± 5.7, and 72.2 ± 4.3 cm^3, respectively. Optimum fermentation times obtained for doughs with 15%, 25% and 35% of flour replacement were 76 ± 3, 88 ± 3, and 109 ± 1 min, respectively. These times were taken as the optimum ones for bread leavening.

The increase in optimum fermentation times and the decrease in dough expansion during leavening can be related to rheological changes in the dough matrix (increased consistency and diminished cohesiveness), previously observed by Bigne et al. (2015), due to the replacement with mesquite flour. Mesquite enriches the formulation with fibre and also exerts a dilution effect on gluten content. Fibre also interacts with gluten proteins inhibiting the free expansion of wheat dough during this stage (Wang, Rosell, & Benedito de Barber, 2002). Salinas, Zuleta, Ronayne, and Puppo (2011) reported that the addition of inulin to wheat flour led to doughs with increased consistency and reduced cohesiveness. This behaviour was also found for other composite doughs containing leguminous flours or isolates. Mohammed et al. (2012) found that the replacement of wheat flour with chickpea flour rendered weaker doughs with less extensibility. In the same way, soy flour and soy protein isolates were unfavourable for gluten formation, affecting dough extensibility and gas retention capacity in composite doughs (Ribotta, Arnulphi, León, & Añón, 2005).

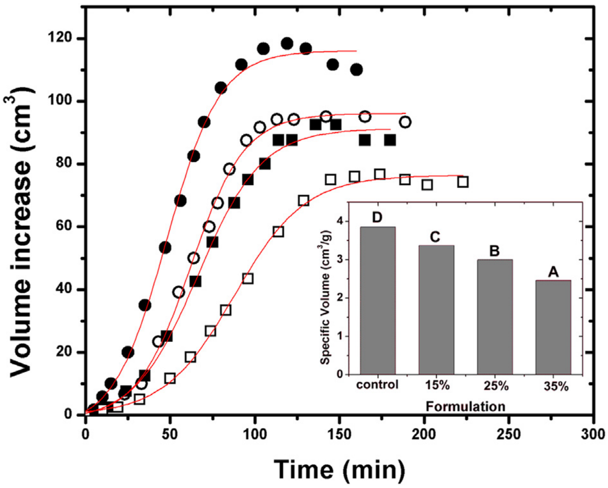

Fig. 1. Typical fermentation curves, corresponding to wheat-mesquite mixtures at different levels of wheat replacement. (●) control, (○) 15% mesquite (w/w), (■) 25% mesquite (w/w) and (□) 35% mesquite (w/w). Lines correspond to fitting with Boltzmann model. Right-inferior corner insert: specific volume of wheat and wheat-mesquite breads (15–35% mesquite). Different letters above bars mean significant differences ($p < 0.05$).

3.2. Fresh bread quality

Values of specific volume for the different breads are included as an insert graph within Fig. 1. Partial replacement of wheat flour with mesquite flour led to significant decreases in the specific volume of breads. The mean value (±SD) obtained for control bread was 3.85 (±0.13) cm^3/g. The replacement of 15%, 25% and 35% of wheat flour led to significantly lower values: 3.37 (±0.13), 3.00 (±0.20), and 2.46 (±0.14) cm^3/g, respectively. Thus, reductions in specific volumes were 12.47%, 22.08%, and 36.10% (against the control). The significant reduction in specific volume when mesquite proportion was increased can be related to the lower expansion of dough during leavening, as shown in Fig. 1.

The quality of bread crumbs corresponding to different formulations can be observed in the photographs of Fig. 2. Less spongy and more compact crumbs were obtained with increasing amounts of mesquite. Size distributions of alveoli were obtained for each bread formulation (histograms in Fig. 2). As expected, the relative frequency of smaller alveoli (<4 mm^2) was higher (89.7%–90.9%) in breads with a high content of mesquite flour (25 and 35 g/100g) than in control bread (85.8%). Concurrently, the frequency of greater size alveoli (>4 mm^2) decreased in the presence of mesquite from 14.2% (control bread) to 10.3% for bread with 35 g/100g of mesquite flour.

Other properties of alveoli such as alveolus density, mean size, void area and circularity were also determined (Table 1). Significant differences were found between control bread and those with the highest levels of mesquite (25 and 35% of replacement). The latter presented higher alveolus density and lower alveolus size and circularity. Nevertheless, with 15% of replacement no significant differences in alveolus parameters with respect to the control sample were detected, with the exception of decreased circularity. Circularity is linked to the capacity of expansion of gas cells that is related, in turn, to the matrix extensibility. In this way, Gan, Galliard, Ellis, Angold, and Vaughan (1992) reported that bran disrupted the gluten matrix and forced the gas cells to expand in a particular dimension, distorting the gas cell structure in bread crumb.

Color is one of the most important attributes of bread crumb. Mesquite flour addition had a decisive influence on final crumb color. Color development can be attributed to the intrinsic brownish color of mesquite flour and to the occurrence of Maillard

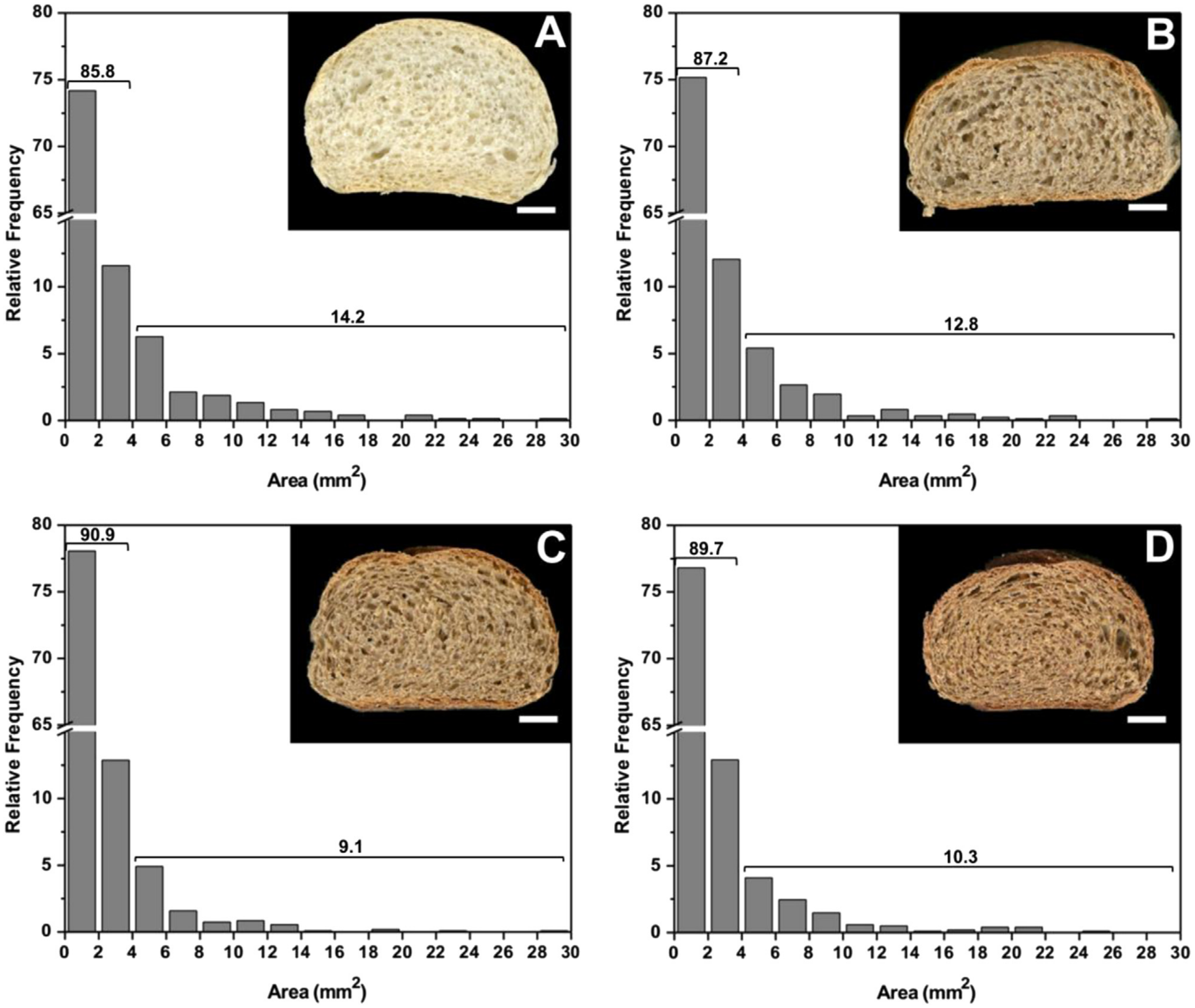

Fig. 2. Histograms of alveoli size distribution in bread crumb for: control (A) and composite breads with 15 (B), 25 (C) and 35% (D) wheat flour replacement by mesquite. Crumb appearance can be observed in pictures (Bar = 10 mm).

Table 1
Alveolate and color properties of composite breads with different replacement fractions of mesquite (*Prosopis*) flour.

Replacement fraction (%)	Alveolus parameters				Crust color			Crumb color		
	Density (nº/mm^2)	Mean size (mm^2)	Void area (%)	Circularity	L*	a*	b*	L*	a*	b*
0	0.17 ± 0.02 a	2.14 ± 0.27 c	36.2 ± 0.5 b	0.45 ± 0.01 b	77.37 ± 1.28 d	1.50 ± 0.51 a	21.64 ± 1.28 d	72.93 ± 2.00 c	−1.10 ± 0.11 a	14.41 ± 0.21 c
15	0.18 ± 0.01 a	1.96 ± 0.17 bc	34.6 ± 2.7 ab	0.39 ± 0.02 a	56.27 ± 0.83 c	8.66 ± 0.28 c	17.26 ± 1.06 c	59.04 ± 1.76 b	2.57 ± 0.16 b	12.16 ± 0.49 a
25	0.22 ± 0.02 b	1.61 ± 0.22 a	34.4 ± 1.5 a	0.39 ± 0.02 a	49.30 ± 0.41 b	8.24 ± 0.57 c	11.95 ± 1.54 b	56.94 ± 1.14 a	4.01 ± 0.07 c	13.22 ± 0.33 b
35	0.20 ± 0.02 b	1.77 ± 0.20 ab	35.6 ± 1.0 ab	0.40 ± 0.01 a	45.46 ± 0.41 a	6.75 ± 0.11 b	6.44 ± 0.27 a	55.34 ± 0.64 a	5.26 ± 0.10 d	14.01 ± 0.41 c

Measurements were made in octuplicate for each bread formulation; mean values ± SD are reported.
Different letters within the same column indicate significant differences ($p < 0.05$); LSD test was used to compare the mean values.

reaction that is favoured by certain mesquite flour components (free sugars and proteins). Values for parameters L*, a* and b* are listed in Table 1. Significant differences were observed in crust and crumb color in all bread formulations. In crust, luminosity and b* progressively decreased when mesquite proportion increased, while parameter a* increased in wheat-mesquite breads. In crumbs, parameter a* also increased but changes in b* were not so marked as in crust and differences were not significant in all cases. These results indicate that, in general, replacement with mesquite led to an increase of the reddish component and a decrease of the yellowish one. Though color development in breads can be a positive aspect for the consumers' acceptance, Maillard reaction renders acrylamide as a by-product, which has been assessed as a carcinogen in humans. Degree of acrylamide formation depends on numerous processing factors and intrinsic characteristics of samples (Virk-Baker, Nagy, Barnes, & Groopman, 2014). This aspect should be further investigated in mesquite breads.

Crumb texture parameters of fresh breads are shown in Fig. 2 (day 0). Crumb moisture values ranged between 32.27 and 33.98 g/100g, thus differences in crumb texture cannot be mainly attributed to changes in this parameter.

Crumb hardness and chewiness gradually and significantly increased ($p < 0.05$) with increasing mesquite flour proportion. As described above, breads with a high content of mesquite exhibited less specific volume and a more compact crumb, which could explain the higher hardness values. The correlation coefficient between hardness and specific volume was 0.986 (negative correlation). Chewiness depends on hardness, so it followed the same trend.

According to Attenburrow, Goodband, Taylor, and Lillford (1989), the compression modulus of a spongy material (like crumb) is directly related to the cell wall modulus and the foam bulk density, and inversely related to the density of the cell wall. Besides the effect on alveolus number and size, mesquite flour could have a hardening effect on alveolus walls, probably related to the high amount of fibre contained in this flour.

Cohesiveness and resilience showed the opposite behaviour. Mesquite led to less cohesive and more disintegrable crumbs but no significant differences ($p > 0.05$) were found among formulations with different levels of replacement with mesquite flour. Resilience, related to the ability of instantaneous recovering after a deformation, diminished after mesquite addition and significant differences ($p < 0.05$) were observed among different levels of replacement. Resilience positively correlated with specific volume ($r = 0.968$).

3.3. *Nutritional qualities of breads*

Results from composition analysis of breads are shown in Table 2. Only slight differences were found in moisture and lipid content between formulations. However, the mineral content increased significantly with mesquite incorporation. Protein content decreased lesser than 1% in bread with the maximum mesquite level.

The most relevant nutritional aspect is related to the fibre content of breads. With only 15/100g replacement of wheat by mesquite flour, total dietary fibre content increased more than twice, reaching a value of 6.94 g/100g. This means that composite wheat-mesquite breads could support the claim “high content of dietary fibre”, according to the provisions of FAO/WHO (ALINORM 09/32/26, 2009), which establish a minimum of 6 g/100g.

3.4. *Changes during storage*

Crumb staling is related to moisture loss and starch retrogradation, particularly amylopectin retrogradation (Gray & Bemiller,

Table 2
Composition analysis of wheat-mesquite breads.

Replacement fraction (%)	Moisture (g/100g)	Ash (g/100g)	Proteins (g/100g)	Lipids (g/100g)	TDF (g/100g)
0	33.98 ± 0.93 b	1.82 ± 0.01 a	9.83 ± 0.12 c	2.34 ± 0.52 ab	2.53 ± 0.54 a
15	33.12 ± 0.30 ab	2.23 ± 0.05 b	9.61 ± 0.01 bc	2.63 ± 053 ab	6.94 ± 0.80 b
25	32.88 ± 0.74 ab	2.45 ± 0.04 c	9.38 ± 0.16 b	2.71 ± 0.03 b	8.43 ± 0.52 c
35	32.27 ± 1.00 a	2.53 ± 0.01 c	8.87 ± 0.04 a	2.24 ± 0.25 a	9.26 ± 0.56 c

Mean values ± SD are reported. TDF: total dietary fibre. Total carbohydrates different from fibre were not determined.
Different letters within the same column indicate significant differences ($p < 0.05$); LSD test was used to compare the mean values.

2003; Ronda, Quilez, Pando, & Roos, 2014). During storage of mesquite-wheat breads, progressive deterioration of crumb texture was observed. Hardness and chewiness increased, while resilience and cohesiveness diminished. The changes of these parameters during storage (day 0–6) can be observed in the line graphs of Fig. 3. Covariance analysis was performed with time as an independent variable and replacement levels as the treatment; it was assessed that storage time had a significant effect ($p < 0.05$) on all the textural parameters of mesquite breads. Several authors have studied changes in hardness or firmness during storage of bread systems, and have reported a nonlinear behaviour, that can be fitted with the Avrami exponential equation (Besbes, Jury, Monteau, & Le Bail, 2014; Le-Bail, Boumali, Jury, Ben-Aissa, & Zuniga, 2009; Le-Bail, Leray, Perronnet, & Roelens, 2011; Ronda & Roos, 2011). In the present work, as the plateau was not probably reached within the storage period, staling rates were analysed only in the initial stage (up to 3rd day) by performing a linear regression of hardness data. The adjusted R^2 were higher than 0.847 in all cases and the slopes obtained for those models were 2.24; 3.28; 2.83 and 3.43 N/day (SE < 0.21 in all cases) for 0, 15, 25 and 35% replacement levels, respectively. Replacement with mesquite led to significant increases in slopes (regarding to control), thus suggesting a promotion of hardening at the initial stages. However, at the end of the storage period hardness value had increased more than fivefold (540%) for control bread while in wheat-mesquite breads this effect was attenuated, particularly at the highest mesquite content (35% of replacement), with an increment in hardness of 286%. Chewiness followed the same trend and after 6 storage days, it had increased 304% and 124% for control and bread with 35% replacement, respectively. Other textural properties were less affected; resilience diminished between 51.3% and 55.1% and cohesiveness between 29.1 and 37.7%, after 6 storage days.

A similar effect on crumb hardening was previously reported by Katina, Salmenkallio-Marttila, Partanen, Forssell, and Autio (2006) for bran-enriched bread where higher firmness values were observed for fresh bread respect to non-enriched bread, but the change during storage was attenuated.

3.5. Thermal behaviour of composite dough and crystallinity

Gelatinization affects the native organization of starch granules, with the consequent loss of initial crystallinity. However, bread crumb is an intermediate moisture system, and thus, gelatinization is restricted, leading to a split of the gelatinization peak in

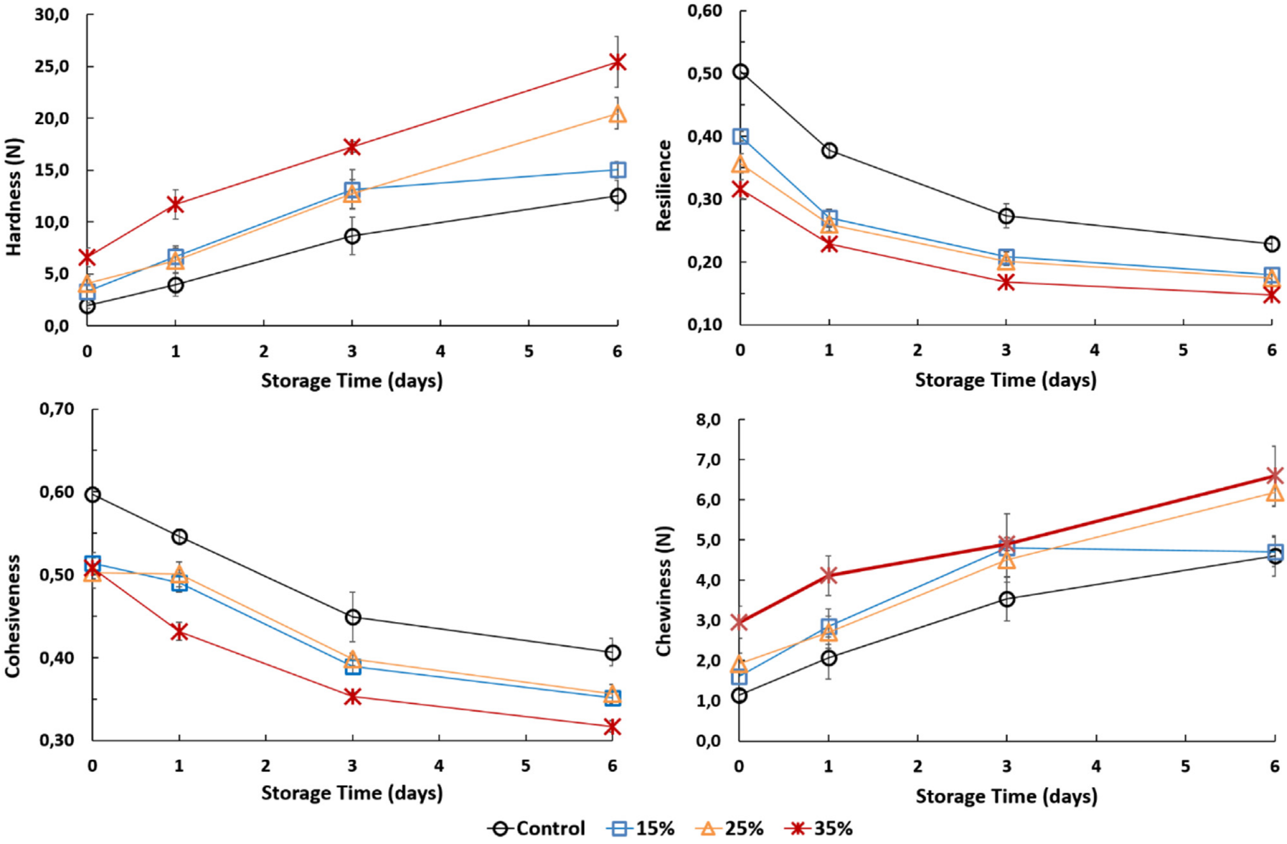

Fig. 3. Textural parameters as function of storage time for different bread formulations.

Table 3
Temperatures and enthalpies of starch gelatinization and amylopectin retrogradation in doughs with different replacement fractions of mesquite flour.

Starch gelatinization

Replacement fraction (%)	T_0 (°C)	T_{pI} (°C)	T_{pII} (°C)	T_f (°C)	ΔH (J/g*)
0	63.57 ± 0.06 a	70.87 ± 0.04 a	90.59 ± 0.30 a	102.18 ± 0.56 a	9.24 ± 0.70 a
15	67.12 ± 0.20 b	74.25 ± 0.12 b	91.69 ± 0.28 a	101.19 ± 0.70 a	11.01 ± 0.56 b
25	70.18 ± 0.11 c	78.54 ± 0.23 c	94.09 ± 0.67 b	102.40 ± 1.06 a	10.99 ± 0.29 b
35	74.71 ± 0.31 d	81.63 ± 0.29 d	ND	102.51 ± 0.97 a	10.33 ± 0.41 b

Amylopectin retrogradation

Replacement Fraction (%)		T_0 (°C)	T_p (°C)	T_f (°C)	ΔH (J/g*)	RI (%)
Day 3	0	50.76 ± 1.11 a	60.45 ± 0.43 a	74.50 ± 1.45 a	1.23 ± 0.09 b	10.82
	35	51.35 ± 1.39 a	64.00 ± 1.68 b	77.30 ± 1.05 b	0.41 ± 0.16 a	3.97
Day 6	0	50.87 ± 0.99 a	59.74 ± 0.39 a	74.03 ± 1.22 a	1.72 ± 0.23 c	18.61
	35	50.26 ± 0.37 a	61.94 ± 1.58 ab	76.35 ± 0.06 ab	0.72 ± 0.004 a	6.97

Measurements were made in triplicate for each dough formulation; mean values ± SD are reported.
*g starch; ND: not determined.
Values with the same letter within the same column are not significantly different ($p > 0.05$); LSD test was used to compare the mean values.

thermograms (Correa & Ferrero, 2015). Gelatinization parameters are shown in Table 3. Enrichment with mesquite flour led to significantly higher values of the onset (T_0) and first peak (T_{pI}) temperatures, which were progressively shifted when mesquite amount was increased. However, final temperatures (T_f) were not affected. These shifts would also indicate a restriction in gelatinization related to the presence of fibres and sugars from mesquite. At 35% of replacement, only one peak was detected. Mesquite flour, and particularly mesquite fibre, would lead to a more cooperative process but at higher temperatures. The extent of starch gelatinization increases in the presence of fibre, as indicated by the higher gelatinization enthalpies. Fibre could affect water distribution, allowing better water availability for gelatinization even in the presence of gluten.

Polarized light microscopy was used to analyse the presence of crystalline structures in mixture components and bread crumb. Micrographs are shown in Fig. 4. It can be observed that wheat and mesquite flours and their mixture (35% p/p mesquite) exhibit a marked birefringence (Fig. 4A, B and C). In wheat flour, crystallinity corresponds to native starch granules. In mesquite (*Prosopis*), birefringence could be assigned to cellulosic and other fibrous materials highly present in cell walls, as reported by Bravo, Grados, and Saura-Calixto (1998) and to other components such as sugars (mainly sucrose). These results are in agreement with the diffraction pattern obtained for mesquite flour, where several peaks in the range from 10 to 30° were detected, indicating crystalline structures (Fig. 5A). These peaks could be assigned to components from mesquite flour. Liu and Hu (2008) analysed cellulosic fibres of

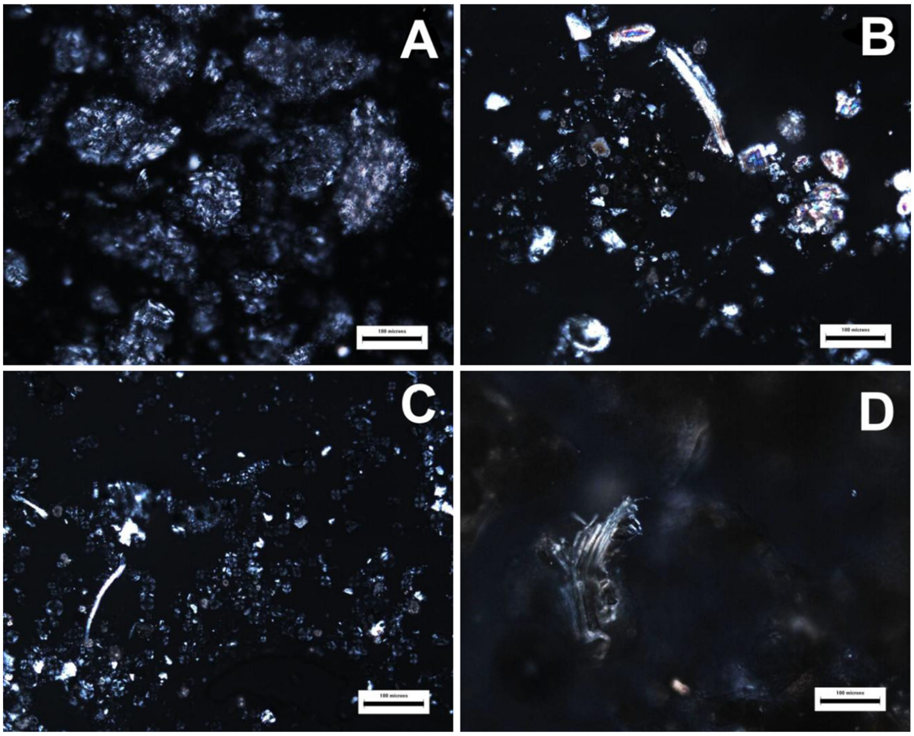

Fig. 4. Polarized light micrographs of: A, wheat flour (20X); B, mesquite flour (20X); C, flour mixture (35% mesquite) (20X); D, lyophilized crumb of bread 35% (20X). Bar in micrographs = 100 μm.

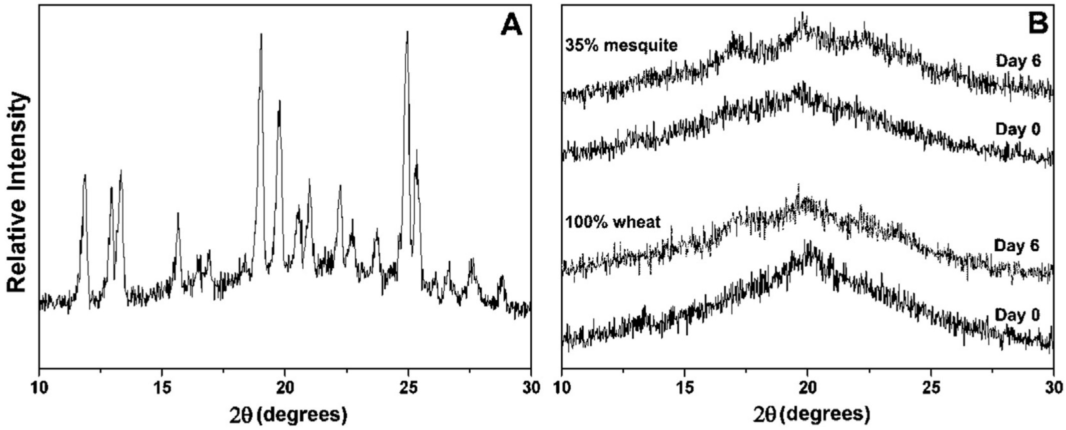

Fig. 5. Typical diffractograms of A) mesquite flour and B) lyophilized control and wheat-mesquite (35%) crumbs without storage and after 6 days of storage.

bamboo using X-ray diffraction and observed crystallinity peaks at 2θ angles of 15, 16.3 and 22.7°, and Tserki, Zafeiropoulos, Simon, and Panayiotou (2005) studied fibres of flax, hemp and wood, rich in cellulose, hemicellulose and lignin, and found characteristic peaks close to 2θ angles of 16° and 22°. Besides, characteristic double strong peaks close to 19 and 25° could be attributed to those reported for sucrose at 18.9, 19.7, 24.8 and 25.2° (Chinachoti & Steinberg, 1986).

Fig. 4D shows the micrograph obtained for crumb of wheat-mesquite bread (35% of replacement) at 0 day of storage. As expected, starch granules have lost their characteristic birefringence, and the residual crystalline structure would correspond to mesquite components. Diffraction patterns of lyophilized crumb from control bread and 35% wheat-mesquite bread, after 0 and 6 storage days, are shown in Fig. 5B. Crystallinity peaks are detected close to 2θ angles at: 13, 15, 17, 20, 22, and 24°. The lowest value of total crystallinity was obtained for fresh control bread (11.40 ± 0.91%). Total crystallinity significantly increased after six days of storage (20.57 ± 1.73%), mainly due to augmented peaks at 17, 22 and 24 of 2θ angles. The increase in the area of these peaks is associated with recrystallization of melted starch (Ribotta, Cuffini, León, & Añón, 2004).

The crystallinity of crumb from wheat-mesquite bread was significantly higher (23.58 ± 2.79%) than that of control bread at day 0 of storage, which is in agreement with the greater presence of crystalline structures from mesquite flour, as commented above.

After 6 days of storage, the increase in crystallinity in these breads (26.65 ± 2.51) was significant but lower than expected, suggesting a certain inhibition of retrogradation. Through DSC assays (Table 3) it could be assessed that the extent of amylopectin retrogradation was 1.72 ± 0.23 J/g starch in wheat systems, but it was limited in the presence of mesquite flour (0.72 ± 0.004 J/g starch). Other works have also remarked the attenuating effect of high contents of insoluble fibre on starch retrogradation (Ronda et al., 2014). This behaviour would also explain the attenuated changes in hardness observed during storage.

4. Conclusions

The addition of mesquite flour markedly improved fibre content (6–9 g/100g) in final products but markedly affected breadmaking and bread characteristics. Mesquite addition led to longer leavening times and also diminished the expansion of dough during leavening (lower final volumes). As a consequence, final specific volumes of composite breads were lower than that of control wheat bread. Diminished volume was related to a change in alveolus characteristics: more alveoli with smaller sizes and loss of circularity led to more compact breads. Crumb texture changed. Hardness and chewiness increased, and cohesiveness and resilience decreased when higher contents of mesquite flour were added (25–35%). The reduction in the technological quality of composite breads, compared with wheat bread, can be associated with the dilution of gluten proteins with flour replacement, and the presence of new components (like fibre) in the system, which can affect the viscoelastic properties of the dough and consequently, the crumb structure. Storage of breads led to increased hardness and chewiness, and loss of cohesiveness and resilience in all formulations. However, these changes were attenuated in composite breads in comparison with wheat bread. This can also be related to less starch retrogradation, as evidenced by DSC and X-ray assays.

Acknowledgements

Authors are grateful to University of La Plata and CONICET (Argentina) for the funds (UNLP Projects X517, A230 and PIP CONICET 0354) and fellowships received. Authors want to thank the kind cooperation of our colleague, Dr. Alicia R. Chaves for the statistical revision of the manuscript.

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