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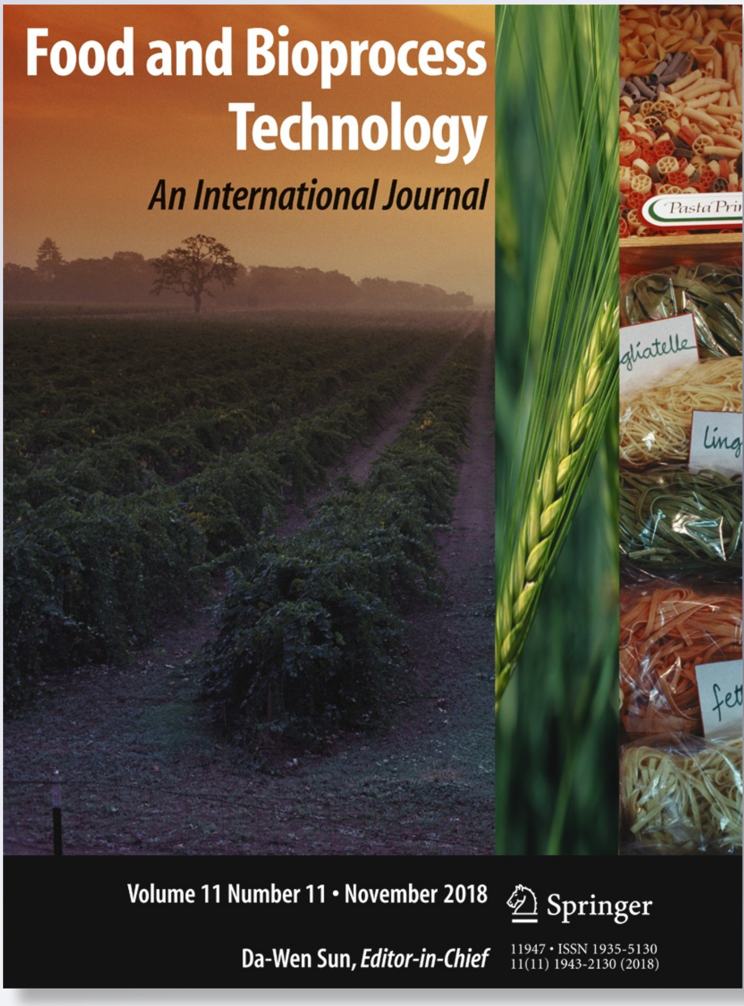

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ORIGINAL PAPER

Bread Staling: Changes During Storage Caused by the Addition of Calcium Salts and Inulin to Wheat Flour

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Abstract
The objective of this work was to study changes in staling parameters of wheat bread formulated with different calcium salts (carbonate, citrate, or lactate) and inulin. The moisture content and texture of crumbs were studied. Moreover, the effect of calcium and inulin levels on crumb firmness during storage was estimated by fitting the Avrami equation. Starch retrogradation and the amylose-lipid complex were evaluated by differential scanning calorimetry (DSC) and X-ray diffraction. Crumbs with high inulin content retained water during storage, regardless of the nature of the calcium salt used. In addition, they presented lower loss of elasticity and cohesiveness than the control crumb. Crumbs with calcium citrate and low inulin content produced the lowest increase in crumb firmness. In addition, elasticity and cohesiveness of crumbs containing low amount of inulin and organic salts were almost maintained. The salt that favored the retrogradation process was calcium lactate with high inulin content, evidenced by the shorter half-time obtained with the kinetic studies. Also, the increase in crystallinity of B-type starch in crumbs with organic calcium salts and high inulin content, correlates with the highest values of retrogradation enthalpy obtained by DSC.

Keywords Wheat bread · Calcium salt-inulin system · Bread staling · Starch retrogradation

Introduction

Bread is one of the most consumed foods around the world, mainly obtained from an industrial way but preferred fresh by consumers. Breads are commonly composed of flour, water, salt, and yeast, and are considered as heterogeneous and unstable solid foams formed by crust and crumb with a cell wall microstructure described as a dispersion of starch granules in a continuous protein matrix (Szczesniak 2002). In staling process besides starch retrogradation, which is responsible for the increase in firmness, other processes such as water migration from inside to outside the bread piece and interactions between bread components leading to changes in protein and/or protein-starch networks occur (Gray and Bemiller 2003; Wilderjans et al. 2013).

The main carbohydrate in bread is starch. When native starch is heated and dissolved in water, the crystalline structure of amylose and amylopectin molecules is lost. When this system is cooled, the linear molecules of amylose and linear parts of amylopectin molecules retrograde and rearrange themselves again to a more crystalline structure. The linear chains place themselves parallel and form hydrogen bridges into a different ordered structure in a process termed retrogradation (Primo-Martin et al. 2007). Retrogradation is a process directly related to the staling of bread responsible to the firmness of the cell walls in bread (Gray and Bemiller 2003; Goesaert et al. 2005; Wilderjans et al. 2013). The recrystallization of amylose molecules is followed by a slow recrystallization of amylopectin. The slow crystallization process of these amylopectin polymers is due to their limited molecular movement, which restricts the formation of molecular arrangements of crystalline structure because they are mostly confined within the gelatinized and hydrated starch granules. This long-term development of crystallinity in processed starch is attributed to the retrogradation of amylopectin (Wang et al. 2015). The starch retrogradation phenomenon

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depends on the temperature and time of storage, and is undesirable because of its contribution to bread staling.

Several authors have studied the kinetics of bread staling by applying the Avrami model and found values of Avrami exponent close to 1 (Kim and d'Appolonia 1977; Russell 1983).

Zobel and Kulp (1996) obtained Avrami exponents different from 1, indicating that the increase in firmness during bread aging is due to the retrogradation of starch (Avrami exponent = 1) together with other several factors such as diffusion of moisture between crumb and crust and starch-gluten interactions.

Starch retrogradation is a complex process involving a series of molecular and physicochemical events. A diversity of physical and chemical methods, such as differential scanning calorimetric (DSC) and X-ray diffraction (XRD), have been applied to investigate the changes that take place in starch properties. When a system is heated, the absorption of heat occurs as a result of phase transitions, such as melting. The realignment of amylopectin molecules to form a partially ordered structure and the exudation of water from starch can be monitored by DSC. In the case of retrograded starch, the DSC endotherm provides quantitative measures of enthalpy change and transition temperatures for the melting of recrystallized amylopectin (Miles et al. 1985; Karim et al. 2000). The retrogradation of several wheat flour systems was previously studied by differential scanning calorimetry and X-ray diffraction by some researchers (Ribotta et al. 2004; Bigne et al. 2016). Studies on the retrogradation of wheat starch in the presence of inulin with different polymerization degrees were carried out by Luo et al. (2017). Gallagher et al. (2003) explored the application of fructooligosaccharide in biscuit dough and found an improvement in the quality and shelf life of biscuits. These authors found that the inclusion of inulin in an extruded flour-based product lowered dough consistency and elasticity due to a lubricating effect of sugars and oligosaccharides; also, different kinetics of starch gelatinization were observed.

On the other hand, calcium carbonate is a salt commonly used as fortifying agent in wheat bread (Ranhotra et al. 2000), and is also conventionally utilized in breadmaking products as dough conditioner to regulate the pH. In addition, it was proved that the calcium carbonate present in wheat bread is better absorbed into the bones of growing rats in the presence of inulin (Salinas et al. 2017). The effect of the addition of inulin to wheat flour on dough has been previously studied by different authors (Liu et al. 2016; Salinas et al. 2016). Inulin increases the development time and stability of wheat dough, and decreases water absorption, leading to modifications in crumb hardness and bread volume (Peressini and Sensidoni 2009; Salinas et al. 2012; Peressini et al. 2017). Nevertheless, until now, studies on the combined effect of calcium salt-inulin systems on starch retrogradation and wheat bread staling have not been performed. Therefore, the aim of this work was to study changes during storage of wheat breads formulated with different calcium salts and inulin blends.

Materials and Methods

Materials

The materials used in this work were wheat flour for Breadmaking (type 0000, Molino Campodónico Ltda., Argentina) (AAC 2018). The composition of wheat flour was 9.92 ± 0.02% of proteins, 0.86 ± 0.02% of lipids, 0.382 ± 0.009% of ash, 11.85 ± 0.03% of moisture, and 3.1 ± 0.1% of total dietary fiber. Alveographic parameters were 132 mm, 47 mm, and 264 for tenacity (P), extensibility (L), and deformation energy of dough (W), respectively. And, the farinographic parameters were 57.9 mL, 18 min, 38 min, and 12 UB for water absorption, development time, stability, and softening degree, respectively. Wet gluten content was 24.4%. Other ingredients used were calcium carbonate (ANEDRA), calcium lactate (Sigma-Aldrich), calcium citrate (Sigma-Aldrich), and inulin enriched with oligofructose (Synergy 1, BENEO Orafti, Belgium), sodium chloride (CELUSAL, Argentina), fresh yeast (CALSA, Argentina), and distilled water.

Methods

Bread Formulation

Wheat flour, sodium chloride (2% wheat flour basis (w.f.b.)), fresh yeast (3% w.f.b.), and some formulations with different levels of inulin and calcium salt (carbonate, citrate, or lactate) were prepared according to Salinas et al. (2012). The different formulations used for breadmaking are shown in Table 1.

Table 1 Bread formulation

	Calcium (g/kg)	Inulin (%)
Control	0	0
Ca, Ci, La	1.80	0
Ca1, Ci1, La1	1.20	1
Ca2, Ci2, La2	2.40	1
Ca3, Ci3, La3	1.20	12
Ca4, Ci4, La4	2.40	12

Different calcium salts: Ca calcium carbonate, Ci calcium citrate, or La calcium lactate. Control bread prepared without calcium salt and inulin (control), bread prepared with 1.8 g of calcium per kilogram of flour (Ca, Ci, or La), bread prepared with 1% of inulin (wheat flour basis (w.f.b.)) and 1.20 g of calcium/kg of flour (Ca1, Ci1, or La1) or 2.40 of calcium/kg of flour (Ca2, Ci2, or La2), and bread with 12% of inulin (w.f.b.) and 1.20 calcium/kg of flour (Ca3, Ci3, or La3) or 2.40 calcium/kg of flour (Ca4, Ci4, or La4)

Bread dough (90 g) was formed and fermented at 30 °C for the optimal time obtained in previous work (Salinas and Puppo 2014; Salinas et al. 2016). Finally, breads were baked in a convection oven (Ariston, Argentina) at 220 °C for 26 min.

Breads were cooled to room temperature, individually wrapped in plastic film, packed in polyethylene bags, and stored up to 7 days at 20 °C.

Quality of Bread Storage

Crumb Moisture The moisture of bread stored up to 7 days was determined according to AACC 44–19 (AACC 2000). Determinations were carried out in triplicate.

Texture of Bread Crumb The texture profile analysis (TPA) of bread slices stored up to 7 days was performed. Two slices (height 2 cm) from the middle part of bread were obtained. A texture analyzer TA.XT2i (Stable Micro Systems, Surrey, UK) equipped with a 25-kg load cell was used to perform the TPA of crumb. The slice was subjected to a double compression cycle (deformation 40%, crosshead speed 0.5 mm/s) with a cylindrical probe (diameter = 2.5 cm). Firmness, springiness, and cohesiveness were determined. Eight replicates were analyzed for each formulation.

Modeling of Staling Avrami Model

For studying the kinetics of bread staling, a modified Avrami equation was applied to changes in crumb firmness along time, according to Armero and Collar (1998). The Avrami equation (Eq. 1) considers that the non-crystallized fraction (θ) is a decreasing exponential function with respect to time. The mathematical parameters of the Avrami equation (Eq. 1) applied for bread firmness (7 days) are based on starch retrogradation:

$$\theta(t) = (F_\infty - F_t)/(F_\infty - F_0) = \exp\left(-k.t^n\right) \tag{1}$$

where $\theta(t)$ is the fraction of recrystallization that is able to occur during time; F_0, F_t, and F_∞ are the firmness values at zero time, t time, and infinite time, respectively; k is the constant rate of the process; and n is the Avrami exponent related to the crystal morphology that describes the order of crystal growth (Hiemenz 1984).

The crystallization half-time ($t_{1/2}$) is defined as the time at which the extent of crystallization is half-completed (50%). It can be determined from the measured kinetic parameters k and n (Eq. 2):

$$t_{1/2} = (\ln 2/k)^{(1/n)} \tag{2}$$

Shorter crystallization half-time is associated with a faster crystallization rate.

Effect of Calcium Salts and Inulin on Bread Staling

The different breads studied were (i) *control*, (ii) with calcium (*Ca*, *Ci*, or *La*), and (iii) with high calcium (2.40 g of calcium/kg flour) and high inulin (12%) content (*Ca4*, *Ci4*, or *La4*).

Differential Scanning Calorimetry Doughs (≈10 mg) were placed in aluminum-coated pans that were hermetically sealed and subjected to one heating cycle using Q100 equipment (TA Instruments, USA). Dough samples were heated from 5 to 105 °C at 10 °C/min (gelatinization). That final temperature (105 °C) was selected because it is the temperature reached in the center of the crumb during baking. After storage at 20 °C for 0, 3, 5, and 7 days, the same pans with the cooked dough were subjected to the second heating cycle from 5 to 140 °C at 10 °C/min. Calibration of the instrument was performed using indium, lauric acid, and stearic acid as standard calibration standards. An empty aluminum-coated pan was used as a reference. Amylopectin retrogradation was characterized by different temperatures: onset (T_0), peak (T_p), and final (T_f). The enthalpy between T_0 and T_f associated with retrogradation enthalpy of amylopectin (ΔH_R) was determined. Determinations were carried out in duplicate.

The retrogradation index (RI) was defined as the ratio of the enthalpy of retrogradation and gelatinization enthalpy (Eq. 3) and was calculated according to Correa and Ferrero (2015).

$$\mathrm{RI} = \Delta H_R/\Delta H_g \times 100 \tag{3}$$

where ΔH_R is the enthalpy associated with amylopectin retrogradation and ΔH_g corresponds to enthalpy associated with starch gelatinization of dough.

X-ray Diffraction Analysis Bread crumbs previously stored at 0 and 5 days were lyophilized. Then, they were ground and pressed for 5 min in the sample container with a plunger of 1000 g. The samples were covered with Kapton film and analyzed on a powder diffractometer X'Pert Pro (PANalytical, Holland) equipped with a data analysis program with crystalline graphite monochromator. XRD spectra were acquired at 40 kV and a current of 40 mA with Cu k_α radiation (λ = 0.154 nm). The diffraction angle (at 2θ) was scanned from 4 to 40° in 0.02° increments with 1-s counting time. The X-ray diffraction patterns were analyzed using the PeakFit version 4.12 software (SeaSolve Software Inc., Framingham, MA, USA), and the relative total crystallinity (C_{total}) was calculated as the ratio of the total crystalline area (sum of all peak areas detected over the amorphous area) and the total area (crystalline and amorphous fractions of the spectrum). The crystalline grade of B-type (15, 17, 22, 24°) and V-type (20°) fractions was calculated by dividing the crystalline fraction of each type by the total area of the diffraction pattern. Determinations were carried out in duplicate.

Statistical Analysis

One-way analysis of variance (ANOVA) was conducted using Statgraphics Plus software (Bitstream, Cambridge, MN, USA). A significance level of 5% was adopted for all mean comparisons. Fisher's least significant difference (LSD) procedure was used to discriminate among the means.

Results and Discussion

Stability of Bread During Storage

During storage, the acceptability of consumers due to the loss of bread freshness decreases. This phenomenon is called bread staling and includes different processes, such as increase in crumb firmness, loss of crust crispness and moisture, and deterioration of organoleptic properties (Cauvain 1998). The moisture of bread crumbs during storage is shown in Fig. 1. The control crumb lost water constantly over time up to 36% at the seventh day, whereas in the presence of calcium and inulin, a sharp decrease up to day 3 was observed. Then, the moisture remained constant until the seventh day.

Crumbs with calcium carbonate with low calcium and inulin content (Ca1) presented the smallest water loss (14%) on the seventh day (Fig. 1a). On the other hand, crumbs with high inulin content showed a similar tendency of water loss, independently of the quantity of calcium used. They lost 18% of water compared to fresh bread (Ca3 and Ca4).

For crumbs with organic calcium salts (citrate and lactate), a different behavior between low and high inulin contents was observed at short times (< 3 days) (Fig. 1b, c). Crumbs with calcium citrate continued losing water up to day 7, although in a minor proportion than control crumb (Fig. 1b), while at the end of storage, crumbs with calcium lactate reached the same moisture independently of the content of calcium and inulin (Fig. 1c). In addition, crumbs with calcium lactate and high inulin content (La3 and La4) did not change their moisture after the third day of storage.

In general, in the presence of inulin, less amount of water was lost in crumb after storage and was independently on the content and the source of the calcium salt (inorganic or organic) utilized.

The interaction between calcium ion and inulin with wheat flour components such as proteins, starch, and water led to a decrease in the moisture content of fresh crumbs ($t = 0$ day) with respect to control bread because dough were prepared with less amount of water. However, water was more bound during time in the presence of calcium and inulin, so the moisture content of these crumbs was higher than that of the control bread at the end of storage, increasing the shelf life of bread.

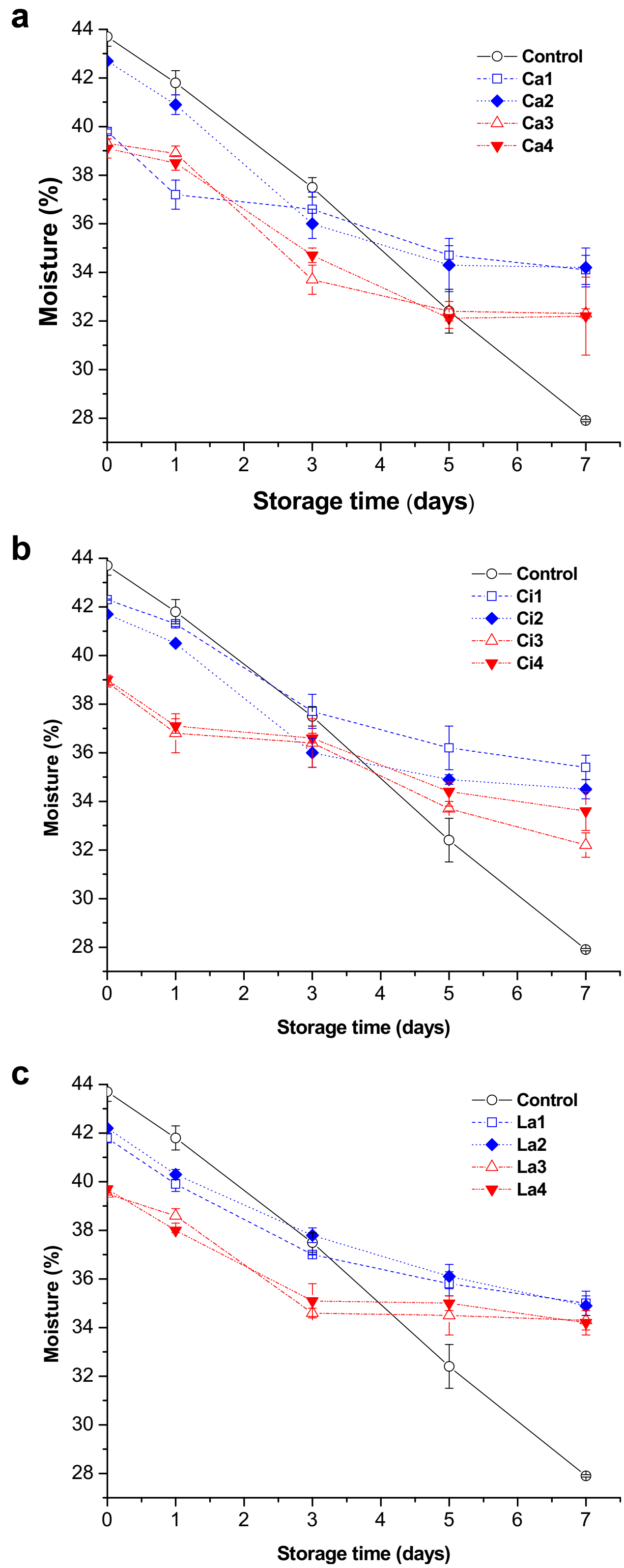


Fig. 1 Moisture of bread crumb during storage. **a** Calcium carbonate. **b** Calcium citrate. **c** Calcium lactate. *Control* without calcium and inulin; bread with 1.20 g calcium/kg flour and 1% inulin (Ca1, Ci1, La1); bread with 2.40 g calcium/kg flour and 1% inulin (Ca2, Ci2, La2); bread with 1.20 g calcium/kg flour and 12% inulin (Ca3, Ci3, La3) and bread with 2.40 g calcium/kg flour and 12% inulin (Ca4, Ci4, La4)

Variations of crumb moisture could directly affect the texture of the bread crumb (Fig. 2). It can be observed that the firmness increased during storage, presenting the control crumb a plateau at the fifth day. The increase in firmness varied according to the calcium salt used. No significant differences in firmness values or in variation of this parameter with respect to control bread were observed for breads with calcium carbonate and inulin (Fig. 2a). However, crumbs with organic calcium salts presented a different behavior. Breads with calcium citrate and 1% of inulin (Ci1 and Ci2) presented crumbs with lower firmness values, while with high contents of inulin (12%), they behaved similarly to the control bread (Fig. 2b). On the other hand, breads with calcium lactate and high inulin content (La3 and L4) exhibited the opposite behavior; i.e., they became harder than control bread (Fig. 2c).

The springiness (elasticity) and cohesiveness of crumbs decreased during storage. Control crumb lost 11% of elasticity at the end of the storage period, while the elasticity of crumbs with calcium carbonate decreased between 11 and 16% with respect to fresh crumb (Fig. 2d). Crumbs with organic calcium salts presented an elastic behavior different from that observed for the inorganic salt. The elasticity of crumbs with low inulin

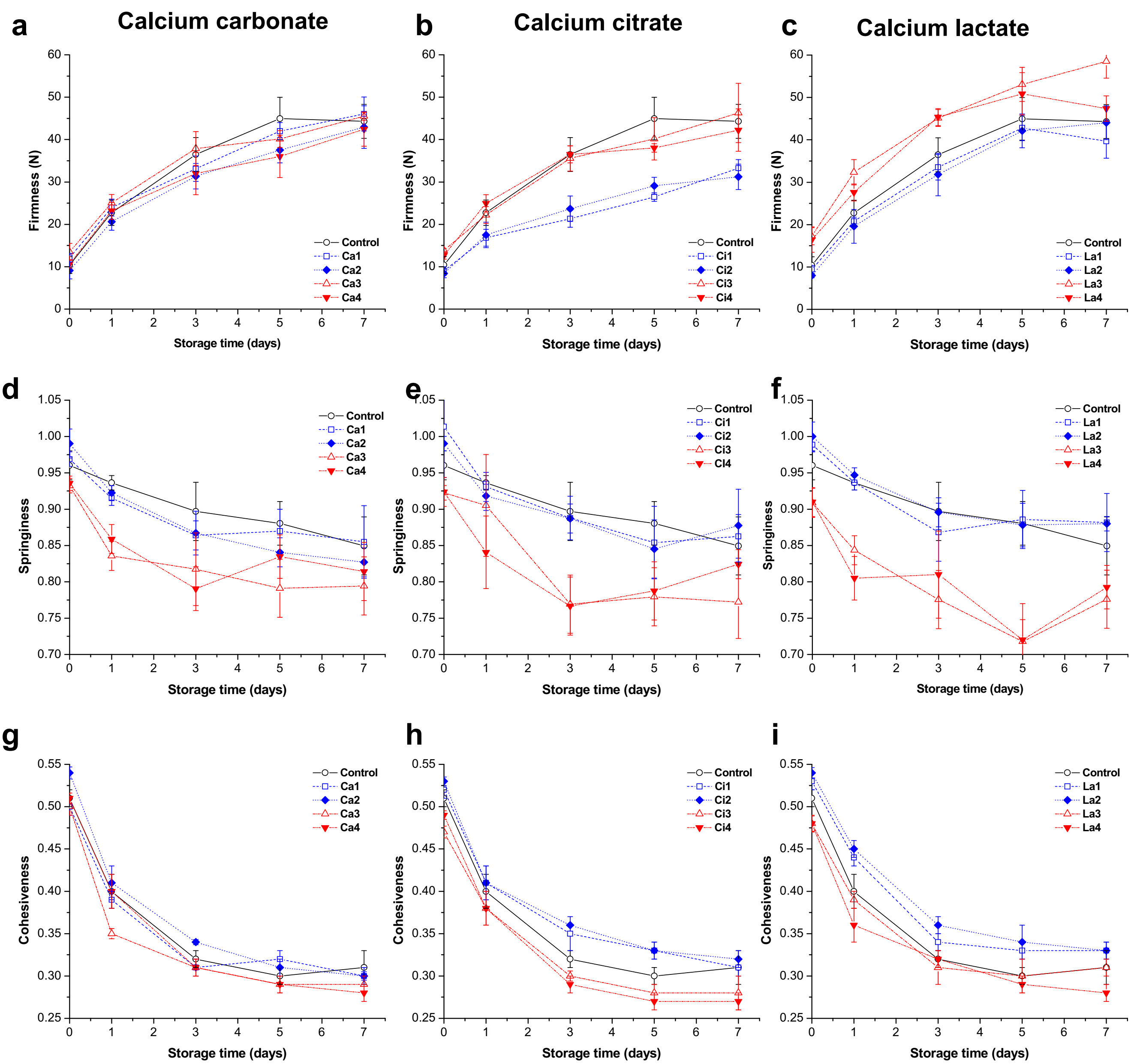


Fig. 2 Textural parameters of bread crumb. **a**–**c** Firmness. **d**–**f** Springiness. **g**–**i** Cohesiveness. Crumbs with calcium carbonate (**a**, **d**, **g**), calcium citrate (**b**, **e**, **h**), and calcium lactate (**c**, **f**, **i**). *Control* without calcium and inulin; bread with 1.20 g calcium/kg flour and 1% inulin (Ca1, Ci1, La1); bread with 2.40 g calcium/kg flour and 1% inulin (Ca2, Ci2, La2); bread with 1.20 g calcium/kg flour and 12% inulin (Ca3, Ci3, La3) and bread with 2.40 g calcium/kg flour and 12% inulin (Ca4, Ci4, La4)

content (1%) was similar to that of control crumb; a higher decrease with a high level of inulin (12%) was observed (Fig. 2e, f). The cohesiveness of all crumbs with calcium carbonate was similar to that of control, while crumbs with organic calcium salts and 1% of inulin (Ci1, Ci2, La1, La2) resulted more cohesive than the control sample. In summary, crumbs with calcium citrate or lactate and low amount of inulin were softer, more elastic, and cohesive.

During storage, firmness of bread considerably increased and did not show any direct or unique relationship with changes in the water content of crumb. This behavior suggests that there are other processes involved in the increase of firmness such as starch retrogradation.

In a complex food matrix, the Avrami model loses great part of its physical sense; nevertheless, it is a useful mathematical model for describing the staling kinetics because it fits well the experimental data of firmness.

Starch retrogradation is a crystallization process that begins with nuclei formed spontaneously, and these nuclei trigger the growth of other crystals. This process can be modeled with the Avrami equation (Eq. 1), and in these conditions, exponent n, which indicates the crystal nucleation/growth ratio, is equal to 1. In Table 2, different kinetic parameters calculated with Eqs. 1 and 2 are shown. In general, the coefficients n obtained were lower than 1 ($n < 1$) for calcium carbonate and citrate, and higher than 1 ($n > 1$) for calcium lactate, mainly with 12% of inulin and a high content of calcium (Ca4, Ci4, and La4) (Table 2). One of the first studies that interpreted the significance of the Avrami n exponent was performed by McIver et al. (1968). An exponent value of $n = 1$ represents a rod-like growth from instantaneous nuclei, $n = 2$, a rod-like growth from sporadic nuclei, while $n = 3$ represents a crystal growth in the form of discs from sporadic nuclei. According to the values of Avrami exponent obtained in this work, crystals of amylopectin in crumbs grow as rod-like; in the case of calcium carbonate and citrate, growth would occur with the mechanism of instantaneous nuclei ($n \leq 1$) while for calcium lactate ($n \geq 1$) by sporadic nuclei. In addition, n values lower than 1 ($n < 1$) would be associated with the formation of few large nuclei, while values greater than 1 ($n > 1$) would indicate the formation of high quantity of nuclei leading to a more rapidly crystallization, as in the case of calcium lactate.

Fig. 3 DSC analysis. **a** Thermograms (a) cooked dough non-stored, (b) dough stored for 7 days after gelatinization at 20 °C. **b** Amylopectin retrogradation during storage with (a, d) calcium carbonate, (b, e) calcium citrate, (c, f) calcium lactate. ΔH_R enthalpy associated with amylopectin retrogradation (a, c, e), RI retrogradation index (b, d, f). Control: without calcium and inulin; bread with 1.80 g calcium/kg flour without inulin (Ca, Ci, La) and bread with 2.40 g calcium/kg flour and 12% inulin (Ca4, Ci4, La4) ▶

For crumbs with calcium carbonate, the kinetic constant (k) presented values between 0.39 and 0.50; this parameter resulted lower (higher values of $t_{1/2}$ of crystallization) in crumbs with low amount of inulin (Ca1 and Ca2) (Table 2), indicating a low rate of starch crystallization or low staling kinetics with respect to the control. Crumbs with high levels of inulin (Ca3 and Ca4) presented values of k higher and values of $t_{1/2}$ similar to those obtained for control crumb. This behavior suggests an antistaling effect of inulin when it is present in low amounts (1%) in crumbs with calcium carbonate.

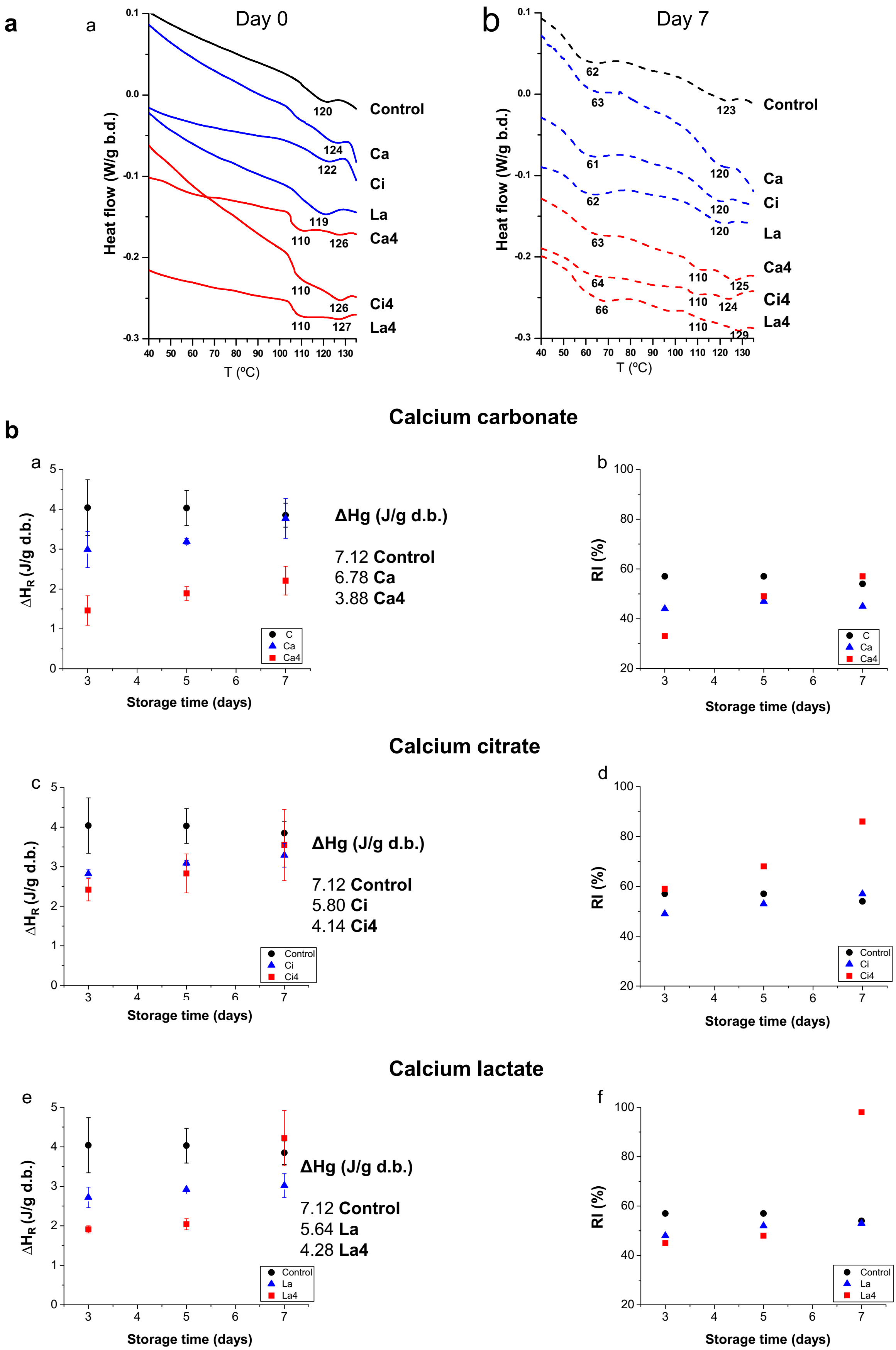
On the contrary, in crumbs with high levels of calcium citrate (Ci2 and Ci4), a higher rate of crystal nucleation and crystallization with the increase in calcium content (higher k and lower $t_{1/2}$) was observed, independently of the content of inulin.

A delay of recrystallization (higher $t_{1/2}$) on breads containing low content of calcium citrate was observed (Ci1 and Ci3). These results suggest that this calcium salt in low amounts would delay the crystallization of starch, increasing the shelf life of bread, which would be attributed to the stabilizing action of citrate anion on gluten proteins. In a previous work, we demonstrated that the calcium citrate incorporates positive charge into dough (Salinas and Puppo 2014); therefore, high amounts of this salt (Ci2 and Ci4) increase the net positive charge leading to greater absorption of water coming from the matrix, and consequently favoring interactions starch-starch increasing crystallization of this polymer (lower $t_{1/2}$). On the other hand, at equal calcium content (low Ci1, Ci3 or high Ci2, Ci4), a high amount of inulin also enhance recrystallization. Moreover, at low inulin content (La1 and La2), an

Table 2 Regression coefficient of Avrami model

	Calcium (g/kg)	Inulin (%)	Calcium carbonate				Calcium citrate				Calcium lactate			
			n	k	$t_{1/2}$	r^2	n	k	$t_{1/2}$	r^2	n	k	$t_{1/2}$	r^2
Control	0	0	1.20	0.42	1.52	0.99	1.20	0.42	1.52	0.99	1.20	0.42	1.52	0.99
1	1.20	1	0.95	0.39	1.81	0.98	0.73	0.36	2.45	0.98	1.21	0.38	1.63	0.98
2	2.40	1	0.91	0.41	1.79	0.99	0.88	0.48	1.52	0.98	1.15	0.35	1.80	0.98
3	1.20	12	0.90	0.47	1.53	0.99	1.08	0.31	2.08	0.99	0.92	0.45	1.61	0.99
4	2.40	12	0.72	0.50	1.56	0.99	0.87	0.56	1.27	0.99	1.43	0.38	1.51	0.99

n Avrami exponent, k constant rate (1/day), and $t_{1/2}$ crystallization half-time (day)


a
a
Day 0
Heat flow (W/g b.d.)
T (ºC)
Control
Ca
Ci
La
Ca4
Ci4
La4
120
124
122
119
110
126
110
126
110
127
b
Day 7
Heat flow (W/g b.d.)
T (ºC)
62
63
123
61
120
62
120
120
63
110
125
64
110
124
66
110
129
b
Calcium carbonate
a
ΔH$_R$ (J/g d.b.)
Storage time (days)
ΔHg (J/g d.b.)
7.12 Control
6.78 Ca
3.88 Ca4
C
Ca
Ca4
b
RI (%)
Storage time (days)
Calcium citrate
c
ΔH$_R$ (J/g d.b.)
Storage time (days)
ΔHg (J/g d.b.)
7.12 Control
5.80 Ci
4.14 Ci4
Control
Ci
Ci4
d
RI (%)
Storage time (days)
Calcium lactate
e
ΔH$_R$ (J/g d.b.)
Storage time (days)
ΔHg (J/g d.b.)
7.12 Control
5.64 La
4.28 La4
Control
La
La4
f
RI (%)
Storage time (days)

increase in calcium lactate decreased k and increased $t_{1/2}$. In addition, crumbs with low content of inulin retained more amount of water (Fig. 1), improving the shelf life ($> t_{1/2}$). In the presence of high levels of inulin, this prebiotic traps a high amount of water from starch-water bonds, favoring starch-starch interactions and therefore the retrogradation of this polymer ($< t_{1/2}$).

Starch Retrogradation and Amylose-Lipid Complex Fusion During Storage

Although phenomena such as water diffusion between crumb and crust and starch-gluten interactions have been proposed as effects that could contribute to the increase in crumb hardness during staling, the main effect of these processes has been attributed mainly to amylopectin recrystallization (Zobel and Kulp 1996).

The effect of calcium and inulin on starch retrogradation and on the amylose-lipid complex dissociation of wheat flour was studied by DSC and XRD. Usually, a dough sample presents two endothermic peaks on DSC thermograms, corresponding to the gelatinization process. For dough without inulin (Ca, Ci, La), those peaks were placed between 60 and 105 °C and for dough with high calcium content (Ca4, Ci4, La4) between 75 and 110 °C (data not shown). Besides, an endothermic peak between 105 and 130 °C for dough without inulin corresponds to the dissociation of the amylose-lipid complex; this peak was split into two peaks in dough with calcium and high inulin content. Tananuwong and Reid (2005) previously studied the corn starch-water interactions during gelatinization and amylose-lipid complex dissociation by DSC. These authors attributed this split to the formation of different structures of the amylose-lipid complex in conditions of water availability. In the present work, dough was previously cooked in the pan at 105 °C with the purpose of simulating the maximum temperature in the center of the crumb; therefore, in the second cycle of heating performed at 0 day, the characteristic endotherm belonging to starch gelatinization (≅ 60 °C) is absent because starch was previously gelatinized in the first cycle of heating (Fig. 3a (a)). An endothermic peak at approximately 120 °C in the control cooked dough was observed, which was assigned to the amylose-lipid complex (Fig. 3a (a)). The cooked dough with different calcium salts in the absence of inulin (Ca, Ci, La) presented similar behavior of that observed for control sample. This endotherm splits into two peaks at 110 and 126 °C due to the presence of 12% inulin (Ca4, Ci4, La4).

At day 7 of storage, retrogradation of amylopectin was observed in all thermograms (Fig. 3a (b)), as can be deduced from the endotherm obtained between 62 and 66 °C. No differences were detected during storage on peaks corresponding to the dissociation of the amylose-lipid complex, because this complex does not dissociate at 105 °C.

Luo et al. (2017) studied the effect of inulin (0–20%) of distinct degree of polymerization (DP; < 10, 2–60, > 23) on the gelatinization and retrogradation of wheat starch. In all cases, these authors found a lower enthalpy of retrogradation at 7 days of storage.

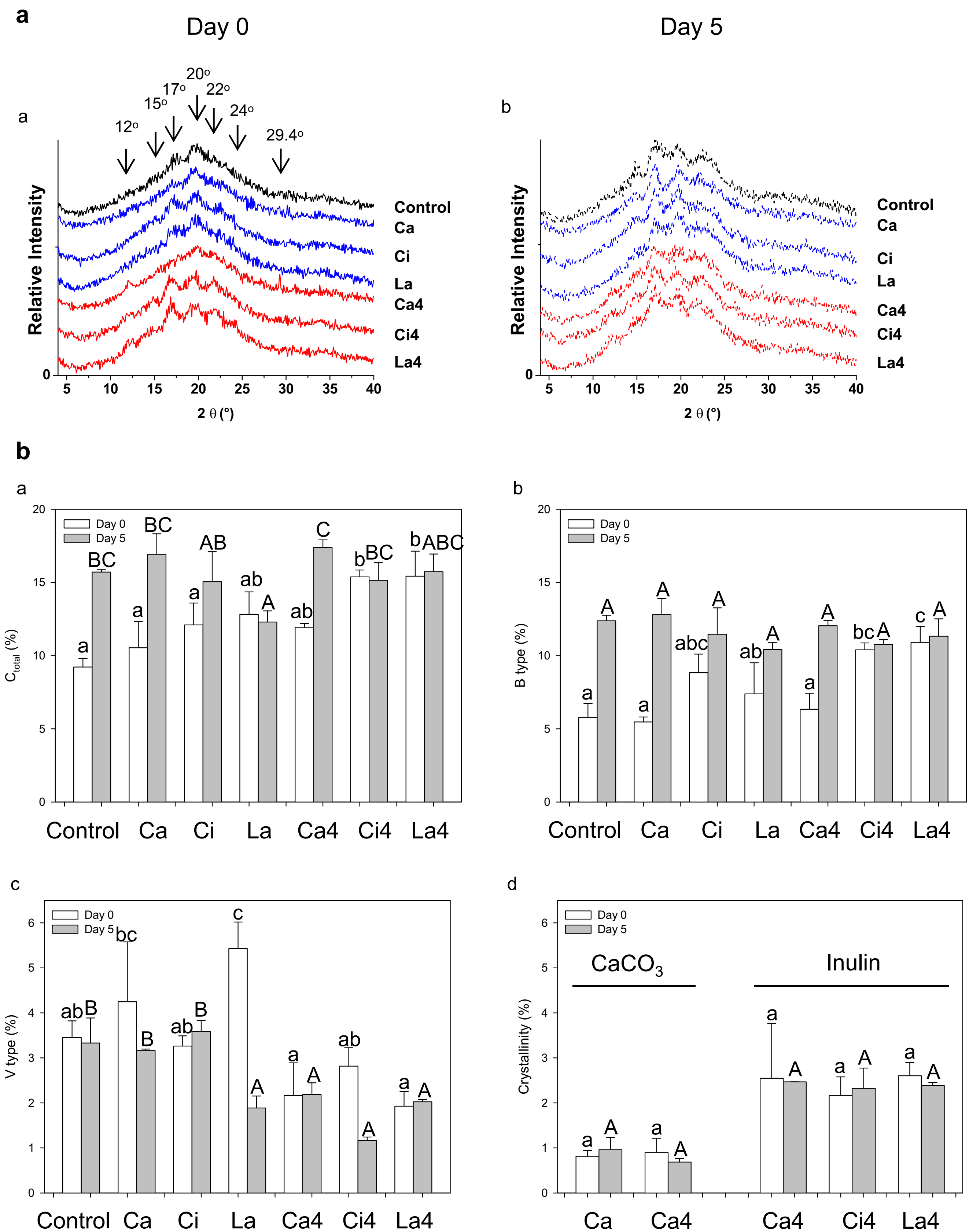
The retrogradation enthalpy (ΔH_R) and RI as a function of storage time are shown in Fig. 3b. Control cooked dough was the sample that presented the highest values of ΔH_R; besides, this parameter did not change with storage time. The values of ΔH_R were significantly lower in the presence of calcium carbonate and inulin (Ca4) (Fig. 3b (a)), and for the same formulation did not significantly change with storage time. Higher values of ΔH_R are associated with greater amylopectin recrystallization, a phenomenon that contributes to the increase in crumb firmness after storage. Dough with calcium carbonate presented lower RI; in the presence of inulin, a linear increase with time was observed, reaching a RI value of 57% (Fig. 3b (b)).

Dough with organic calcium salts exhibited a similar retrogradation behavior (Fig. 3b (c, e)). In the absence of inulin, the presence of calcium (Ci, La) decreased ΔH_R with respect to control. The presence of inulin further decreased the value of ΔH_R up to day 5; a significant increase at the seventh day was observed, mainly when calcium lactate was used (La4). No significant differences in RI were observed between control and samples with calcium (Ci, La) during storage (Fig. 3b (d, f)). The variation of RI in dough containing inulin followed the same tendency as that observed for ΔH_R, reaching the sample with calcium lactate (La4), a RI value of 98% (Fig. 3b (f)).

Calcium carbonate was the salt that together with inulin was able to better prevent amylopectin retrogradation, while along storage time, calcium lactate was the salt with the lowest capacity of preventing this process.

The increase in crystallization of the crumb components after the fifth day of storage was studied by X-ray diffraction. Figure 4a shows X-ray diffraction patterns of lyophilized fresh bread (Fig. 4a (a)) and breads stored during 5 days (Fig. 4a (b)). The intensity of the peaks varied with the type of formulation and day of storage. Bread crumbs presented different peaks at 2θ angles: 12°, 15°, 17°, 20°, 22°, 24°, and 29.4°. The peak of 12° was present in crumbs with inulin (Ca4, Ci4, and La4). Several authors attributed this peak to this molecule (Ronkart et al. 2006; Aravind et al. 2012). The peaks at 2θ

Fig. 4 X-ray diffraction pattern of bread crumb. **a** Non-stored (a) and stored for 5 days at 20 °C (b). **b** (a) Total crystallinity (C_{total}), (b) B-type mass crystallinity, (c) V-type mass crystallinity, and (d) calcium and inulin mass crystallinity of bread crumb fresh and stored. *Control* without calcium and inulin; bread with 1.80 g calcium/kg flour without inulin (Ca, Ci, La) and bread with 2.40 g calcium/kg flour and 12% inulin (Ca4, Ci4, La4). Different letters indicate significant differences between samples ($p < 0.05$): lowercase letters for fresh crumbs (day 0) and capital letters for stored crumbs (day 5)


a
Day 0
Day 5
a
b
12°
15°
17°
20°
22°
24°
29.4°
Relative Intensity
2 θ (°)
Control
Ca
Ci
La
Ca4
Ci4
La4
b
a
b
c
d
Day 0
Day 5
C_{total} (%)
B type (%)
V type (%)
Crystallinity (%)
CaCO3
Inulin
Control
Ca
Ci
La
Ca4
Ci4
La4

angles of 15°, 17°, 22°, and 24° are commonly associated to B-type crystalline phase (Ribotta et al. 2004), while the peak at 20° is associated with V-type crystalline phase that corresponds to the amylose-lipid complex. Finally, in breads with calcium carbonate (Ca, Ca4), a peak at 29.4° was observed. This peak was previously found by Díaz-Ramírez et al. (2016) in foods containing calcium carbonate.

Figure 4a (a) shows that fresh bread crumbs with organic calcium salts (Ci, La, Ci4, La4) presented a peak at 17° more pronounced than the peak at 20°. After the fifth day of storage (Fig. 4a (b)), crystallinity represented by the area of the peaks at 17° and 24° increased.

During baking, most of the starch gelatinize, but because dough is a water-limited system, a low fraction of starch granules remains in crystalline state. In addition, starch retrogradation begins instantly after baking. Therefore, an initial value of total crystallinity (C_T) of 9.2% was obtained for control fresh crumb (Fig. 4b (a)). Comparing to control sample, crumbs with calcium in the absence of inulin (Ca, Ci, and La) presented similar values of C_T. However, in crumbs with organic calcium salts and high amounts of inulin (Ci4 and La4), the percentage of C_T was higher; this behavior can be attributed to crystals of inulin (peak 12°) and starch (peaks 15° and 17°). After 5 days of storage, period in which amylopectin recrystallizes (aging of bread), C_T increased for all formulations studied, except for crumbs with organic calcium salts and high inulin content (Ci4 and La4) and for calcium lactate without inulin (La) in which the value of C_T was similar to that observed at day 0 (Fig. 4b (a)).

The sum of the crystallinity of peaks at 15°, 17°, 22°, and 24°, associated to crystallization of B-type starch, is shown in Fig. 4b (b), and individual values for each peak are shown in Table 3. The B-type crystallinity of fresh crumbs with calcium carbonate (Ca and Ca4) and with organic calcium salts (Ci and La) was statistically similar to that obtained for control crumb. Crumbs with organic salts and inulin (Ci4 and La4) presented a higher percentage of B-type crystallinity (Fig. 4b (b)).

Table 3 shows that in fresh breads (day 0), the crystallinity of peaks at 15° and 17° of crumbs with calcium carbonate without inulin (Ca) was similar to that of control crumb. However, a significant increase with respect to control in the area of peaks at 15° and 17° was observed for the other formulations. No significant differences in the area of peaks 22° and 24° were observed between all formulations.

Starch crystallinity increased at the fifth day (Fig. 4b (b)), mainly due to the increase of the area of peaks at 17° and 24°, except for Ci4 and La4 crumbs that presented similar crystallinity to that of fresh bread (Table 3).

Bigne et al. (2016) found an increase in C_T during storage of wheat breads complemented with algarroba flour, with an increase in peaks of 17°, 22°, and 24°. These authors associated this behavior with starch recrystallization. An increase in B-type crystallinity of starch in crumbs with organic calcium salts and high inulin correlates with the highest values of ΔH_R obtained by DSC assays (Fig. 3b (c, e)). In stored wheat bread crumbs, Ribotta et al. (2004) found a correlation between amylopectin retrogradation (measured by DSC) and the B-type crystalline structure of starch (analyzed by XRD). Higher values of ΔH_R and percentage of crystallinity were obtained by these authors.

Figure 4b (c) shows crystallinity associated with the amylose-lipid complex (V-type). V-type crystallinity of **control** fresh crumb (day 0) was similar to that obtained for crumbs with calcium and inulin, except for the sample with calcium lactate (**La**); the last one presented higher V-type crystallinity in the absence of inulin.

An increase of starch crystallization (B-type) in La4 with respect to La (Fig. 4b (b)) would be associated with the high inulin content capable of holding considerable amounts of water, decreasing starch gelatinization, and therefore, less

Table 3 Percent crystallinity obtained by X-ray diffraction of fresh (day 0) and stored (day 5) crumbs at 20 °C

	Calcium (g/kg)	Inulin (%)	Starch crystallinity (%)							
			Day 0				Day 5			
			15°	17°	22°	24°	15°	17°	22°	24°
Control	0	0	0.8 ± 0.3 a	1.2 ± 0.8 a	1.7 ± 0.6 a	1.5 ± 0.6 a	1.8 ± 1.1 a	4.3 ± 0.3 b	3.0 ± 0.1 ab	5.6 ± 0.9 ab
Ca	1.8	0	0.6 ± 0.1 a	1.3 ± 0.4 a	1.4 ± 0.8 a	2.0 ± 1.1 a	2.3 ± 0.0 a	3.7 ± 0.4 ab	2.2 ± 0.6 ab	2.9 ± 0.5 ab
Ci	1.8	0	2.2 ± 0.7 b	2.4 ± 0.8 ab	1.7 ± 1.2 a	2.0 ± 1.5 a	1.9 ± 0.8 a	3.6 ± 0.7 ab	2.5 ± 0.8 ab	2.7 ± 0.3 a
La	1.8	0	2.1 ± 0.8 b	2.1 ± 0.5 ab	2.1 ± 1.1 a	1.4 ± 0.3 a	1.7 ± 0.3 a	2.9 ± 0.3 a	2.0 ± 0.8 ab	3.5 ± 1.4 ab
Ca4	2.4	12	2.3 ± 0.4 b	1.3 ± 0.6 a	1.1 ± 0.1 a	2.0 ± 0.3 a	1.2 ± 0.8 a	4.2 ± 0.6 b	3.7 ± 0.0 b	4.6 ± 2.5 ab
Ci4	2.4	12	1.6 ± 0.1 ab	3.1 ± 0.1 bc	2.8 ± 0.4 a	3.0 ± 0.9 a	1.1 ± 0.6 a	3.9 ± 0.8 ab	2.4 ± 1.8 ab	5.8 ± 0.4 b
La4	2.4	12	2.6 ± 0.5 b	3.8 ± 0.6 c	2.1 ± 0.1 a	1.3 ± 0.1 a	1.6 ± 0.3 a	4.7 ± 0.1 b	1.5 ± 0.6 a	3.4 ± 1.2 ab

Crumb crystalline grade B-type at 15, 17, 22, and 24°. Mean ± standard deviation. Different letters in the same column indicate significant differences ($p < 0.05$)

proportion of amylose would be available for forming the amylose-lipid complex, leading to low V-type crystallinity. Figure 4b (c) shows that no changes in crystallinity were observed at the fifth day of storage with respect to day 0 for most of the formulations, except for La and Ci4 for which this parameter decreased.

The crystallinity of calcium carbonate (peak at 29.4°) was statistically similar in crumbs with and without inulin and did not significantly change with storage (Fig. 4b (d)). A similar tendency was observed for the peak belonging to inulin (12°), suggesting that the crystallinity of both ingredients does not change during storage time.

Conclusions

The incorporation of different calcium salts (carbonate, citrate, or lactate) and inulin to wheat flour produced distinct changes in breads during storage. The loss of moisture is delayed in the presence of calcium and inulin. The water was retained fundamentally when organic calcium salts jointly with high content of inulin were used. The firmness of crumb increased during storage, while elasticity and cohesiveness decreased. Crumbs with calcium citrate and lactate and low level of inulin resulted softer, more elastic, and cohesive. This calcium salts produced the lowest increase in crumb firmness, mainly at low inulin content. Kinetic parameters obtained from Avrami models in crumbs with calcium carbonate depended on inulin level, while for citrate, it was affected by the calcium content. In crumbs with high inulin amount, this carbohydrate holds water, leading to recrystallization of starch contributing to bread staling. However, crumbs with calcium carbonate or lactate and low inulin content presented higher shelf life than control breads and those with high inulin level. The salt that led to less retrogradation process was calcium carbonate, independently of inulin content; the opposite behavior was observed for crumbs with calcium lactate and inulin, evidenced by the shorter half-time obtained with the Avrami model. Only fresh crumbs with organic salts and high inulin content presented a high percentage of B-type crystallinity of starch that increased with 5 days of storage, which was also evidenced by the increase in the area of 24° peak. The increase in crystallinity of B type in crumbs with organic calcium salts and high inulin content correlates with the highest values of ΔH_R obtained by DSC assays. It can be finally concluded that the mechanism of bread aging depends on several processes that are dependent on the nature of calcium salt and the level of inulin that is necessary to incorporate. The greatest retardation of staling was achieved with organic calcium salts in the presence of a low content of inulin; therefore, this combination is suitable for formulating nutritionally enhanced breads with a longer shelf life.

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