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Effect of organic calcium salts–inulin systems on hydration and thermal properties of wheat flour

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ABSTRACT

The aim of this work was to study the effect of calcium acid salts–inulin systems on hydration and thermal properties of wheat flour dough. Wheat flour was enriched with calcium lactate ($CaLa_2$) or calcium citrate (Ca_3Ci_2) (1080–2520 ppm Ca) and inulin (*In*) (1%–13%, w/w flour basis). Water absorption (W_{abs}), moisture content (M_{cont}), water activity (a_w) and relaxation time (λ) of dough were analyzed. Pasting properties during heating–cooling process were studied: peak viscosity (PV), breakdown (BD), final viscosity (FV) and setback 1(SB1) were determined. Temperatures (T_{pI}, T_{pII}) and enthalpy (ΔH_{gel}) of gelatinization of dough were analyzed by DSC. Samples with *Ca* and *In* presented lower W_{abs} than control sample with an In^2 dependence, with slight difference between both surface responses. More time for dough development (t_d) was necessary with Ca_3Ci_2 than with $CaLa_2$, being t_d independent of calcium content at *In* level ($\geq$6.5%). Dough with Ca_3Ci_2 was more stable with less degree of softening than $CaLa_2$-dough, due to the protein stabilizing effect of citrate ion (Hofmeister series) with a maximum at 6.5% *In*. M_{cont} and λ decreased with the increase of *In*, independently of calcium. Hydration properties directly influenced pasting parameters. The increase in *In* content decreased viscosity (PV, FV) without affecting BD. SB1 behavior suggests the formation of pastes with low and high stability with $CaLa_2$ and Ca_3Ci_2, respectively. Gelatinization degree decreased (40%) and retarded ($\Delta T = 10$ °C) at high levels of both ingredients. $CaLa_2$ had more influence in hydration and thermal properties of wheat flour–inulin blends, enhancing a high degree of inhibition of gelatinization and leading to pastes with low viscosity after cooling. This behavior was influenced by the presence of inulin.

1. Introduction

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

Some foods, like white bread are lacking of calcium content. Processing of wheat grain into flour can modify the content of nutrients. The germ and aleuronic layers are particularly rich in minerals and vitamins. These nutrients are discarded with the hull during milling, leading to low nutritional value white flour (Rosell, 2003). Hull produces low quality bread; therefore, fortification of white wheat flours with vitamins and minerals has become a current strategy in the food industry. Wang and Tang (1995) reported the effect of fortification with calcium and zinc lactate on the rheological and baking properties of wheat flour. They showed that the supplementation improved the dough expansion without adverse effects on bread quality, which retained 87 and 93% of the added calcium and zinc, respectively. The content of calcium in foods is not only an important factor, but also the absorption of this mineral in the body determines its bioavailability. Some functional food ingredients, such as inulin, a carbohydrate considered as dietary fiber (Roberfroid, 1993), may enhance calcium bioavailability from foods (Roberfroid, 2005; Weaver & Liebman, 2002; Whiting & Wood, 1997).

Although there is no information available about the influence of calcium–inulin mixtures on rheological characteristics of wheat flour dough, previous studies have explored in a separate form the effect of inulin (Gomez, Ronda, Blanco, Cabailero, & Apesteguía, 2003; O'Brien, Mueller, Scannell, & Arendt, 2003; Peressini & Sensidoni, 2009) and different calcium salts (Sudha & Leelavathi, 2008) on dough and bread quality. The effect of different calcium salts (calcium carbonate, calcium lactate, calcium citrate and calcium phosphate) (range: 800–1600 ppm Ca) on farinograph (water absorption, dough development time, and dough stability) and alveograph (tenacity, extensibility, and deformation energy of dough) properties were

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studied. A tendency to form dough with low stability and with high extensibility-to-tenacity ratio with calcium carbonate and lactate salts was observed. The opposite behavior was detected for calcium citrate. No significant differences in pasting profiles for the three blends were observed by these authors. However, little information is available on how calcium salts together with inulin affect the thermal and hydration properties of the mixtures.

Nowadays, food innovation is built on the concept of functional food. The concept of functional foods is based in the use of nutrition knowledge in the food industry with the aim of improving consumers' health (Peressini & Sensidoni, 2009). Bread is one of the foods consumed by a large proportion of the population, from children to elderly, and constitutes a good matrix for including nutritional ingredients like calcium and fiber. From a technological point of view, bread quality is closely related with dough quality; therefore, the aim of this work was to study the effect of calcium organic salts (calcium citrate and calcium lactate) combined with inulin on the hydration and thermal properties of wheat flour blends. Hydration was conducted by farinographic water absorption, moisture content, water activity and molecular mobility (RMN) assays. Pasting properties during heating (peak and final viscosity, breakdown and setback) and thermal properties (gelatinization temperatures and enthalpy) were studied through viscoamilographic (RVA) and differential scanning calorimetry (DSC) assays, respectively. For studying interaction between factors, calcium and inulin content, a central composite design (CCD) was used; and data was analyzed through response surface methodology (RSM).

2. Materials and methods

2.1. Materials

The wheat flour used in this work (type 0000, Molino Campodónico Ltda., Argentina) (ACA, 1992) was adequate for breadmaking with a protein content of 9.7%, lipids 1.12%, ash 0.36% and moisture 12.6%. Other ingredients used were sodium chloride (CELUSAL, Argentina), calcium citrate (Ca_3Ci_2, Sigma Aldrich), calcium lactate ($CaLa_2$, Sigma Aldrich) and oligofructose-enriched inulin (Synergy 1, BENEO Orafti, Belgium, containing 92.7% d.b.).

2.2. Experimental design and statistical analysis

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

Central composite designs consist of a factorial design (the corners of a cube) together with center and star points that allows the estimation of second-order effects.

In this work we used two designs, one for $CaLa_2$ and the other for Ca_3Ci_2. Calcium and inulin levels for the experimental design were selected according to Salinas, Zuleta, Ronayne, and Puppo (2011). The daily intake of calcium (USDA, 2008) and the maximum levels of this mineral allowed in bread, were considered. In addition, inulin levels were selected according to the amount of this fiber needed to ensure calcium bioavailability.

Some parameters were analyzed by RSM using 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. Significantly different means were determined according to Fisher's least significant differences (LSD) test. Mean was calculated for each parameter. The second order model proposed (Khuri & Cornell, 1996) for each parameter was (Eq. (1)):

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

where Y is the variable response (water absorption, moisture content, and ^{1}H-NMR molecular mobility); $b_{1,}$ $b_{11,}$ b_{22} and b_{12} are regression coefficients; X_1 and X_2 are coded variables that represent calcium (*Ca*) and inulin (*In*), respectively.

The model adequacies were checked by the variance analysis (F test) and R^2 values. Variable effects were represented using surface graphs. Parameters (Y) selected for RSM were those whose R^2 was higher than 0.600. The root-mean-square error (RMSE) frequently used, is a good measure of RSM accuracy, and measure differences between predicted and estimated values predicted.

2.3. Formulation of blends

Each flour blend consisted of wheat flour, NaCl 2% (flour basis, f. b.), calcium salt (calcium citrate or calcium lactate) at levels (mg Ca/1 kg flour) of 1080, 1200, 1800, 2400 and 2520. The inulin was incorporated at levels of 0, 1, 6.5, 12 and 13% (g In/100 g flour). Levels for both calcium ion and inulin ingredients were selected according to the design utilized in a previous work (Salinas et al., 2011).

2.4. Structure of dough during development

Development of dough was followed by farinographic assays. A Brabender farinograph (300 g capacity) (Brabender, Duigsburg, Germany) was utilized for measuring water absorption (W_{abs}), development time (t_d), stability (Stb) and softening degree (Soft) of dough (AACC 54-21).

Blend with $CaLa_2$ (2400 ppm Ca) and 12% *In* presented a special farinograph form; therefore it was selected for microstructure analysis. Dough was analyzed at different stages of mixing. Solid ingredients and water (farinographic absorption value) were mixed in a kneader at 90 rpm. Cylindrical pieces of dough (2 mm diameter × 0.5 cm height) were taken at different times (8, 16, 29, 45 and 100 min) and stored in aqueous glutaraldehyde solution (10% v/v) for scanning electron microscopy (SEM) analysis. Samples were washed twice with 0.5 M phosphate buffer and dehydrated with a graded acetone series: 25%, 50%, 75% and 100%. 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).

2.5. Preparation of dough

Each flour blend consisted of commercial wheat flour, NaCl 2% flour basis, and the amount of calcium salts and inulin according to the design proposed. The optimum quantity of water (W_{abs}) and mixing time (t_d) were established by farinographic assays. Ingredients were mixed in a small scale kneader (Keenwood Major, Italy) at 90 rpm. Final dough temperature was 23–25 °C. Dough was laminated (4 passes), and let rest for 15 min at 25 °C covered with a film to avoid water loss. Dough without calcium and inulin, used as control dough (C), was analyzed outside each 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.

2.6. Dough hydration properties

2.6.1. Moisture content and water activity

Dough moisture content was determined according to the AACC Approved Method 44-19 (AACC International, 2000) as the difference between weights measured before and after drying at 135 °C in a 2-hour period. Dough water activity 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.

2.6.2. Molecular mobility

The molecular mobility of the different dough was analyzed by relaxation assays with RMN Brücker Minispec equipment (Brücker, USA). A dough portion 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.

2.7. Thermal properties of blends

Blends used were: C (0 ppm Ca, 0% *In*), La1 or Ci1 (1200 ppm Ca, 1% *In*), La2 or Ci2 (2400 ppm Ca, 1% *In*), La3 or Ci3 (1200 ppm Ca, 12% *In*) and La4 or Ci4 (2400 ppm Ca, 12% *In*).

2.7.1. Pasting properties

Pasting properties of blends were determined according to AACC Approved Method 76-21(AACC International, 2000) using the Rapid Visco Analyzer (RVA) (Serie 4, Newport Scientific, Australia). Mixtures composed of the mentioned blends (3.5 g based on 14% moisture) and 25 ml of distilled water were heated to 50 °C for 1 min and then ramped to 95 °C at a rate of 12.16 °C/min. After holding at 95 ºC for 2.5 min, the temperature was decreased during 3.8 min to 50 °C and kept under this temperature for 1 min. In the first 10 s process, the suspension was stirred at a speed of 960 rpm and then immediately at 160 rpm until the end. The total run time was 13 min. Results were analyzed using the Thermocline software (version 2.4 Windows),

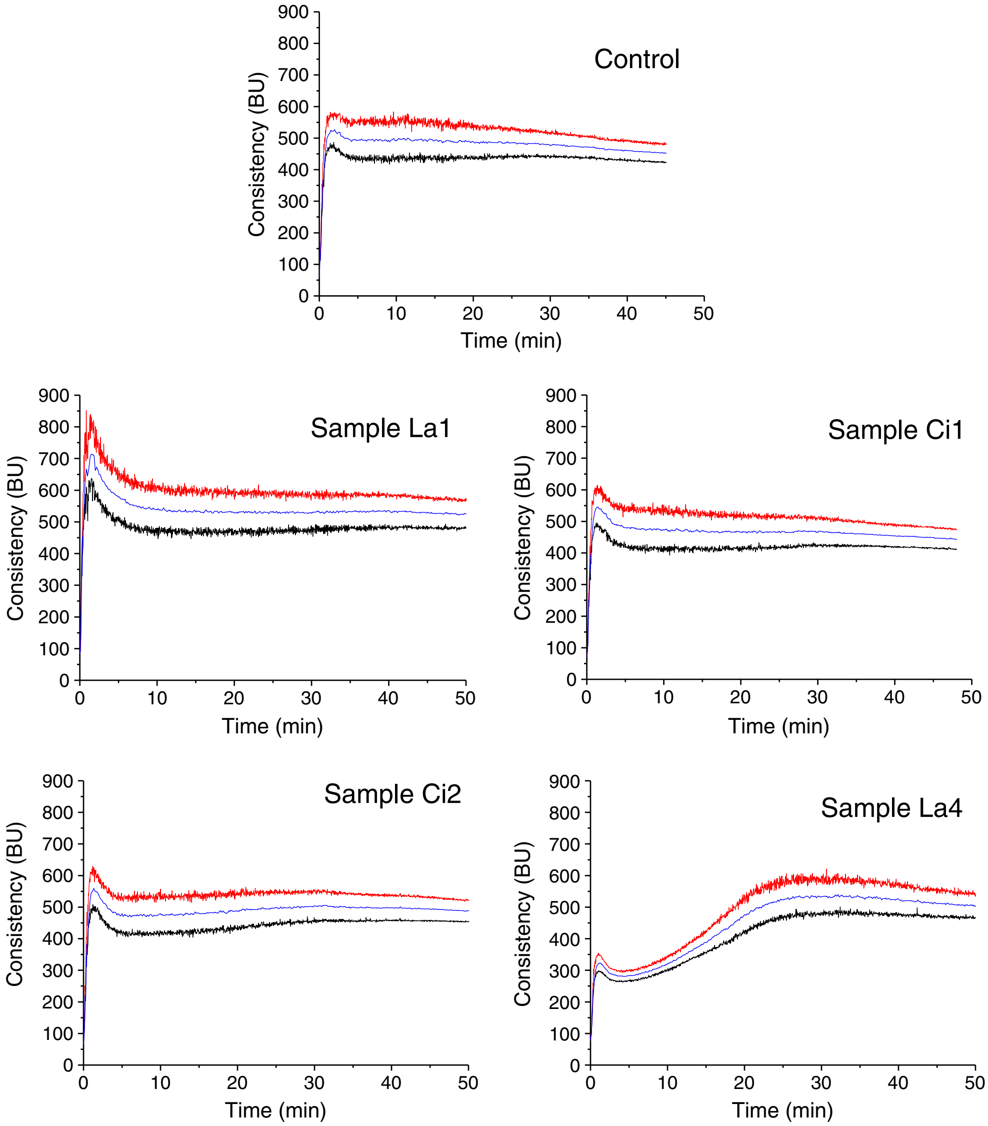

Fig. 1. Farinograms of wheat flour–salt–inulin–calcium salts blends. a) Control sample; b) sample La1: 1200 ppm Ca ($CaLa_2$), 1% Inulin; c) sample Ci1: 1200 ppm Ca (Ca_3Ci_2), 1% Inulin; d) sample Ci2: 2400 ppm Ca (Ca_3Ci_2), 1% Inulin; e) sample La4: 2400 ppm Ca ($CaLac_2$), 12% Inulin.

with the temperature profile STD 1 (Standard Analysis 1). Viscosity of samples was monitored during the thermal treatment (pasting profiles). The parameters obtained were: peak viscosity (PV, maximum viscosity during heating), breakdown (BD = peak viscosity − trough viscosity), final viscosity (FV, maximum viscosity after cooling) and setback 1 (SB1 = FV − PV). All samples were analyzed by triplicate.

2.7.2. Thermal properties

The thermal properties of the blends were determined by differential scanning calorimetry (DSC) using a Q100 equipment (TA Instruments, USA). About 10 mg of dough was placed in aluminum capsules that were hermetically sealed and subjected to one heating cycle. Dough samples were heated from 5 °C to 140 °C at a rate of 10 °C/min. Starch gelatinization was characterized by different temperatures: onset (T_0), peak (T_{pI} and T_{pII}) and final (T_f). The enthalpy associated to starch gelatinization (ΔH_{gel}) was determined between T_0 and T_f. All samples were analyzed by duplicate.

3. Results and discussion

Fig. 1 shows the farinographs of representative samples of the design. The farinograph of control flour presents two peaks, one at short times (2 min) corresponding to the hydration of flour components with water, and a second one (12 min) belonging to the optimum consistency of the dough formed. Calcium salts and inulin modified in a certain degree, this farinographic pattern. Differences in the shape of the first peak of samples with low Ca level (1200 ppm) added as lactate (La1) or citrate (Ci1) and low level of *In* (1%), were observed. La1 peak was sharper than Ci1 peak, probably due to the different interaction of each anion with water. The ability of certain ions, mainly anions, of altering water organization and consequently protein structure is well known.

Kinsella and Lee Hale (1984) studied how hydration of different anions affects protein hydrophobic interactions, contributing to dough consistency. They found that fluoride (F^-) anion enhances the hydrogen-bonded structure of water and thus strengthens hydrophobic effects on protein, slightly reducing the consistency after the first farinographic peak, although developing a normal gluten network with prolonged dough stability. On the other hand, the chaotropic thiocyanate (SCN^-) anion alters the normal water structure, disturbing hydrogen bonds (reducing hydrophobic interactions), favoring a great first peak of consistency, but with an accelerated breakdown of gluten. In our case, the citrate anion showed a similar behavior of that presented by fluoride in the Hofmeister series, among the strongly hydrated more stabilizing anions (Schwierz, Horinek, & Netz, 2010).

Water structure-maker ions (citrate > acetate; Mg^{+2}) enhance the tetrahedral coordinated hydrogen-bond structure of water; and water structure-breaker ions, such as K^+ ion, disrupt water tetrahedral coordination (Calligaris & Nicoli, 2006).

Farinograms of flour with thiocyanate (SCN^-) ion presented by Kinsella and Lee Hale (1984) and with lactate ion of this work, were similar. Therefore, we supposed that lactate ion has the same destabilizing effect as SCN^-, being this last anion at the final position of the Hofmeister series, corresponding to the most destabilizing ions (Schwierz et al., 2010). An increase of calcium level (2400 ppm), with no modification of inulin (1%) content did not modify the farinogram, independently of the type of salt used. Samples Ci2 and La2 presented similar farinograms, therefore in Fig. 1 only the Ci2 sample is shown (Fig. 1, sample Ci2). In the presence of both types of calcium salts (lactate and citrate), a high *In* level (12%) caused a change in the typical shape of the farinogram, with the second peak of consistency greatly high. In Fig. 1 only La4 is shown. This effect may be attributed to the structuring effect of inulin in the presence of enough calcium level. This behavior was similar to that observed with inorganic salt such as calcium carbonate (Salinas et al., 2011), suggesting a predominance of the prebiotic effects over that of calcium salts.

Due to the special characteristics of the farinogram of the samples with high calcium–inulin levels, dough structure was studied by SEM at different stages of kneading (Fig. 2) and sample La4 was selected for this purpose. Analysis of dough structure at different stages of mixing allows better understanding of the nature of interactions between blend components with water. Wheat flour generally shows two populations of starch granule, one of high and the other of smaller size. At 8 and 16 min of kneading, the granules mixed with gluten proteins can be visualized without a definite structure.

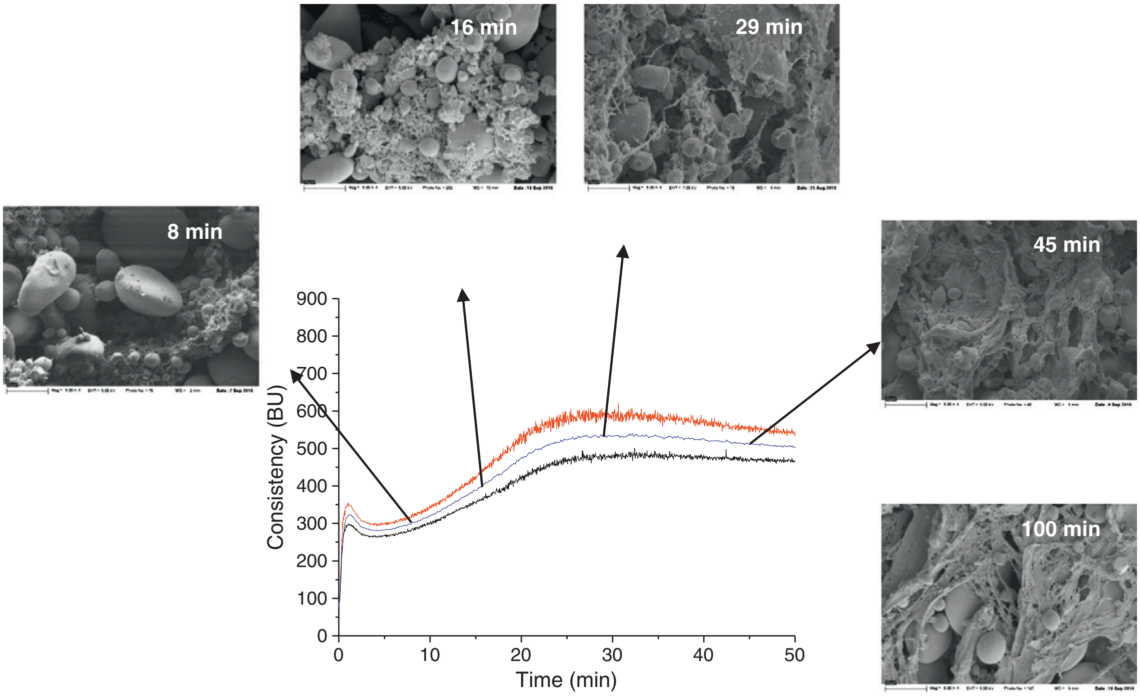

Fig. 2. Scanning electron micrographs (SEM) of sample La4 obtained from dough with different mixing times: 8 min; 16 min; 29 min; 45 min; and 100 min. Magnification: 5000×.

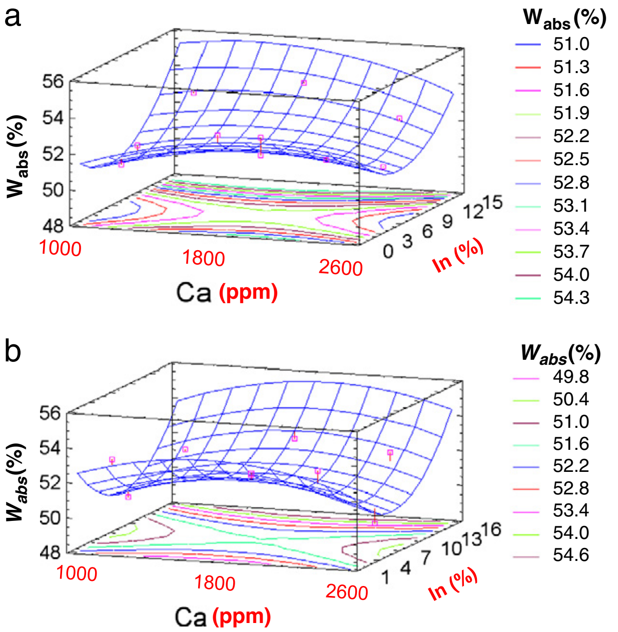

Fig. 3. Response surface graphs of water absorptions of blends a) $CaLa_2$; b) Ca_3Ci_2. Point over surface belongs to average experimental data.

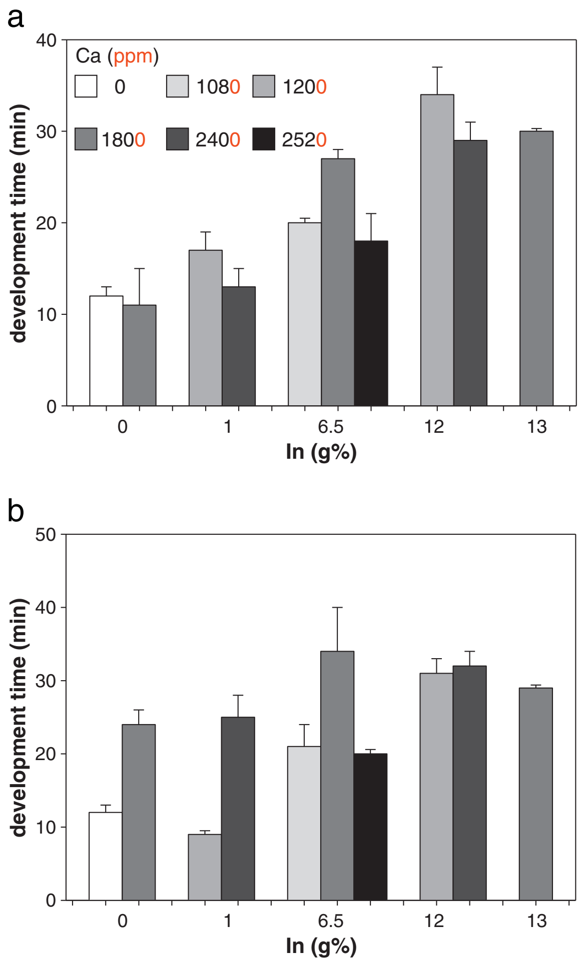

Fig. 4. Development time of wheat flour–salt–Inulin–calcium salts blends a) $CaLa_2$; b) Ca_3Ci_2. Error bars: standard deviations.

At this stage, the mix has a liquid consistency. More kneading allows the mix to acquire optimum dough consistency, reached at 29 min (development time, t_d). At this time, the granules are immersed in a laminar structured protein network, over which a white and thin chain of filament structure is observed, possibly belonging to inulin. At 45 min a more disrupted network is observed and an important over kneading (100 min) confers the network a filament aspect.

Fig. 3 shows the response surface of the farinographic water absorption, W_{abs}. For $CaLa_2$ mixtures, W_{abs} varied with In^2 and no significant dependence was observed with the calcium added (Fig. 3a, Table 1). W_{abs} of the Ca_3Ci_2 samples also presented a direct dependence with In^2 (Fig. 3b and Table 1). Control sample C, showed a W_{abs} higher (56.4%) than that presented by both kinds of blends. A decrease in water absorption with the increase of inulin content was also reported by several authors included in the Morris and Morris (2012).

Variations in development time (t_d) can be observed in Fig. 4. When the source of calcium was lactate (Fig. 4a), the t_d increased with fiber content; however, no significant differences were observed for samples without inulin. Fiber may be affecting the gluten network formation, needing more time to form elastic dough, perhaps due to its interaction with water through the high number of hydroxyl groups, competing in this way with the other components (proteins and starch) for water. Calcium citrate, compared to lactate salt, presented a different behavior on t_d (Fig. 4b), mainly at low *In* levels (0 and 1%), being this parameter significantly higher with high calcium content. In general, more time was required for the formation of gluten network in the presence of calcium citrate. Moreover, at high inulin levels (>6.5%), t_d resulted independent of salt content. In addition, for both salts at intermediate concentration of Ca and *In* (1800 ppm and 6.5%, respectively), higher t_d values were obtained. This behavior was opposed to that obtained with inorganic salt such as $CaCO_3$, suggesting the different interaction among organic (lactate, citrate) and inorganic (carbonate) anions with proteins and water. Carbonate ion may stabilize the gluten network through electrostatic unions, while organic anions would have an effect on water structure and indirectly on hydrophobic interactions with proteins.

Table 1
Analysis of variance and regression coefficients for the second-order polynomial model.

Source	Calcium lactate						Calcium citrate					
	W_{abs} (%)		M_{cont} (%)		λ (ms)		W_{abs} (%)		M_{cont} (%)		λ (ms)	
	b	*p*-value	b	*p*-value	b	*p*-value	b	*p*-value	b	*p*-value	b	*p*-value
Constant (b_0)	51.79		39.65		10.71		51.6		38.92		10.27	
Ca	−0.01	0.97	0.12	0.22	−0.06	0.26	−0.01	0.97	−0.03	0.70	−0.05	0.73
In	0.42	0.08	−1.13	0.00	−0.56	0.00	−0.49	0.21	−1.28	0.00	−0.72	0.00
Ca^2	−0.55	0.11	−0.14	0.29	0.05	0.42	−0.75	0.19	0.18	0.15	−0.42	0.04
In^2	1.25	0.01	0.32	0.02	−0.02	0.80	1.56	0.03	1.31	0.00	−0.21	0.29
Ca.In	−0.42	0.16	−0.19	0.13	−0.33	0.00	0.11	0.81	0.08	0.47	−1.35	0.00
R^2		0.907		0.852		0.819		0.82		0.867		0.6
Root MSE		0.489		0.428		0.27		0.864		0.388		0.468

Significant differences at *p*-value <0.05.

Table 2
Farinographic parameters of calcium–inulin wheat flour blends.

Sample			Calcium lactate		Calcium citrate	
# Blend	Ca (ppm)	In (%)	Stb (min)	Soft (BU)	Stb (min)	Soft (BU)
1	1200	1	31.9 c	25 f	46 c	10 b
2	2400	1	39.7 e	14 cde	>46	nd
3	1200	12	29.5 bc	18 e	30.6 ab	15.5 c
4	2400	12	32 cd	12 abc	27.0 a	37 d
CP	1800	6.5	45.5 f	13 abcd	44.6 c	5 a
5	1080	6.5	46.2 f	8 a	>46	nd
6	1800	13	22.0 a	24 f	26.4 a	19 c
7	2520	6.5	33.5 cd	16 de	>46	nd
8	1800	0	25.4 ab	32.5 g	>46	nd
C	0	0	36.5 de	10 ab	36 bc	10 b

Farinographic parameters: stability (Stb), softening degree (Soft). CP: Central Point (3 replicates), C: control blend (without inulin and calcium). Different letters in the same column indicate significant differences ($p<0.05$). Ingredients: calcium (Ca), and inulin (In).

The stability and softening degree of dough not only were affected by fiber but also by $CaLa_2$ levels used. Without inulin (0 and 1800 ppm Ca) the $CaLa_2$ addition produces a decrease in stability and a significant increase of softening (Table 2). The highest stability values were obtained at inulin levels of 6.5% with 1080 and 1800 ppm Ca ($CaLa_2$). When the maximum fiber level was used (13% *In*), the minimum stability was obtained, having a strong disrupting effect on gluten structure. Without fiber, comparing to $CaLa_2$, an opposite behavior was observed, dough stability increased with Ca_3Ci_2 (samples C and 8, Table 2). Independently of this salt content, inulin (>6.5%) considerably decreased stability and increased softening degree.

Wehrle, Grau, and Arendt (1997) studied the influence of lactic acid on dough prepared with wheat flour–sodium chloride blends. These authors found that the lactic acid decreased t_d and stability, increasing softening of dough.

Samples corresponding to each salt due to each different chemical formula; contain the same percentage of calcium cation but different proportion and anion type. According to Hofmeister series, the increase of dough stability would be the consequence of the stabilizing effect of citrate ion on protein structure (Table 2). Similar calcium content implies a higher number of lactate moles ($3\times CaLa_2$) than citrate (Ca_3Cit_2), being the relationship: three La^- ions for each Ci^{-3} ion. Nevertheless, due to the different degree of protonation, the negative charge provided by lactate is −3, instead of the −2 offered by the citrate ion. The explanation of this supposition is that the pKa of –COOH group of lactic acid is 3.08 (Weast, 1976) and the pKa values of the three –COOH groups of citric acid are 3.14, 4.77 and 6.39 (Weast, 1976). All dough presented similar pH, containing either $CaLa_2$ (5.57–5.68) or Ca_3Ci_2 (5.66–5.72) but lower than the pH of the control sample (5.80). At these pH values, lactate is deprotonated and citrate still has a carboxyl group protonated. This different negative charge proportion may influence the water molecule structuring and/or the interaction with these ions with positive net charge of gluten proteins, thus generating structures with different consistency and stability observed during kneading.

3.1. Calcium salts and inulin effect on dough hydration variables

Fig. 5 shows the response surface graph (RSG) for moisture content (M_{cont}) and molecular mobility (λ) of dough for both designs. The moisture of the control dough was the highest one (43.4%). The moisture of dough made with both types of salts (Fig. 5a,b, and Table 1) showed a dependence with In^2, mainly for Ca_3Ci_2, and with the inverse of *In* reflected in the different shapes of surfaces.

Water activity (a_w) of control dough was 0.971. Dough with $CaLa_2$ showed a_w values between 0.966 and 0.971, while Ca_3Ci_2 values were in the range 0.963–0.975. The lowest values of a_w belong to dough with high levels of *Ca* and *In*.

The molecular mobility (λ) of control dough was 12.20 ms. Molecular mobility of dough with calcium salts (Figs. 5c and d, and Table 1) varied inversely with *In* and $Ca\cdot In$. This behavior implies that at low *In* level, an increase of *Ca* content incremented λ, however at high *In* levels a decrease of λ was observed. For calcium citrate-dough, dependence with Ca^2 was also observed (Table 1). Differences in RSG form suggest greatest influence in λ of calcium citrate over lactate; the latter being more influenced by fiber content. High values of this parameter suggest high water mobility of the system, being in a high-energy state, leading to a more labile dough structure (Salinas et al., 2011).

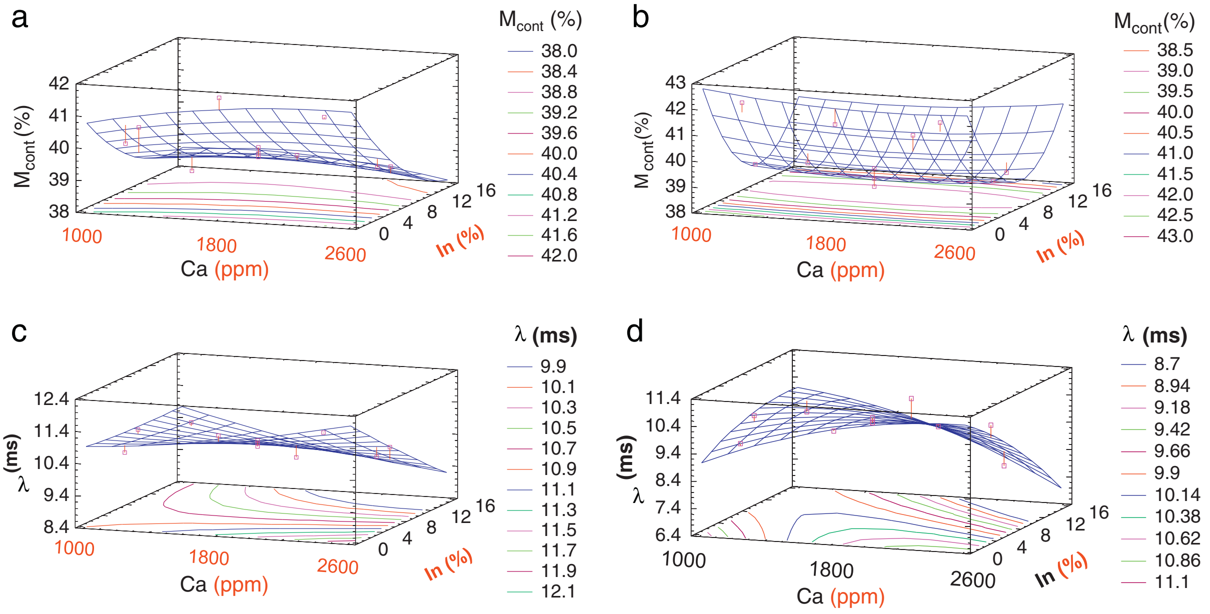

Fig. 5. Response surface graphs of moisture content (M_{cont}) (a, b) and ^1H spin-spin NMR relaxation time (λ) (c, d) of dough with $CaLa_2$ (a, c) and Ca_3Ci_2 (b, d). Point over surface belongs to average experimental data.

3.2. Pasting and thermal properties of blends

RVA is a technique used to observe changes in pasting properties of starch during heating and cooling processes. These changes would affect the quality of final starch-based products. RVA pasting properties are related to the gelatinization and short-term retrogradation of starch-based samples during heating and cooling (Chantaro & Pongsawatmanit, 2010). When starch granules are heated in water excess, granules irreversibly swell and lose their native birefringence and crystalline order and form a paste. In contrast, pasting is the phenomenon following gelatinization, involving granular swelling, exudation of molecular components from the granule and eventually, total disruption of the granule (Atwell, Hood, Lineback, Varriano-Marston, & Zobel, 1988). When cooled, pastes will form a viscoelastic gel; the molecular re-association that occurs during the cooling and storage of gelatinized starch molecules to form an ordered structure is defined as starch retrogradation or setback (Arjona-Roman et al., 2011). In our case, all blends showed a similar RVA profile (graphs not shown). The parameters obtained are shown in Fig. 6.

The peak viscosity (PV) decreased with high *In* levels (12%) with both calcium salts (Fig. 6a and b). The PV decrease could be due to the inulin competition with starch granules for water absorption; the less hydration starch granules would conduct to less viscosity. Brennan and Samyue (2004) studied pasting properties of wheat starch with different inulin levels (2.5, 5 and 10%) and also found a decrease in PV with *In*, attributing this behavior to the inhibiting starch gelatinization and hence pasting of this fiber. Similar results were observed with hi-maize flour added with inulin (Brennan, Monro, & Brennan, 2008).

The breakdown (BD) of samples with high inulin level (12%) was similar to control blend, while high values of BD with 1% inulin was obtained (Fig. 6c and d). Results suggest that calcium salts enhance

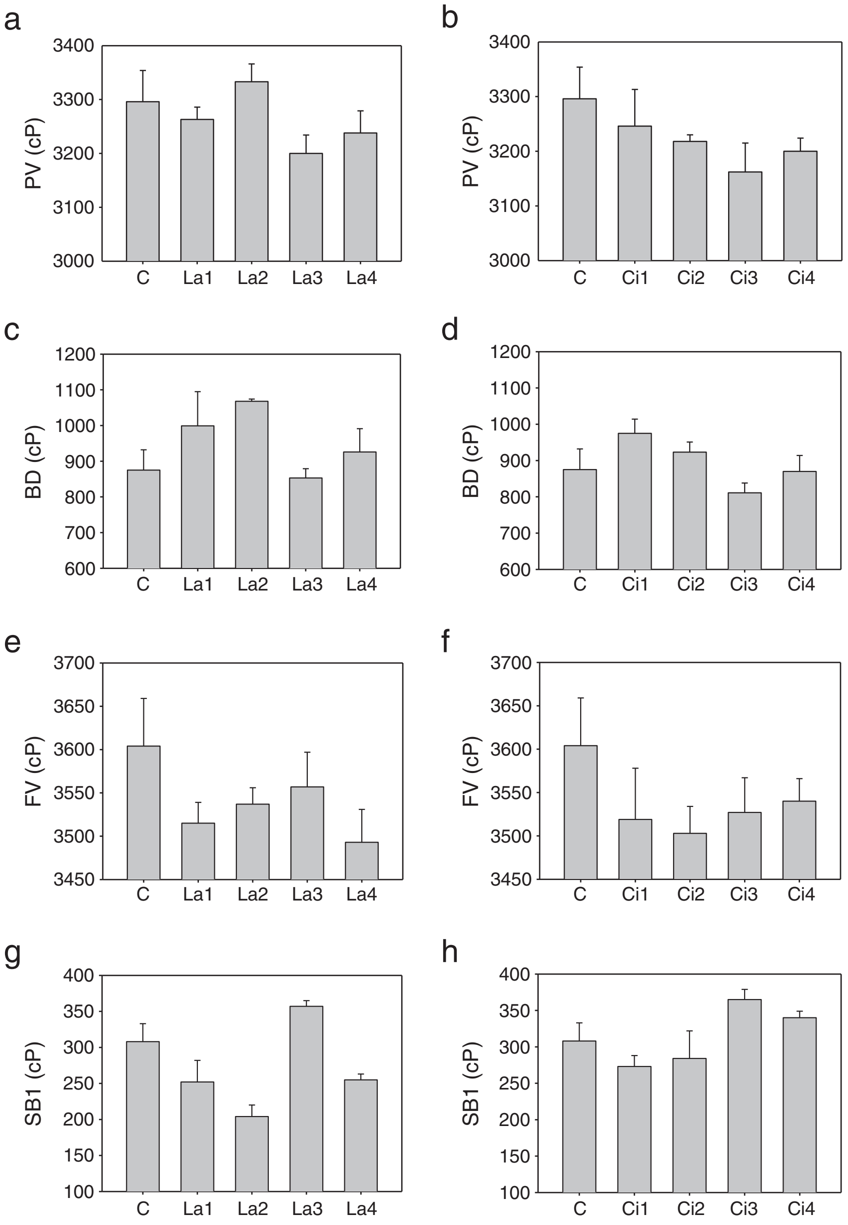

Fig. 6. RVA parameters of wheat flour–salt–Inulin–calcium salt blends. Peak viscosity, PV (a, b); breakdown, BD (c, d); final viscosity, FV (e, f); setback 1, SB1 (g, h). Error bars: standard deviations. Samples with: CaLa$_2$ (a, c, e, and g), Ca$_3$Ci$_2$ (b, d, f, and h). C = control sample. La1, Ci1:1200 ppm Ca–1% In; La2, Ci2: 2400 ppm Ca–1% In; La3, Ci3:1200 ppm Ca–12% In; La4, Ci4: 2400 ppm Ca–12% In.

breakdown but inulin contributes to gel stability during heating. No significant difference in FV between samples was observed, suggesting the formation of a low viscosity gel with the presence of fiber and calcium salts.

The highest final viscosity (FV), after cooling, was obtained with the control blend (Fig. 6e and f).

At the same level of inulin, the increase of calcium lactate diminished the setback 1 (SB1) (Fig. 6g); therefore pastes with low stability were obtained. Gupta, Shimray, and Rao (2012) related setback values to the easiness of cooking and the tendency to amylose retrogradation. These authors also found that organic acids decreased setback values, and these were affected by the nature of acid more than its concentration. Similar observations have been reported by Wu, Tsai, Wei, Sun Pan, and Huang (2010) in rice flour from different varieties.

On the other hand, inulin content increment in calcium citrate-wheat flour blends produced an increase in SB1 (Fig. 6h). A more stable paste was formed after cooling. This behavior could be caused not only by starch retrogradation, but also by inulin gel forming properties.

Besides the fine structure of amylopectin, the amylose/amylopectin ratio and water content, starch setback was influenced by inulin and the type and content of calcium. Nevertheless, Arjona-Roman et al. (2011) found that PV and setback were more influenced by citric acid addition than by other factors.

Gelatinization behavior of dough was analyzed by differential scanning calorimetry (DSC). Control dough presented two gelatinization endothermic peaks at 72 (T_{pI}) and 90 °C (T_{pII}) (Fig. 7). Dough with different salt levels presented similar DSC profiles. At a high calcium level and due to considerable content of fiber, a shift of 10 °C for both peaks to high temperature values was observed. Gelatinization enthalpy significantly decreased respect control dough value for both kinds of salts, mainly with the presence of inulin. Results suggest that dough with fiber had less water absorption, leading to a low quantity of available water for gelatinization phenomenon. These results agree with those observed of PV in RVA assays (Fig. 6a and b).

4. Conclusions

Blends with organic calcium salts absorbed less water quantity and yield dough with low moisture content in the presence of inulin, with lower pH than control dough. In general, when $CaLa_2$ was used, dough was formed in less time and was less stable than with Ca_3Ci_2, resulting in a greater molecular mobility. This behavior can be attributed to the destabilizing effect of the lactate anion on the structure of gluten proteins. The interaction of this ion with the flour components resulted in pastes of high viscosity, both during heating and after cooling, at low inulin concentration. Pastes with $CaLa_2$ also showed minor setback 1, indicating the formation of a less structured gel after cooling, related to the short-time retrogradation process. These results were reflected in gelatinization parameter values obtained by calorimetry: lower enthalpy and higher peak temperature than control dough. Nevertheless, in this last test, no significant differences between two salts studied were detected.

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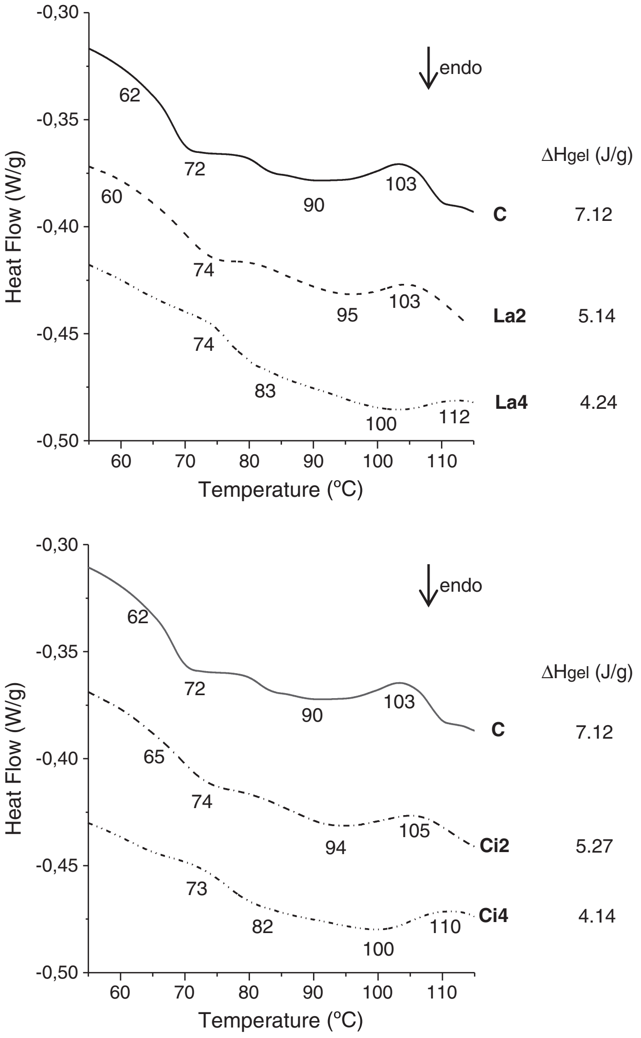

Fig. 7. DSC thermograms of dough with $CaLa_2$ and Ca_3Ci_2. C = control dough. La2, La4, Ci2, and Ci4: see legend of Fig. 6.

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