Food Bioprocess Technol (2014) 7:1618–1628
DOI 10.1007/s11947-013-1180-y

ORIGINAL PAPER

Rheological Properties of Bread Dough Formulated with Wheat Flour–Organic Calcium Salts–FOS-Enriched Inulin Systems

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Received: 27 March 2013 / Accepted: 23 August 2013 / Published online: 7 September 2013
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Abstract The objective of this work was to study the effect of organic calcium salts–fructooligosaccharide (FOS)-enriched inulin systems on dough structure and rheological properties of wheat flour dough. Wheat flour was enriched with calcium lactate ($CaLa_2$) or calcium citrate (Ca_3Ci_2) (from 1,080 to 2,520 ppm Ca) and FOS-enriched inulin (In) (from 0 to 13 %, *w*/*w* flour basis). Alveographic, texture, relaxation, and viscoelasticity properties of dough were analyzed. Wet and dry gluten quantity, related to scanning electron microscopy structure, was also determined. Tenacity, extensibility, and deformation energy of dough decreased with the increment of In content. When $CaLa_2$ was employed, they changed mainly with fiber, hiding the effect of lactate; whereas with Ca_3Ci_2, these parameters were not only affected by the fiber, but also by the salt. At In 6.5 %, high calcium content (2,520 ppm Ca) produces an increase in hardness and a decrease in cohesiveness for citrate ion; the opposite effect was detected with lactate ion. These parameters decreased at high content of In (13 %). Adhesiveness was dependent only on In level; more adhesive dough at In>6.5 % were obtained, mainly in the case of $CaLa_2$. Ca_3Ci_2 prevents the decrease in adhesiveness at In>12 %. At high calcium levels, high In produced more elastic dough only in the presence of citrate; for lactate, the predominant factor was In. Lactate anion destabilizes protein structure, and together with In favors formation of a less elastic gluten network. On the other hand, the stabilizing effect of citrate ion in a firm network was enhanced by FOS-enriched inulin.

Keywords Calcium lactate · Calcium citrate · Fructo-oligosaccharides/inulin · Wheat flour · Dough rheology

Introduction

Calcium is an essential nutrient required in substantial amounts in human nutrition. The two salts used in this work, calcium lactate and citrate, are widely used as food additives. Calcium lactate is generally recognized as safe with no limitation when used in accordance with good manufacturing practice. It can be used as a firming, flavor, and leavening agent and also as thickener and nutrient supplement. The calcium, sodium, and potassium salts of citric acid are generally recognized as safe and can be used in non-alcoholic beverages, dairy product analogs, infant formulae, among other food sources (fish, meat, poultry, milk) at levels not to exceed good manufacturing practice. Calcium citrate can be used in these products as gelling agents (Brannen et al. 2002).

Bread is a suitable food matrix for inclusion of this mineral. In the UK, the Department of Health (1998) proposed mandatory fortification of white flour with calcium carbonate contributing to approximately with 14 % of total calcium intake. Arabic breads fortified with equal contents of different calcium salts (carbonate, sulfate, citrate), presented similar bread quality as the bread without calcium. Nevertheless, due to the distinct nature of the anion, calcium content provided by each salt was different: 744, 576, and 334 mg Ca/100 g flour for calcium carbonate, calcium sulfate, and calcium citrate, respectively (Ziadeh 2002). Ranhotra et al. (1999) established that wheat flour can be fortified with high levels of calcium (924 and 1412 mg Ca/100 g wheat flour) without adversely affecting white bread quality. Besides, the selection of the appropriate calcium salt for a specific food application should be based on the calcium content of salt, pH, solubility,

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taste, and bioavailability. Calcium carbonate, citrate, lactate, and gluconate present 40, 21, 14, and 9.3 % of calcium, respectively (Berdanier 2002). Currently, the dominant anions in the calcium supplement market worldwide are carbonate and citrate (Thomas et al. 2008). It has been reported that calcium as citrate is better absorbed than calcium as carbonate, causing a greater rise in serum calcium and a greater fall in serum parathyroid hormone (Thomas et al. 2008; Tondapu et al. 2009).

Prebiotics, such as inulin and oligofructose, favor calcium bioavailability. They are non-digestible polysaccharides that are fermented by the gut microbiota, leading to a reduction of pH (Levrat et al. 1991). At low pH, more minerals become soluble and are more readily absorbed from the gut mucosa cell (Ohta et al. 1995). For this reason, it is recommended to include fiber together with calcium in bread formulation. However, the incorporation of fiber to wheat flour produces a negative effect on structure and rheological properties of dough (Collar et al. 2007) due to the dilution of starch and gluten proteins in the blend, leading to the formation of a less elastic matrix. Changes in the technological process must be performed to resolve this issue.

Rheological measurements are commonly used as rapid and sensitive indicators of polymer molecular structure. They are also being applied to bread dough as indicators of the gluten structure and predictors of its functional behavior in breadmaking (Armero and Collar 1997; Dobraszczyk and Morgenstern 2003; Angioloni et al. 2008). Rheological properties of wheat dough with calcium carbonate and inulin were studied (Salinas et al. 2012). Dough hardness, adhesiveness, and elasticity increased with calcium and mainly by inulin. At high inulin content (12 %), $CaCO_3$ acted as dough strengthener.

In another previous work, we have studied hydration and thermal properties of wheat dough formulated with organic calcium salts (lactate and citrate) and inulin (Salinas and Puppo 2013). Water absorption decreased with the presence of calcium and inulin. Dough with calcium lactate ($CaLa_2$) presented lower development time, was less stable and exhibited greater molecular mobility than dough with Ca_3Ci_2. High paste viscosity was also observed for $CaLa_2$ blends. Hydration of components would influence the structure and rheological properties of dough. Therefore, the objective of this work was to study the effect of organic calcium salts (lactate and citrate)–inulin systems on microstructure and rheological properties (viscoelasticity, texture, relaxation) of wheat bread dough.

Materials and Methods

Materials

Wheat flour (type 0000, Molino Campodónico Ltda., Argentina) (A.A.C 2013) for breadmaking with a protein content of 9.7 %, lipids 1.12 %, ash 0.36 % and moisture 12.6 %, was used. Alveographic parameters were 108 mm, 74 mm, and 188 for tenacity (P), extensibility (L), and deformation energy of dough (W), respectively. Farinographic parameters of this flour were 56.4 ml, 12 min, 36.5 min, and 10 UB for water absorption, development time, stability and softening degree, respectively. Wet gluten content was 29.3 %. Other ingredients used were sodium chloride (CELUSAL, Argentina), calcium citrate: $Ca_3(C_6H_5O_7)_2{\cdot}4H_2O$ (Ca_3Ci_2, Sigma-Aldrich, S.A.), calcium lactate: $Ca(C_3H_5O_3)_2$ ($CaLa_2$, Sigma-Aldrich), and oligofructose-enriched inulin at a ratio of fructooligosaccharide (FOS)/inulin (70:30) (Synergy 1, BENEO Orafti, Belgium, containing 92.7 % d.b.).

Experimental Design and Statistical Analysis

Mixtures of wheat flour, calcium salts, and inulin were prepared following the factor levels proposed by Salinas et al. (2012). A factorial design (central composite design, CCD) with two factors (calcium and inulin) was utilized. Two experimental designs were performed, according to the calcium salt used (lactate or citrate). Levels of calcium between 1,080 and 2,520 ppm, and inulin between 0 and 13 % were analyzed (see Table 1). Response surface methodology (RSM) was applied to design experimental parameters in order 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 linear and quadratic effects and interactions between those factors. A second-order model was proposed (Salinas et al. 2012) (Eq. 1):

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

Table 1 Second-order design matrix used for evaluating rheological properties variables of dough

Runs	Coded		Uncoded	
	Ca	In	Ca (ppm)	In (%)
1	−1	−1	1,200	1
2	1	−1	2,400	1
3	−1	1	1,200	12
4	1	1	2,400	12
CP	0	0	1,800	6.5
8	−1.2	0	1,080	6.5
9	0	+1.2	1,800	13
10	1.2	0	2,520	6.5
11	0	−1.2	1,800	0
C	0	0	0	0

CP central point (three replicates), *C* control (outside of the design) where $\overline{Y}$ is the variables response (G', tan δ, and E_3); b_0, b_1, b_2, b_{11}, b_{22}, b_{12} are regression coefficients; X_1 and X_2 are coded variables representing calcium (Ca) and inulin (In) levels, respectively. The model adequacies were checked by the variance analysis (F test) and R^2 values. Variables effects were represented using surface graphs. Parameters (Y) selected for RSM were those whose R^2 was higher than 0.754. We also analyzed control dough, without calcium and inulin, not belonging to the CCD. Data obtained (G', tan δ, and E_3) were analyzed using response surface methodology by Statgraphics plus for Windows 5.1 software (Cambridge, MN, USA). Parameters were subjected to one-way ANOVA according to the general linear model procedure with least-square mean effects. Significantly different means ($p<0.05$) were determined according to Fisher's least significant differences test. Mean and standard deviation were calculated for each parameter.

Dough Formulation

Each flour blend consisted of wheat flour, NaCl (2 %), $CaLa_2$ or Ca_3Ci_2 and inulin in levels established in the designs (Salinas et al. 2012). Quantity of water and mixing time was established by farinographic assays (Salinas and Puppo 2013). Solid ingredients were mixed in a small-scale kneader (Kenwood Major, Italy) for 1 min. Water was added to the solids. The blend was kneaded for 1 min at 50 rpm until reaching development time at 90 rpm (speed 2). Final dough temperature was 24±1 °C. Dough was laminated (four passes), and was allowed to rest for 15 min at 25 °C covered with a plastic film to avoid water loss. Finally, it was laminated to 1 cm thick before cutting. Cylindrical pieces of dough (3 cm in diameter) were cut for analysis. Control dough (C), without calcium salts and inulin, was analyzed outside each central composite design.

Rheological Properties of Dough

Dough Tenacity and Extensibility Tenacity (P), extensibility (L), and deformation energy of dough (W) were measured in a Chopin alveograph (Chopin, France) (A.A.C.C International 2000) at a constant quantity of 2.5 % NaCl solution, corresponding to that obtained for wheat flour was utilized for all blends.

Dough Texture Dough texture was analyzed by performing a texture profile analysis. Twenty cylindrical pieces of dough were used. Dough texture parameters were evaluated using a TA.XT2i Texture Analyzer (STABLE MICRO SYSTEMS, Surrey, U.K.) 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=75 mm). Force–time curves were obtained at a crosshead speed of 0.5 mm/s. Hardness, cohesiveness, and adhesiveness of cylindrical dough pieces were calculated. Hardness is defined as the maximum force registered 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. Adhesiveness is the negative area obtained during the first compression cycle.

Dough Relaxation Stress-relaxation tests were performed in a texture analyzer equipped with a 25-kg load cell together with a 75-mm-diameter cylindrical probe. Each cylindrical dough piece 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. Semisolid 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) a regression exponential decay of second-order was performed. A generalized Maxwell model (Steffe 1996) consisting of two Maxwell elements with a residual spring in parallel (Rodriguez-Sandoval et al. 2009; Salinas et al. 2012) was applied (Eq. 2). The equation obtained from the generalized Maxwell model was shown as:

$$\sigma(t) = \sigma_1 * \exp(-t/T_1) + \sigma_2 * \exp(-t/T_2) + \sigma_3 \qquad (2)$$

Where σ (t) represents the stress measured at any time during the relaxation test, t representing the time. The relaxation time T_i is defined as the ratio between the viscosity and the elastic modulus (Eq. 3).

$$T_i = \eta_i / E_i \qquad (3)$$

The elastic relaxation modulus E_i is defined as the ratio between the stress and constant strain (Eq. 4).

$$E_i = \sigma_i / \varepsilon_0 \qquad (4)$$

Where ε_0 is a constant strain calculated as the ratio of deformation to the initial height of the sample.

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 of the different dough 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 serrated surface sensor system (35 mm diameter) with 1.5 mm gap between plates. The upper plate was lowered and the excess of sample was trimmed off. The exposed surface was covered with a thin layer of semisolid silicone to prevent moisture loss during testing. Samples were rested for 15 min, allowing them to relax before testing. Two types of rheological tests were performed as follows: (a) deformation sweeps at constant frequency (1 Hz) to determine the maximum deformation (γ_{max}) that a sample can experience in the linear viscoelastic range, and b) frequency sweeps (from 0.005 to 100 Hz) at constant deformation (5 Pa) within the linear viscoelastic range. Mechanical spectra were obtained by recording the dynamic moduli G', G'', and tan δ as frequency function. G', the dynamic elastic or storage modulus, is related to the material response as a solid; while G'' standing for the viscous dynamic or loss modulus, is related to the material response as a fluid. Tan δ (G''/G') is related with the overall viscoelastic response: low values of this parameter indicate a more elastic sample.

Gluten Quantity and Dough Structure

Gluten Quantity Wet and dry gluten were determined according to AACC 38-12 method with some modifications. A Glutomatic 2200 (Perten Instrument, Sweden) was utilized. The maximum time for dough development in glutomatic (75 s) was lower than the minimum farinographic development time (8 min). Therefore, dough was prepared in a kneading and then introduced in the glutomatic equipment for washing and gluten obtaining. Dough quantity equivalent to 10 g of flour was placed into the ordinary test chamber equipped with the fine 80-μm sieve. Dough was kneaded 30 s and washed for 8 min. Quantity of wet gluten (WG) and wet gluten/dry gluten ratio (DG) (WG/DG) were assayed.

Microscopy of Dough Microstructure of dough was analyzed by scanning electron microscopy. Cylindrical dough pieces (2 mm diameter×5 mm height) were stored in 2.5 %*v*/*v* glutaraldehyde solution and then washed twice with 0.5 M phosphate buffer before dehydration with a graded acetone series: 25, 50, 75, and 100 % (three times). Drying of samples was performed at the critical point with the intermediate CO_2 fluid. 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).

Results and Discussion

Dough Tenacity and Extensibility

Results of tenacity, extensibility, and deformation energy were not suitable for predicting baking quality as in the case of wheat flour, but allowed us to study at constant water level, the influence of inulin and organic calcium salts on these parameters.

Calcium lactate did not modify the tenacity P (Fig. 1a) nor the extensibility L (Fig. 1b) of blends, at the same content of fiber, with the exception of 6.5 % FOS-enriched inulin. At intermediate In level (6.5 %) P significantly decreased ($p<0.05$) with the increase of calcium content (2,520 ppm Ca). In contrast, P and also L at constant inulin content were modified by the increase in calcium citrate level. At 1 % In, P increased, while at 12 % of fiber, it decreased with calcium salt content (Fig. 1c). At inulin levels ≤6.5 %, L significantly decreased with the increment of Ca_3Ci_2 (Fig. 1d). Sudha and Leelavathi (2008) studied the effect of calcium salts (carbonate, lactate, citrate, phosphate) at different levels (800, 1,200, 1,600 mg Ca/kg wheat flour) on alveographic parameters, but without fiber addition. Significant differences in P value of dough with Ca_3Ci_2 in comparison to control sample were not reported by these authors; however, a lower value of P was found for $CaLa_2$.

Deformation energy (W) of dough with $CaLa_2$ and Ca_3Ci_2 are also shown in Fig. 1a, c (black circles), respectively. In the absence of inulin, W with both calcium salts was significantly higher than the value observed for control dough. At 6.5 % of inulin and intermediate calcium level (1,800 ppm Ca) both doughs presented a maximum value of W, contrary to what happened when using an inorganic calcium salt ($CaCO_3$) (Salinas et al. 2012).

All parameters were negatively affected by the presence of inulin. As wheat flour absorbs less quantity of water in the presence of FOS/In, blends with the prebiotic (≥6.5 %) were subjected to an excess of water content, leading to low values of P, L, and W. For the same quantity of calcium, lactate contributed with a negative charge of −1, while citrate contributed with a net charge of −2; in addition, more lactate than citrate anions were incorporated (Salinas and Puppo 2013). Differences in tenacity and extensibility can be attributed to the distinct amount of negative charge of both organic anions and to the relation cation/anion of each calcium salt. When $CaLa_2$ was employed, parameters (P, L, and W) changed mainly with fiber, hiding the effect of lactate; whereas with Ca_3Ci_2, these parameters were not only affected by the fiber, but also by the salt.

Dough Texture

Texture profile analysis parameters are shown in Fig. 2. In the absence of In, calcium salts produced an increase in hardness (Fig. 2a, b) mainly with Ca_3Ci_2, coincident with the high deformation energy of dough (W) obtained. At 1 % In, dough hardness increased with $CaLa_2$ content; but at high levels of In (≥6.5 %), higher levels of $CaLa_2$ decreased this parameter. An increase of inulin content (Fig. 2a) resulted in an increase in

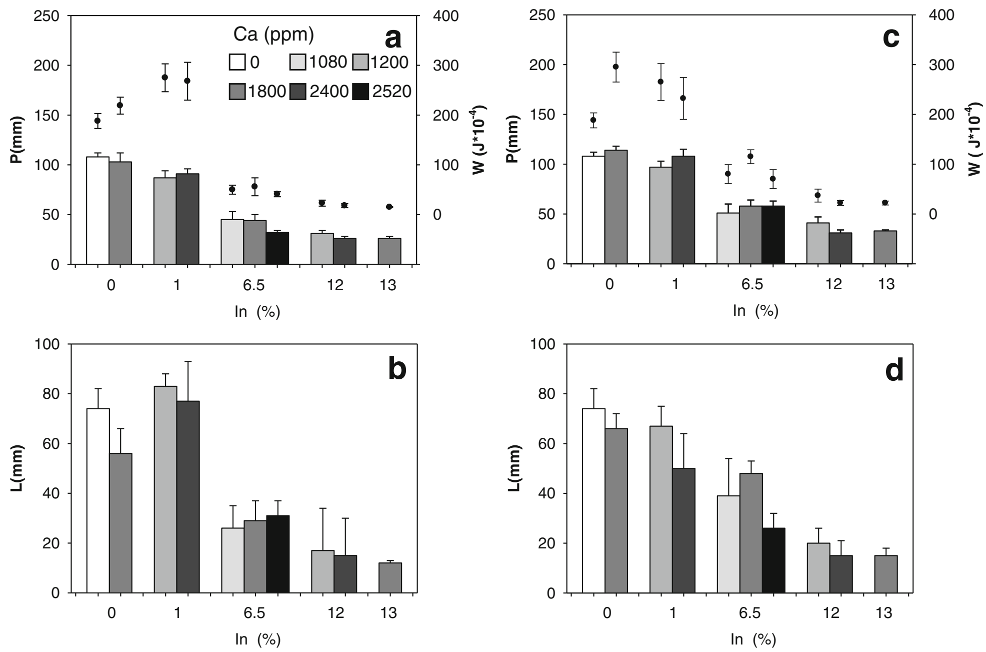

Fig. 1 Alveographic parameters of wheat flour–inulin–calcium salt blends. Tenacity, *P* (*bars*) and deformation energy, *W* (*dots*) (**a**, **c**); extensibility, *L* (**b**, **d**). *Errors bars* standard deviations. Blend with $CaLa_2$ (**a**, **b**), Ca_3Ci_2 (**c**, **d**)

hardness, the control sample having the softer dough than others. In a previous work (Salinas and Puppo 2013), we observed that inulin produced low molecular mobility with a less water availability, favoring dough structuring.

Citrate anion, accompanying the high level of calcium (2,520 ppm Ca), unlike lactate, produced hard dough, suggesting the formation of a more firm network as a consequence of the stabilizing effect of this ion (Fig. 2b). At high content of In (13 %), this effect was diminished.

In the absence of In, the incorporation of $CaLa_2$ decreased dough cohesiveness (Fig. 2c); a low cohesiveness is associated with a less integration of components within dough. However, at intermediate levels of In (6.5 %), a high calcium content produces an increase in cohesiveness. The contrary effect was observed with Ca_3Ci_2 (Fig. 2d).

Although adhesiveness (Fig. 2e, f) depended mainly on FOS-enriched inulin level, differences were observed between salts. At 6.5 % In, adhesiveness was not modified by the content of $CaLa_2$ and Ca_3Ci_2, although this parameter was high in the case of calcium citrate. More adhesive dough at In 12–13 % were obtained with $CaLa_2$.

The most influence on texture parameters was observed at 6.5 % In. Lactate and citrate are anions with different hydration abilities, modifying in distinct manner interactions between proteins (Salinas and Puppo 2013). Lactate is a chaotropic anion which alters the normal water structure, modifying in a negative form interactions between gluten proteins, leading to a low rigid network and therefore to a softer dough. Dough was more cohesive because components were more integrated. On the contrary, citrate is a non-chaotropic anion; its interaction with water strengthens hydrophobic interactions of proteins leading to a more structured network, and hence harder and less cohesive dough. FOS enriched with inulin is a soluble component that would be less implicated in the gluten matrix. These molecules, due to its lubricant effect, are able to migrate to the dough surface, contributing to high adhesiveness.

Dough Viscoelasticity

Samples presented G' values higher than G'' throughout all frequency range, suggesting that dough are predominantly elastic systems. Control dough presented the lowest value of G' (12.4 kPa). For dough with $CaLa_2$, a direct dependency with Ca and Ca^2 and mainly with the inverse with Ca*In was observed, being the last one the more significant dependence (Fig. 3a, Table 2). This dependency means that at low levels of $CaLa_2$ (1,200 ppm Ca) an increase in In produced the increment of this parameter. Peressini and Sensidoni 2009 found that an increment in inulin content resulted in higher and lower values of G' and tan δ, respectively, suggesting that dough became more elastic with the fiber. On the other hand, at a high level of calcium (2,400 ppm Ca) the increment of inulin, decreased G'. When Ca_3Ci_2 was employed, a very different surface response was obtained; G' increased with In, independently of salt content (Fig. 3b, Table 2).

The parameter that better represents viscoelasticity of dough is tan δ, because it is the ratio between both moduli (G''/G'). Control dough had maximum viscosity, with the highest value of tan δ (tan δ=0.350). Surface responses of this parameter were quite different depending on which salt ($CaLa_2$ or Ca_3Ci_2) was incorporated to blends. For lactate the main influence was observed for Ca*In interaction, while for citrate the predominant factor was In (Fig. 3c, d,

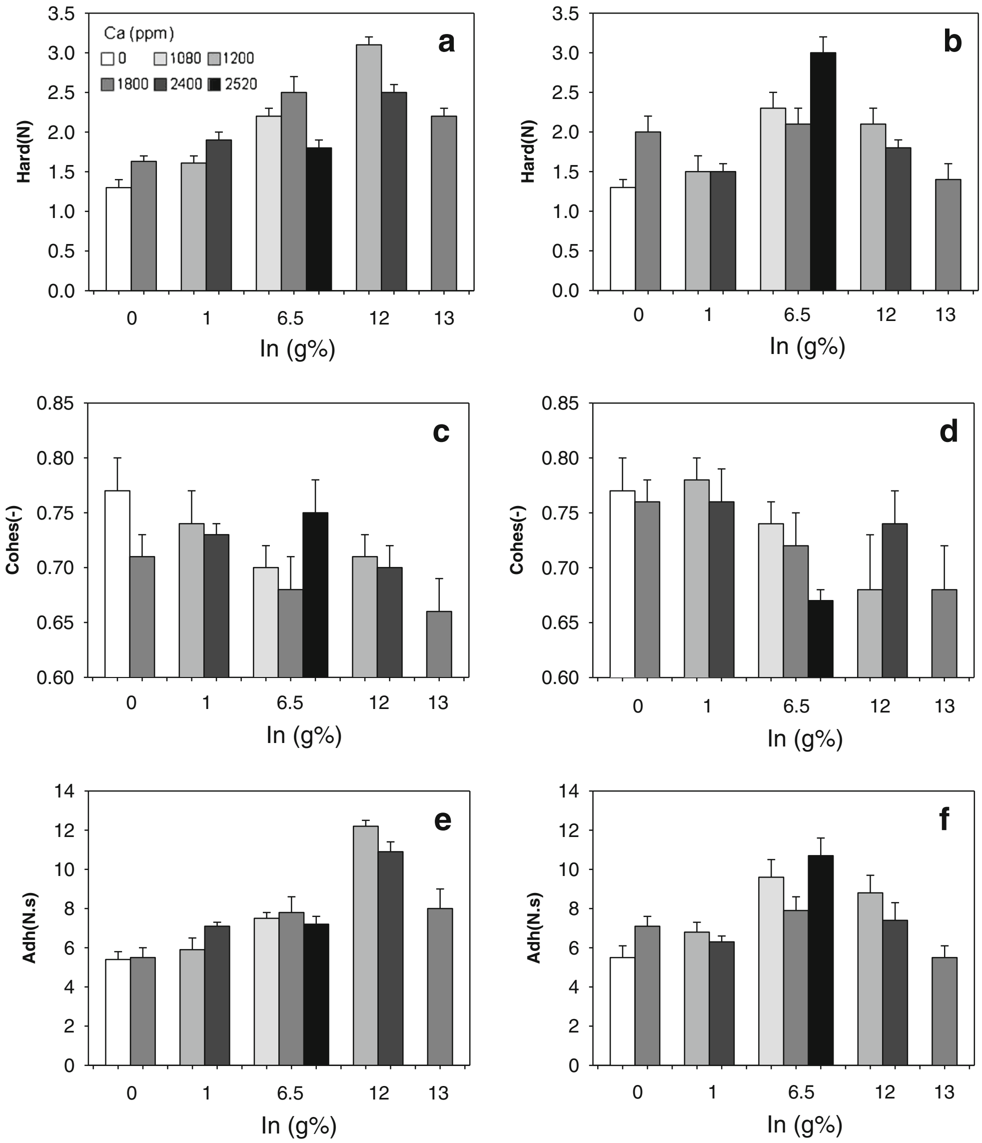

Fig. 2 Texture parameters of dough prepared with wheat flour–inulin–calcium salts blends: hardness (**a**, **b**), cohesiveness (**c**, **d**), adhesiveness (**e**, **f**). *Errors bars*: standard deviations. Blend with $CaLa_2$ (**a**, **c**, **e**), Ca_3Ci_2 (**b**, **d**, **f**)

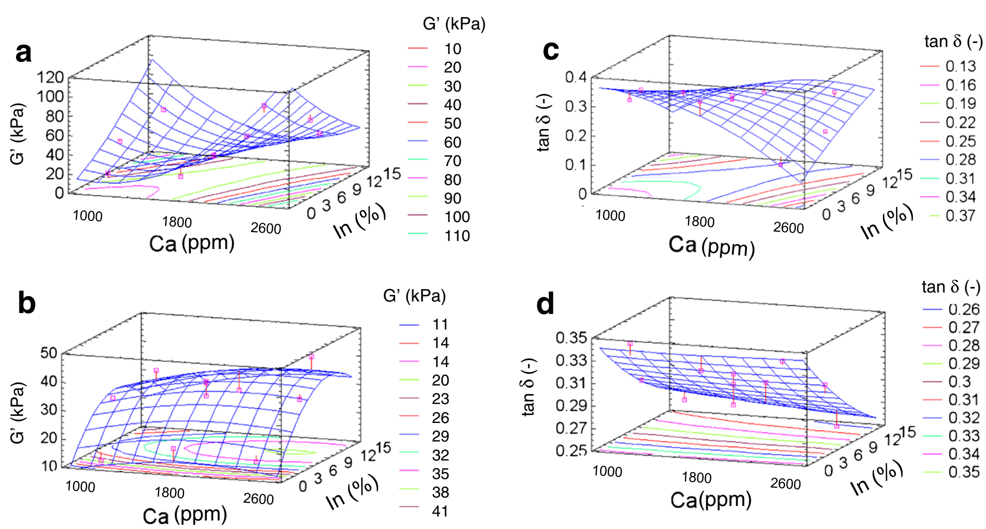

Fig. 3 Response surface graphs of dough prepared with wheat flour–inulin–calcium salts blends: storage modulus, G' (**a**, **b**) and loss tangent, tan δ (**c**, **d**). Blend with $CaLa_2$ (**a**, **c**), Ca_3Ci_2 (**b**, **d**). Point over surface belongs to average experimental data. Contour plots are shown at the bottom of the response surface graphs

Table 2 Analysis of variance and regression coefficients for the second-order polynomial models

Source	Calcium lactate						Calcium citrate					
	G' (kPa)		tan δ (−)		E3 (kPa)		G' (kPa)		tan δ (−)		E3 (kPa)	
	b	p value	b	p value	b	p value	b	p value	b	p value	b	p value
Constant (b_0)	26.48		0.296		0.389		34.38		0.298		0.313	
Ca	16.05	0.001	−0.039	0.03	−0.013	0.79	2.43	0.29	−0.002	0.80	0.091	0.07
In	−2.41	0.39	−0.004	0.75	0.228	0.01	8.61	0.008	−0.030	0.005	0.088	0.07
Ca^2	21.68	0.001	−0.038	0.08	0.012	0.82	−2.11	0.48	8.10^{-4}	0.93	0.09	0.14
In^2	42.15	0.38	−0.007	0.71	0.05	0.48	−7.12	0.049	0.0046	0.61	−0.042	0.46
Ca*In	−23.61	0.001	0.055	0.02	−0.055	0.43	1.27	0.66	−0.02	0.82	−0.05	0.37
R^2		0.963		0.832		0.825		0.840		0.818		0.754
Root MSE		6.73		0.003		0.129		5.39		0.014		0.103

Significant differences at p value<0.05

Table 2). In consequence, at high calcium levels, high contents of In produced more elastic (less tan δ) dough only in the presence of citrate ion. Increasing contents of inulin of different degree of polymerization resulted in an increase of G' and a decrease in tan δ, indicating greater dough elasticity, effect that was less pronounced for the shorter chain inulin (Peressini and Sensidoni 2009). Other authors (Wang et al. 2002) reported a decrease in elasticity upon addition of 3 % chicory inulin to wheat flour.

Dough Relaxation

Dough relaxation was analyzed in a previous work in dough prepared with calcium carbonate (Salinas et al. 2012). Applying the Maxwell general model to dough relaxation curve, an elastic modulus for each zone (E_1, E_2, E_3) and two relaxation times (T_1, T_2) ($T=\eta/E$) were obtained. Relaxation times (T_1 and T_2) are shown in Table 3 and RSM of elastic modulus (E_3) is shown in Fig. 4 and regression coefficients in Table 2. E_1 and T_1 governs stress relaxation at the beginning of the test, attributed to the orientation of molecules of low size; while E_2 and T_2 (intermediate zone) represent relaxation of polymeric molecules. If stress does not change with deformation achieving the equilibrium, the E_3 term predominates and represents the stored energy. For a better understanding of relaxation phenomenon, only T_1, T_2, and E_3 parameters were analyzed.

Control dough presented the low values of T_1 and T_2, on the other hand dough with $CaLa_2$ (2,400 ppm Ca, 12 % In) presented a high value of these parameters (Table 3); although they were not significantly different with respect to other samples. No significant differences in T_1 and T_2 parameters of dough with Ca_3Ci_2 were observed. Results suggest that in the case of calcium lactate, at high inulin level, there is a major degree of relaxation of polymers, coincident with the high viscosity of dough.

Table 3 Relaxation parameters of calcium salts–inulin wheat flour dough

# Blend		Relaxation			
		$CaLa_2$		Ca_3Ci_2	
Ca (ppm)	In (%)	T_1 (s)	T_2 (s)	T_1 (s)	T_2 (s)
1,200	1	311±14 bc	8.6±0.7 bcd	316±31 bc	9.0±1.8 ab
2,400	1	277±12 ab	7.4±0.7 ab	336±48 c	10.3±2.7 b
1,200	12	312±24 bc	9.0±0.4 cde	319±30 c	8.3±1 ab
2,400	12	357±5 c	10.3±0.9 e	297±28 bc	8.0±0.4 ab
1,800	6.5	275±32 ab	7.0±0.9 a	317±50 c	8.5±1.2 ab
1,080	6.5	308±35 bc	8.4±1.2 bcd	322±10 c	8.4±0.5 ab
1,800	13	331±37 bc	9.5±0.5 de	340±9 c	8.5±0.7 ab
2,520	6.5	278±6 abc	7.0±0.4 ab	327±38 c	8.6±0.7 ab
1,800	0	282±23 abc	7.9±1.2 abc	256±6 ab	6.8±0.5 ab
0	0	227±15 a	6.1±0.5 a	227±15 a	6.1±0.5 a

Relaxation parameters: Relaxation time (T). (1,800 ppm Ca; 6.5 % In): central point (three replicates). Different letters in the same column indicate significant differences (p<0.05). Ingredients: calcium (Ca), inulin (In). All blends had 2 % NaCl

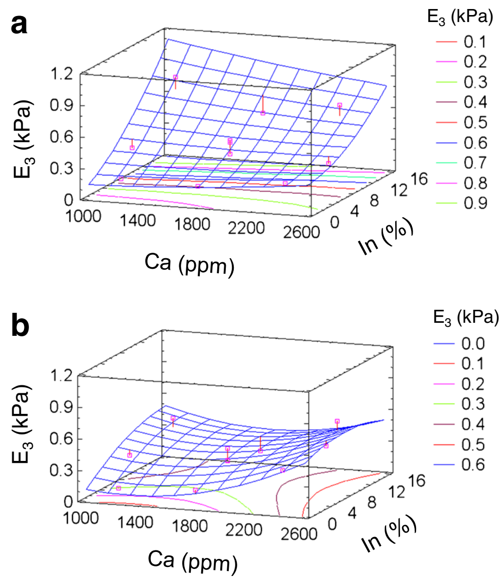

Fig. 4 Response surface graphs of the relaxation elastic modulus (E_3) of dough. Blends with **a** $CaLa_2$, **b** Ca_3Ci_2. Point over surface belongs to average experimental data. Contour plots are shown at the bottom of the response surface graphs

In the absence of inulin, the E_3 value of dough with $CaLa_2$ (0.2 kPa) was significantly higher than the value obtained for control dough (0.11 kPa), in concordance with the major value of hardness and G'. The elastic modulus E_3 of dough with $CaLa_2$, as it can be observed in RSM (Fig. 4a), proportionally increased with inulin. The same behavior was observed in dough with calcium carbonate (Salinas et al. 2012). RSM of dough with Ca_3Ci_2 is shown in Fig. 3b. The regression model did not present terms with statistical significance ($p<0.05$), but E_3 exhibited a slight tendency to augment with the increment of calcium and inulin levels.

The rheological properties of different dough varied with the level of FOS-enriched inulin but also, and in a great manner, with the type of organic calcium salt. At constant level of In (6.5 %) and the highest content of Ca (2,520 ppm Ca), dough with $CaLa_2$ presented less hardness and tan δ than dough with Ca_3Ci_2. In addition, dough with Ca_3Ci_2 showed higher value of the equilibrium elastic modulus (E_3), suggesting higher stored energy, consistent with the stabilizing effect of this anion on gluten network. A more structured gluten matrix, like in the case of calcium citrate dough, is desirable for resisting the different technological stages during breadmaking, so as to ensure high quality breads.

Gluten Content and Dough Structure

WG and wet to dry gluten ratio (WG/DG) are shown in Table 4. In the absence of inulin, comparing to control sample, WG of $CaLa_2$ and Ca_3Ci_2 decreased and increased, respectively. The lowest value of WG was observed for dough with 1 % In–2,400 ppm Ca and 6.5 % In–2,520 ppm Ca. At low inulin level (≤6.5 %), calcium lactate disfavored gluten formation, confirming low degree of protein interaction due to disestablishing effect of this anion. This effect was evident at 1 % In. At 12 % In, an increase in salt content did not augment WG, suggesting a protector effect of the fiber against gluten destructuring due to lactate, favoring gluten entanglement. The same tendency was observed for WG/DG, obtaining the lowest value for dough with the highest content of $CaLa_2$ and 1 % In. Values of WG/DG of around 3 was obtained for all samples, an optimum value for breadmaking dough. WG values of dough with Ca_3Ci_2 were higher than the value of control dough. At intermediate inulin concentration (6.5 %), more WG was obtained with citrate than with lactate. These results support the stabilizing effect of citrate ion on gluten proteins. In calcium citrate systems, inulin enhances the increment of WG/DG.

Quality of gluten is an important factor since this polymer is the base component of dough structure. As it was previously observed for calcium carbonate–inulin systems (Salinas et al. 2012), a gluten matrix involving starch granules can be also observed. Dough with the same content of WG formed a more homogeneous gluten structure (Fig. 5, La1, Ci1, Ci2). A less

Table 4 Wet gluten quantity and gluten ratio of calcium salts–inulin–wheat flour dough

# Blend		$CaLa_2$		Ca_3Ci_2	
Ca (ppm)	In (%)	WG (%)	WG/DG	WG (%)	WG/DG
1,200	1	31.7±0.5 e	3.25±0.02 bc	31.7±0.3 bc	3.10±0.02 ab
2,400	1	25.5±0.1 a	2.59±0.11 a	31.6±0.3 bc	3.15±0.06 abc
1,200	12	30.7±0.1 de	3.50±0.12 d	30.8±0.0 ab	3.28±0.06 cd
2,400	12	31.2±0.3 e	3.41±0.06 cd	31.1±0.4 b	3.31±0.03 d
1,800	6.5	28.3±1.4 bc	3.23±0.12 b	31.2±0.5 b	3.22±0.04 bc
1,080	6.5	29.4±0.7 cd	3.26±0.03 bc	31.1±0.3 b	3.28±0.05 cd
1,800	13	28.2±0.3 bc	3.41±0.05 cd	32.9±0.5 c	3.29±0.00 cd
2,520	6.5	24.5±0.5 a	3.31±0.04 bc	30.3±0.6 ab	3.18±0.09 bc
1,800	0	27.4±0.3 b	3.29±0.02 bc	31.6±0.4 bc	3.07±0.06 a
0	0	29.3±1.4 cd	3.21±0.09 b	29.3±1.4 a	3.21±0.09 bcd

WG Wet gluten, *WG/DG* gluten ratio (wet gluten/dry gluten). Different letters in the same column indicate significant differences ($p<0.05$). Ingredients: calcium (Ca), inulin (In). All blends had 2 % NaCl

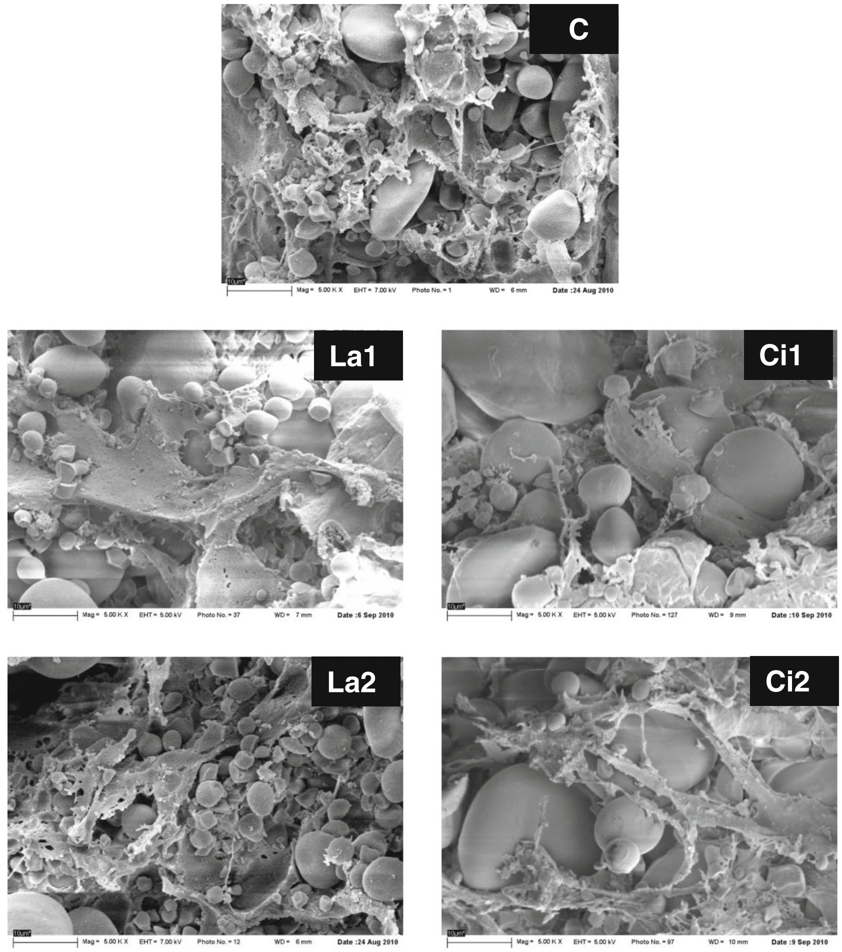

Fig. 5 Scanning electron micrographs (SEM) of dough prepared with wheat flour–inulin–calcium salts blends. *C* control dough. Samples with: $CaLa_2$ (*La1*, *La2*), Ca_3Ci_2 (*Ci1*, *Ci2*). La1, *Ci1*: 1,200 ppm Ca–1 % In; La2, *Ci2*: 2,400 ppm Ca–1 % In. *C* 0 ppm Ca–0 % In. Magnification, ×5,000

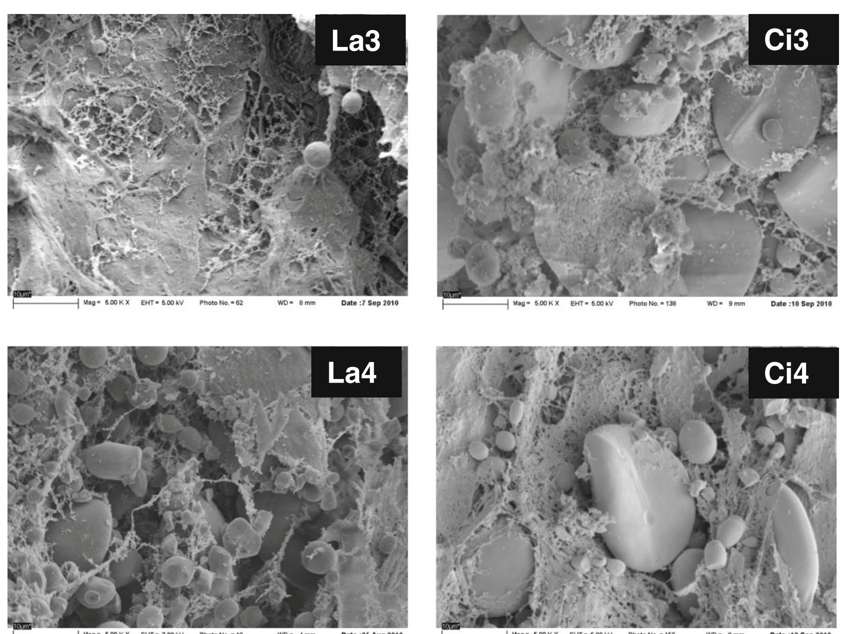

Fig. 6 Scanning electron micrographs (SEM) of dough prepared with wheat flour–inulin–calcium salts blends. Samples with: $CaLa_2$ (*La3*, *La4*), Ca_3Ci_2 (*Ci3*, *Ci4*). La3, *Ci3*: 1,200 ppm Ca–12 % In; La4, *Ci4*: 2,400 ppm Ca–12 % In. Magnification, ×5,000

laminar gluten network was observed for control dough but mainly for La2 dough, presenting these two dough values of WG less than 30 %.

Structures of dough with high content of In (12 %) are shown in Fig. 6.

As in Fig. 5, dough with a gluten matrix in which starch granules are immersed was also observed. For organic salts, as in the case of the inorganic salt (Salinas et al. 2012), dough structure in which a string of molecules of FOS-enriched inulin over gluten matrix, was also obtained. Dough with high content of In also presented the same quantity of wet gluten, being approximately 31 % in all samples. The gluten network appears to be more homogeneous in the presence of calcium citrate. Nevertheless, La4 dough seems to exhibit a more opened structure due to destabilizing effect of lactate.

Conclusions

Gluten quantity, microstructure, and rheological properties of dough were, at the same content of calcium, dependent on the type of anion (citrate or lactate) of the organic salt. These properties were also dependent on inulin content. Differences in rheological behavior can be attributed to distinct kinds of interactions between the fiber and the organic calcium salts. When $CaLa_2$ was employed, dough tenacity and extensibility changed mainly with fiber, hiding the effect of lactate; whereas with Ca_3Ci_2, these parameters were also affected by the salt. At intermediate content of FOS-enriched inulin and the highest level of Ca_3Ci_2, the hardest dough with the lowest cohesiveness and the highest adhesiveness was obtained. $CaLa_2$ exhibit the opposite behavior. With high amounts of In, Ca_3Ci_2 was more effective in preventing dough adhesiveness than $CaLa_2$. Viscoelasticity of dough was dependent on inulin level for Ca_3Ci_2 and on the interaction between calcium and inulin for $CaLa_2$. Ca_3Ci_2 also favored the formation of more quantity of gluten leading to a more homogeneous matrix.

Gluten structure and different rheological properties are influenced by calcium content and mainly by the type of the anion that accompany at this element in the salt. These properties were also dependent on the organic salt–inulin interaction. While lactate anion destabilizes protein structure and, jointly with FOS-enriched inulin, favored the formation of a less elastic gluten network, citrate ion helped in stabilizing the protein matrix, which was further enhanced by the presence of FOS-enriched inulin.

In conclusion, from the mineral point of view, not only calcium level is important, but also the type of calcium salt due to the different nature of the anion that accompanies this ion, affecting rheological properties of dough. The evaluation of the technological performance of these formulations during bread making and the physical, sensory, and nutritional bread quality still remains.

Acknowledgments The authors would like to acknowledge CONICET and FCAyF (UNLP) of Argentina for the financial support and Molino Rio de la Plata S.A. of Argentina for technical assistance.

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