Food Bioprocess Technol (2012) 5:888–896
DOI 10.1007/s11947-010-0381-x

ORIGINAL PAPER

Gluten-free Bread Based on Tapioca Starch: Texture and Sensory Studies

Laura B. Milde · Laura A. Ramallo · María C. Puppo

Received: 29 December 2009 / Accepted: 6 May 2010 / Published online: 22 May 2010

Abstract In the present work, gluten-free formulations for breadmaking, destined to celiac people, were studied. A base blend of tapioca starch and corn flour (80:20) and typical bread ingredients such as yeast, salt, sugar and water were utilised. Ingredients such us vegetable fat, hen egg, and soybean flour were incorporated in different levels by means of an experimental design of three factors. Bread quality was analysed throughout physical (specific volume, weight loss percentage) and textural (firmness, elasticity and firmness recovery) parameters. The optimum bread selected, the bread with highest levels of fat and soybean flour and one egg, presented low values of firmness (≤100 N) and elasticity (>65%) and the lowest variation of these parameters with storage. Overall acceptability of this bread was 84% for habitual consumers of wheat bread and 100% by celiac people. Therefore, tapioca starch-based breads with spongy crumb, high volume and a good sensory acceptance were obtained.

L. B. Milde · L. A. Ramallo
Facultad de Ciencias Exactas, Químicas y Naturales-Universidad Nacional de Misiones,
Mariano Moreno,
1375, 3300 Posadas, Argentina

M. C. Puppo
Facultad de Ciencias Agrarias y Forestales-Universidad Nacional de La Plata,
60 y 119,
1900 La Plata, Argentina

M. C. Puppo (✉)
CONICET–Facultad de Ciencias Exactas UNLP (CIDCA),
47 y 116,
1900 La Plata, Argentina
e-mail: mcpuppo@quimica.unlp.edu.ar

Keywords Gluten-free bread · Tapioca · Soybean · Bread texture · Sensory quality

Introduction

Technological advancement promotes the development of new functional foods. Due to additive and new ingredients incorporation, this kind of foods sometimes can be related to non-natural foods. Therefore, sensory tests must be performed (Sabanis and Tzia 2009). Celiac disease is an inflammatory disease, manifested by genetically susceptible individuals, of the upper small intestine which results from gluten ingestion (Kelly et al. 1999; Mc Carthy et al. 2004). People with this disease require gluten-free foods; those that do not contain wheat, oat, barley and rye. Gluten-free (GF) bread needs to be prepared with non-cereal flour, so as to avoid gluten (specifically gliadins) content. Wheat gluten proteins are the unique proteins that form a distinctive cohesive and viscoelastic dough capable of retaining carbon dioxide during fermentation and contribute to the appearance and crumb structure of bread (Gallagher et al. 2003; Gallagher et al. 2004); consequently, additives must be included to improve gluten-free bread volume (Di Cagno et al. 2004). Common bread is traditionally the more consumed food by humans, because it is a product of low price with high nutritional value. Following this objective, formulation of gluten-free bread presents a challenge to both cereal technologists and bakers, because of the absence of gluten network that is the responsible for physical and sensory properties of bread. Gluten-free bakery products can be produced with alternative ingredients such as starches, gum and hydrocolloids, dairy products, leguminous proteins, prebiotics and several combinations (Gallagher et al. 2004).

The common flours used to replace wheat in breadmaking for celiac people may be corn, soybean and rice (Machado 1996; Nishita et al. 1976; Sanchez et al. 2002, 2004; Specher Sierra 2005; Rosales-Juárez et al. 2008) and in some cases, maize (Brites et al. 2009). Cassava starch could be a suitable option of gluten-free bread ingredient. Mandioca or Tapioca (*Manihot esculenta* Crantz) is a regional plant typical of South America, which grows in Misiones province of Argentina and surroundings. This crop grows in wet and tropical weather, and also under low nutrients availability. It also survives in drought conditions (Burrell 2003). Tapioca is a naturally GF ingredient; however, GF formula requires polymeric substances, like proteins or hydrocolloids, for reproducing viscoelastic properties of gluten to provide structure and retain gas (McCarthy et al. 2005).

Soybean flour and soy protein concentrate were first used by Ranhotra et al. (1975) to prepare soy-fortified GF bread; and later by other researchers (Sanchez et al. 2002, 2004; Sciarini et al. 2010). Dairy ingredients were used in GF bread formulas to increase water absorption and to improve shape, volume and crumb firmness of loaves, especially those with high protein/low lactose content (Gallagher et al. 2003). Sanchez et al. (2004) used, in GF bread formulation, both ingredients: soy flour and dry milk. They could increase protein content of GF bread by modifying in small degree the specific volume and sensory quality of loaves.

The objective of this work was to obtain gluten-free bread with local tapioca starch and to analyse the influence of the addition of fat, whole egg and soybean flour on texture and sensory properties of loaves.

Materials and Methods

Materials

Materials used in this work were all gluten free and regional (Argentina). Tapioca starch (Ranchito, Misiones), corn flour (Indelma, Santa Fe), fresh yeast (Calsa, Buenos Aires), salt (Celusal, Tucumán), sugar (Ledesma, Jujuy), soybean flour (Instituto, Misiones), whole egg and vegetable fat (Margadán, Buenos Aires), were utilised.

Experimental Design and Statistical Analysis

Preliminary assays were performed to achieve adequate experimental conditions to prepare GF bread with tapioca as main ingredient. Changes were followed with a sensory panel of habitual consumers of wheat bread. The tapioca starch-corn flour ratio and the type of ingredients incorporated (fat, whole egg and soybean flour) were selected according to different sensory attributes: colour, flavour, texture and overall acceptability. The optimum dry mix was found to be composed of tapioca starch-corn flour 80:20. Salt (1.4%), sugar (5%), yeast (5%), vegetable fat (2–6%), egg (0–2 units) and soybean flour (0–10%) as protein ingredients were also incorporated. A quantity of water (50–58%) sufficient to give optimum dough was aggregated to the mix before kneading. Percentage of ingredients is expressed as grams ingredient/100 g tapioca starch-corn flour mix. The optimum dough consistency was determined by measuring extensibility in a hand-made way.

In spite of the experimental limitations of this study, a final formulation of GF bread was obtained from an experimental design that allowed acquiring optimal amounts of each ingredient. A random experimental design of three factors was used: fat (F), whole egg (E) and soybean flour (S). Twelve formulations for preparing bread (b1-b12) were defined and are shown in Table 1.

Full factorial designs are the optimal experimental strategy to simultaneously study the effect of several factors on the answer and its interactions. In the Response Surface Method, the experiment is designed to estimate interaction and even quadratic effects of factors and to obtain an optimal response (Montgomery 1997; Khuri and Cornell 1996). Central composite design is an experimental design, useful in response surface methodology, for building a second order (quadratic) model for the response variable (Corzo and Gomez 2004; Cortes-Gómez et al. 2005; Toufeili et al. 1994).

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

Table 1 Experiment design of bread formulations

Bread	Coded variable levels			Decoded variable levels		
	F	*E*	*S*	*F* (g)[a]	*E* (unit)	*S* (g)[a]
1	−1	−1	−1	10	0	0
2	+1	−1	−1	30	0	0
3	+1	+1	−1	30	2	0
4	+1	+1	+1	30	2	50
5	−1	+1	+1	10	2	50
6	−1	−1	+1	10	0	50
7	−1	+1	−1	10	2	0
8	+1	−1	+1	30	0	50
9	0	0	0	20	1	25
10	+1	0	+1	30	1	50
11	+1	0	0	30	1	25
12	0	0	+1	20	1	50

F fat, *E* whole egg, *S* soybean flour

[a] F, S: g ingredient (F or S)/500 g tapioca starch-corn flour mix

distance from the centre of the design space to a factorial point is ±1 unit for each factor, the distance from the centre of the design space to a star point is ±α with |α|>1. The precise value of α depends on certain properties desired for the design and on the number of factors involved. Since the amount of eggs to incorporate had to be an integer number, it was not possible to apply the popular rotatable central composite design. Fortunately, easy-to-use software, for example Statgraphics plus, for desired function methodology implementation is available. Due to these modifications, the applied response surface model is not the optimal one, but it allowed finding a relation between the independent variables and the response.

All treatments were performed randomly and data obtained (Tables 1 and 2) from mechanical parameters (firmness, elasticity, firmness recovery) were analysed using response surface methodology by Statgraphics plus for Windows 5.1 software. Data obtained from physical parameters (specific volume, weight loss) were subjected to analysis of variance (ANOVA). Average parameters and standard deviation were calculated (Table 2). The second order model proposed (Khuri and Cornell 1996; Sanchez et al. 2002) for each textural and physical parameter was (Eq. 1):

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}{X_1}^2 + b_{22}{X_2}^2 + b_{33}{X_3}^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 \quad (1)$$

Where Y is the response (firmness (f), elasticity (e) and firmness recovery (fr)); b_0, b_i, b_{ii}, and b_{ij} are regression coefficients; X_1, X_2 and X_3 are coded variables that represent the F, E and S, respectively.

The model adequacies were checked by the variance analysis (F test) and R^2 values. The effect of variables was registered using surface graphs.

Breadmaking

Ingredients were mixed in a home kneader Philips 32 Serie (Philips, Brasil) at 160 rpm for 2 min. Kneading was continued by hand up to obtaining homogeneous dough (10 min). Dough was incorporated in greased stainless rectangular moulds (29.3×10.2×9.5 cm) and fermented 30 min at 35°C. Breads were baked at 240°C for 20 min and at 280°C for 10 min, with steam.

Physical and Mechanical Evaluation

Specific Volume of Bread Volume of bread pieces, that were almost regular in shape, was calculated by measuring height, width and length of the loaves, with a ruler. As the loaves were not regular in width, the last one was measured in the bottom and in the top of each cross-section of the bread. The average width between both measurements was calculated for all pieces of bread. Specific volume (V_e) was obtained dividing each volume by its respective weight (Reyes Aguilar et al. 2004). Three replicates were acquired for each formulation.

Weight Loss of Bread Weight loss (WL) of bread was determined according to method utilised by Da Mota Zanella et al. (2005) (Eq. 2):

$$\%\mathrm{WL} = [(\text{weight of dough} - \text{weight of bread})/\text{weight of dough}] \times 100 \quad (2)$$

Texture Evaluation Texture Profile Analysis of bread was performed using a Universal Dynamometer (Adamel Lhomargy DY32, Roissy en Brie, France) provided with a 1,000 N cell (with a sensitivity of tenth of N). Each sample was subjected to two cycles of compression up to 50% of

Table 2 Physical and texture parameters of breads

	Physical parameters[a]		Texture parameters[a]		
Bread	V_e (cm^3/g)	%WL	f (N)	e (%)	fr (%)
1	1.88±0.04bc	9.38±0.04a	132±0.1a	70.9±0.1a	89.0±0.5d
2	1.75±0.03a	11.35±0.06b	183±6.6b	73.0±4.0bc	90.2±0.2e
3	2.07±0.04d	13.68±0.03c	108±9.8c	67.7±2.8d	90.8±1.4f
4	2.68±0.01e	25.58±0.05d	81±0.7d	63.6±0.4e	88.2±1.4c
5	2.23±0.02f	17.07±0.02e	98±11e	72.9±5.1b	85.9±0.7a
6	1.61±0.06g	14.29±0.03f	152±13f	48.1±1.6f	89.1±1.7cd
7	2.10±0.00d	15.38±0.04g	158±22g	73.6±2.1c	91.3±0.4g
8	1.51±0.02h	13.95±0.00h	329±35h	71.1±2.5a	86.0±1.1a
9	1.81±0.04ab	14.29±0.03f	207±19i	60.1±1.7g	87.5±2.3b
10	2.01±0.01d	15.91±0.02i	101±3.6j	67.6±3.5d	89.0±1.5cd
11	1.91±0.01c	12.20±0.06j	101±7.0j	67.8±0.1d	91.8±1.4g
12	1.72±0.03a	11.11±0.05k	110±16k	60.2±1.7g	92.7±0.9h

Different letters in the same column indicate significant differences ($p<0.05$).

V_e specific volume, *%WL* percentage of weight loss, f firmness, e elasticity, *fr* firmness recovery, 30°C testing temperature, *2 h* time after baking

[a] Average values and standard deviations.

the original height with a rectangular probe (20×20 cm; Bourne 2002). Force time curves were obtained at a crosshead speed of 100 mm/min. Samples were cut in blocks of 9 cm width×9 cm length×7 cm height. Product firmness, elasticity and firmness recovery were determined in triplicates for each formulation of the experimental design. Firmness is defined as the maximum force registered during the first compression cycle and was measured according to AACC standard method (AACC 2000). Elasticity was calculated as of the ratio of L2/L1, expressed as percentage; being L2 and L1 the distances between the beginning and the maximum force of the second and first compression cycle, respectively. Firmness recovery was calculated as the percentage of F2/F1; where F2 and F1 are maximum forces of the second and first compression peaks, respectively.

All evaluations were performed with fresh bread and the same bread was then stored at 25°C for 24 h, in order to study the effect of storage time on mechanical properties of different bread formulations. Samples were stored in sealed plastic containers to avoid moisture loss.

Sensory Evaluation

Two separate consumer studies were performed. Sensory analysis tests with habitual consumers of bread from wheat flour (non-celiac people) and celiac people were performed with a selected bread formulation. The more suitable bread formula for sensory evaluation was selected according to the best textural (low firmness, high elasticity and high firmness recovery) and physical (high specific volume, low weight loss) parameters. All breads were baked and packaged in the morning of the day of testing, and were tested within 4 h from baking (fresh bread) and tested within approximately 26 h from baking (stored bread). Sensory attributes evaluated were firmness, cohesiveness and overall acceptability. In order to analyse overall acceptability, a five-point hedonic scale was utilised. Some authors have assigned values to each score, assuming equal intervals (Carpenter et al. 2000; Watts et al. 1989). The higher rating reflected good quality attributes. Scores ranged from "Like very much" (score 5) to "Dislike very much" (score 1; Meilgaard et al. 1999; Gallagher et al. 2003).

The nine-point hedonic scale is recommended for use in sensory evaluation of food product. Its use has been validated in the scientific literature (Stone and Sidel 1993). However, in some instances, adaptations of the nine-point hedonic scale were found useful (Pittia et al. 1999; Zandstra et al. 1999; Abdullah and Cheng 2001). We must choose scales that are easy for the panellists to use; thus, they can concentrate on the product evaluation. A five-point scale was used in order to simplify the respondent's task. In addition, five-point hedonic scale provided all the answers needed for our objectives.

Texture parameters Sensory hardness and cohesiveness were evaluated by applying three-point scale: 1, "hard"; 2, "firm"; 3, "soft" for hardness. For the cohesiveness evaluation, the sensory scale was: 1, "brittle"; 2, "tender"; 3, "gummy". For evaluation of bread texture, approximately 50 g of each bread sample were presented to judges in individual white plastic plates under white light at room temperature.

Bread consumers Sixty students and staff from the University of Misiones, Argentina, aged between 20 and 55 years, participated in this taste test. Sensory evaluation was conducted in the Sensory Laboratory of the Department of Food Science-University of Misiones, and was performed on fresh bread and the same bread that was stored at 25°C for 24 h. Two coded samples were presented to the judges simultaneously, and the judges were asked to indicate their hedonic response to each sample on the scale.

Celiac people Sensory evaluation with 20 celiac people was performed with the same bread formulation (only fresh bread) as was tasted by consumers. Celiac patients from the Dr. Ramón Madariaga Hospital (Posadas, Argentina) participated in the sensory studies on the acceptability of the bread by celiac individuals.

Panellists of both sensory evaluations were not trained (Zacarías et al. 1985; Hellemann et al. 1990; Hamad and Fields 2006).

Results and Discussion

V_e and WL of different formulated breads (Table 1), are shown in Table 2. Statistical analysis (ANOVA) showed that significant differences ($p<0.05$) in Ve and WL were observed between samples.

Bread 4 (b4) presented the highest V_e and WL. Soybean proteins usually present high water imbibing capacity. Nevertheless, their interaction with starch (tapioca and corn) and in the presence of high levels of egg is not able to retain water in bread.

Diminishing level of F but maintaining E and S (b5), produced a slight decrease in V_e and WL. This decrease was intensified when E was eliminated from formulation (b6), presenting one of the lowest values of V_e. The egg must not be absent if we want to obtain bread with an acceptable volume.

When E and S are present together, there is a synergic effect, because the highest values of both parameters (V_e and WL) were obtained. Bread maintained water in crumb structure better if low levels of fat were used (b4 vs. b5). Comparing formulations F and S in intermediate and high levels (b9, b10, b11, b12) and with only one egg, better

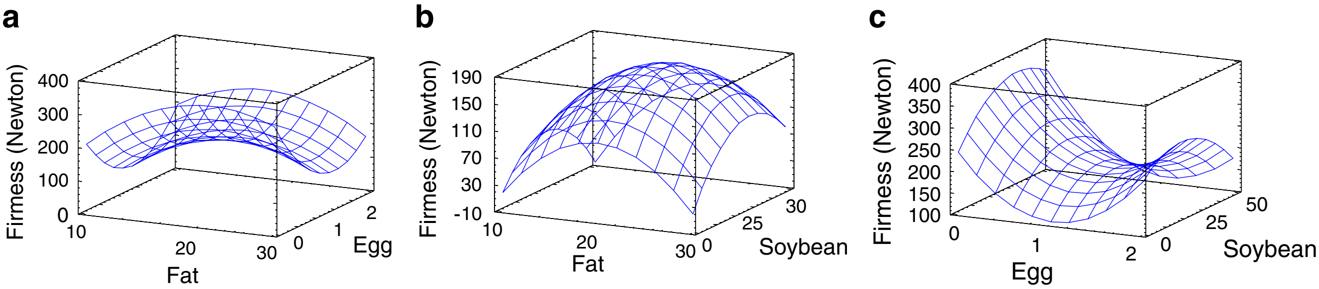

Fig. 1 Response surface for firmness *(f)* of gluten-free breads as a function of **a** fat (F) and egg (E), **b** fat (F) and soybean flour (S), **c** egg (E) and soybean flour (S)

volume was obtained when F and S were in their high level (b10). In this work, a low WL level was searched, in order to ensure moisture content that impede bread dehydration and to prevent a great increase of hardness and elasticity of product (Esparza Rivera et al. 2005).

Formulations without soybean flour and with two whole eggs (2 units) developed good volume (b3 and b7). Since tapioca starch and corn flour contain virtually no protein, soybean flour was included to ensure a nutritionally balanced product with enriched in protein content (Sanchez et al. 2004).

Eggleston et al. (1992) reported an increase in bread volume in products made with fat, tapioca and soybean flour. A uniform distribution of size of gas cells were obtained, allowing a soft and sponge crumb texture. In our case, breads not only with soybean flour and fat, but also by one or two whole eggs in the formulation presented high specific volume with a soft crumb texture (low firmness). Edema et al. (2005) reported, in bread formulated with soybean flour and corn starch, a regular cell distribution with small crust cracking, due to high hydration capacity of soybean proteins.

Due to benefit properties of soybean proteins, through S, and of E, and acceptable V_e and low WL values, b10 was considered as one of the optimum bread. Bread 10 presented a similar response than the best breads (b4 and b5), but with only one egg in its formulation. Simultaneously, b10 were selected by non-trained consumers (data not shown), as a good bread.

Mean values and standard deviation of texture parameters (firmness, elasticity and firmness recovery) of all bread samples are also shown in Table 2. The bread with lowest firmness, that is, with softer crumb, was bread 4 (b4). On the other hand, the bread with highest firmness and lowest elasticity and firmness recovery was b8. These results agree with specific volume and water loss results. Both breads have the same and highest content of fat and soybean. However, b4 was formulated with two eggs, while b8 did not contain egg in its formulation. These results indicate that egg is an important ingredient in formulating gluten-free bread with adequate physical and textural properties.

Surface response (SR) graphs shows variation of texture parameters as function of two independent variables, maintaining the third variable at the middle level (code variable= 0). Figure 1 shows surface response of *f*. Breads with fat, at middle level, presented maximum firmness values, with the exception when F was combined with the middle level of E (Fig. 1a). This effect was accentuated with soybean addition, presenting a maximum at level 0 of S (Fig. 1b). On the other hand, with level 0 of E and the highest level of S, lowest firmness values were observed (Fig. 1c).

Figure 2 shows SR graph of *e*. The presence of fat did not provoke a significant influence in elasticity values (Fig. 2a, b), whereas soybean addition decreased bread elasticity probably due to interaction with fat (Fig. 2b). The egg addition produced a maximum in elasticity at middle level (one egg; Fig. 2a); the elasticity decreased with the presence of soybean flour (Fig. 2c).

Edema et al. (2005), in concordance with our results, evaluating bread with corn flour reported moderate

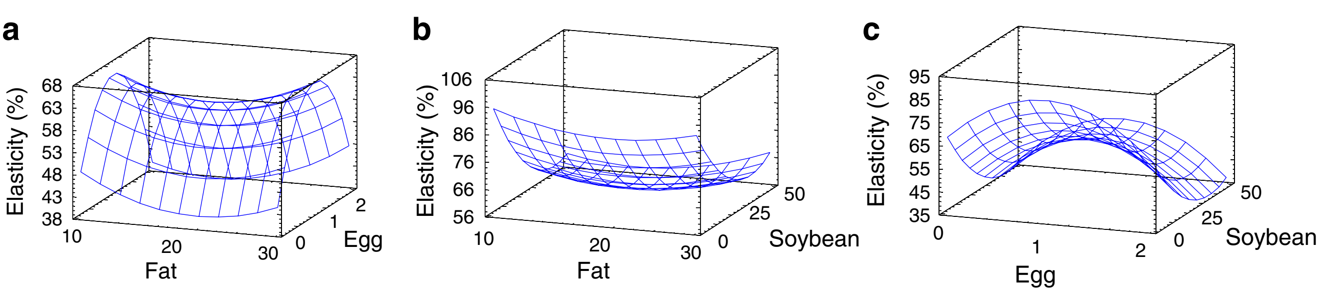

Fig. 2 Response surface for elasticity *(e)* of gluten-free breads as a function of **a** fat (F) and egg (E), **b** fat (F) and soybean flour (S), **c** egg (E) and soybean flour (S)

Table 3 Analysis of variance and regression coefficients for the second-order polynomial model

Source	Firmness		Elasticity		Firmness recovery	
	Coefficient	p value	Coefficient	p value	Coefficient	p value
Constant (b_0)	187.86		60.83		89.4217	
b_1	21.01	0.0151	0.77	0.6274	−0.06	0.9293
b_2	−42.84	0.0001	−1.13	0.4810	0.19	0.7757
b_3	10.94	0.1688	−14.17	0.0001	−1.58	0.0331
b_{11}	−88.93	0.0013	5.46	0.2469	0.51	0.7923
b_{22}	125.18	0.0001	−21.09	0.0005	−4.03	0.0593
b_{33}	−70.07	0.0066	12.89	0.0133	2.98	0.1454
b_{12}	−37.81	0.0002	2.91	0.0842	0.53	0.4377
b_{13}	21.25	0.0130	2.56	0.1177	−0.35	0.6035
b_{23}	−32.79	0.0008	0.44	0.7637	−0.44	0.5224
R^2		90.4%		88.3%		43.2%

Significant differences at p value <0.05

elasticity values when soybean was incorporated to formulation.

Surface response of fr was similar to those obtained for elasticity due to relationship between parameters.

Variation of texture parameters of fresh breads, with the level of different ingredients (F, E, S) according to experimental design showed in Table 1, was analysed with a second order polynomial model, and regression coefficient was obtained (p<0.05; Table 3). The model well predicted firmness and elasticity variations (r^2>85%), while for firmness recovery, the model did not render a good correlation (r^2<50%).

Fat and eggs showed significant linear effects on firmness (Table 3). All variables showed significant quadratic and interaction effects on this parameter. Only soybean flour showed significant linear effects on elasticity and firmness recovery, while eggs exhibited significant quadratic effect on elasticity and firmness recovery. These results indicate that fat does not affect the elasticity of the fresh bread.

The optimum bread formula was selected according to firmness and elasticity values of fresh bread and according to the lowest variation with storage, of firmness (Δf) and elasticity (Δe). Low and intermediate values of firmness (≤100 N) and elasticity (>65%), respectively, were preferable to be chosen, due to avoiding crumb disaggregation.

Ageing of bread involves physicochemical and sensory changes, such as crumb firmness increase, loss of flavour, crust hardening, formation of more opaque crumb and a decrease in starch solubility (Kulp and Ponte 1981). Inagaki and Seib (1992) proposed that the more important change occurs in crumb firmness.

Table 4 shows changes in texture parameters due to bread storage (24 h at 25°C). Breads b5 and b10 presented the lowest firmness increase and elasticity loss. This behaviour could be due to the fact that both breads have the highest content of soybean flour and also they have egg in their formulations. Bread b2, that not contained egg nor soybean flour, presented the highest firmness increase. It can be observed that the addition of egg and soybean proteins

Table 4 Variation of firmness (Δf), elasticity (Δe) and firmness recovery (Δfr) of gluten-free bread with storage

	Texture parameters		
Bread	$\Delta f = f_{24} - f_0$ (N)	$\Delta e = e_{24} - e_0$ (%)	$\Delta rf = fr_{24} - fr_0$ (%)
1	234.6^{ab}	−34.8^{a}	−13.4^{a}
2	619.0^{e}	−13.8^{b}	−9.1^{b}
3	321.2^{d}	−30.1^{c}	−6.2^{c}
4	125.1^{f}	−2.4^{d}	−3.7^{d}
5	83.2^{f}	−2.3^{d}	−7.4^{e}
6	274.7^{bc}	−10.5^{e}	−9.2^{b}
7	241.8^{ab}	−24.4^{f}	−12.1^{f}
8	210.9^{a}	−3.3^{g}	−11.3^{g}
9	216.7^{a}	14.9^{h}	−10.2^{h}
10	109.1^{f}	−0.7^{i}	−11.8^{g}
11	259.5^{bc}	−4.2^{j}	−16.0^{i}
12	291.8^{cd}	7.4^{k}	−18.6^{j}

Different letters in the same column indicate significant differences (p<0.05)

Storage conditions: T. Average texture parameters of fresh bread: firmness (f), elasticity (e), firmness recovery (fr). Fresh breads: f_0, e_0, fr_0. Stored brads: f_{24}, e_{24}, fr_{24}

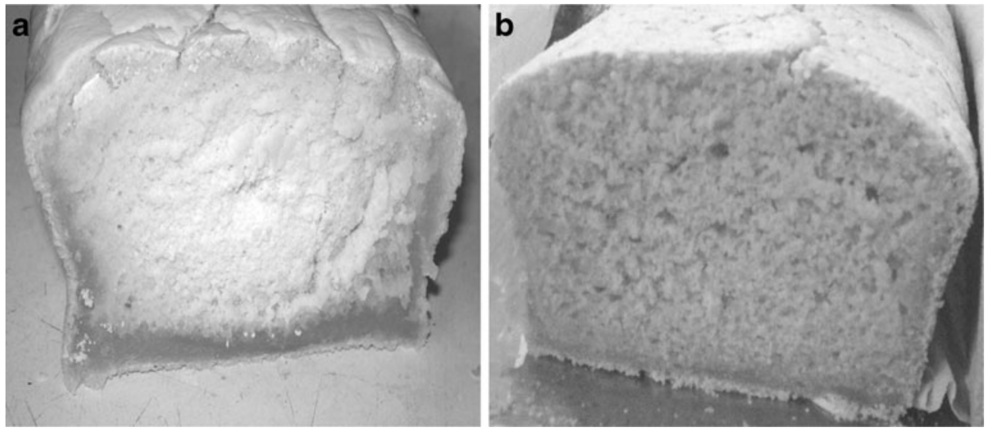

Fig. 3 Control bread (**a**) and bread prepared with the optimum formulation, b10 (**b**)

contribute to the decrease of ageing. Soybean, specially accompanied by egg at the highest level (b4, b5), did not favour the increase of bread firmness in a great extent.

An intermediate level of egg (1 unit) does not contribute in the same manner with the different levels of fat or soybean flour. Breads with similar high firmness and elasticity increase, was obtained with intermediate levels of E and F; and medium and high level of S (b9 and b12). The lowest variation with storage, of firmness (Δf) and elasticity (Δe) was obtained with b10, that is, with the highest levels of F and S.

Esparza et al. (2005) found in wheat bread, in which part of wheat flour was replaced by soybean flour; that firmness of bread did not change with storage time (1-48 h). Nevertheless, breads with soybean retained more water during time, due to the high water holding capacity and the ability of these proteins to reinforce crumb structure. The use of egg in addition with soybean maintained the elasticity characteristic of tapioca. The presence of egg favours bread with more soft texture (Torres and Pacheco 2007). Studies performed by Eggleston et al. (1992) suggest the use of tapioca-soybean flour mixtures, with the aim to increase nutritional value of bread. They also proposed the addition of fat and egg albumen for reducing the extent of starch solubilisation and gelatinization, increasing the quantity of air trapped during batter mixing. The combination E+S could interfere with starch retrogradation, inhibiting firmness increase and elasticity changes.

Textural changes during storage were also considered in the selection of the best tapioca-corn bread used in sensory analysis by celiac people.

Sensory analysis with habitual consumers of bread (non-celiac people) and celiac people was performed with a one selected bread formulation. The bread chosen was the bread that presented suitable physical and textural characteristics fresh and with 1-day storage. Figure 3 shows that bread 10 (b10) presented also a good crust and crumb structure.

With respect to the sensory evaluation of each product, quantitative scores information was analysed by frequencies. Table 5 shows percentage of different attributes: overall

Table 5 Frequency of terms (scores) for sensorial attributes of b10 formulation (fresh and stored by 24 h): Overall acceptability, hardness and cohesiveness evaluated by habitual consumers of bread and celiac people

Sensorial parameters	Score	Bread consumers		Celiac people
		Fresh	Stored (24h)	Fresh
Overall acceptability				
Like very much	5	5	14	45
Like moderately	4	42	26	45
Neither like nor dislike	3	37	39	10
Dislike moderately	2	10	18	0
Dislikes very much	1	6	3	0
Hardness				
Soft	3	60	19	0
Firm	2	39	74	100
Hard	1	1	7	0
Cohesiveness				
Gummy	3	60	27	10
Tender	2	32	39	85
Brittle	1	8	34	5

acceptability (OA), hardness and cohesiveness, for bread 10 (b10). Scores can be grouped into two levels: acceptable (3-5) and non-acceptable (1 and 2). B10 exhibited equal OA for categories 4 and 5. Bread 10 was well accepted by celiac people. Major part of consumers, habitual consumers of wheat flour bread (non-celiac people; 84%) accepted the fresh bread and that percentage was slightly reduced (79%) when they evaluated the stored bread.

For hardness attribute, scores 3 and 2 were grouped as acceptable; therefore, b10 was totally accepted by celiac people. This sensory attribute, hardness, had good acceptability in an elevated percentage of bread consumers (98%), still through samples with 24 h of storage (93%). The increase in firmness of bread with storage time was observed by consumers, but without arriving to rejection.

Cohesiveness scores gummy and tenderness (3 and 2) were considered both acceptable; therefore, b10 obtained 95% of acceptation by celiac people. Habitual bread consumers considered this attribute 93% acceptable in fresh bread, and 66% in storage bread. Storage period (24 h) downgraded cohesiveness characteristics of bread b10.

Conclusions

This study has demonstrated that gluten-free breads with high acceptability can be made from tapioca starch and corn flour. These breads were totally natural, without chemical additives. Analysis of interaction between components (fat, egg, soybean flour) at different levels of fresh and stored breads was performed. Bread with the highest content of F, E and S showed the highest specific volume and lowest crumb firmness and elasticity, but a great weight loss. The optimum formulation (b10), selected according to physical and texture data, presented a similar response than the best breads but with only one egg in its formulation. In addition, b10 presented low firmness and elasticity changes after 1-day storage. Sensory evaluation with bread consumers and especially celiac people confirmed the proposed objectives. Bread with 100% of acceptance, prepared with local raw materials, enriched in proteins due to soybean flour incorporation and natural (free of additives and preservatives), was able to be obtained.

Acknowledgements The authors would like to acknowledge SeCyT (UNAM) and FCAyF (UNLP) of Argentina for the financial support.

References

AACC International. (2000). *Approved methods of the American Association of Cereal Chemists (10th ed) Methods 72-10, 74-09*. St. Paul: The Association.

Abdullah, A., & Cheng, T. C. (2001). Optimization of reduced calorie tropical mixed fruits jam. *Food Quality and Preference, 12*, 63–68.

Bourne, M. (2002). *Food texture and viscosity. Concept and measurement* (pp. 182–186). New York, USA: Academic Press, Elsevier Science.

Brites, C., Trigo, M. J., Santos, Collar, C., & Rosell, C. M. (2009). Maize-based gluten-free bread: influence of processing parameters on sensory and instrumental quality. *Food and Bioprocess Technology*, doi:10.1007/s11947-008-0108-4 (in press)

Burrell, M. M. (2003). Starch: the need for improved quality or quantity an overview. *Journal of Experimental Botany, 54*(382), 451–456.

Carpenter, R., Lyon, D., & Hasdell, T. (2000). *Análisis sensorial en el desarrollo y control de la calidad de alimentos* (p. 191). Zaragoza, España: Acribia.

Cortes-Gómez, A., San Martín-Martínez, E., Martínez-Bustos, F., & Vázquez-Carrillo, G. M. (2005). Tortillas of blue maize (*Zea mays* L.) prepared by a fractionated process of nixtamalization: analysis using response surface methodology. *Journal of Food Engineering, 66*, 273–281.

Corzo, O., & Gomez, E. (2004). Optimization of osmotic dehydration of cantaloupe using desired function methodology. *Journal of Food Engineering, 64*, 213–219.

Da Mota Zanella, V., Mireles Mendoza, C., Camarena Aguilar, E., & Bautista Justo, M. (2005). Efecto del uso de masas congeladas sobre las características y textura en pan blanco. *Revista Salud Pública y Nutrición, 13*, 456–465.

Di Cagno, R., De Angelis, M., Auricchio, S., Greco, L., Clarke, C., De Vincenzi, M., et al. (2004). Sourdough bread made from wheat and nontoxic flours and started with selected lactobacilli is tolerated in celiac sprue patients. *Applied and Environmental Microbiology, 70*(2), 1088–1096.

Edema Mojisola, O., Sanni Lateef, O., & Sanni Abiodun, I. (2005). Evaluation of maize-soybean flour blends for sour maize bread production in Nigeria African. *Journal of Biotechnology, 4*(9), 911–918.

Eggleston, G., Omoaka, P., & Oihedioha, D. (1992). Development and evaluation of products from cassava flour as new alternatives to wheaten breads. *Journal of the Science of Food and Agriculture, 59*(3), 377–385.

Esparza Rivera, J., Meza Velásquez, F., Meza Velásquez, M., & Meza Velásquez, J. (2005). Efecto de la adición de proteína de soja en la textura de un pan blanco. *Agrofaz, 5*(1), 775–782.

Gallagher, E., Gormley, T. R., & Arendt, E. K. (2003). Crust and crumb characteristics of gluten-free breads. *Journal of Food Engineering, 56*(2–3), 153–161.

Gallagher, E., Arendt, E. K., & Gormley, T. R. (2004). Recent advances in the formulation of gluten-free cereal-based products. *Trends in Food Science and Technology, 15*(5), 143–152.

Hamad, A. M., & Fields, M. L. (2006). Nutritional and sensory evaluation of bread made from fermented wheat meal and corn chips made from fermented corn meal. *Journal of Food Science, 44*(5), 1514–1516.

Hellemann, U., Barylko-Pikielna, N., & Matuszewska, I. (1990). Interaction between bread and butter with varying NaCl contents: hedonic responses and sensory characteristics. *Food Quality and Preference, 2*(3), 167–176.

Inagaki, T., & Seib, P. A. (1992). Firming of bread Crumb with cross-linked waxy barley starch substituted for wheat starch. *Cereal Chemistry, 69*(3), 321–325.

Kelly, C. P., Feighery, C., Gallagher, R. B., & Weir, D. G. (1999). The diagnosis and treatment of gluten-sensitive enteropathy. *Advances in Internal Medicine, 53*(1), 341–364.

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

Kulp, K., & Ponte, J. G. (1981). Staling of white pan bread: fundamental causes. *Critical Reviews in Food Science and Nutrition, 15*(1), 1–48.

Machado, L. M. P. (1996). Pão sem glúten: Otimização de algumas variáveis de processamento. In: Dissertação de Mestrado em Tecnologia de Alimento (pp. 186). Brasil: Campinas.

Mc Carthy, D. F., Gallagher, E., Gormley, T. R., Schober, T. J., & Arendt, E. K. (2004). Formulation of gluten-free bread using response surface methodology. In Lafiandra, Masci, & D'Ovidio (Eds.), *The gluten proteins* (pp. 379–382). Cambridge, UK: The Royal Society of Chemistry.

McCarthy, D. F., Gallagher, E., Gormley, T. R., Schober, T. J., & Arendt, E. K. (2005). Application of response surface methodology in the development of gluten-free bread. *Cereal Chemistry, 82*(5), 609–615.

Meilgaard, N., Civille, G., & Carr, B. (1999). *Sensory evaluation techniques* (p. 416). Florida, USA: CRC Press.

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

Nishita, K., Roberts, R., Bean, M. M., & Kennedy, B. M. (1976). Development of a yeast-leavened rice–bread formula. *Cereal Chemistry, 53*(5), 626–635.

Pittia, P., Nicoli, M. C., Comi, G., & Massini, R. (1999). Shelf-life extension of fresh-like ready-to-use pear cubes. *Journal of the Science of Food and Agriculture, 79*, 955–960.

Ranhotra, G. S., Loewe, R. J., & Puyat, L. U. (1975). Preparation and evaluation of soy fortified gluten-free bread. *Journal of Food Science, 40*(1), 62–64.

Reyes Aguilar, M., de Palomo, P., & Bressani, R. (2004). Desarrollo de un producto de panificación apto para adulto mayor a base de harina de trigo y arroz. *Archivos Latinoamericanos de Nutrición, 54*(3), 314–321.

Rosales-Juárez, M., González-Mendoza, B., López-Guel, E. C., Lozano-Bautista, F., Chanona-Pérez, J., Gutiérrez-López, G., et al. (2008). Changes on dough rheological characteristics and bread quality as a result of the addition of germinated and non-germinated soybean flour. *Food and Bioprocess Technology, 1*(2), 152–160.

Sabanis, D., & Tzia, C. (2009). Effect of rice, corn and soy flour addition on characteristics of bread produced from different wheat cultivars. *Food and Bioprocess Technology, 2*(1), 1–12.

Sanchez, H. D., Osella, C. A., & de la Torre, M. A. (2002). Optimization of gluten-free bread prepared from cornstarch, rice flour, and cassava starch. *Journal of Food Science, 67*(1), 416–419.

Sanchez, H. D., Osella, C. A., & de la Torre, M. A. (2004). Use of response surface methodology to optimize gluten-free bread fortified with soy flour and dry milk. *Food Science Technology International, 10*(1), 5–9.

Sciarini, L. S., Ribotta, P. D., León, A. E. & Pérez, G. T. (2010). Influence of gluten-free flours and their mixtures on batter properties and bread quality. *Food and Bioprocess Technology*, doi:10.1007/s11947-008-0098-2 (in press).

Specher Sierra, M. (2005). *Desarrollo de un producto de panificación apto para ser consumido por personas con esprue celíaco. PhD Thesis*. Ciudad de Guatemala, Guatemala: Universidad del Valle de Guatemala.

Stone, H., & Sidel, J. L. (1993). *Sensory evaluation practices*. San Diego: Academic Press, Inc.

Torres, E., & Pacheco, D. (2007). Evaluación nutricional, física y sensorial de panes de trigo, yuca y queso llanero. *Revista Chilena de Nutrición, 34*(5), 133–141.

Toufeili, I., Dagher, S., Shadarevian, S., Noureddine, A., Sarakbi, M., & Farran, M. (1994). Formulation of gluten- free pocket-type flat breads: optimization of methilcellulose, gum arabic, egg albumen levels by response surface methodology. *Cereal Chemistry, 71*(6), 594–601.

Watts, B., Ylimaki, G., Jeffery, L., & Eías, L. (1989). *Métodos sensoriales básicos para la evaluación de alimentos* (p. 170). Ottawa, Canadá: CIID.

Zacarías, I., Yáñez, E., Araya, E., & Ballester, D. (1985). Sensory evaluation and acceptability study, at the consumer level, of bread supplemented with sweet lupine flour. *Archivos Latinoamericanos de Nutrición, 35*(1), 119–129.

Zandstra, E., Graaf, C., van Trijp, H., & van Staveren, W. (1999). Laboratory hedonic next term ratings as predictors of consumption. *Food Quality and Preference, 10*, 411–418.