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Effect of glutens of different quality on dough characteristics and breadmaking performance

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ABSTRACT

Glutens of different quality were extracted from commercial flours of distinct breadmaking performance and employed as improvers at a level of 1 g/100 g. The same flours used as a source of gluten were employed for testing the gluten effect. Flours were characterized by farinographic and alveographic assays and their protein profile was determined by SDS-PAGE. Rheology of each dough without and with gluten addition was studied by empirical and fundamental assays. Breadmaking performance was evaluated by loaf volume measurements and crumb texture.

Though protein content was similar for all flours (11 g/100 g), dough exhibited different breadmaking characteristics which could be related to a different gliadin/glutenin proportion and a different protein profile. The weakest flour lacked two glutenin subunits (83 and 64.5 kDa) and showed a lower number of bands of gliadins respect to the other ones. Adding any of the three types of gluten to the weakest flour resulted in an increase of farinographic stability. The medium and inferior quality flours showed an increase in dough elasticity when the strongest gluten was added. In breadmaking assays the medium quality flour and its mixtures with gluten showed the highest specific volumes.

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1. Introduction

Cereal grains and flour based products represent the main source of calories in human diet since ancient times until nowadays. Among cereals, rice and wheat are the most extended. In spite of their poor nutritional value, cereals also remain as the main source of proteins in many developing regions (Ruiz Camacho, 1981).

The unique breadmaking aptitude of wheat flour is related to its complex protein composition. Variation in the composition of the high molecular weight prolamins (the HMW subunits of glutenin) has been strongly correlated with differences in the breadmaking quality. The major part of these variations in breadmaking performance can be related to variations in the profile of gluten proteins, gliadins and glutenins (Shewry, Tatham, Barro, Barcelo, & Lazzeri, 1995; Wieser, 2007). These macromolecules are capable of imbibing water even up to three times their dry weight, and under mechanical stress, rendering a viscoelastic network that is responsible for gas retention during leavening and the further expansion of loaves during baking.

The quality and proportion of these storage proteins widely vary with the wheat type (genetic aspects), the environmental conditions and the interaction genotype-environment, thus conducting to marked differences on breadmaking performance.

In order to obtain a constant level of quality in breadmaking products, the industry usually employs several types of additives of diverse chemical structure to reinforce the gluten network (Stauffer, 1990). Among these additives, vital gluten represents a widely used alternative.

Vital wheat gluten is a valuable co-product of the wet milling process that yields wheat starch (Bergthaller, 1997; Grace, 1988; Maningat & Bassi, 1999; Maningat, Bassi, & Hesser, 1994). According to the Codex Standard of the Food and Agriculture Organization (FAO), wheat gluten must contain a minimum of 80 g/100 g (N × 6.25) protein, which is ~73 g/100 g when calculated by the N × 5.7 conversion factor used for wheat and wheat products. Typically, commercial wheat glutens have 5–10 g/100 g moisture and 73–82 g/100 g protein (N × 5.7), 3–20 g/100 g carbohydrates (primarily starch), 5–8 g/100 g total lipids (1–2 g/100 g free lipids), 0.5–1.5 g/100 g ash, and ≈1 g/100 g pentosans (Maningat et al., 1994; Wadhawan, 1988).

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The vital gluten (VG) can be used as a flour improver and being itself a flour component, there are no restrictions on its use, as it occurs with other additives. Normally, the production of VG is based on the formation of wheat dough until the wet gluten mass is obtained and then washed for splitting the starch granules and other soluble components from it while the gluten matrix stays insoluble (McDermott, 1985; Stauffer, 1990). Afterward, wet gluten must be dried; the initial moisture must be reduced up to 6–8 g/100 g. The drying step is the critical one because drastic heating conditions can lead to protein denaturation (called in this case "devitalization"). The critical temperature for this procedure is around 55–60 °C.

Commonly, the gluten that is commercialized and used as an additive is not classified according to its protein profile or quality. However, it would be expected that glutens from flours of different quality had a distinct effect on dough quality. In the present work an extraction technique was applied to obtain gluten at a laboratory scale.

The objectives of the present work were: a) to analyze the effect of glutens extracted from three Argentinean commercial flours of different breadmaking performance when used as improvers on each one of the original flours; b) to relate this effect to the physicochemical characteristics of each gluten.

2. Materials and methods

2.1. Flours

Three different types of flours, of high (H4), intermediate (H3) and inferior (H3i) qualities were employed, provided by Molino Campodónico S.A. (La Plata, Argentina). According to the Código Alimentario Argentino (CAA, 2011), H4 could be classified as a 0000 type flour (ash content less than 0.492 g/100 g) and the other two were both 000 type (ashes between 0.492 and 0.65 g/100 g). The 000 type flours exhibited different breadmaking aptitude: one of them was of standard breadmaking quality (H3) and the other one of inferior quality (H3i). Quality was determined according to the alveographic performance of each flour. Values of alveographic work (W) were: 341 x 10^{-4}, 283.5 x 10^{-4} and 226 x 10^{-4} J for H3, H4 and H3i respectively. Tenacity to extensibility ratios (P/L) were: 1.45; 3.20 and 1.13 for H3, H4 and H3i respectively.

2.2. Flour characterization

Moisture content was determined according to AACC 44-15A (2000). Proteins were measured, following the Kjeldahl method, with a Digestion Unit K-435 and a distillation unit K-350 Büchi Labortechnik AG (Adapted from AACC method 46-11A) (2000). Kjeldahl factor used was 5.7. Ashes were measured according to AACC 8-03 (2000). Wet and dry gluten were determined using a Glutomatic-Glutork equipment (Method AACC 38-12A, 2000). All measurements were performed by duplicate.

2.3. Quality rheological tests

Farinographic tests in a 100-g capacity farinograph (Brabender, Duisburg, Germany) (IRAM 15855, 2000) were conducted for rheological characterization of samples. Replicates were performed according to the IRAM regulations.

2.4. SDS-PAGE

Proteins were sequentially extracted from flours according to the method of Singh, Shepherd, and Cornish (1991) modified as in Nieto-Taladriz, Perretant, and Rousset (1994). Three solutions were used for protein extraction. A NaCl solution (9 g/L) was used to separate albumins and globulins. Flour samples (20 mg) were extracted with 1 ml of this solution at 50 °C for 10 min and centrifuged at 12100 × *g* (4 °C). The supernatant was disregarded and the precipitate was extracted with a 1-propanol solution for gliadin separation (500 mL/L) containing 0.08 mol/L Tris–HCl buffer (pH 8.0)). Sample with the extraction buffer was heated at 50 °C for 30 min, with stirring at 10-min intervals, and then centrifuged for 2 min at 12100 × *g*. Supernatants were collected and dried at 50 °C overnight. The precipitate was washed twice with NaCl (9 g/L) solution.

For glutenin extraction, the residue from the previous step was extracted with 500 mL/L 1-propanol containing Tris–HCl (0.08 mol/L), 4-vinyl-piridine (14 g/L), dithiothreitol (DTT) (10 g/L). After incubating 30 min at 50 °C, samples were centrifuged and supernatants were kept. Supernatants were used for SDS-PAGE.

Protein determination by Kjeldahl method was used to quantify the amount of protein on each extract and on the final residue. Thus, amount of gliadin, glutenin and the insoluble residue (g/100 g) were calculated on dry basis (db).

Gels of 10 g/100 g of acrylamide 1-mm thick were prepared. Samples (10 μL) were solved in 10 μl of sample buffer, composed of SDS (20 g/L), glycerol (400 g/L), and bromophenol blue (0.2 g/L). From each sample solution, 10 μL was loaded per well on gels. Running time was ≈1 h (Mini-PROTEAN 3, Bio-Rad Laboratories). The running buffer was composed of glycine (0.192 mol/L), Trisma base (0.025 mol/L), and SDS (1 g/L), pH 8.3. Two replicates of each extract (gliadins and glutenins) were assayed.

Gels were stained for 24 h with an aqueous coloring solution containing acetic acid (16 g/100 g), methanol (40 g/100 g), Coomasie blue R (2 g/100 g). The same solution without the colorant was used for discoloring the gels. Low molecular mass standards (Amersham, GE) of 94 kDa (phosphorylase b), 67 kDa (albumin), 45 kDa (ovalbumin), 30 kDa (carbonic anhydrase), 20.1 kDa (trypsin inhibitor), and 14.4 kDa (α-lactalbumin) were used.

2.5. Gluten extraction

Vital glutens, G4, G3 and G3i were obtained from the flours H4, H3 y H3i, respectively. A water-dough hand washing process (Sayaslan, 2004) was adapted to obtain only the protein fraction. First of all 1 part of flour plus 0.6 parts of water were mixed in a kneader (Arno, Brazil) for 9 min to obtain a firm dough and left to rest for 15 min. Then the dough was washed to eliminate the starch. Finally, the wet gluten was freeze-dried (Heto FD-4, Denmark) to obtain a sample with the minimum structure damage.

2.6. Dough formulation

Flours were added with the different glutens in order to obtain an increase of 1 g/100 g on original protein concentration of each flour. Level of added gluten was selected according to the usual practices in mill industry. Twelve samples were prepared (3 controls plus 9 gluten added flours) as indicated in Table 1. The control within each group was the flour without added gluten. Final protein concentrations of flours with added gluten (H3 group, H4 group and H3i group) were 12.2 g/100 g, 11.8 g/100 g and 11.9 g/100 g respectively.

For rheology assays, doughs were prepared mixing the gluten added flours with the optimum amount of water (determined from farinographic assays) in a small scale kneader. To obtain optimum consistency, the farinographic development time was applied.

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Table 1
Formulations prepared with the different flours (H3, H4 and H3i) and their extracted glutens (G3, G4 and G3i, respectively).

Group	No gluten added	Gluten added		
		G3	G4	G3i
H3 group	H3 (control)	H3 + G3	H3 + G4	H3 + G3i
H4 group	H4 (control)	H4 + G3	H4 + G4	H4 + G3i
H3i group	H3i (control)	H3i + G3	H3i + G4	H3i + G3i

H3 = standard quality flour, H4 = high quality flour, H3i inferior quality flour, G3 = gluten extracted from H3, G4 gluten extracted from H4, G3i = gluten extracted from H3i.

2.7. Dough characterization

2.7.1. Texture Profile Analysis (TPA)

Ten cylindrical samples of 2 cm diameter and 1 cm height were obtained from dough. Dough texture parameters were evaluated using a texture analyzer (TA.XT2i, Stable Micro Systems, Surrey, U.K.) with Texture Expert for Windows (v.1.2) software. Dough was allowed to relax 15 min at 20 °C before testing and then was submitted to two cycles of compression up to 40% of the original height with a cylindrical probe (diameter = 7.5 cm). Force time curves were obtained at a crosshead speed of 0.5 mm/s. Product hardness, adhesiveness, elasticity, and cohesiveness were determined. Hardness is defined as the maximum force registered during the first compression cycle. Adhesiveness is the negative area obtained during the first cycle. Cohesiveness was determined as the ratio between the positive area of the second cycle and the positive area of the first cycle. Elasticity (springiness) was measured as the distance of the detected height of the second compression divided by the original compression.

2.7.2. Dough relaxation assays

Relaxations assays were performed by applying a compression up to 40% in a single pulse of 20 min. A texture analyzer (TA.XT2i, Stable Micro Systems, Surrey, U.K.) with Texture Expert for Windows (v.1.2) software were used. Dough was allowed to relax 15 min at 20 °C before testing. Three replicates per sample were done. The decay curves (Force as a function of time) were obtained and fitted to a Maxwell model with two elements in parallel with a spring (Steffe, 1996) (Eq. (1)). The coefficients were obtained in order to characterize the behavior of the system.

$$\sigma = f(t) = A_1 e^{\frac{-t}{\lambda_1}} + A_2 e^{\frac{-t}{\lambda_2}} + \sigma_e \quad (1)$$

where σ = stress (Pa), $A_{1,2}$ = pre-exponential factors (Pa), t = time (min), $\lambda_{1,2}$ = relaxation times (G/μ) (min) and σ_e = equilibrium stress ($\gamma_o G_o$) (Pa).

2.7.3. Dynamic rheometry

Measurements were performed in an oscillatory rheometer (RS600, Haake, Germany) at 30 ± 0.1 °C using a serrated plate–-plate sensor system with a gap of 1.5 mm between plates. Dough was laminated up to a height of 0.5 cm and cylindrical pieces 2 cm diameter were obtained. After mounting on the rheometer measurement sensor, dough was left to rest 15 min before testing. Frequency sweeps (0.005–100 Hz) at constant deformation within the linear viscoelastic range were performed on two replicates of each sample. Dynamic moduli G' (elastic or storage modulus), G'' (viscous or loss modulus) and tan δ (G''/G') related to overall viscoelastic response were calculated.

2.8. Breadmaking

Pure flour with or without extracted gluten (400 g) was mixed with NaCl (2 g/100 g flour) and then the optimum amount of water, determined by farinographic assays was added. Fresh yeast (3 g/100 g flour) was dispersed in part of the total water. The amount of added gluten was 1 g per 99 g flour. No other improvers were used. Salt and yeast were previously dissolved into the amount of the corresponding farinographic water. Ingredients were mixed by applying the development time obtained from farinograph. Temperature of the dough reached up to 23–25 °C. Dough was covered with plastic film to avoid desiccation and rested 15 min at 30 °C. Then dough was laminated 4 times, turning dough 90° after two consecutive passages and rested 15 more min. After resting, 80 g-spherical pieces were cut and shaped in equipment MPZ (Argentina) at 1400 rpm. Dough pieces were placed in baking trays, and leavened in a fermentation chamber, previously sprayed with water, at 30 °C for 80 min. Another water spray was done before entering the oven. Pieces were baked in an oven without steam at 200 °C for 23 min.

2.8.1. Bread characterization

2.8.1.1. Volume. Bread volume was determined by seed displacement in a loaf volume meter. To calculate the specific volume (cm^3/g), the ratio between bread volume and the weight of each piece was determined. Four replicates were performed for each formulation.

2.8.1.2. Crumb texture. Texture profile analysis (TPA) assays were performed on 6 replicates of each sample as described above for dough samples, with a degree of compression of 40% and with an SMSP/25 probe. A texture analyzer (TA.XT2i, Stable Micro Systems, Surrey, U.K.) with Texture Expert for Windows (v.1.2) software were used. Force time curves were obtained at a crosshead speed of 0.5 mm/s. Hardness, cohesiveness, elasticity and resilience were determined. Resilience is "an instant springiness", calculated as the ratio between the area during the withdrawal of the first compression and the area of the first compression.

2.8.1.3. Crumb moisture. Crumb moisture was performed on two replicates of each sample and determined in an oven at 105 °C up to constant weight. Samples were taken from the center of each bread slice.

2.9. Statistical analysis

Analyses of variance were conducted on each group (control + gluten added formulations) separately on the dependent

Table 2
Flour composition and farinographic parameters of flours: WA (water absorption) DT (dough development time), DS (dough stability), S (Softening degree).

Sample	Composition						Farinographic parameters			
	Moisture (g/100 g)	Ashes (g/100 g)	Proteins N × 5.7 (g/100 g)	Wet Gluten (g/100 g)	Dry Gluten (g/100 g)	WG/DG	WA(g H_2O/ 100 g of flour)	DT (min)	DS (min)	S (FU)
H3	$13.7^a \pm 0.2$	$0.64^a \pm 0.02$	$11.2^a \pm 0.2$	$29.9^b \pm 0.4$	$9.1^a \pm 0.2$	3.3	$59.8^b \pm 0.3$	$23.0^b \pm 0.7$	$31.3^b \pm 1.0$	$28.8^b \pm 1.8$
H4	$13.9^a \pm 0.1$	$0.48^b \pm 0.04$	$10.8^a \pm 0.1$	$30.5^b \pm 0.2$	$9.0^a \pm 0.3$	3.4	$59.3^b \pm 0.4$	$28.8^a \pm 0.4$	$40.5^a \pm 0.0$	$17.5^c \pm 3.5$
H3i	$13.3^b \pm 0.1$	$0.62^a \pm 0.02$	$10.9^a \pm 0.1$	$32.9^a \pm 0.3$	$9.0^a \pm 0.3$	3.7	$61.7^a \pm 0.4$	$8.5^c \pm 0.7$	$14.3^c \pm 1.0$	$45.0^a \pm 0.0$

Means within columns followed by the same letter were not significantly different ($P > 0.05$) ± SD.
H3 = standard quality flour, H4 = high quality flour, H3i inferior quality flour.

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variables studied considering each formulation as a level in a one-way factorial design. For simultaneous pairwise comparisons, least significance differences (LSD) test was chosen. Differences in means and F-tests were considered significant when $P < 0.05$. All statistical procedures were computed using the SYSTAT software (SYSTAT, Inc., Evanston, IL). Experimental data were reported as mean values. Standard deviations (SD) were given into each table.

3. Results and discussion

3.1. Flour characterization

3.1.1. Physicochemical and rheological characterization

In Table 2, results from flour composition and farinographic assays are shown. Higher values for ashes in flours H3 and H3i are expected since these flours are less refined than H4 flour (000-type according to the Código Alimentario Argentino, 2011).

Though total protein content is similar for all flours the relationship between wet gluten-dry gluten differs from each other. H3 and H4 values are the closest to the optimum one (≈3) (Ashokkumar, 2009)

The farinograph water absorption of H3i (61.7) was significantly higher than absorption values of H3 and H4 (59.8 and 59.3, respectively). This result is in agreement with the results of gluten determination, where H3i showed a higher WG/DG relationship.

Development times of the three flours were also different. The highest value corresponded to H4 flour (28.8 min). Long development times are undesirable in the industrial breadmaking because they are related with more energy input. H3i development time was the lowest (8.5 min). Lower development times are characteristic of a poor breadmaking quality.

It can be observed that the values for stability and softening degree for H4 are characteristic of a strong and stable flour. On the other hand, H3i showed a farinograph profile associated with a poorer quality. This characterization confirmed alveographic parameters of the employed flours.

3.1.2. Protein profile

The differences among flours evidenced by their farinographic and alveographic behaviors can be related to their different protein profiles, particularly the different proportions of gliadins and glutenins. Table 3 shows the percentage of each fraction and the insoluble protein residue obtained for each flour.

The inferior quality flour (H3i) exhibited a higher amount of gliadins respect to H4 and H3. On the other hand, the strongest flour (H4) had a higher level of glutenins and also exhibited the lowest quantity of gliadins.

In Fig. 1 the SDS-PAGE profiles for gliadins (a) and glutenins (b) are shown.

Profiles for H3 and H4 were similar, indicating the presence of the same type of subunits.

Gliadins had a molecular weight (MW) distribution between 31 and 74 kDa, assuming an average MW of 31 kDa for α- and β-gliadins, 35 kDa for γ- gliadins and 44 kDa up to 74 kDa for ω-gliadins (Fido, Békés, Grass, & Tatham, 1997). Even though gliadins are considered monomeric, polymeric components have been identified by SDS-PAGE under reducing conditions (Qi, Wei, Yue, Yan, & Zheng, 2006). In Fig. 1a, bands at 107 kDa in H3 and H4 lanes could correspond to these polymeric components. Weak bands of MW between 30 and 45 kDa could be attributed to α-, β-, γ-gliadins and fast ω-gliadins (Khatkar, Fido, Tatham, & Schofield, 2002; Shewry, Tatham, Forde, Kreis, & Miflin, 1986). A marked band at 25 kDa is observed in both flours (H3 and H4) and also three bands of lower intensity can be appreciated at lower MW (less than 22 kDa). Despite most of gliadins have MW within the range cited above, several authors (Alaedini & Latov, 2006; Anderson, Hsia, & Adalsteins, 2001; Prasada Rao, Prasad, & Nigam, 2002) have reported the existence of molecular weight gliadins under 30 kDa.

Table 3
Gliadins, glutenins and insoluble residue for the different commercial flours.

Sample	Gliadins (g/100 g on db)	Glutenins (g/100 g on db)	Insoluble residue (g/100 g on db)
H3	$4.3^{b} \pm 0.1$	$4.0^{b} \pm 0.2$	$0.9^{a} \pm 0.08$
H4	$3.7^{c} \pm 0.1$	$4.4^{a} \pm 0.1$	$1.0^{a} \pm 0.06$
H3i	$4.8^{a} \pm 0.2$	$3.5^{c} \pm 0.0$	$0.6^{b} \pm 0.0$

Means within columns followed by the same letter were not significantly different ($P > 0.05$) ± SD.
H3 = standard quality flour, H4 = high quality flour, H3i inferior quality flour.

The inferior quality flour H3i exhibited a different profile (Fig. 1a). It can be seen that bands at 107 and 48 kDa are not present. On the other hand, it can be observed two bands at 92 kDa and 65 kDa, corresponding probably to ω-gliadins or polymeric fractions. Two weak bands are observed at 25.8 kDa and 24.6 kDa for H3i. In the same region only one band at 25 kDa is observed for H3 and H4, probably because the higher amount of protein may have hindered the resolution of the run.

In Fig. 1b, bands between 67.5 and 107 kDa correspond to high molecular weight glutenin subunits (HMW-GS) (Shewry *et al.*, 1986). Bands ranging between 30 and 45 kDa are low MW glutenin subunits (LMW-GS). Other peptides appearing at lower MW could be assigned to contamination.

It can be observed that H3i glutenin profile was also different from the profiles of the other two flours. In H3i bands at 83 and 64.5 kDa, corresponding to HMW-GS were absent. In the region of LMW-GS the three flours exhibited a similar profile. The absence of at least two subunits of high molecular weight glutenins in H3i profile could explain the tendency to render weaker dough, less resistant to mechanical work in comparison to H3 and H4.

3.2. Gluten isolation

Protein content in dry gluten by Kjeldahl method was determined, obtaining proteins contents of 82.2 g/100 g, 79.0 g/100 g and 81.0 g/100 g for glutens extracted from H3, H4 and H3i respectively. The values found were similar or higher than the values reported by Martin extraction process, which were between 75 g/100 g and 80 g/100 g (N × 5.7) (Sayaslan, 2004). Glutens extracted from the respective flours (H4, H3, H3i) were identified as G4, G3 and G3i, respectively.

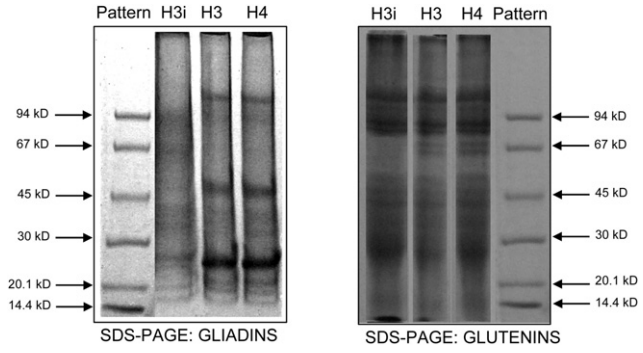

Fig. 1. SDS-PAGE for gliadins and glutenins extracted from the different commercial flours.

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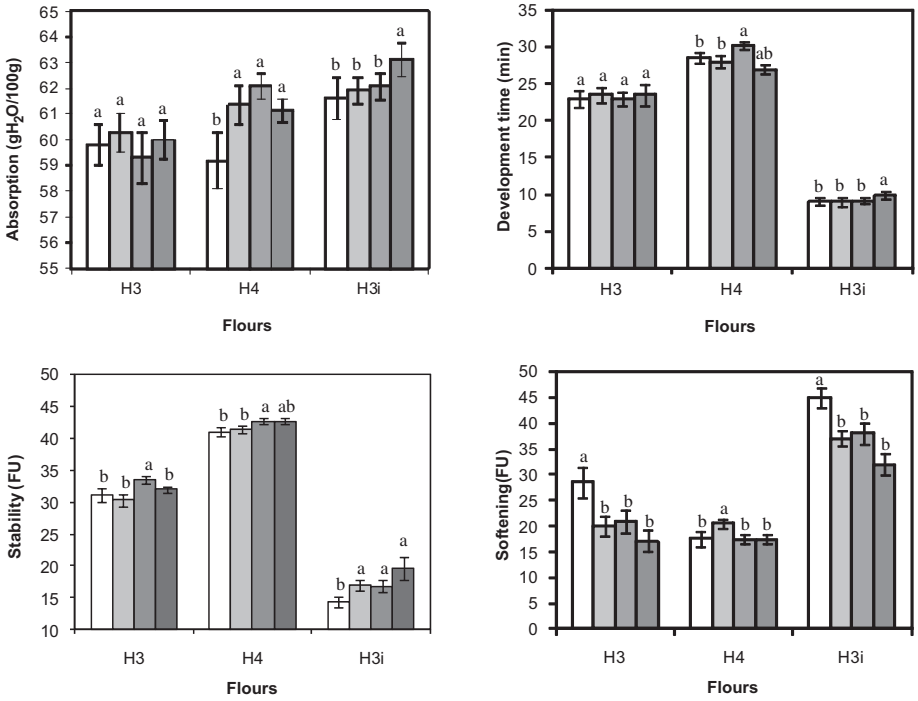

Fig. 2. Farinographic parameters for flour samples (H3, H4 and H3i) without (white columns) and with added gluten: G3 (light gray), G4 (gray) and G3i (dark gray). Different letters indicate significant differences within each group ($p < 0.05$). Error bars represents the standard deviation of the assays. H3 = standard quality flour, H4 = high quality flour, H3i inferior quality flour, G3 = gluten extracted from H3, G4 gluten extracted from H4, G3i = gluten extracted from H3i.

3.3. Rheological characterization of gluten added dough

In Fig. 2, farinographic results for the gluten added flours are shown. Gluten, from all sources increased H4 water absorption (A) up to 2 g/100 g. The other formulations were less affected in this parameter, except for H3i + G3i which exhibited a significative increase respect to H3i.

The flour H3 significantly improved its stability by adding G4 and significantly decreased the softening degree when adding any source of gluten. However, its development time was not affected. The strong flour H4 suffered minor changes in its parameters (except for water absorption).

The flour H3i was the most affected by the addition of gluten, showing a tendency to increase the development time. Besides, a significant enhancement in the stability was observed and a decrease in the softening degree of H3i dough when any of the three types glutens were added. According to these results, there is not a clear effect of the type of gluten, probably because H3i, an inferior quality flour, can be just improved by increasing the gluten content.

The differences observed by adding gluten from H3 or H4, with similar electrophoretic profiles, may be due to the different proportions of subunits (Table 2). Uthayakumaran, Stoddard, Gras, and Bekes (2000) reported that development time is positively affected by increasing the ratio of glutenin high and low MW (HMW/LMW). Tömösközi et al. (2002) also reported that the addition of gliadins led to smaller mixing times and glutenin.to higher development times.

3.3.1. TPA

In Fig. 3 the results of texture profile analysis (TPA) are shown. TPA showed differences among flours, in the same trend as results from farinograph. H4 led to harder and more elastic dough than H3; in turn H3 rendered harder and more elastic doughs than H3i. According to results of TPA reported by Ram and Nigam (1983), glutenins are the main protein component affecting hardness of dough. Thus, it is expected that flours with better protein profiles of glutenins (like H4 and H3) can render harder doughs than flours like H3i.

Gluten addition exhibited different effects depending on each flour. When H4 was added with either G3 or G3i, i.e. less elastic glutens, hardness was affected but not elasticity. This fact is in coincidence with farinographic results since no significant effect was observed on stability and softening degree when H4 was added with G3 and G3i.

When adding gluten to and inferior quality flour like H3i, there was a tendency to increase elasticity but differences were only significant when gluten from the maximum quality flour was added (G4). Hardness was not affected. The medium quality flour, H3, only exhibited significant differences in hardness when G3 and G4 were added. A similar trend was observed in elasticity.

Though adhesiveness was the textural parameter more affected by measurement errors, among the three assayed flours without gluten addition, the highest adhesiveness was found for H4 while the lesser values corresponded to H3i (results not shown). Gliadins are the unique gluten proteins that have a significant contribution to adhesiveness (Ram & Nigam, 1983). According to this and taking into account the electrophoretic profile of each flour, the higher adhesiveness of H3 and H4 respect to H3i is in accord to the different profile of gliadins. When different gluten were added no clear trends were detected respect to each control.

Cohesiveness is related to the degree of integration among flour components in the dough matrix. No significant differences were found in this parameter when gluten was added.

3.3.2. Relaxation assays

These assays show how macromolecules in dough submitted to a strain can reorganize and tend to a diminished energetic level. Relaxation curves were fitted with an exponential decay model of second order according to Eq. (1) obtaining values for the coefficients A_1, A_2, λ_1, λ_2 and σ_e for each sample. In all cases the R^2

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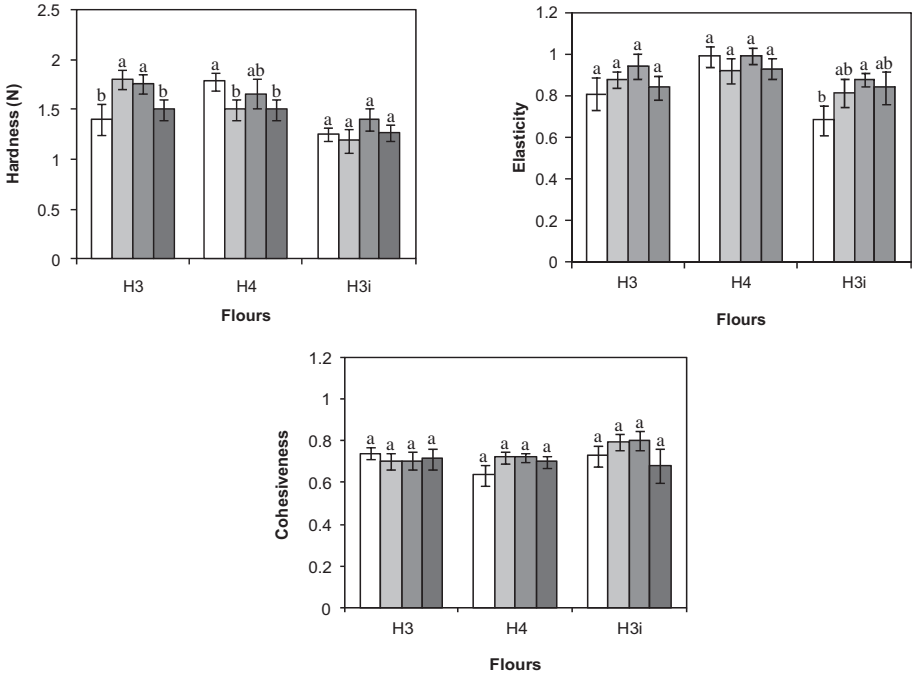

Fig. 3. TPA for dough from commercial flours (H3, H4 and H3i) without (white columns) and with added gluten: G3 (light gray), G4 (gray) and G3i (dark gray). Different letters indicate significant differences within each group ($p < 0.05$). Error bars represents the standard deviation of the assays. H3 = standard quality flour, H4 = high quality flour, H3i inferior quality flour, G3 = gluten extracted from H3, G4 gluten extracted from H4, G3i = gluten extracted from H3i.

obtained were over 0.98. No significant differences were detected for A_1, A_2, and σ_e, but differences in the parameters λ_1, λ_2 were observed. The average of all samples for the pre-exponential factor A_1 was 1128.4 Pa; for the coefficient A_2 was 442.9 Pa and for σ_e, was 612.1 Pa.

In Table 4, H4 without or with added gluten exhibited the highest values for λ_1 but no significant differences among samples with the different added glutens (G3, G3i or G4) were found. H3 samples showed lower values of relaxation time λ_1 when compared to H4; a significant increment of relaxation time was only observed when G4 was added. The inferior quality flour H3i exhibited the lowest values for λ_1, with a significant increment when G4 was added. The relaxation time λ_2 exhibited a similar trend to λ_1 but with less marked differences among samples.

A lower relaxation time is related with less time to reach the equilibrium. Thus, H3i was the dough that reorganizes its structure more rapidly, probably indicating a weaker and more labile gluten network. On the other hand, H4 required longer times to reach equilibrium. Addition of gluten from de strongest flour (G4) had a significant effect on this phenomenon.

3.3.3. Viscoelastic behavior

Rheometric assays were applied to evaluate viscoelastic behavior which is related to the quality of gluten network. Though many factors (breadmaking procedure, water amount, among others) may influence rheological properties of dough (Xu, Bietz, Craig, & Carriere, 2007), its typical viscoelastic behavior is mainly determined by the microstructural characteristics of components particularly gliadins and glutenins. Gliadins are related to viscous response and extensibility of dough, thus contributing to viscous modulus (G'') while glutenins are polymeric proteins responsible for elastic response and contribution to storage or elastic modulus (G') (Goesaert et al., 2005).

It was observed that both moduli depended on frequency and G' was also higher tan G'' in the analyzed range of frequencies. This behavior is far from a gel-like one (Steffe, 1996) and it can be related to the structural characteristics of bread dough where the protein

Table 4
Rheometric (G', G'' and tan δ measured at 1 Hz) and relaxation parameters for flours and gluten added flours.

Sample	G' (Pa)	G'' (Pa)	tan δ (ad)	λ_1 (min)	λ_2 (min)
H3	$11050^{d} \pm 80$	$3800^{de} \pm 150$	$0.35^{d} \pm 0.05$	$1.22^{b} \pm 0.08$	$0.21^{bc} \pm 0.03$
H3 + G3	$14900^{c} \pm 200$	$4600^{c} \pm 100$	$0.31^{e} \pm 0.04$	$1.20^{b} \pm 0.10$	$0.22^{abc} \pm 0.02$
H3 + G4	$12700^{cd} \pm 150$	$4480^{c} \pm 90$	$0.35^{d} \pm 0.03$	$1.48^{a} \pm 0.04$	$0.23^{ab} \pm 0.01$
H3 + G3i	$9900^{f} \pm 100$	$3220^{g} \pm 60$	$0.33^{de} \pm 0.03$	$1.30^{b} \pm 0.09$	$0.23^{ab} \pm 0.03$
H4	$18500^{a} \pm 300$	$3700^{ef} \pm 200$	$0.20^{g} \pm 0.07$	$1.41^{a} \pm 0.06$	$0.22^{ab} \pm 0.05$
H4 + G3	$15120^{bc} \pm 90$	$3500^{f} \pm 100$	$0.23^{f} \pm 0.03$	$1.43^{a} \pm 0.07$	$0.23^{ab} \pm 0.02$
H4 + G4	$16300^{b} \pm 200$	$4000^{d} \pm 110$	$0.25^{f} \pm 0.04$	$1.41^{a} \pm 0.03$	$0.24^{a} \pm 0.04$
H4 + G3i	$15100^{bc} \pm 120$	$3590^{f} \pm 90$	$0.24^{f} \pm 0.03$	$1.48^{a} \pm 0.05$	$0.24^{a} \pm 0.03$
H3i	$10300^{e} \pm 130$	$5400^{b} \pm 170$	$0.53^{b} \pm 0.04$	$1.05^{c} \pm 0.03$	$0.19^{c} \pm 0.02$
H3i + G3	$10100^{e} \pm 120$	$5630^{a} \pm 90$	$0.56^{a} \pm 0.03$	$1.18^{b} \pm 0.06$	$0.198^{bc} \pm 0.009$
H3i + G4	$11500^{d} \pm 300$	$5590^{a} \pm 70$	$0.49^{c} \pm 0.04$	$1.22^{b} \pm 0.03$	$0.22^{bc} \pm 0.04$
H3i + G3i	$10000^{e} \pm 400$	$5700^{a} \pm 110$	$0.57^{a} \pm 0.06$	$1.10^{c} \pm 0.10$	$0.20^{bc} \pm 0.02$

Means within columns followed by the same letter were not significantly different ($P > 0.05$) ± SD.
H3 = standard quality flour, H4 = high quality flour, H3i inferior quality flour, G3 = gluten extracted from H3, G4 gluten extracted from H4, G3i = gluten extracted from H3i.

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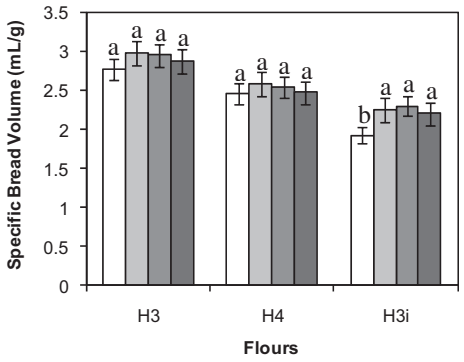

Fig. 4. Specific volume of breads from commercial flours (H3, H4 and H3i) without (white columns) and with added gluten: G3 (light gray), G4 (gray) and G3i (dark gray). Different letters indicate significant differences within each group ($p < 0.05$). Error bars represents the standard deviation of the assays. H3 = standard quality flour, H4 = high quality flour, H3i inferior quality flour, G3 = gluten extracted from H3, G4 gluten extracted from H4, G3i = gluten extracted from H3i.

matrix is "filled" with starch granules. The frequency sweeps allowed to determine G′, G″ and the tangent of loss angle (tan δ = G″/G′), related to the global response of the material. Results are shown in Table 4.

He and Hoseney (1991) suggested that the higher value of tan δ, characteristic of inferior quality flours could be attributed to a gluten network with lower quantity of crosslinking or weaker crosslinking that could be more easily dissociated. On the other hand, stronger flours exhibit lower values of tan δ when compared to weaker or medium flours (Miller, 1999).

As it can be observed from Table 4, all samples with H4 flour, presented higher G′ values according to the stronger and more elastic characteristics of this flour. On the other hand, H3i showed a more viscous character as evident from tan δ.

When gluten was added, no clear trends could be observed. No significant changes were evident in H3i, as it was evidenced by rheological assays involving greater deformations.

3.4. Breadmaking

Specific volumes of bread are shown in Fig. 4. H4 exhibited lower volumes than all H3 samples that were added with gluten. This low volume can be attributed to the stronger character of this flour and its low extensibility, as observed by alveographic results. Besides, the better breadmaking performance observed in the case of H3 and H4 when compared with H3i is in agreement with the different electrophoretic profile of those flours, specially the glutenin profile. Within H3 and H4 groups no significant differences were observed when any of the assayed glutens were added (G4, G3 and G3i). H3i breads showed the lowest volumes as expected by the inferior quality of this flour. When gluten was added, significant differences were observed respect to control. Besides, dough made with this flour exhibited a tendency to flow during the fermentation period. Similar results were reported by Aamodt, Magnus, and Færgestad (2005) with flours with a major proportion of gliadins. Breads obtained with H3i had a shape relationship different from breads obtained with H3 or H4, being more flattened breads (results nor shown). Crumb was also softer and with a less uniform air cell distribution than breads obtained from H3 or H4. As seen in the electrophoretic profiles, H3i exhibited important differences in certain glutenin subunits of high molecular weight (HMW-G) when

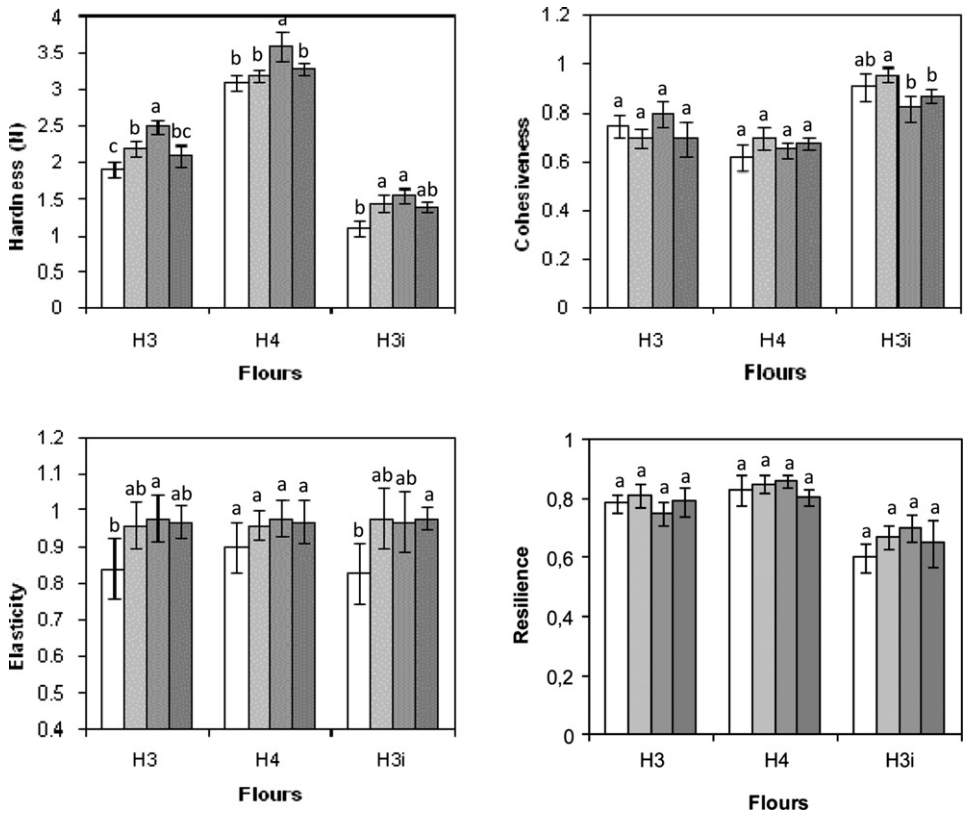

Fig. 5. TPA for crumbs of breads from commercial flours (H3, H4 and H3i) without (white columns) and with added gluten: G3 (light gray), G4 (gray) and G3i (dark gray). Different letters indicate significant differences within each group ($p < 0.05$). Error bars represents the standard deviation of the assays. H3 = standard quality flour, H4 = high quality flour, H3i inferior quality flour, G3 = gluten extracted from H3, G4 gluten extracted from H4, G3i = gluten extracted from H3i.

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compared to H3 and H4. These differences could lead to a weaker network without the necessary tenacity to support air cells, leading to collapse and a less uniform distribution. Though specific volume was improved when adding G3, G4 or G3i to H3i, other poor quality characteristics of breads obtained with this flour could not be modified by gluten addition.

In Fig. 5, TPA results for crumb are shown. A harder crumb was obtained for H4 samples, and an increase in hardness was achieved when G4 were added. H3 exhibited a less hard crumb than H4 and hardness was also increased when G3 or G4 were added. As it was observed in other parameters, H3i behavior is modified by the three types of gluten. The resistance to strain related to crumb hardness can be attributed to the quality of gluten network (Attenburrow, Goodband, Taylor, & Liliford, 1989). At the beginning of the compression cycle, tension is absorbed by air cell walls that are elastic but as the compression progresses, a critical point is reached where the structure collapses by different mechanisms; a stronger gluten leads to a more resistant air cell wall. According to this, H4 is the hardest sample, as expected by the more elastic gluten. On the other hand, even though H3i led to more compact breads, it also exhibited the lowest hardness of crumb in agreement with the poorer gluten quality.

Comparing H3, H4 and H3i samples, cohesiveness was higher in H3i samples with or without added gluten. Elasticity was similar for H3, H4 and H3i and showed a tendency to increase when any type of gluten was added. However, significant differences respect to each control were obtained only in some cases. Resilience or “instant elasticity” reflected differences among groups, but addition of gluten had no significant effect on this parameter. H3i samples, with or without gluten showed the lowest resilience values.

4. Conclusions

Glutens extracted from flours of different breadmaking performance showed different proportions of gliadins and glutenins and a distinct protein profile by electrophoresis. The inferior quality flour used as a source of gluten lacked certain gliadin and glutenin subunits. These differences in gluten quality led to dissimilar rheological behaviors of doughs and different breadmaking performance. Adding any of the three types of gluten to the weakest flour resulted in an improvement of several quality indicators as farinographic stability, elasticity of dough and crumb and specific volume of bread. The viscoelastic character of dough from the weakest flour was significantly affected by the strongest gluten that rendered systems with a reduced G''/G' relationship. The middle quality flour tended to improve when gluten of the strongest flour was added. These results show that the physicochemical and functional characterization of gluten could help to adequate the type of added gluten to a specific quality of flour. Besides, it is possible to achieve particular changes in dough rheology and breadmaking performance according to the selected gluten-flour couple.

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