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ORIGINAL PAPER

Wheat Flour Enriched with Calcium and Inulin: A Study of Hydration and Rheological Properties of Dough

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Abstract The aim of this work was to study the effect of calcium (Ca) carbonate-inulin (In) systems on hydration and rheological properties of wheat flour dough. Wheat flour, Ca carbonate from 108 to 252 (mg Ca/100 g flour) content, and enriched In oligofructose at levels of 1% to 13% (flour basis), were used. Hydration dough properties were researched analyzing water absorption (W_{abs}), moisture content (M_{cont}), water activity (a_w), and relaxation time (λ). W_{abs} and a_w decreased with increasing In levels independently of Ca content. Dough development time increased with the amount of Ca. In the presence of In, samples with the lowest content of Ca were those showing the highest development time values. Inulin was the main component that controled W_{abs} in dough. In the presence of $CaCO_3$, although water seemed to be in a free state according to the high a_w value measured (>0.975), the low value of relaxation time obtained suggests less molecular mobility. Rheological properties of dough were studied by texture, relaxation, and viscoelasticity assays. Dough hardness and consistency significantly increased with Ca and mainly with In content. At high In content, dough texture was enhanced by $CaCO_3$ due to the fact that this salt could behave as dough strengthener. Adhesiveness of dough was not modified by $CaCO_3$ at low In levels. However, Ca affected adhesiveness at intermediate In levels. Adhesiveness was significantly increased by In presence. Calcium and In both diminished dough cohesiveness. The In presence increased dough elasticity, independently of Ca content. A second-order polynomial model and response surface methodology were used for studying hydration dependence and rheological parameters (R^2> 0.771) on Ca and In. Dough M_{cont} varied with In^2 and mainly inversely proportional to In. An inverse dependence of λ on In was detected. Dynamic and relaxation elastic moduli (G' and E_3) showed a linear dependence on In.

Keywords Calcium–inulin system · Wheat flour dough · Dough hydration · Dough rheology

Introduction

Nutrition has progressed from the prevention of dietary deficiency and the institution of nutrition standards, to the promotion of a state of well-being and health and disease risk reduction (Roberfroid 2000). Foods that are expected to have a specific health effect due to relevant constituents, or foods from which allergens have been removed, are known as functional foods. Among the most common functional foods are those that contain prebiotics (Roberfroid 2000). Prebiotics are food components that increase the population of health-promoting microorganisms such as bifidobacteria and lactobacilli that are already resident in the human colon (Gibson and Roberfroid 1995; Gibson et al. 2000). Inulin (In) and fructose-containing oligosaccharides (FOS) are molecules that have prebiotic activity (Roberfroid 1993; Gibson et al. 1995; Roberfroid 2005a).

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Inulin, oligofructose, and FOS are fructans with a degree of polymerization of two to 60 fructose moieties connected by β-(2→1)-type linkages. Due to their structural conformation, these short-chain carbohydrates resist the hydrolytic action of enzymes present in the human digestive tract, and when reaching the colon, these are fermented exclusively by bifidobacteria and lactobacilli (Roberfroid 1993). Fermentation causes fecal bacterial biomass increase and ceco-colonic pH decrease, producing a large amount of fermentation products such as short-chain fatty acids that exert systemic effects on lipid metabolism.

Thus, In can be considered as a dietary fiber (DF). Increased intake of DF through foods is actually being recommended for good health because DF is likely to help reduce coronary heart-related diseases, diabetes, and intestinal diseases (Rastall et al. 2000; Peressini and Sensidoni 2009).

Calcium (Ca) is considered to be a functional ingredient because an adequate life-long intake of this mineral can reduce osteoporosis risk in elders (Cashman and Flynn 1999). In the elderly, Ca supplementation, in combination with vitamin D, may reduce bone loss and fracture incidence (Dawsom-Hughes et al. 1997).

Besides the amount of Ca in foods, the absorption of this mineral from diet is also a determining factor of its bioavailability. Thus, there is a need to identify functional food ingredients that may enhance Ca absorption in order to optimize its bioavailability from foods (Whiting and Wood 1997; Weaver and Liebman 2002). It is well known that In, among other beneficial effects, increases Ca absorption (Roberfroid 2005b).

Calcium and In are both nutritional ingredients and can be included in several foods. Previous studies have explored In effect (Gomez et al. 2003; O'Brien et al. 2003; Peressini and Sensidoni 2009) and different Ca salts (Sudha and Leelavathi 2008) on dough and bread quality. However, information about the influence of Ca–In mixtures on rheological characteristics of wheat flour dough is not available. In consequence, the objective of this work was to study the effect of Ca carbonate–In systems on the hydration and rheological properties of wheat flour dough.

Materials and Methods

Materials

Materials used in this work were wheat flour (type 0000, Molino Campodónico Ltd., Argentina) (ACA 1992) for bread making, Ca carbonate ($CaCO_3$, ANEDRA S.A, Argentina), and oligofructose-enriched In (Synergy 1, BENEO Orafti, Belgium, containing 92.7% d.b.). Wheat flour was characterized according to AACC methods (AACC International 2000) regarding its composition (moisture, ash, protein, lipid, and total DF content) and breadmaking-quality descriptors such as wet and dry gluten content and farinographic and alveographic parameters.

Experimental Design and Statistical Analysis

Response surface methodology (RSM) was applied to design the experiment and to obtain an optimal response (Montgomery 1997; Khuri and Cornell 1996). Full factorial designs are the optimal experimental strategy to simultaneously study the effect of several factors on sample response and to estimate interaction between them and even quadratic effects. Central composite design is an experimental design, useful in RSM, for building a second-order (quadratic) model for the response variable (Khuri and Cornell 1996).

Central composite designs consist of a factorial design (the corners of a cube) together with center and star points that allow the estimation of second-order effects (Fig. 1). If the distance from the center of the design space to a factorial point is ±1 unit for each factor, the distance from the center of the design space to a star point is $\pm\alpha$ with $|\alpha|>1$. The precise value of α depends on certain properties desired for the design and on the number of factors involved.

Mixtures of wheat flour, Ca carbonate, and In were prepared according to a central composite design. Levels of Ca were selected according to maximum levels of Ca allowed to be incorporated into bread, responding to the recommended daily intake of Ca (USDA 2008). Inulin levels were selected according to the amount needed to ensure Ca bioavailability. Calcium and In levels selected for the experimental design are shown in Fig. 1.

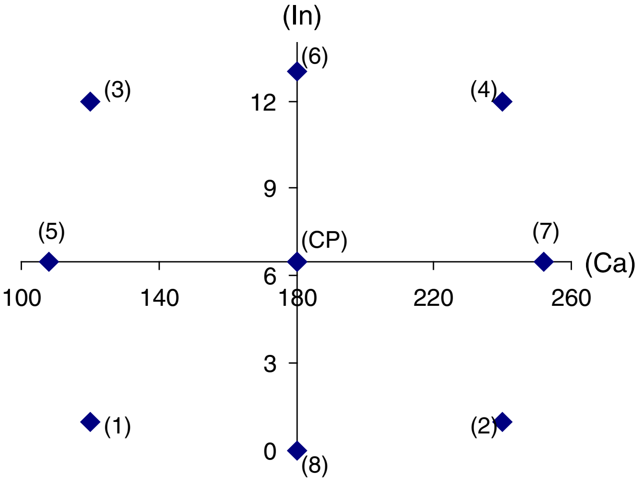


Fig. 1 Experimental central composite design. Calcium (*Ca*)-inulin (*In*) blends: *1* 120:1, *2* 240:1, *3* 120:12, *4* 240:12, *CP* 180:6.5, *5* 108, 6.5, *6* 180:13, *7* 252:6.5, *8* 180:0, (Control) 0:0. Levels: Ca (mg% f.b.) and In (g% f.b.); *f.b.* flour base

Data obtained for all the hydration and rheological parameters were analyzed using RSM by Statgraphics plus for Windows 5.1 software. Parameters were subjected to one-way ANOVA according to the general linear model procedure with least-square mean effects. Different means were determined according to Fisher's least significant differences test. Mean and standard deviation were calculated for each parameter. The second-order model proposed (Khuri and Cornell 1996) for each parameter was (Eq. 1):

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{11}X_1^2 + b_{22}X_2^2 + b_{12}X_1X_2 \qquad (1)$$

where Y is the dough response (moisture content (M_{cont}), ^{1}H-NMR relaxation time, relaxation elastic modulus, and dynamic storage modulus); b_0, b_i, b_ii, and b_ij are regression coefficients; X_1 and X_2 are coded variables that represent Ca and In, respectively.

The model adequacies were checked by the variance analysis (F test) and R^2 values. Variables effect were represented using surface graphs. Parameters (Y) selected for RSM were those whose R^2 was higher than 0.771.

Dough Formulation

Each flour blend consisted of wheat flour (400 g), NaCl 2% flour basis (8 g), the amount of $CaCO_3$ and In established in the design (Fig. 1), and the optimum quantity of water established in farinographic assays, water absorption (W_{abs}, %). Ingredients were mixed according to farinographic development time, in a small-scale kneader (Keenwood Major, Italy) at 90 rpm. Final dough temperature was 23–25 °C. Dough was laminated (four passes) and let rest for 15 min at 25 °C covered with a film to avoid water loss. Finally, it was laminated to 1 cm thick before cutting. Dough without Ca and In, used as control dough (Control), was analyzed outside the central composite design.

Dough pH was measured using a pH meter (SevenMuli, Mettler Toledo, USA) with a puncture tip electrode that was introduced into the dough. This assay was performed despite the absence of the required conditions to obtain a meaningful pH reading (e.g., low concentration of hydrogen ions or liquid medium).

Hydration Properties of Dough

Water Absorption Farinograph assays were performed according to AACC Approved Method 54-21(AACC International 2000) using a Brabender equipment (Duisburg, Germany). W_{abs} (%) was determined as the water volume to be added to 300 g of mixture (flour, $CaCO_3$, and In) to reach a maximum consistency of 500 Brabender units (BU).

Moisture Content and Water Activity Moisture content of dough was determined according to AACC Approved Method 44-19 (AACC International 2000) as the difference between weights measured before and after drying at 135 °C in a period of 2 h. Water activity of dough was measured with a Water Activity Meter Aqualab series 3 (Decagon Devices Inc., Washington, USA). Values correspond to the average of three determinations in both cases.

Molecular Mobility The molecular mobility of the different doughs was analyzed by relaxation assays with a RMN Brucker Minispec equipment (Brucker, USA). A portion of dough was introduced into glass tubes (10 mm diameter) up to 3 cm height, and tubes were closed to avoid dehydration. ^{1}H spin–spin relaxation times (λ) were measured using the Carr–Purcel–Meiboon–Gill pulse sequence. Assays were performed in quadruplicate.

Dough Microstructure The microstructure of doughs was analyzed by scanning electron microscopy (SEM) (Puppo et al. 2005). Cylindrical dough samples (2 mm diameter× 2 cm height) were immersed in 2.5% glutaraldehyde and then washed twice with 0.5 M phosphate buffer before dehydration. Samples were dehydrated using a graded acetone series: 25%, 50%, 75%, and three times with 100% acetone, covering the entire sample. Drying of samples was performed at the critical point with the intermediate CO_2 fluid (Bray 2000). Samples were then coated with gold in a sputter coater (Pelco, Redding, USA), and were observed at 5 kV in a JEOL JSM 35 CF scanning electron microscope (Tokyo, Japan).

Rheological Properties of Dough

Dough Development Development of dough was followed by farinographic and alveographic assays. A Brabender farinograph (300 g capacity; Brabender, Duigsburg, Germany) was utilized for measuring W_{abs}, development time (t_d), stability, and softening degree of dough (AACC 54-21). A Chopin alveograph (Chopin, France) was used for the rheological characterization of wheat flour blends through tenacity measurement (P), extensibility (L), and alveographic force (W). A modified technique (AACC 50-30A) was used. The same quantity of 2.5% NaCl solution, corresponding to that obtained for wheat flour was utilized for all blends.

Dough Texture Dough texture was analyzed through texture profile analysis (TPA). Cylindrical samples of dough (20 pieces) of 3 cm in diameter and 1 cm in height were obtained. Dough texture parameters were evaluated using a TA.XT2i Texture Analyzer (Stable Micro Systems, Surrey, UK) with a load cell of 25 kg and a Texture Expert for Windows version 1.2 software. Each sample was subjected 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. Hardness, consistency, adhesiveness, cohesiveness, and springiness of cylindrical dough pieces were calculated. Hardness is defined as the maximum force registered during the first compression cycle. Consistency is the sum of the areas under the force vs. time curve corresponding to the first and second compression cycles. Adhesiveness is the negative area obtained during the first compression cycle. Cohesiveness is calculated as the ratio between the positive area of the second cycle and the positive area of the first cycle. Springiness is calculated as the d_2/d_1 ratio, being d_2 and d_1 the distances between the initial and the maximum forces of second and first compression peaks, respectively.

Dough Relaxation Stress-relaxation tests were performed in a texture analyzer equipped with a 25-kg load cell together with a 100-mm diameter cylindrical probe. Each cylindrical dough sample (3 cm diameter, 1.0 cm height) was placed on the center of the aluminum base and compressed with the probe up to 40% of its original height (40% strain level) with a crosshead speed of 0.5 mm/s. The constant compressive strain applied to the sample was maintained for 1,200 s. Solid silicone was placed at the lateral border of the dough to prevent dehydration. Tests were conducted at room temperature (25 °C) on three dough replicates per formula. Stress-relaxation curves were fitted using Origin Pro 8 software (OriginLab Corporation, MA, USA) and a nonlinear regression analysis was performed. A generalized Maxwell model (Steffe 1996) consisting of two Maxwell elements with a residual spring in parallel (Rodriguez-Sandoval et al. 2009) was applied (Eq. 2).

$$\sigma(t) = f(t) = A_1{*}\exp(-t\,E_1/\eta_1) + A_2{*}\exp(-t\,E_2/\eta_2) + \gamma_0 E_3 \qquad (2)$$

Where σ (t) represents the stress measured at any time during the relaxation test, γ_0 is the deformation applied, A_1 and A_2 are pre-exponential factors, t representing the time, E_1, E_2, and E_3 standing for the elastic relaxation moduli, and η_1 and η_2 are the viscosities.

The relaxation time T is defined as the ratio between the viscosity and the elastic modulus (Eq. 3).

$$T_{\mathrm{i,rel}} = \eta_{\mathrm{i}}/E_{\mathrm{i}} \qquad (3)$$

By applying this model, elastic relaxation moduli (E) and relaxation times (T) were obtained for the first and second exponential terms. Modulus E_3 corresponds to the equilibrium modulus at infinite time.

Dough Viscoelasticity Cylindrical pieces (3 cm diameter, 0.5 cm height) of the different doughs were subjected to dynamic rheological measurements. Measurements were performed in a Haake RS600 oscillatory rheometer (Haake, Germany) at 25±0.1 °C, using a plate–plate sensor system (35 mm diameter) with 1.5 mm gap between plates. Two types of rheological tests were performed as follows: (a) deformation sweeps at constant frequency to determine the maximum deformation ($\gamma_{\max}$) that a sample can experience in the linear viscoelastic range and (b) frequency sweeps (from 0.005 to 100 Hz) at constant deformation within the linear viscoelastic range. Mechanical spectra were obtained by recording the dynamic moduli G', G'', and tan δ (G''/G') as frequency function. G' corresponding the dynamic elastic or storage modulus, related to the material response as a solid while G'' standing for the viscous dynamic or loss modulus, related to the material response as a fluid. tan δ is related with the overall viscoelastic response: low values of this parameter indicate a more elastic sample.

Results and Discussion

The analysis of the breadmaking wheat flour used for dough preparation in this work revealed the following composition (%): proteins, 9.7±0.4; lipids, 1.12±0.08; ash, 0.361±0.001; and moisture, 12.6±0.2. Total DF was 2.8±0.3, with a total percentage of carbohydrates of 73.3 (calculated by difference).

This flour produced 29±1% and 9.1±0.2% of wet and dry gluten, respectively; with a ratio of 3.21±0.09 between these parameters. As measured by farinographic assays, the flour presented a W_{abs} value of 56±2%, a stability of 36±4 min, and a softening degree of 10±1 BU. Tenacity and extensibility were 108±4 and 74±8 mm, respectively, with an alveographic work of 188±15 ($\mathrm{J}\times10^{-4}$).

Development of Dough

Figure 2 shows farinographs corresponding to samples identified as 1 to 4 in the experimental design (Fig. 1). Samples with low content of In (Fig. 2a, b) presented farinograms similar to those of the corresponding control flour (not shown). Inulin level increase substantially changed the farinogram profile (Fig. 2c, d), so that samples showed a highly steep second peak (centered at 500 BU) as compared with the first one. Changes in farinogram shape would be related to the type of gluten structure that is formed during mixing in the presence of In and also to the nature of In–water interaction in dough. Inulin at levels higher than 6.5% significantly decreased W_{abs} of wheat flour, and this effect was independent of Ca content (Table 1). A decrease in W_{abs} in the presence of In has been previously reported by several authors (Wang et al. 2002; O'Brien et al. 2003; Peressini and Sensidoni 2009).

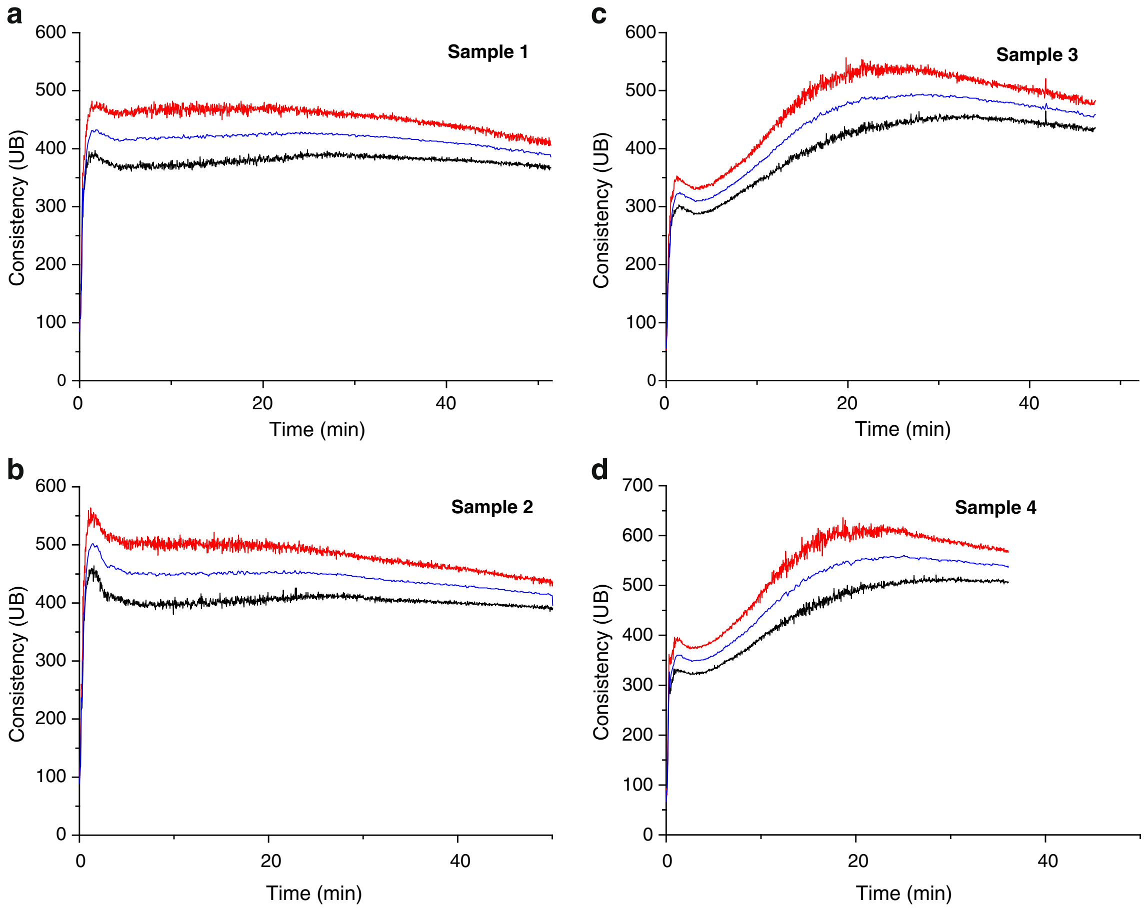


Fig. 2 Farinograms of wheat flour–In–Ca carbonate blends. **a** *Sample 1* 120 mg% Ca and 1 g% In; **b** *sample 2* 240 mg% Ca and 1 g% In; **c** *sample 3* 120 mg% Ca and 12 g% In; **d** *sample 4* 240 mg% Ca and 12 g% In

Table 1 Farinographic parameters of calcium–inulin wheat flour blends

Sample			W_{abs} (%)	t_d (min)	Stb (min)	Soft (BU)
Blend no.	Ca[a]	In[b]				
1	120	1	54.1±0.8 c, d	24±1 b, c, d	42±6 c	16±1 b
2	240	1	54.7±0.4 d	21±1 b	42±4 c	17±8 a, b
3	120	12	50±3 a	26±1 d, e	20±3 a	15±6 a, b
4	240	12	51±2 a, b	21±2 b	22±1 a	22±4 b, c
CP	180	6.5	51.2±0.3 a, b	20±2 b	>50	<6
5	108	6.5	49.8±0.0 a	25±1 c, d, e	>50	<6
6	180	13	51.8±0.5 a, b, c	22±1 b, c	23±3 a	22±6 b, c
7	252	6.5	49.5±0.2 a	29±1 e	>50	13±4 b
8	180	0	53.6±0.1 b, c, d	22±3 b	35±5 b	28±2 c
Control	0	0	56±2 c	12±1 a	36±4 b, c	10±1 a

Farinographic parameters: water absorption (W_{abs}), development time (t_d), stability (Stb), softening degree (Soft). Different letters in the same column indicate significant differences ($p<0.05$). Ingredients: calcium (Ca) and inulin (In)

CP central point (three replicates), *Control* control dough (without inulin and calcium)

[a] mg Ca/100 g wheat flour

[b] g In/100 g wheat flour

This behavior is related to high water-retention capacity of this molecule (De Gennaro et al. 2000).

Time (t_d) necessary for the optimum dough development increased with the amount of Ca, reaching a value of 29 min for sample 7 (252 mg Ca/100 g flour) (Table 1). In the presence of In, samples with the lowest content of Ca were those presenting the highest t_d values, suggesting a depressor effect of Ca in the presence of fiber. Samples with the highest stability and lowest softening degree were those containing intermediate In level (samples CP, 5, and 7).

Values of alveographic parameters are shown in Fig. 3. In the traditional assay, the volume of 2.5% NaCl solution to be incorporated in this technique is generally calculated according to M_{cont} of flour. In this case, determining moisture of Ca–In–wheat flour blends proved difficult due to their hygroscopicity; therefore, all doughs were prepared with the amount of 2.5% NaCl solution required for wheat flour alone. Ca addition did not modify the tenacity (Fig. 3a) or extensibility (Fig. 3b) of samples as compared with the control sample (Control), either in the absence of In or in the presence of low levels (1%) of this substance. These parameters were directly influenced by the presence of the fiber. At intermediate and high levels of In (6.5%) a significant decrease in *P* and *L* was observed, mainly at In levels of 12% and 13% (Fig. 3a, b). The same tendency was observed for *W*, reaching very low values (Fig. 3c), due to water excess incorporated to the blend (Fig. 3c).

Hydration Properties of Dough

Doughs with high content of In (doughs 3, 4, and 6) presented the lowest M_{cont} and, for a given fiber content, M_{cont} was independent of the amount of Ca incorporated as Ca carbonate (Table 2). Inulin has been the main determinant of W_{abs} in dough. The high water-retention capacity of In allowed reaching the optimum dough consistency with lower water content. This phenomenon allows In to form gels (Kim et al. 2001), and gelation led to the formation of a more structured dough.

The polynomial model applied to the moisture parameter (M_{cont}) resulted in the response surface shown in Fig. 4. Moisture content varied with In^2 and was inversely proportional to In, the latter being the most important variation factor.

Water activity (a_w) of dough was high (>0.967) indicating that dough is a system containing high-energy water. Dough with high In content (≥6.5%) presented a_w values significantly lower than control dough (Table 2). Among samples with 6.5% of In (dough CP, 5 and 7), a_w increased with increasing $CaCO_3$ amounts, suggesting that this salt contributes to augment dough high-energy water content.

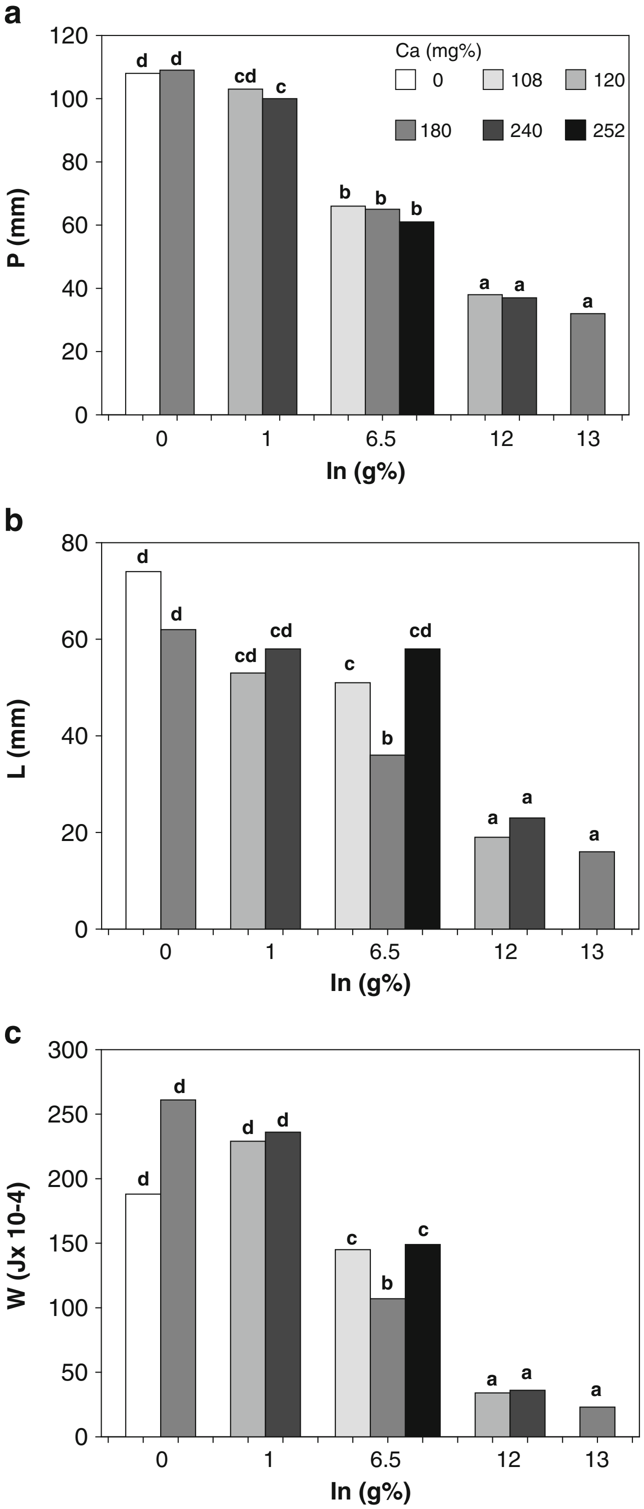


Fig. 3 Alveographic parameters of wheat flour–In–Ca carbonate blends. **a** Tenacity (*P*), **b** elasticity (*L*), and **c** alveographic work (*W*). Different letters indicate significant differences ($p<0.05$)

Molecular mobility has been studied in cereal systems using the ^1H NMR relaxation technique (Leung et al. 1979; Chen et al. 1997; Ruan et al. 1997). Nuclei are excited for a few miliseconds and when the pulse stops, they return to ground state emitting a signal. Relaxation curves of the

Table 2 Hydration parameters of calcium–inulin wheat flour dough

Dough			M_{cont} (%)	a_w (−)	λ (ms)	pH
Blend no.	Ca[a]	In[b]				
1	120	1	40.9±0.2 d	0.971±0.001 b	11.3±0.2 d	6.35±0.00 d, e
2	240	1	41.3±0.1 d	0.975±0.001 c	12.1±0.2 e	6.36±0.01 e
3	120	12	38.2±0.2 b	0.969±0.000 a, b	10.2±0.3 b	6.23±0.01 b, c
4	240	12	38.0±0.2 a, b	0.967±0.001 a	10.1±0.2 b	6.28±0.01 c, d
CP	180	6.5	39.2±0.4 c	0.974±0.005 b	10.8±0.3 c	6.31±0.06 d, e
5	108	6.5	39.0±0.0 c	0.970±0.000 a, b	10.9±0.2 c	6.20±0.01 b
6	180	13	37.7±0.1 a	0.970±0.002 a, b	10.9±0.2 c	6.29±0.02 c, d, e
7	252	6.5	39.0±0.3 c	0.975±0.002 c	9.7±0.0 a	6.29±0.00 c, d, e
8	180	0	42.7±0.1 e	0.984±0.001 d	12.3±0.2 e	6.28±0.03 c, d
Control	0	0	43.4±0.1 f	0.971±0.003 b, c	12.2±0.1 e	5.80±0.03 a

Parameters: moisture content (M_{cont}), water activity (a_w), ^{1}H spin–spin relaxation time (λ), pH. Different letters in the same column indicate significant differences ($p<0.05$). Ingredients: calcium (Ca) and inulin (In)

CP central point (three replicates), *Control* control dough (without inulin and calcium)

[a] mg Ca/100 g wheat flour

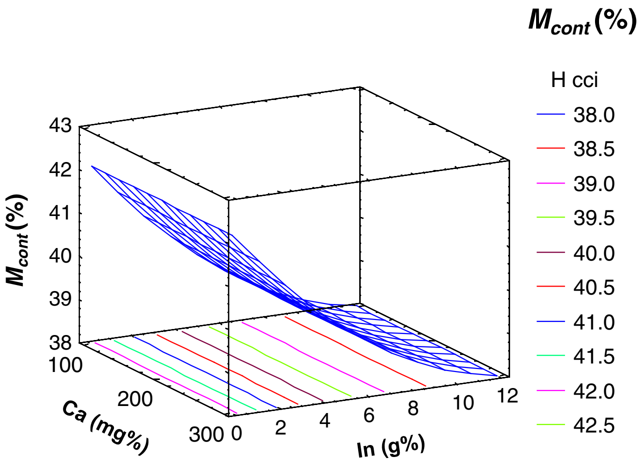
[b] g In/100 g wheat flour

proton (^{1}H) signal intensity vs. time have exponential decays and can be fitted with equations having one, two, or more exponential terms. Each term, represented by a relaxation time (λ_1, λ_2, $\lambda_{3\ldots}$ λ_i), can be associated to distinct populations of molecules having different mobilities. Species with shorter relaxation times are less mobile (solid-like state) than those with longer relaxation times (liquid-like state). The spin echo signal at $t=0$ is proportional to the number of hydrogen nuclei of each species. In the present work, the decay curves were fitted to a one-term exponential model according to Eq. 4:

$$I = A\exp(-t/\lambda) \tag{4}$$

Where I represents the ^{1}H signal intensity (proportional to mobile water fraction in the sample), t being time, λ being the relaxation time (a constant parameter), and A being the signal intensity of protons at $t=0$.

Using this technique, Leung et al. (1976) investigated different systems based on corn starch, pectin, casein, and sodium alginate. Corn starch exhibited two different populations with distinct mobility, while the other systems presented a mono exponential decay. The mono phase behavior is detected when: (a) the exchange rate of water between phases (less mobile or more mobile) is fast compared with the relaxation rate, (b) one phase is present in small amount, (c) one of the relaxation times is very short, or (d) both relaxation times are very similar. Leung et al. (1979) reported for dough a double-exponential decay assigning two different mobile water fractions, although other authors (Lopes-da-Silva et al. 2007) have applied a simple exponential decay equation for modeling dough NMR relaxation curves. In dough, λ is a parameter related to the water mobility of the system. Higher λ values denote higher molecular mobility; water seems to be more loosely linked to the other molecules and consequently in a high-energy state. This phenomenon depends on the molecular structure of dough components; for example, molecular mobility of water in dough was found to be affected by hydrocolloid-water interactions (Linalud et al. 2011).



M cont (%) =39.21-1.663*In + 0.512*In^2
R^2 = 0.969

Fig. 4 Response surface graphs of moisture content (M_{cont}) of dough and the representative model containing the significant regression coefficients

In the present study, samples with the highest λ values were those that contained In in a very low proportion (1 g In/100 g wheat flour) (Table 2). Inulin is a polymer of fructose that retains water through hydrogen bonds with the OH groups of the fructose molecules. High levels of $CaCO_3$ did not modify In behavior, except in dough 7, in which 252 mg of Ca allowed a higher water immobilization. In the presence of $CaCO_3$, water is immobilized despite being in a high-energy (free) state. This behavior could be attributed to the influence of the salt in the restructuring of the gluten matrix, leading the formation of a stiffer dough. Friedli and Howell (1996) proposed that deamidation of soluble wheat proteins conferred them a high density of negative charge due to the increase in glutamic and aspartic acids. In the presence of Ca, these proteins form strong but coarse gels. Calcium enhances the formation of a three-dimensional network by forming bridges between the negatively charged proteins.

The response surface that explains the λ behavior at different $CaCO_3$ and In levels is shown in Fig. 5. This response surface reflects an inversely proportional dependence of In content.

Dough hydration would also be influenced by pH. Control dough (Control) presented acid pH (pH 5.8) due to the type of amino acids composing gluten proteins. About 8% of the total amino acid residues of gluten are ionizable (Kasarda et al. 1971). These ionic residues are distributed between acidic (3.2%) and basic (4.8%) amino acids. Acidic residues consist mostly of glutamic acid (pK_a 4.6), with a smaller contribution of aspartic acid. The basic amino acid residues are distributed among arginine (≅2.2%, pK_a 12.5), histidine (≅1.5%, pK_a 6.3), and lysine (≅1.1%, pK_a 10). Polar and non-polar amino acids represent about 40% and 38%, respectively, of the total amino acid residues (Kasarda et al. 1971). At pH 5.8 acid groups are negatively charged while basic groups are protonated. Basic amino acids are present in higher amount, therefore wheat proteins in dough exhibit net positive charge (Table 2). The $CaCO_3$ salt (sample 8) decreased dough acidity (pH 6.28) due to the alkaline properties of the $CO_3^{=}$ ion. Variation of dough pH was independent of the fiber content (Table 2).

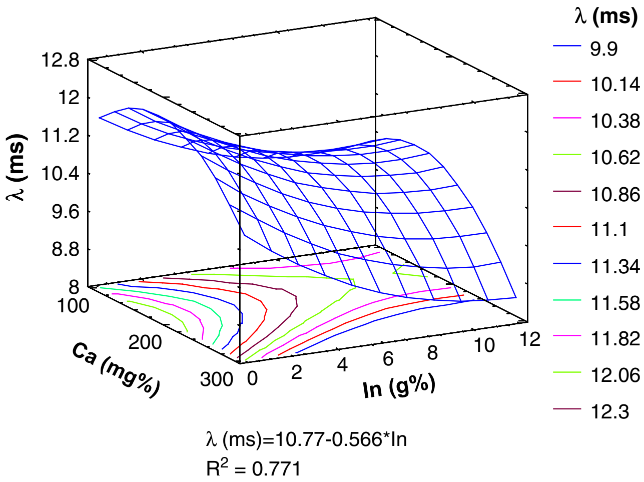


Fig. 5 Response surface graphs of ^{1}H-NMR relaxation time (λ) of dough and the representative model containing the significant regression coefficients

As mentioned before, the wet gluten content of the control sample was 29±1%. For all samples, this parameter varied from 28.6±0.6% (sample 3) to 31.0±0.2% (sample 1). The dry gluten presented the same tendency, with values of 9.1±0.2% for the control sample, and 9.2±0.1% (sample 3) to 10.3±0.2% (sample1) for samples of the experimental design. These values suggest that Ca and In have no effect on gluten formation.

Not only is quantity but also quality of gluten important since this polymer is the base of dough structure. Figure 6 shows the microstructure, analyzed by SEM, of five doughs of the experimental design. A gluten matrix involving starch granules can be observed. The gluten network formed in the presence of Ca was homogeneous (Fig. 6a, b). In doughs with high content of In, an entanglement of this fiber over the gluten network can be observed (Fig. 6c, d). The gluten matrix behind the In network of dough 4 seems to be also more homogeneous than that of dough 3. Peressini and Sensidoni (2009) observed by CSLM the presence of a higher concentration of protein phase in doughs prepared with In than in the reference sample, attributing this phenomenon to lower W_{abs} of the former. In our case, doughs 3 and 4, which presented lower W_{abs} (Table 1), also exhibited a more uniform and concentrated protein matrix (Fig. 6).

Rheological Properties of Dough

In the absence of In, hardness and consistency of dough (Control sample) increased with Ca concentration (180 mg %) (Fig. 7a). The incorporation of In significantly increased these parameters. At a constant level of In (6.5%), increasing the amount of Ca provoked a substantially increment of hardness and consistency, with maximum values obtained at 252 mg% of Ca (Fig. 7a, b). Results suggest that In alone confers consistency to dough, probably due to its gel-forming and structuring capacity (Izydorczyk et al. 2001; Peressini and Sensidoni 2009). In addition, this effect is potentiated by $CaCO_3$ that may also behave as a dough strengthener.

Similar tendency for hardness and consistency was observed for adhesiveness, which increased significantly with increasing In levels. At 12% of In, adhesiveness of dough decreased significantly with $CaCO_3$ incorporation (Fig. 7c). Cohesiveness is a parameter related to the force linking together product particles. Both ingredients, Ca and

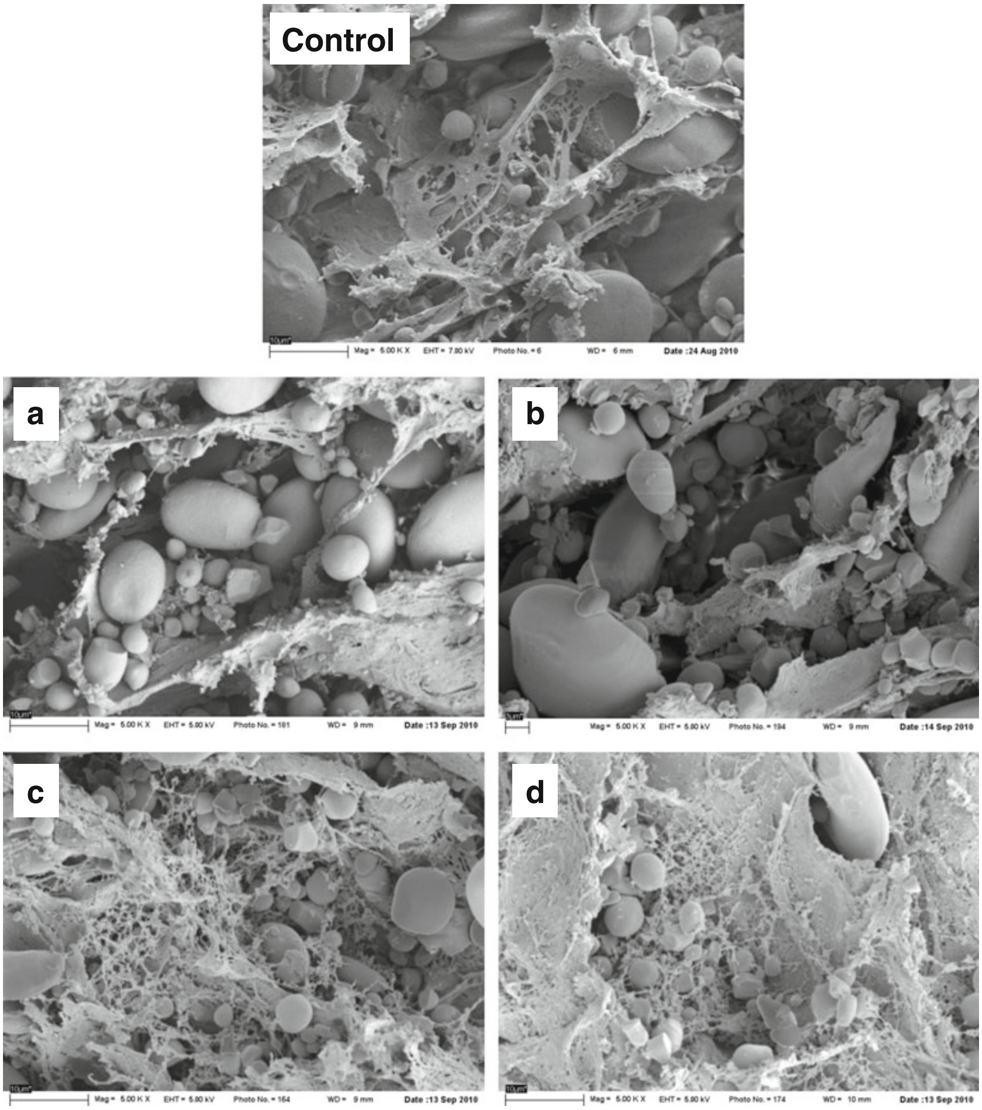


Fig. 6 SEM of dough. *Control* control dough. **a** *Dough 1* 120 mg% Ca and 1 g% In; **b** *dough 2* 240 mg% Ca and 1 g% In; **c** *dough 3* 120 mg% Ca and 12 g% In; and **d** *dough 4*: 240 mg% Ca and 12 g% In. Magnification, ×5,000

In, diminished dough cohesiveness (Fig. 7d). Likewise, doughs with the highest consistency were those that developed less cohesiveness, suggesting that an increase in hardness would interfere with dough particles linkages. The presence of In at low concentration (1%) decreased springiness (0.859±0.007 for dough 1 and 0.858±0.009 for dough 2) in comparison to the control dough (0.867±0.009). At high concentration (12% and 13%), In increased dough springiness independently of Ca content, with values of 0.88±0.01, 0.884±0.005, and 0.886±0.004 for doughs 3, 4, and 6, respectively.

In spite of the similar water content of doughs with 6.5% of In, the sample with the highest Ca content (252 mg%) presented the lowest molecular mobility. This water immobilization led to the formation of matrices of greater hardness and consistency. These more structured and elastic doughs were also more adhesive. This behavior agrees with the state of water in the dough since high-energy water, capable of migrating to the surface, contributes to adhesiveness. For doughs from different flours, Lopes-da-Silva et al. (2007) assigned differences in the molecular mobility of protein/water matrices to differences in network rigidity.

Stress-relaxation assays were previously used for studying the rheological properties of doughs (Rodriguez-Sandoval et al. 2009; Correa et al. 2010). In viscoelastic solids like doughs, stress decays to an equilibrium value. According to Yadav et al. (2006), stress-relaxation curves of dough exhibit three zones: a first high slope zone, an intermediate decaying zone, and a third one with a negligible slope that reaches an equilibrium stress value. Relaxation is a phenomenon related to the molecular and structural reorientation of the system. Parameters obtained applying the two-term Maxwell model, the elastic modulus *E* and the relaxation time *T*, thus reflected this structural orientation of dough components. Dough relaxation behavior may be described through two processes: a fast

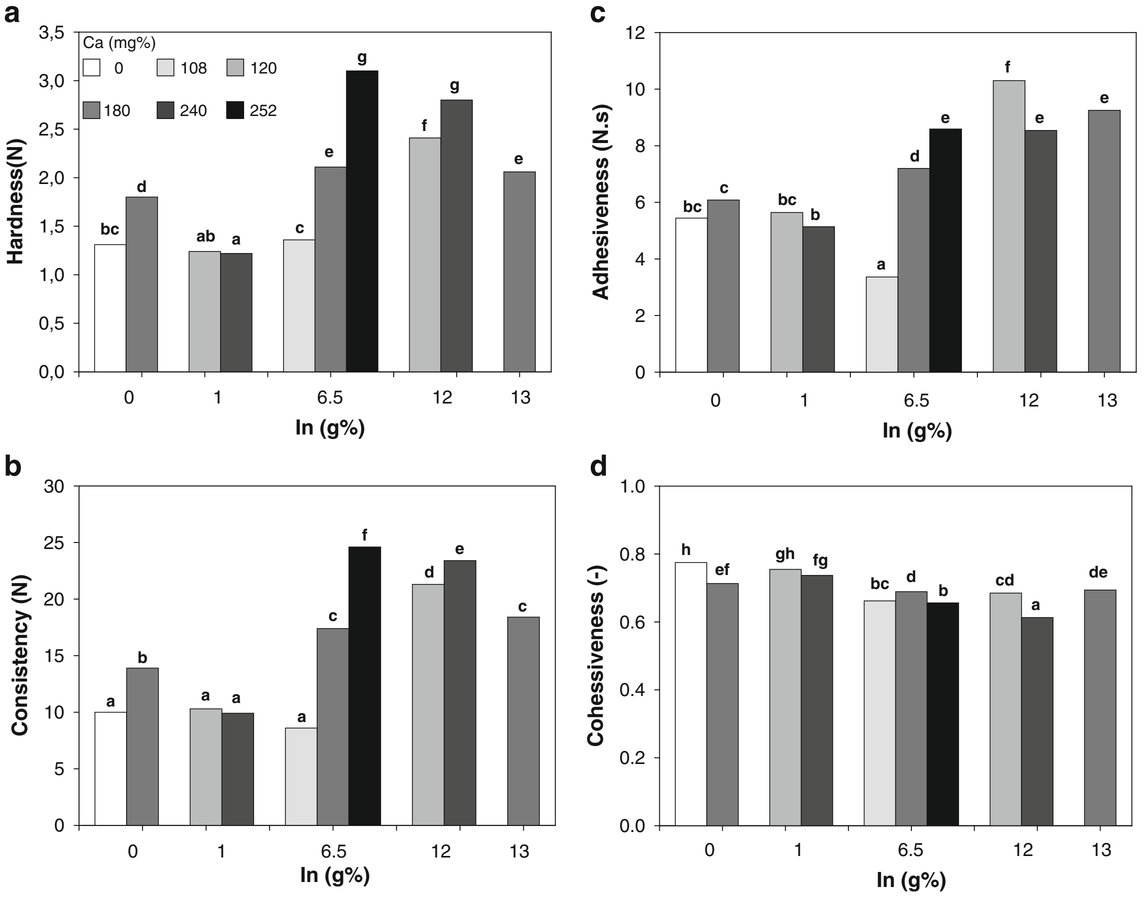


Fig. 7 Texture parameters of dough prepared with wheat flour–In–Ca carbonate blends: **a** hardness, **b** consistency, **c** adhesiveness, and **d** cohesiveness. Different letters indicate significant differences ($p<0.05$)

Table 3 Relaxation parameters of calcium–inulin wheat flour dough

Dough			Relaxation[c]				
Blend no.	Ca[a]	In[b]	E_1 (kPa)	E_2 (kPa)	E_3 (kPa)	T_1 (seg)	T_2 (seg)
1	120	1	0.4±0.1 a, b, c	3.9±0.9 a, b	0.15±0.03 a	294±27 b, c, d	7±1 a, b
2	240	1	0.37±0.08 a, b	3.2±0.4 a	0.13±0.02 a	315±38 d	7.6±0.9 a, b, c
3	120	12	0.58±0.09 d, e	4.3±0.7 a, b	0.69±0.04 d	337±48 d, e	9±1 c, d
4	240	12	0.70±0.05 e	4.2±0.2 a, b	0.65±0.01 d	371±31 e	9.7±0.0 d
CP	180	6.5	0.5±0.1 c, d	5±1 b	0.35±0.04 b	316±31 d	7.9±0.8 b, c
5	108	6.5	0.64±0.03 e	6.6±0.4 c	0.46±0.02 c	304±5 b, c, d	7.6±0.5 a, b, c
6	180	13	0.54±0.04 c, d, e	4.3±0.3 a, b	0.66±0.03 d	335±21 d, e	8.9±0.3 d
7	252	6.5	0.58±0.02 d, e	6.7±0.4 c	0.51±0.04 c	266±22 a, b	7.1±0.2 a, b
8	180	0	0.49±0.06 b, c, d	4.4±0.5 a, b	0.16±0.01 a	275±16 a, b, c	7.3±0.7 a, b
Control	0	0	0.33±0.06 a	4.4±0.3 a, b	0.11±0.00 a	227±15 a	6.5±0.5 a

Relaxation parameters: elastic modulus (E), relaxation time (T). Different letters in the same column indicate significant differences ($p<0.05$). Ingredients: calcium (Ca) and inulin (In)

CP central point (three replicates), *Control* control dough (without inulin and calcium)

[a] mg Ca/100 g wheat flour

[b] g In/100 g wheat flour

[c] Correlation coefficients were between 0.979 (dough 7) to 0.986 (dough 4)

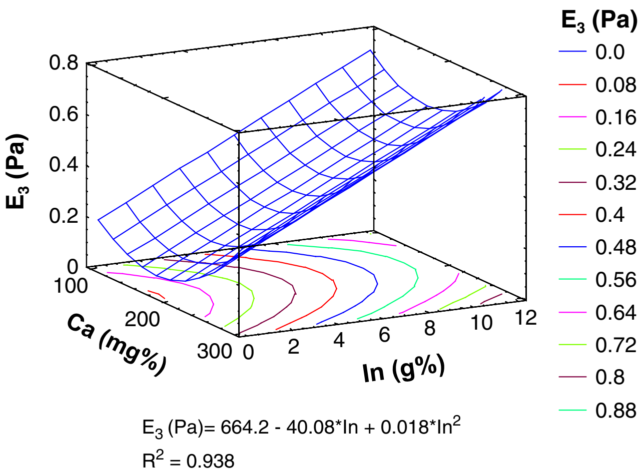


Fig. 8 Response surface graphs of the relaxation elastic modulus (*E₃*) of dough and the representative model containing the significant regression coefficients

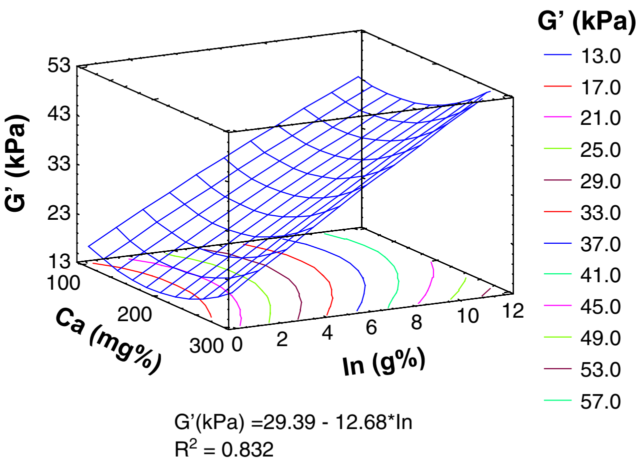


Fig. 9 Response surface graphs of the storage modulus (G') of dough and the representative model containing the significant regression coefficients

relaxation (0.1–10 s) associated with small molecules that relax faster, and a slow process (10–10,000 s) related to the relaxation of high molecular mass polymers that comprise gluten (Dobraszczyk and Morgenstern 2003; Li et al. 2003).

Applying the Maxwell model, an elastic modulus for each zone (E_1, E_2, and E_3) and two relaxation times (T_1 and T_2) were obtained for the doughs under analysis in the present study. These relaxation parameters are shown in Table 3. Doughs of experimental design presented values of E_1 higher than the Control dough, and these differences were significant in the case of dough 4. Regarding the E_2 modulus, the highest values were obtained for doughs 5 and 7. Values of E_3 significantly increased only when In was present; the higher proportion of fiber, the higher E_3 values. The E_3 behavior is represented by the regression coefficients of the second-order polynomial model (Table 3) and the response surface (Fig. 8), with In being the most important term.

Parameters E_1 and E_3 were of the same magnitude order while values of E_2 were almost one order greater. These results suggest that gluten polymeric proteins that are relaxing in zone 2, represented by E_2, are greatly contributing to dough elasticity.

As expected, the relaxation time T_1 (zone 1) was higher than T_2 (zone 2). In addition, T_1 increased with increasing In concentrations, independently of Ca carbonate level

Table 4 Viscoelasticity parameters of calcium–inulin wheat flour dough

Dough			Viscoelasticity		
Blend no.	Ca[a]	In[b]	G' (kPa)	G'' (kPa)	tan δ (–)
1	120	1	14±2 a	4.7±0.7 a	0.335±0.008 b, c
2	240	1	15.2±0.9 a	5.0±0.5 a	0.33±0.01 b, c
3	120	12	42±5 c, d	11±2 d, e	0.27±0.02 a
4	240	12	46±1 d	12.1±0.6 e	0.26±0.02 a
CP	180	6.5	29±7 b	8±1 b, c	0.29±0.02 a, b
5	108	6.5	35±2 b, c	9.9±0.5 c, d	0.29±0.01 a, b
6	180	13	49±5 d	12±1 e	0.253±0.004 a
7	252	6.5	37±3 c	11±2 d, e	0.30±0.03 a, b
8	180	0	19.2±0.4 a	6.4±0.4 a, b	0.33±0.01 b, c
Control	0	0	12.4±0.0 a	4.3±0.1 a	0.350±0.008 c

Viscoelasticity parameters: storage modulus (G'), loss modulus (G''), loss tangent (G''/G'). Different letters in the same column indicate significant differences ($p<0.05$). Ingredients: calcium (Ca) and inulin (In)

C control dough (without inulin and calcium), *CP* central point (three replicates), *Control* control dough (without inulin and calcium)

[a] mg Ca/100 g wheat flour

[b] g In/100 g wheat flour

(Table 3). Results suggest that In favored a higher degree of dough relaxation at short times, coincident with the higher values of E_1, and associated with the ordering of small molecules. Comparing with samples CP and 5, the dough with the highest Ca content (sample 7) presented the lowest degree of relaxation (lowest T_1), indicating that $CaCO_3$ exerts a strengthening effect on dough, as it was evidenced by TPA assays. In the second phase, the relaxation degree was lower, probably due to the difficulty in reordering larger molecules (Table 3). Higher T_1 and T_2 values were observed for samples with the highest In content (dough 3, 4, and 6).

Dynamic elastic modulus G' followed the same behavior than E_3 (Table 4). Likewise, for all doughs G' was higher than G'' in the whole frequency range, and curves were almost parallel, with a predominance of a gel-like behavior (not shown). The RSM applied to G' (Fig. 9) shows that the dynamic elastic modulus presented a positive linear variation with In content, independent of Ca level, without any interaction between both variables.

The G' and G'' values obtained determined tan δ values of approximately 0.3, with doughs 3, 4, and 6 being the most elastic ones (lowest tan δ) (Table 4).

Conclusions

Inulin limited W_{abs} in dough due to its high water-retention capacity. In an excess of water content, In promoted a considerable decrease in dough tenacity and extensibility. The amount of water corresponding to farinographic W_{abs} permitted, in the presence of In, the formation of doughs that needed more time to develop and presented lower M_{cont}. Calcium, in the presence of In, acted as dough strengthener. Doughs obtained under such conditions presented less molecular mobility, suggesting that In entraps and immobilizes water within the gluten matrix. This behavior was enhanced in the presence of a very high Ca content (252 mg Ca/100 g flour) due to the restructuring of gluten network by the divalent cation, leading to stiffness, springiness, and a more homogeneous matrix.

The conformation and, particularly, flexibility of the combined gluten–In–Ca–water matrix would finally determine the degree of water binding. Therefore, if the final spatial conformation of proteins is modified by the presence of In–Ca carbonate, it will produce different matrices with distinct rigidity/flexibility and capacity water-binding ability, and, therefore, with distinct rheological properties.

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