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Optimization of the formulation of nutritional breads based on calcium carbonate and inulin

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ABSTRACT

The purpose of this work was to optimize nutritional wheat bread formulation based on calcium carbonate and inulin (*In*); and to study dough fermentation properties and bread quality. During fermentation, time (t_f) and dough volume (V_{max}) were analyzed. Specific volume (Vs), crust colour, moisture, texture and alveolus properties of crumb are studied. Dough with high *In* content experienced a fast although less expansion. Furthermore, calcium fortification decreased t_f. Crust colour was dependent on inulin content. At 13 g/100 g *In*, the lowest dough V_{max} but the highest Vs bread was obtained. Crumb firmness and chewiness increased with 6.5 g/100 g *In* although high level of prebiotic did not modify these parameters. At the same *In* quantity, an increase of calcium carbonate resulted on softer and more elastic crumbs. Crumb moisture, decreased with the increment of prebiotic. Without *In* and in the presence of Ca, crumbs presented low alveolus area. The highest Vs of 13 g/100 g *In* breads was due to the high number of alveolus with a large void area. Based on the response surface of multiples variables (moisture, cohesiveness, chewiness, Vs) was optimized using a desirability function; the optimum calcium-prebiotic fortified bread obtained was that one that contained 2.196 g/kg Ca and 9.635 g/100 g *In*.

1. Introduction

Wheat bread is a major component of people's diet all over the world. There has been an increasing demand for food products with additional health benefits. During milling of wheat grain, a high proportion of minerals and vitamins are lost resulting in a reduction in the nutritional value of the flour. Lost nutrients (mainly thiamine, niacin and iron) may be reinstated in the flour without being harmful to human health (Rosell, 2003). In Argentina the fortification of wheat flour with iron, thiamine, riboflavin, niacin and folic acid is mandatory. Therefore, deficiencies of some essential nutrients are covered, but not all such as calcium. According to the Nutrition and Health National Inquest (NHNI, 2007) of Argentina, calcium was one of the most critical nutrients; this mineral was deficient in 45.6% of children (2–5 years old) and in 94.3% of women (10–49 years old). Women had lower intakes respect to the recommended daily intake (1 g/day); being independent of their geographic location, socioeconomic status or age. A diet with adequate calcium supplements could help to control diseases caused by deficiency of this element such as osteopenia and osteoporosis. Because the calcium content of white flour, and hence breads made with them, is very low, the contribution to the diet is negligible. Bread is an adequate food for providing calcium to people due to it is widely consumed throughout the world.

Several authors have added minerals as calcium and magnesium to improve nutritional quality of bread (Berdanier, 2002; Sudha & Leelavathi, 2008; Ziadeh, 2002). Not only the calcium quantity but rather its bioavailability is important. For this reason, it is recommended to include prebiotic together with calcium in bread formulation. Prominent among prebiotics is inulin, a water soluble carbohydrate formed with 2–250 fructose subunits ($\beta_{2\rightarrow1}$) with a terminal glucose (Roberfroid, 2007). Inulin is a soluble and fermentable dietary fibre that is not digested by the enzymes of the human digestive tract, stimulates the growth of beneficial bacteria in the colon, suppressing in turn the activity of undesirable bacteria (Fuller & Gibson, 2005; Gibson, Beatty, Wang, & Cummings, 1995; Meyer & Stasse-Wolthuis, 2009; Saad, 2006; Wang, 2009). Inulin

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has also been studied due to other possible beneficial effects to health such as increasing bone absorption of calcium (Lobo et al., 2009; Weaver, 2005), enhancing resistance to gastrointestinal infections (Sauer, Richter, & Pool-Zobel, 2007; Yap, Mohamed, Jamal, Diederick, & Manap, 2008) and colon cancer (Davis & Milner, 2009; Pool-Zobel & Sauer, 2007). Therefore, for an adequate calcium bioavailability, the addition of inulin is recommended.

Rheological properties of wheat dough with calcium carbonate and inulin were previously studied (Salinas, Zuleta, Ronayne, & Puppo, 2012). It was found that hardness, adhesiveness and elasticity of dough increased with the level of calcium but mainly with inulin content. Rheological properties of dough would influence the structure and breadmaking quality of bread. Krupa-Kozak, Altamirano-Fortoul, Wronkowska, and Rosell (2012) studied the effect of inulin and different calcium salts (lactate, citrate, carbonate and chloride) on gluten-free bread; although there has been no previous report on wheat breads. Therefore, the objective of this work was to study the effect of calcium carbonate-inulin systems on breadmaking quality of wheat flour and to optimize a formulation of wheat bread fortified with these nutrients of high quality.

2. Materials and methods

2.1. Materials

A wheat flour (type 0000, Molino Campodónico Ltda., Argentina) (AAC, 2014, chap. IX) for breadmaking (9.92 g/100 g proteins, 0.86 g/100 g lipids, 0.382 g/100 g ash, 11.8 g/100 g moisture) was used. Farinographic parameters of this flour were 57.9 ml, 18 min, 38.0 min and 12 UB for water absorption, development time, stability and softening degree, respectively. Other ingredients used were sodium chloride (CELUSAL, Argentina), fresh yeast (CALSA; Argentina), calcium carbonate ($CaCO_3$, ANEDRA S.A, Argentina), and inulin enriched with oligofructose (Synergy 1, BENEO Orafti, Belgium, containing 92.7% d.b.).

2.2. Experimental design

Mixtures of wheat flour, calcium carbonate 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. Levels of calcium between 1.08 and 2.52 g/kg, and inulin between 0 and 13 g/100 g were analyzed. Response Surface Methodology (RSM) was applied to design experimental parameters in order to obtain optimal ingredients levels for producing baked wheat bread (Khuri & Cornell, 1996; Montgomery, 1997).

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 according to Salinas et al. (2012).

The model adequacies were checked by the variance analysis (*F* test) and R^2 values. Variables effects were represented using surface graphs. We also analyzed control bread, without calcium and inulin, not belonging to the CCD. Data obtained 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 (LSD) test. Mean and standard deviation were calculated for each parameter.

2.3. Dough formulation

Each flour blend consisted of wheat flour, NaCl (2 g/100 g wheat flour basis, w.f.b), fresh yeast (3 g/100 g w.f.b). Optimum quantity of water (W_{abs}) and mixing time (t_d) were established according to Salinas et al. (2012) (farinographic assays, Table 1). Solids ingredients were mixed in a small scale kneader (Kenwood Major, Italy) for 1 min. Water with fresh yeast dispersed was added to the solid blend. The first minute was kneaded at 50 rpm (speed 1) and the rest of the time at 90 rpm (speed 2) until reaching the development time. Dough was rested for 10 min at 25 °C, covered with a plastic film to avoid water loss; then was laminated (4 passes) and again let repose for 10 min.

2.4. Breadmaking process

2.4.1. Fermentation time optimization

Dough (50 g) was placed in a 500 ml graduated cylinder with a plunger mobile and inserted in a fermentation cabinet (Brito Hnos, Argentina) at 30 °C for 240 min. Dough volume (ml) was recorded during 4 h: every 10 min the first 2 h and the ulterior 2 h, volume was measured every 30 min. Measurements were performed in duplicate. The increase in volume (ΔV) was registered as a function of time and curves were adjusted by the Chapman model using the Sigmaplot 10.0 Software.

$$\Delta V = V_{max}[1 - \exp(-bt)]^c \quad (1)$$

where ΔV is the volume increment, t is the time and V_{max} correspond to the maximum volume increment achieved; b and c are constants. Fermentation time (t_f) is the time required for achieving 3/4 of V_{max}, because during the beginning of the baking process the fermentation continues until the structure is established.

2.4.2. Baking process

Dough (90 g) was rounded, left resting for 15 min and then shaped into a bread shipowning (MPZ, Argentina). These pieces were proofed at 30 °C according to their t_f and baked (26 min at 210 °C) in a convection oven (Ariston, Argentina). Bread quality was evaluated 2 h after baking.

2.4.3. Bread quality evaluation

2.4.3.1. Specific volume. Four breads of each formula were analyzed. The specific volume was determined as a ratio of volume and weight (AACC, 2000).

Table 1
Second-order designs matrix used for evaluating bread quality of blends.

Runs #	Coded		Uncoded		W_{abs} (g/100 g)	t_d (min)
	Ca	*In*	Ca (g/kg)	*In* (g/100 g)		
1	−1	−1	1.20	1	54.1	24
2	1	−1	2.40	1	54.7	21
3	−1	1	1.20	12	50.0	26
4	1	1	2.40	12	51.0	21
CP	0	0	1.80	6.5	51.2	20
8	−1.2	0	1.08	6.5	49.8	25
9	0	+1.2	1.80	13	51.8	22
10	1.2	0	2.52	6.5	49.5	29
11	0	−1.2	1.80	0	53.6	22
C	0	0	0	0	56.0	12

CP: central point (3 replicates). C: control (outside of the design). W_{abs}: water absorption. t_d: development time. W_{abs} and t_d utilized for breadmaking were obtained from Salinas et al. (2012).

2.4.3.2. Colour of crust. Colour measurements of bread crust (40 measurements/formulation) were performed with a tristimulus (L^*, a^*, b^*, CIE) colour analyzer (Chroma Meter CR 400, Konica Minolta, Osaka, Japan). Results were expressed as browning index (BI) according to the following equations (2) and (3) (Buera, Retriella, & Lozano, 1985; Saricoban & Yilmaz, 2010):

$$BI = \frac{100(X - 0.31)}{0.172} \quad (2)$$

$$X = \frac{a^* + 1.75L^*}{5.645L^* + a^* - 3.012b^*} \quad (3)$$

2.4.3.3. Texture of bread crumb. The texture profile analysis (TPA) of bread slices was performed on fresh bread at 20 °C. From the middle part of each bread piece, two slices of 2 cm height 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 slices were subjected to a double compression cycle (deformation: 40%, crosshead speed: 0.5 mm/s) with a cylindrical probe (diameter = 2.5 cm). Firmness, cohesiveness, consistency, springiness, resilience and chewiness were determined. Eight replicates were analyzed for each formulation.

2.4.3.4. Moisture content. Moisture of bread crumb was determined according to AACC 44-19 (AACC, 2000). Values obtained were the mean of three replicates.

2.4.3.5. Crumb image analysis. Crumb grain characteristics of bread were assessed using a digital image analysis system. Images (10 × 10 cm^2) were previously acquired at 138 dpcm with an HP scanner 4070 model. The analysis was performed on the centre of the slice. Images were processed using ImageJ software. The image of the centre of each slice was cropped to a square of 300 × 300 pixels (equivalent to 217 × 217 mm^2) and converted to grey-level image (8 bits). The image was binarized using the algorithm Isodata employing a threshold value of 209. Black dots represent alveoli. Image imperfections were considered; with an alveolar threshold of 0.005 cm^2, minor threshold values were not counted as alveoli. Crumb grain characteristics studied were: numbers of cell per area (N) (cm^{-2}), mean cell area (A_M) (cm^2) and the void fraction or total area occupied by alveoli, that is the ratio between the cells and the total crumb area selected (A_T) (%).

2.5. Optimization of bread formulation and quality verification

The multi response optimization process was selected according a desirability option. Four responses (moisture, Vs, chewiness and cohesiveness) were simultaneously optimized by this desirability function. Several authors have used this methodology for the optimization of various food formulations (Gan et al., 2007; Vatsala, Saxena, & Rao, 2001).

The desirability function approach is one of the most widely used methods in industry for the optimization of multiple response processes (Islam, Alam, & Hannan, 2012). It is based on the idea that the quality of a product or process depends on multiple quality variables; all of them should be inside of the "desired" limits for being acceptable.

A desirability function [$d_j(Y_j)$] assigns numbers between 0 and 1 for each response: completely undesirable (value 0) and ideal response (value 1). Depending on whether a particular response is to be maximized, minimized, or assigned a target value, different desirability functions $d_j(Y_j)$ can be used. A useful class of desirability functions was proposed by Derringer and Suich (1980).

The individual desirabilities are then combined using the geometric mean (Derringer & Suich, 1980; Harrington, 1965), which gives the overall desirability (D).

The levels of calcium and inulin for the optimum formulation were obtained using the maximum D value. Optimum values of each factor (Ca and In) were utilized in the model generated by RSM for calculating water absorption, mixing and fermentation times, according to:

$$W_{abs} = 49.83 + 5.63\cdot10^{-3} \times Ca - 1.01 \times In - 1.53\cdot10^{-6} \times Ca^2 + 3.03\cdot10^{-5} \times CaIn + 0.057 \times In^2 \quad (4)$$

$$t_d = 50.02 - 0.033 \times Ca + 0.41 \times In + 9.3\cdot10^{-6} \times Ca^2 - 1.5\cdot10^{-4} \times CaIn + 6.5\cdot10^{-3} \times In^2 \quad (5)$$

$$t_f = 61.27 - 0.17 \times Ca - 8.89 \times In - 8.91 \times Ca^2 - 4.5 \times CaIn - 1.27 \times In^2 \quad (6)$$

Considering these variables, the optimum bread was prepared. This bread was experimentally analyzed and the results were statistically compared to the predicted values by the mathematical model.

3. Results and discussion

3.1. Fermentation curves

Fig. 1 shows as example, the fermentation curves of control dough (C), dough with 12 g/100 g In – 2.40 g/kg Ca (#4) and 1.80 g/kg Ca in the absence of inulin (#11). It can be observed an increase in volume of all dough as a function of time; but volume of dough C increased in greater proportion than the dough with calcium and with Ca + In.

Table 2 shows V_{max} coefficients and fermentation time (t_f) of dough. The highest V_{max} was obtained for control dough (C). In the absence of inulin, V_{max} value decreased with calcium carbonate (1.80 g/kg Ca). With high inulin content (≥6.5 g/100 g) V_{max} decreased mainly for 12 and 13 g/100 g In. Results suggest that in the presence of inulin there is a low expansion of dough.

Dough with the highest fermentation times were those with 0 and 1 g/100 g In, being the exception sample #CP (Table 2). The

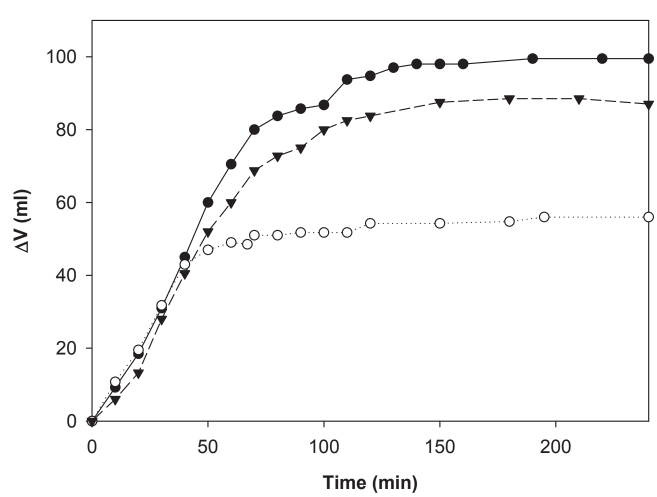

Fig. 1. Fermentation curves of dough: control sample (C, —●—); Sample #4: 2.40 g/kg Ca, 12 g/100 g In (···○···); Sample #11: 1.80 g/kg Ca, 0 g/100 g In (— ▼—).

Table 2
Maximum volume and fermentation time of dough.

Runs #	Ca (g/kg)	*In* (g/100 g)	V_{max} (ml)	t_f (min)
1	1.20	1	90.4 ± 0.8 ef	57 ± 4 c
2	2.40	1	85.8 ± 0.8 f	66 ± 5 d
3	1.20	12	63 ± 1 bc	48 ± 2 b
4	2.40	12	53.8 ± 0.4 a	39 ± 1 a
CP	1.80	6.5	71 ± 2 d	63 ± 10 cd
8	1.08	6.5	65.1 ± 0.4 c	46 ± 3 ab
9	1.80	13	58.2 ± 0.8 ab	47 ± 5 ab
10	2.52	6.5	67 ± 1 c	47 ± 2 ab
11	1.80	0	88 ± 1 e	68 ± 3 d
C	0	0	99.9 ± 0.8 g	67 ± 4 d

Different letters in the same column indicate significant differences ($p < 0.05$). V_{max}: maximum volume, t_f: fermentation time.

addition of calcium carbonate did not change the t_f of the dough in the absence of *In* (# 11). In contrast, for dough with 1 g/100 g *In*, the t_f increased with the incorporation of calcium carbonate. At high inulin level (≥12 g/100 g *In*) the lowest t_f were obtained; this effect is desirable for the baking process because it shortens operative time industrial bread preparation.

Peressini and Sensidoni (2009) found that the addition to a strong flour of Raftiline® HP, similar to Synergy 1®, at levels of 2.5 g/100 g, 5 g/100 g and 7.5 g/100 g, the V_{max} also gradually decreased with increasing levels of this inulin.

3.2. Quality of bread

The colour of the bread crust is an important parameter in the choice of the bread by consumers; very light colours or extremely dark are undesirable. Principally, the crust colour exhibited by a bread loaf depends on a number of factors such as the type of flour, the quality and quantity of the ingredients used (Komlenić et al., 2010), and also the baking temperature and time (Shittu, Raji, & Sanni, 2007). Browning index (BI) is shown in Table 3. This index indicates the level of brown colour and is reported as an important parameter of bread quality, related to enzymatic and non-enzymatic (Maillard reaction) browning reactions (Palou, Lopez-Malo, Barbosa-Canovas, Welti-Chanes, & Swanson, 1999; Saricoban & Yilmaz, 2010). Due to the issue that baking time and temperature was the same for all formulations, we can infer that the change in formulation caused plainly visible change in the colour of the functional bread, compared to the control bread (without calcium and inulin). In the absence of inulin, the BI parameter slightly increased with calcium content (# 11). This effect could be due to Maillard reaction, which is favoured at alkaline pH (Martins, Jongen, & Van Boekel, 2000), considering that pH were 5.80 and 6.28 for C and #11, respectively (Salinas et al., 2012). The crust colour was independent of the calcium content at *In* ≥ 6.5 g/100 g. The crust of breads with inulin showed a reduction in luminosity (L^*) and an increase of the parameters a^* and b^* respect to control sample (data not shown), which results in a greater BI suggesting a reddish colouration in the presence of the prebiotic. In addition, BI increased with inulin concentration; at high inulin contents (12 and 13 g/100 g) the highest values were observed. At each inulin level, the crust colour was independent of the content of calcium. Similar results were reported by other authors (Frutos, Guilabert-Antón, Tomás-Bellido, & Hernández-Herrero, 2008; Hager et al., 2011; Peressini & Sensidoni, 2009). Frutos et al. (2008) studied the effects on bread quality of fibre artichoke employing levels of 0, 3, 6, 9 and 12 g/100 g (wheat flour basis). They found that an increment in the fibre incremented the crust colour. A darker curst colour was reported for all levels of addition (2.5 g/100 g; 5 g/100 g and 7.5 g/100 g) and 2 types of inulin (Peressini & Sensidoni, 2009). An enhancement of bread crust colouration was also reported for breads prepared with inulin content in the range of 3 g/100 g to 10 g/100 g (Hager et al., 2011; Poinot et al., 2010).

Specific volume (Vs) of the bread is shown in Table 3. At same inulin content, an increase in the calcium salt did not cause significant difference in Vs. The highest Vs was obtained with 13 g/100 g *In*, indicating a high capacity of dough, in the presence of inulin, to retain gas produced during fermentation due to an adequate development of the gluten network during kneading process. Results suggest that a high V_{max} not necessarily generate a high bread volume. The lowest V_{max} but the highest Vs at high inulin content (13 g/100 g) were obtained. These results suggest a certain prebiotic effect during dough growing, leading to high retention of CO_2 during baking; possibly by the formation of a gelled matrix network interpenetrated with gluten.

Texture attributes of fresh bread crumbs are shown in Figs. 2 and 3. In the absence of *In*, calcium salt did not produce modifications in firmness (Fig. 2a). The softest crumbs were obtained in the absence or 1 g/100 g *In*. Nevertheless, at high levels of *In* (≥6.5 g/100 g), an increase in levels of calcium decreased this parameter, associated with a softer crumb; this behaviour could correspond to greater alveolar area, parameter that will be discussed later. Consistency values followed the same trend as firmness (data not shown). Cohesiveness is related to the integration of components, high values of this parameter are desired in fresh bread crumbs. At same inulin values (≤6.5 g/100 g *In*), cohesiveness was incremented with a major quantity of calcium added (Fig. 2b). High levels of inulin significantly decreased springiness (Fig. 2c); while resilience, related to the instantaneous ability of crumb for recovering the original geometry (instantaneous elasticity), was significantly higher for samples with calcium carbonate, indicating a positive effect of the salt on the crumb matrix. At ≥6.5 g/100 g *In*, lower resilience was obtained (Fig. 2d).

Chewiness values (Fig. 3a) changed mainly directly with firmness (see Fig. 2a) and inversely with cohesiveness (see Fig. 2b). The model obtained applying RSM for chewiness was: $7.756 - 0.669 \times Ca - 1.721 \times In^2$ ($R^2 = 0.898$, RMSE = 0.592); an inverse relationship with Ca and In^2 was obtained. High levels of calcium would facilitate mastication work of bread due to the softer crumb obtained.

These results are coincident with those reported in bread formulated with Artichoke fibre (Frutos et al., 2008). These authors studied the effect of inulin on bread quality and they found an increased in hardness and chewiness but a decreased in resilience and Vs with higher quantity of prebiotic aggregated.

The moisture of fresh crumb of the different formulations was also analyzed. Control bread crumb was the wettest (Fig. 3b).

Table 3
Physical properties of breads.

Runs #	Ca (g/kg)	*In* (g/100 g)	BI (−)	Vs (cm^3/g)
1	1.20	1	62 ± 10 c	2.5 ± 0.2 bc
2	2.40	1	75 ± 11 c	2.7 ± 0.2 cd
3	1.20	12	97 ± 9 f	2.5 ± 0.1 cd
4	2.40	12	97 ± 9 f	2.5 ± 0.2 cd
CP	1.80	6.5	84 ± 10 e	2.1 ± 0.2 ab
8	1.08	6.5	84 ± 10 e	2.15 ± 0.05 ab
9	1.80	13	102 ± 9 f	3.2 ± 0.4 e
10	2.52	6.5	86 ± 10 e	2.5 ± 0.2 bcd
11	1.80	0	55 ± 7 b	2.6 ± 0.1 cd
C	0	0	41 ± 6 a	2.8 ± 0.3 d

Different letters in the same column indicate significant differences ($p < 0.05$). BI: browning index for bread crust; Vs: specific volume of bread.

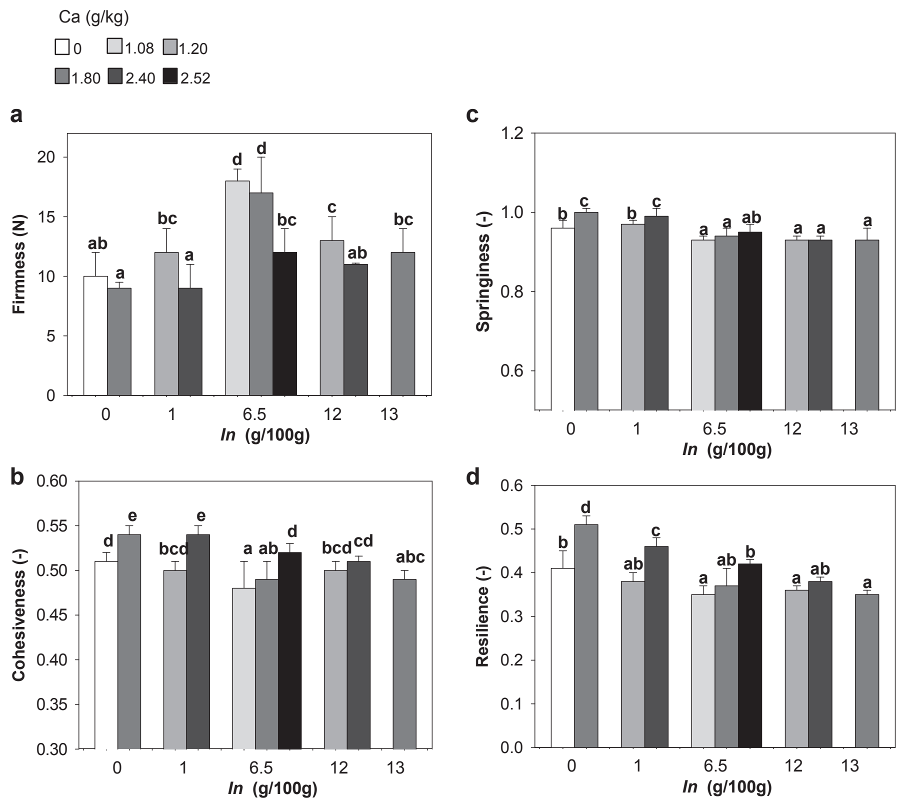

Fig. 2. Texture parameters of crumb bread: a) firmness, b) cohesiveness, c) springiness and d) resilience. Errors bars: standard deviations. Different letters in the same graphics indicate significant differences ($p < 0.05$).

Calcium carbonate in the absence of the inulin (#11) caused a decrease in moisture content, consistent with lower water absorption (Table 1). However, at low inulin level (1 and 6.5 g/100 g), the salt provoked an increase of crumb moisture (Fig. 3b), indicating the influence of calcium carbonate in the water retention in the crumb. At high inulin contents (≥12 g/100 g), crumbs moisture decreased. This phenomenon is straightly related with farinographic water absorption (W_{abs}); water quantity needed for dough formation was less with the increment of prebiotic (see Table 1). The model obtained applying RSM for crumb moisture was: $39.29 + 0.549 \times Ca - 1.119 \times In + 0.927 \times In^2 - 0.775 \times CaIn$ ($R^2 = 0.984$, RMSE = 0.228). This model shows that moisture was directly affected by Ca and In^2 and indirectly with In and its interaction with calcium (CaIn).

O'Brien, Mueller, Scannell, and Arendt (2003) replaced on breads, fat with different types of inulin (2.5 and 5 g/100 g). They found that with prebiotic, hardness and crust colour increased, but Vs diminished. Besides the increment in firmness, chewiness and elasticity; a decrease in Vs with high quantities of fibre artichoke was observed by Frutos et al. (2008). Other authors found similar results; with the increment of inulin level increased hardness and crust colour, and diminished crumb moisture (Peressini & Sensidoni, 2009).

3.3. Crumb structure

Structural properties of bread crumb are summarized in Table 4. The void percentage (A_T), which measures the proportion of the cross-sectional area of the crumb containing gas cells (Zghal, Scanlon, & Sapirstein, 1999), was slightly lower for samples with high inulin content. Mean alveolus area (A_M) of bread crumbs were similar, therefore a greater number of alveoli (N) will cause a higher percentage of total air trapped (A_T) in the crumb. In the absence of inulin, crumb with calcium carbonate (#11) presented lower N and less A_T than control (C) crumb (Table 4). At low In (1 g/100 g), A_M decreased without increasing N, yielding lower A_T when calcium was added.

3.4. Optimization of the formulation and verification of the model

Contour plots from response surface for four variables (Vs, moisture, chewiness, cohesiveness) were superimposed (Fig. 4). Considering all these response variables, maximum and minimum limits of each response were established. The following variables were selected on a desired range of good quality for breads: moisture (40.2–42.0 g/100 g), Vs (2.5–3.0 cm^3/g), cohesiveness (0.400–0.500) and chewiness (5.50–6.40 N). An area of optimum performance (yellow area), that included the limit values of the four variables was obtained and is shown in Fig. 4. Within this area, the formulation with the highest overall desirability ($D = 0.96$) was selected (star point) as the optimum one for breadmaking. This formulation contained 2.196 g/kg Ca and 9.635 g/100 g of inulin.

Table 5 shows the predicted and experimental values for each response variables. Moisture and cohesiveness were within the range predicted ($p < 0.05$); while specific volume and chewiness were outside the range. Experimental Vs was higher than the maximum value determined by the range. In addition, chewiness of this bread was also lower than the predicted value, a desirable condition for bread consumers. Thus, the model can be used to optimize the basic formulation of nutritional breads of high quality.

4. Conclusions

In general, the incorporation of prebiotic increased crumb firmness (until 6.5 g/100 g In) and crust colour. Nevertheless, cohesiveness, elasticity and moisture of crumbs resulted reduced.

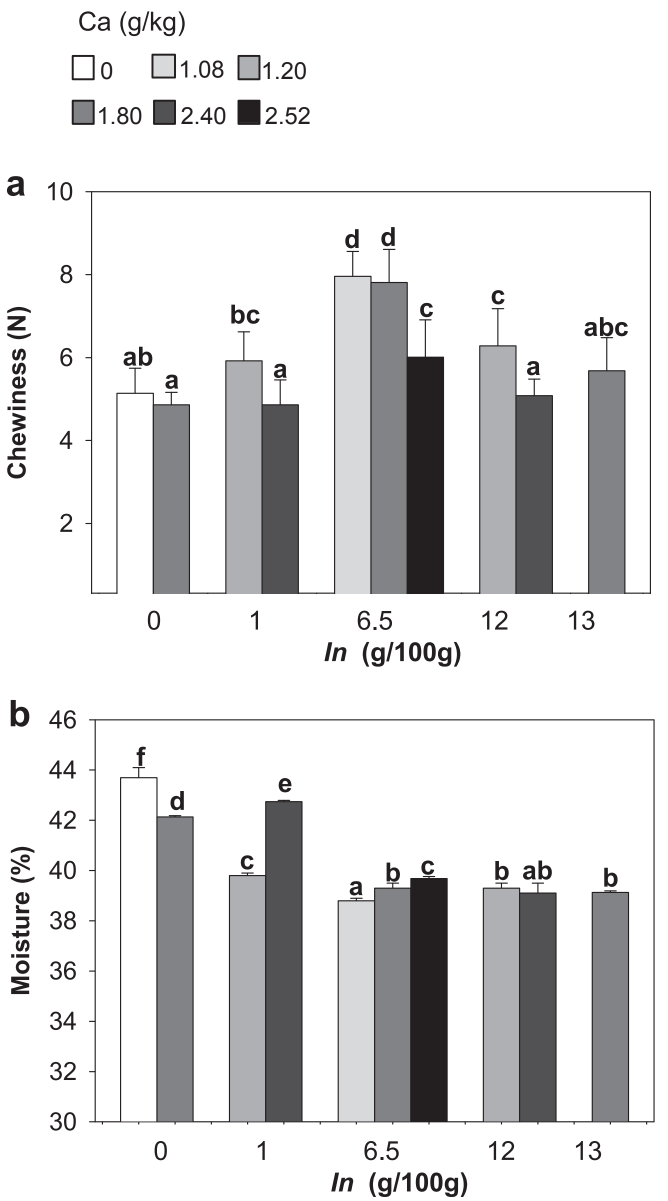

Fig. 3. Crumb bread properties: (a) chewiness, (b) moisture. Errors bars: standard deviations. Different letters in the same graphics indicate significant differences ($p < 0.05$).

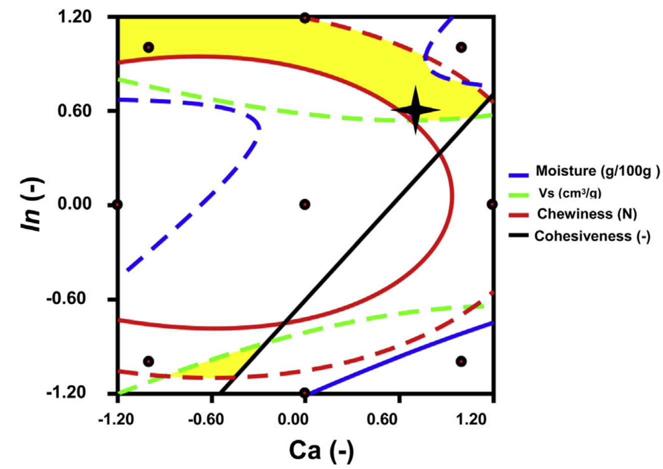

Fig. 4. Graph of superimposed plots of the four responses variables of bread. Yellow area: feasible region; ———: Upper bound; - - - -: lower bound. Black star on the yellow area: optimal formulation. The desirability value (*D*) was 0.96. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 4
Analysis of alveolus properties of crumb grain.

Runs #	Ca (g/kg)	*In* (g/100 g)	*N* (cm^{-2})	$A_M \cdot 10^3$ (cm^2)	A_T (%)
1	1.20	1	156 ± 17 d	25 ± 5 b	17 ± 4 f
2	2.40	1	160 ± 18 d	19 ± 4 a	13 ± 4 de
3	1.20	12	79 ± 16 ab	21 ± 4 ab	8 ± 3 abc
4	2.40	12	111 ± 22 c	21 ± 4 ab	11 ± 3 cd
CP	1.80	6.5	72 ± 26 a	19 ± 6 a	6 ± 2 abc
8	1.08	6.5	73 ± 16 ab	21 ± 2 ab	7 ± 1 abc
9	1.80	13	140 ± 34 d	22 ± 7 ab	13 ± 3 de
10	2.52	6.5	101 ± 31 bc	21 ± 5 ab	9 ± 3 bc
11	1.80	0	112 ± 29 c	21 ± 5 ab	10 ± 2 cd
C	0	0	138 ± 15 d	25 ± 6 b	15 ± 4 ef

Different letters in the same column indicate significant differences ($p < 0.05$). *N*: numbers of alveolus per area; A_M: mean alveolus area and A_T: area occupied by alveoli.

Table 5
Predicted and experimental value of the responses variables at optimum formulation.

Responses variables	Optimum bread		
	Predicted value	Experimental value	Range
Moisture (g/100 g)	39.02	39.09 ± 0.04	38.72–39.33
Cohesiveness (−)	0.506	0.524 ± 0.008	0.48–0.53
Chewiness (N)	6.65	4.34 ± 0.31	5.84–7.44
Specifics volume (cm^3/g)	2.49	2.98 ± 0.21	2.06–2.91

Mean ± SD. Range: confidence interval of the numerical optimization (95%).

Specific volume of all breads was almost the same (2.5 cm^3/g). Sample with the highest content of inulin (1.80 g/kg Ca, 13 g/100 g *In*) presented the highest Vs with the lowest firmness. Although the prebiotic negatively affected breadmaking quality, the presence of calcium not only softened crumbs, becoming less firm and chewable, also favoured crumb elasticity and particle integration.

In addition, optimum bread with high levels of calcium and inulin was able to be obtained. Values of bread quality parameters were further experimentally validated evidencing the efficacy of the model based on desirability functions.

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References

AAC. (2014). *Argentinean Alimentarius Codex* (Art 661-Res.167) http://www.anmat.gov.ar/alimentos/normativas_alimentos_caa.asp Accessed in April 2014.
AACC. (2000). *American Association of Cereal Chemists* (10th ed.). St. Paul, MN: The Association.
Berdanier, G. D. (2002). *Handbook of nutrition*. Boca Raton. USA: CRC Press.
Buera, M. P., Retriella, C., & Lozano, R. D. (1985). Definition of colour in the non-enzymatic browning. *Die Farbe, 33*, 316–326.
Davis, C. D., & Milner, J. A. (2009). Gastrointestinal microflora, food components and colon cancer prevention. *Journal of Nutritional Biochemistry, 20*, 743–752.
Derringer, G. C., & Suich, R. (1980). Simultaneous optimization of several response variables. *Journal of Quality Technology, 12*, 214–219.
Frutos, M. J., Guilabert-Antón, L., Tomás-Bellido, A., & Hernández-Herrero, J. A. (2008). Effect of artichoke (*Cynara scolymus* L.) fiber on textural and sensory qualities of wheat bread. *Food Science and Technology International, 14*(5), 49–55.

Fuller, R., & Gibson, G. (2005). Probiotics and prebiotics. Definition and role. In *Encyclopedia of human nutrition* (pp. 1633–1639).

Gan, H. E., Karim, R., Muhammad, S. K. S., Bakar, J. A., Hashim, D. M., & Rahman, R. A. (2007). Optimization of the basic formulation of a traditional baked cassava cake using response surface methodology. *LWT – Food Science and Technology, 40*(4), 611–618.

Gibson, G. R., Beatty, E. R., Wang, X. I., & Cummings, J. H. (1995). Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin. *Gastroenterology, 108*(4), 975–982.

Hager, A., Ryan, L. A. M., Schwab, C., Gaenzle, M. G., O'Doherty, J. V., & Arendt, E. K. (2011). Influence of the soluble fibres inulin and oat beta-glucan on quality of dough and bread. *European Food Research and Technology, 232*(3), 405–413.

Harrington, E. (1965). The desirability function. *Industrial Quality Control, 21*, 494–498.

Islam, M. A., Alam, M. R., & Hannan, M. O. (2012). Multiresponse optimization based on statistical response surface methodology and desirability function for the production of particleboard. *Composites Part B: Engineering, 43*(3), 861–868.

Khuri, A. I., & Cornell, J. A. (1996). *Response surfaces: Designs and analyses* (2nd ed.). New York, USA: Marcel Dekker.

Komlenić, D. K., Ugarčić-Hard, Ž., Jukić, M., Planinić, M., Bucić-Kojić, A., & Strelec, I. (2010). Wheat dough rheology and bread quality effected by *Lactobacillus brevis* preferment, dry sourdough and lactic acid addition. *International Journal of Food Science and Technology, 45*(7), 1417–1425.

Krupa-Kozak, U., Altamirano-Fortoul, R., Wronkowska, M., & Rosell, C. M. (2012). Breadmaking performance and technological characteristic of gluten-free bread with inulin supplemented with calcium salts. *European Food Research and Technology, 235*(3), 545–554.

Lobo, A. R., Cocato, M. L., Jorgetti, V., de Sá, L. R. M., Nakano, E. Y., & Colli, C. (2009). Changes in bone mass, biomechanical properties, and microarchitecture of calcium- and iron-deficient rats fed diets supplemented with inulin-type fructans. *Nutrition Research, 29*(12), 873–881.

Martins, S. I., Jongen, W. M., & Van Boekel, M. A. (2000). A review of Maillard reaction in food and implications to kinetic modelling. *Trends in Food Science & Technology, 11*(9), 364–373.

Meyer, D., & Stasse-Wolthuis, M. (2009). The bifidogenic effect of inulin and oligofructose and its consequences for gut health. *European Journal of Clinical Nutrition, 63*(11), 1277–1289.

Montgomery, D. C. (1997). *Design and analysis of experiments* (4th ed.). New York: John Wiley and Sons.

NHNI. (2007). *Nutrition and health national inquest*. Buenos Aires, Argentina: Ministerio de Salud. www.msal.gov.ar Accessed in July 2013.

O'Brien, C. M., Mueller, A., Scannell, A. G. M., & Arendt, E. K. (2003). Evaluation of the effects of fat replacers on the quality of wheat bread. *Journal of Food Engineering, 56*(2), 265–267.

Palou, E. A., Lopez-Malo, G. V., Barbosa-Canovas, J., Welti-Chanes, J., & Swanson, B. G. (1999). Polyphenoloxidase activity and color of blanched and high hydrostatic pressure treated banana puree. *Journal of Food Science, 64*(1), 42–45.

Peressini, D., & Sensidoni, A. (2009). Effect of soluble dietary fibre addition on rheological and breadmaking properties of wheat doughs. *Journal of Cereal Science, 49*(2), 190–201.

Poinot, P., Arvisenet, G., Grua-Priol, J., Fillonneau, C., Le-Bail, A., & Prost, C. (2010). Influence of inulin on bread: kinetics and physico-chemical indicators of the formation of volatile compounds during baking. *Food Chemistry, 119*(4), 1474–1484.

Pool-Zobel, B., & Sauer, J. (2007). Overview of experimental data on reduction of colorectal cancer risk by inulin-type fructans. *The Journal of Nutrition, 137*(11), 2580S–2584S.

Roberfroid, M. B. (2007). Inulin-type fructans: functional food ingredients. *Journal of Nutrition, 137*(11), 2493s–2502S.

Rosell, C. M. (2003). The nutritional enhancement of wheat flour. In S. P. Cauvain (Ed.), *Bread making improving quality*. Cambridge, England: CRC Press, Woodhead Publishing Ltd.

Saad, S. M. I. (2006). Probiotics and prebiotics: the state of the art. *Brazilian Journal of Pharmaceutical Science, 42*(1), 1–16.

Salinas, M. V., Zuleta, A., Ronayne, P., & Puppo, M. C. (2012). Wheat flour enriched with calcium and inulin: a study of hydration and rheological properties of dough. *Food Bioprocess and Technology, 5*(8), 3129–3141.

Saricoban, C., & Yilmaz, M. T. (2010). Modelling the effects of processing factors on the changes in colour parameters of cooked meatballs using response surface methodology. *World Applied Sciences Journal, 9*(1), 14–22.

Sauer, J., Richter, K. K., & Pool-Zobel, B. L. (2007). Products formed during fermentation of the prebiotic inulin with human gut flora enhance expression of biotransformation genes in human primary colon cells. *British Journal of Nutrition, 97*(5), 928–937.

Shittu, T. A., Raji, A. O., & Sanni, L. O. (2007). Bread from composite cassava–wheat flour I. Effect of baking time and temperature in some physical properties of bread loaf. *Food Research International, 40*(2), 280–290.

Sudha, M. L., & Leelavathi, K. (2008). Influence of micronutrients on rheological characteristics and bread-making quality of flour. *International Journal of Food Sciences and Nutrition, 59*(2), 105–115.

Vatsala, C. N., Saxena, C. D., & Rao, P. H. (2001). Optimization of ingredients and process conditions for the preparation of puri using response surface methodology. *International Journal of Food Science and Technology, 36*(4), 407–414.

Wang, Y. (2009). Prebiotics: present and future in food science and technology. *Food Research International, 42*(1), 8–12.

Weaver, M. (2005). Inulin, oligofructose and bone health: experimental approaches and mechanisms. *British Journal of Nutrition, 93*(S1), S99–S103.

Yap, W. K., Mohamed, S., Jamal, M. H., Diederick, M., & Manap, Y. A. (2008). Changes in infants faecal characteristics and microbiota by inulin supplementation. *Journal of Clinical Biochemistry and Nutrition, 43*(3), 159–166.

Zghal, M. C., Scanlon, M. G., & Sapirstein, H. D. (1999). Prediction of bread crumb density by digital image analysis. *Cereal Chemistry, 76*(5), 734–742.

Ziadeh, G. N. (2002). *Determination of sensory thresholds of selected calcium salts and formulation of calcium-fortified Arabic bread* (M.Sc. thesis). Beirut, Lebanon: American University of Beirut, Arabic.