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Incorporation of several additives into gluten free breads: Effect on dough properties and bread quality

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ABSTRACT

The objective of this work was to assess the effect of emulsifiers, hydrocolloids and enzymes on gluten-free dough rheology and thermal properties and bread quality, while relating dough properties parameters to bread technological quality. Breads were based on rice flour, cassava starch and full-fat active soy flour, with 65% or 75% (flour-starch basis) of water incorporation. Additives used were emulsifiers (diacetyl tartaric acid ester of monoglycerides – DATEM and sodium stearoyl lactylate – SSL), enzymes (glucose oxidase and α-amylase) and hydrocolloids (xanthan gum, carboxymethylcellulose, alginate and carrageenan). Results showed that additive incorporation modified dough behavior, evidenced by different calorimetric and rheological properties. Besides, the electrophoretic pattern of dough extracted proteins changed with glucose oxidase addition. These modifications resulted in breads with different characteristics, such as specific volume, firmness and firming rate, and crumb structure. Nonetheless, they did not necessarily show better quality parameters than the control bread. The control dough displayed good performance for obtaining gluten-free breads of acceptable volume, crumb structure and, principally, with lower hardening rate during storage. Contrary to widespread opinion, this work shows that the presence of additives is not essential for gluten-free bread production. This fact provides new perspectives to the gluten free market at the moment of selecting raw materials and technological parameters, reducing production costs and facilitating gluten free products development.

1. Introduction

Celiac people are unable to consume certain gluten proteins from cereals – such as wheat, rye, barley, kamut, spelt – and hybrids like triticale. The most common cereal flours used for gluten free bread production are rice (Gujral and Rosell, 2004a,b; Marco and Rosell, 2008; Renzetti and Arendt, 2009), sorghum (Schober et al., 2005) and corn flours (Renzetti et al., 2008). Andean crops (Torbica et al., 2010) and tubers such as potato and cassava (Sánchez et al., 2002; Ballesteros López et al., 2004; Ribotta et al., 2004) have also been used. In general, breads formulated with gluten free raw materials include high water incorporation; in the literature water addition ranges between 65% (Ribotta et al., 2004) and 110% (Marco and Rosell, 2008).

Nevertheless, the absence of gluten produces technological problems in the production of baked goods. To counteract these technological problems, several additives have been employed to mimic gluten properties. Emulsifiers are used in the baking industry because of their ability to interact with different flour components and other dough ingredients, resulting in softer crumbs (Demirkesen et al., 2010). They are composed of hydrophilic and hydrophobic residues which allow the interaction of two chemically different phases. Thus, surface tension between two immiscible phases is reduced by emulsifier presence allowing the formation of an emulsion (Krog, 1981; Flack, 1987; Dziezak, 1988). When emulsifiers are used in breadmaking, they contribute to increase the stability of a thermodynamically unstable system (Gómez et al., 2004). Some emulsifiers have already been incorporated into gluten free formulations. Onyango et al. (2009) made gluten free bread based on pregelatinized cassava starch and found that emulsifier addition reinforced dough structure and decreased crumb firmness as well. This behavior was also reported by Demirkesen et al. (2010). Enzymes are currently being added to gluten-free systems as a means of modifying protein functionality (Gujral and Rosell, 2004a,b; Moore et al., 2006; Renzetti et al., 2008), as the formation of a continuous protein network is considered a key factor in enhancing gluten free flours performance for breadmaking. Thus, glucose oxidase incorporation has been studied as a polymerizing agent with varying results, depending on the raw material employed (Gujral and Rosell, 2004b; Renzetti and Arendt, 2009). The α-amylase has been extensively used to delay amylopectin retrogradation. Different hydrocolloids have been added to leavened gluten free products with positive results on crumb structure, taste, global acceptability and shelf life. It has been reported that

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hydrocolloids improve dough development and gas retention through the increase in system viscosity, producing loaves with higher specific volume (Lazaridou et al., 2007; Marco and Rosell, 2008; Peressini et al., 2011). However, it is worth highlighting that the effect of different additives is highly dependent on the raw material used, the nature and quantity of additive used and water availability, being very difficult to predict the real effect of each additive on different formulations. Thus, the objective of this work was to assess the effect of emulsifiers, hydrocolloids and enzymes on gluten-free dough rheology and thermal properties and bread quality, while relating dough properties parameters to bread technological quality.

2. Materials and methods

2.1. Materials

Gluten free breads were formulated with rice flour (Nora's Skills, Argentina; 8.11% proteins, 0.23% ash, 0.28% crude fiber, 0.80% lipids, 79.63% carbohydrates, 10.95% moisture), cassava starch (Señor de Sipan, Argentina; 0.24% proteins, 0.09% ash, 0.21% crude fiber, 0.01% lipids, 86.59% carbohydrates, 12.87% moisture) and full-fat active soy flour (NICCO, Argentina; 36.41% proteins, 4.72% ash, 2.83% crude fiber, 19.80% lipids, 30.26% carbohydrates, 5.98% moisture); compressed yeast (Dánica, Argentina), shortening (Dánica, Argentina) and salt (Dos Anclas, Argentina). The additives employed were: emulsifiers: sodium stearoil-2-lactilate (SSL) and diacetyl tartaric acid ester of monoglyceride (DATEM) were obtained from Alpha emulsionantes (Argentina). Enzymes: glucose oxidase (GOX) and α-amylase (Am) were purchased from Novozyme (Denmark). Hydrocolloids and emulsifiers were of food grade, and enzymes were of analytical grade. Hydrocolloids: xanthan gum (X), carboxymethylcellulose (CMC), carrageenan (C) and alginate (Al). X, C and Al were provided by Saporiti S.A. (Argentina), and CMC was obtained from Latinoquímica Amtex S.A. (Argentina).

2.2. Breadmaking

Basic bread formulation consisted in 45 g of rice flour, 45 g of cassava starch, 10 g of soy flour, 2 g of salt, 2 g of shortening, 3 g of compressed yeast and 65 g of water (except in breads with hydrocolloid addition, where 75 g of water were used). The level of additive incorporation was selected according to preliminary results (Table 1). Ingredients were put together and mixed in a planetary mixer (Arno, Brazil) for 1 min at 156 rpm and 2 min at 214 rpm. The dough obtained was proofed for 30 min (30 °C and 85% relative humidity). After this process, dough was mixed again for 1 min at 156 rpm (this second mixing is carried out to redistribute air cells and nutrients to improve yeast's activity, and to increase air incorporation into the dough); dough was weighed into aluminum cups (60 g) and proofed again under the same conditions (30 min, 30 °C, and 85% relative humidity). Finally, they were put into a rotational oven (Ciclo Ingeniería, Argentina) and baked at 180 °C for 30 min. Once baked, breads cooled for 2 h (until room temperature was reached). Breadmaking was performed in duplicate.

2.3. Dough properties

2.3.1. Large deformation rheology: resistance to penetration

The force required to penetrate the dough was determined using a TA-XT2i texturometer (Stable Micro Systems, United Kingdom) equipped with a 25 kg cell. Samples were prepared as for breadmaking, and 40 g of the resultant dough were weighed into plastic flasks and proofed (60 min, 30 °C, 85% relative humidity). To determine penetration force, fermented dough was compressed until the probe (35 mm diameter) disrupted the dough surface structure, penetrating into the sample, at 5 mm/s. Fig. 1 shows a representative penetration plot. In the first part of the curve, probe is considered to compress the dough without disrupting its structure, up to the point where a threshold force is achieved, and dough resistance to penetration is broken. To obtain this threshold value, two linear regressions were carried out in each of the two parts of the curve; these regressions represented the ideal behavior of the dough. The intersection of both straight lines was considered as dough resistance to penetration under ideal conditions. Dough preparation was performed in duplicate, and three determinations were performed in each dough batch.

2.3.2. Small deformation rheology: frequency sweep

Rheometric experiments were performed with an oscillatory rheometer (Anton Paar, Germany). Frequency sweeps were carried out at 0.1–10 Hz, 0.05% strain and 30 °C (viscoelastic linear range was determined with a previous strain sweep from 0.1% to 100%, at a constant frequency of 1 Hz). Plate-plate geometry (25 mm diameter) was used, with 2 mm gap. Samples were prepared as for breadmaking, but without yeast addition. Dough was allowed to rest for 15 min and then put between plates, and sample excess was carefully trimmed. To avoid water loss during the determination, the exposed edges of dough were covered with vaseline. Before starting the assay, samples were rested for 5 min to allow residual stresses relaxation. Dough preparation was performed in triplicate.

2.3.3. Differential scanning calorimetry (DSC)

For starch gelatinization studies, dough was prepared as for breadmaking but without shortening addition; then, it was proofed (60 min, 30 °C, 85% relative humidity). Approximately 30 mg of sample were weighed into aluminum pans and hermetically

Table 1
Additives employed in gluten-free bread formulations.

Group	Additive	Code	g/100 g flour-starch
Emulsifiers	Diacetyl tartaric acid ester of monoglyceride	DATEM	1
	Sodium stearoil-2-lactilate	SSL	
Enzymes	Glucose oxidase	GOX 1	0.003
		GOX 2	0.03
	α-Amylase	Am 1	0.0006
		Am 1	0.001
Hydrocolloids	Xanthan gum	X	0.5
	Carboxymethylcellulose	CMC	
	Carrageenan	C	
	Alginate	Al	

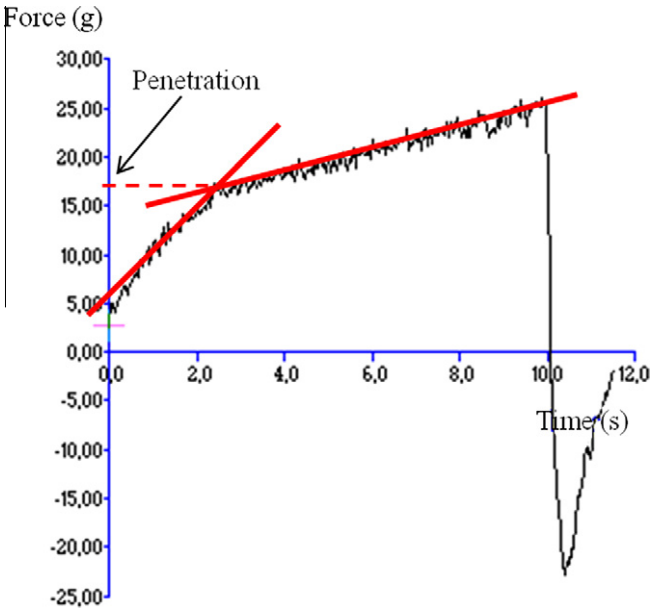

Fig. 1. Representative penetration test plot. The intersection of the two straight-lines was considered as the force required for the probe to penetrate the dough.

sealed. Pans were then heated in the DSC using a temperature profile similar to that measured in the crumb core during baking (León et al., 1997). Temperature profile was as follows: 2 min at 30 °C for sample stabilization, heating from 30 to 110 °C at a heating rate of 11.7 °C/min, and 5 min at 110 °C. Starch gelatinization parameters (To, peak width and ΔH) were obtained from the transition endotherm. To analyze amylopectin retrogradation, pans were stored at 4 °C for 7 days. After this period, pans were reheated in the DSC from 30 to 120 °C at a heating rate of 5 °C/min. To, peak width and ΔH were also obtained. Dough was prepared in duplicate and three pans were prepared in each dough batch.

2.3.4. Dough protein extraction and separation

Dough was prepared as for breadmaking. Protein extraction was carried out with two different solutions: TRIS/HCl 0.5 M, pH 8.8; and TRIS/HCl + 2% SDS. Dough:solvent ratio was 1:10 (w/v). Samples were vortexed for 30 min and then centrifuged 20 min at 3000×*g*. The supernatant was mixed with sample buffer (without 2-mercaptoethanol) and proteins were separated using SDS–PAGE under non-reducing conditions (Ribotta et al., 2005).

2.4. Bread quality

2.4.1. Specific bread volume (SBV)

The volume of each bread loaf was determined by rapeseed displacement. Specific volume was obtained by dividing bread volume/bread weight. Three measurements of each breadmaking replication were performed.

2.4.2. Crumb firmness

To assess crumb firmness, breads were longitudinally cut 2 h after baking, and two slices of 15 mm thick were obtained from the center of each loaf. Firmness was measured using an Instron Universal Texture Machine (Instron, USA) equipped with a 25 mm diameter probe, at a rate of 5 mm/s and 40% compression. Firmness was defined as the maximum force obtained during compression. To obtain hardening rate, firmness measurement was performed 2, 24 and 72 h after baking. Breads were stored in sealed plastic bags at 25 °C. Hardening rate was calculated as the slope of the straight-line obtained from the regression of the three measured points in a force-time plot. Four slices from two different bread loaves were analyzed in each breadmaking batch.

2.4.3. Crumb structure

Digital images from breads were obtained from slices of 15 mm thick using a scanner (HP Scanjet G3010, Palo Alto, USA), with 600 dpi resolution. Images were analyzed using ImageJ Software 1.41o (National Institutes of Health, USA). Image binarization was carried out according to Ribotta et al. (2010). Cell average area (mm^2) and the number of cell/mm^2 were determined. The ratio of small cells ($0.15 < x < 2.00\ mm^2$) to large cells ($2.00 < x < 10.00\ mm^2$) was calculated and it was used as a measure of crumb uniformity. Six slices from three different bread loaves were analyzed in each breadmaking batch.

2.5. Statistical analysis

A completely randomized design was used, with a classifying and a response variable (in this design, it is assumed that the error is normally distributed with a mean = 0, and constant variance). Mean values ± standard deviation are presented. The data obtained were statistically treated by analysis of variance (ANOVA) and the means were compared by the Fisher LSD test at a significance level of 0.05 (coefficient of variation due to sample preparation was lower than 10%). A correlation test was made to evaluate the relationship between variables ($p < 0.05$). These tests were carried out with INFOSTAT statistical software (2011).

3. Results and discussion

3.1. Dough analyses

3.1.1. Rheological studies

Table 2 shows the effect of different additives on dough rheological properties. As shown, emulsifier incorporation affected dough behavior during fermentation, though the effect was different from one emulsifier to another. Thus, when SSL was used, dough resistance was higher than the control (no additive), while the result was just the opposite when DATEM was added. For all gluten-free doughs, G' was higher than G'' in all the frequency range studied, which was indicative of a solid-elastic behavior (Fig. 2). Doughs with both emulsifiers presented higher G' and G'' values than the control. SSL dough had higher G' than DATEM, while the latter one had lower $\tan\delta$. The higher values found in dynamic moduli (G' and G'') of doughs with emulsifiers regarding control dough, clearly indicate that the presence of emulsifiers introduces new interactions into the system, and that their effect will also depend on the type of hydrophilic and hydrophobic interactions established. It has been reported that in wheat based systems, emulsifiers facilitate the interaction between lipids, proteins and starch (Jacobsberg et al., 1976), possibly due to their amphiphilic nature, and that these interactions are responsible for dough reinforcement. The same phenomenon could take place in gluten-free systems, were starch and proteins are the main dough components.

Enzyme addition significantly reduced dough resistance after fermentation. Nevertheless, when analyzing frequency sweeps, it is observed that both GOX doses increased dough consistency (given by higher G' and G'' values), and that $\tan\delta$ was significantly reduced. GOX catalyzes the oxidation of glucose to give gluconolactone and H_2O_2. The H_2O_2 thus formed oxidizes sulfhydryl groups present in proteins, inducing protein cross-linking through the formation of disulfide bonds. H_2O_2 is also proposed to be involved in the oxidative gelation of water soluble pentosans (Hoseney and Faubion, 1981), which induces the formation of a protein/polysaccharide

Table 2
Resistance values after fermentation; elastic (G') and viscous (G'') moduli and tan δ values at 1 Hz for gluten-free doughs.

Additive group	Additive	Resistance (g)	Rheometry		
			G' (kPa)	G'' (kPa)	tan δ
Emulsifiers	None	46.3 ± 3.5b[a]	29.8 ± 1.6a	6.8 ± 0.2a	0.230 ± 0.006b
	DATEM	28.7 ± 1.8a	84.6 ± 6.9a	15.9 ± 0.5a	0.188 ± 0.009a
	SSL	58.8 ± 1.4c	165.5 ± 17.5b	41.1 ± 4.6b	0.246 ± 0.014b
Enzymes	None	46.3 ± 3.5c	29.8 ± 1.6bc	6.8 ± 0.2b	0.230 ± 0.006a
	GOX 1	27.4 ± 1.23a	38.2 ± 4.3c	7.9 ± 1.1b	0.209 ± 0.008ab
	GOX 2	33.9 ± 2.3b	51.1 ± 2.1d	10.3 ± 0.2c	0.201 ± 0.001a
	Am 1	27.3 ± 0.4a	20.8 ± 1.3ab	4.5 ± 0.0a	0.217 ± 0.001bc
	Am 2	24.7 ± 1.1a	17.4 ± 2.2a	3.9 ± 0.4a	0.223 ± 0.002cd
Hydrocolloids	None	46.3 ± 3.5c	29.75 ± 1.62a	6.8 ± 0.2a	0.230 ± 0.006b
	X	35.6 ± 4.3b	30.57 ± 4.24a	7.5 ± 2.7a	0.243 ± 0.003bc
	CMC	28.5 ± 1.2a	24.15 ± 3.31a	6.5 ± 0.8a	0.266 ± 0.002d
	C	22.9 ± 0.7a	60.82 ± 4.18b	12.9 ± 1.3b	0.209 ± 0.006a
	Al	25. 1 ± 1.42a	21.64 ± 2.15a	5.4 ± 0.4a	0.245 ± 0.019c

[a] Different letters within a column and within the same additive group are significantly different ($p < 0.05$).

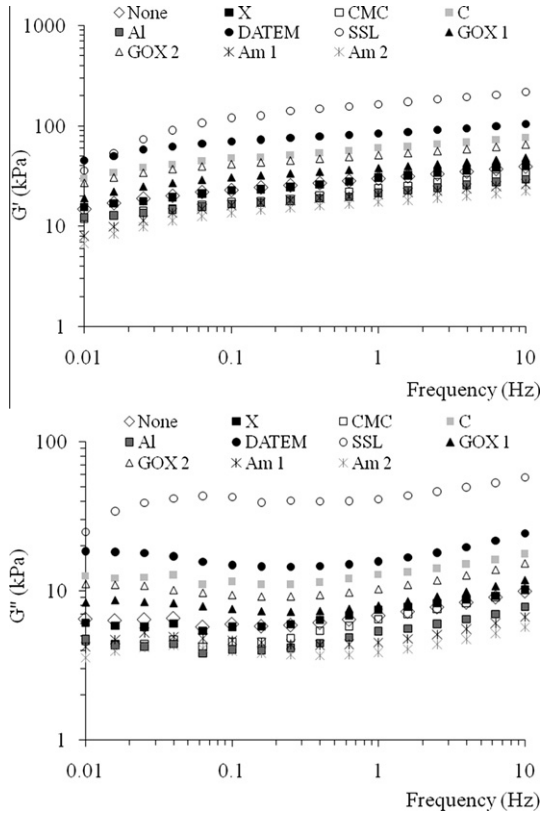

Fig. 2. Dynamic moduli (G' and G'') as a function of frequency for all studied samples.

cross-linked entity, responsible for the increment in the consistency of the system. Thus, the increase in G' and G'' could be due to protein cross-linking and/or pentosans oxidative gelation. α-Amylase hydrolyzes α-(1–4) bonds present in starch, producing low molecular weight α-dextrins. Am incorporation led to a reduction in dough resistance during fermentation. G' and G'' were also reduced, especially for the highest dose. Damaged starch is a starch fraction resulting from the milling process, and is susceptible to enzyme hydrolysis, although native starch can, as well, turn into enzyme substrate during gelatinization (Ferrand, 1964). Thus, it was not surprising to find lower resistance and G' values as a consequence of Am addition/action on susceptible starch fraction.

Doughs were formulated with 65% and 75% (flour/starch basis) of water addition; doughs evaluated were those used for bread-making. Thus, considering that dough with hydrocolloids included higher water amount, it was expected to present lower resistance to penetration. Among doughs with hydrocolloids, X showed the highest resistance, followed by CMC, Al and C ($p < 0.05$). Xanthan gum is known for its thickening properties; it is accepted that in aqueous systems it adopts a helix conformation which turns the molecule rigid, and that this conformation plays an important role on xanthan solution behavior, including high viscosities (Millane and Wang, 1990).

Considering frequency sweeps, hydrocolloids, with the exception of C, did not lead to a significant change in G' and G'' values (1 Hz) with respect to the control dough. Lazaridou et al. (2007), working with rice flour and corn starch based doughs with different water amounts (130, 140 and 150 g/100 g solids), found a decrease in elastic modulus as the water amount increased. This behavior is well documented in wheat systems (Phan-Thien and Safari-Ardi, 1998; Autio et al., 2001), where higher water ratios lead to a diminution in G' and G'', without modifying tan δ, thus concluding that water has mainly a plasticizing effect, while dough structure is unaltered. The C dough had higher G' values than control dough; the same trend was found for viscous modulus (G''); tan δ values were different from one sample to another: they were higher for CMC, followed by Al, the control and X ($p > 0.05$), and lowest for C. Molina Ortiz et al. (2004) studied the behavior of gels based on soy protein isolate and carrageenan, and found a specific interaction between proteins and the hydrocolloid which led to the formation of a more viscoelastic gels.

The correlation between rheology at small and large deformations is still controversial in the literature (Tronsmo et al., 2003; Dobraszczyk and Salmanowicz, 2008; Angioloni and Collar, 2009). Different interactions are established between dough components. If the molecular interactions that are established are strong enough, they may result intact even at high deformation conditions. On the contrary, weak interactions may disrupt under these deformation conditions. Thus, the relationship between both types of assays may be considered a function of the interactions established among dough components. In this work, no correlation was found.

3.1.2. Effect of GOX on protein fraction

Fig. 3 shows SDS–PAGE gel under non-reducing conditions from TRIS/HCl and TRIS/HCl + SDS dough-extracted protein fractions. In GOX samples, a high molecular weight band (arrow) which is absent in the control sample, possibly due to enzyme action, can

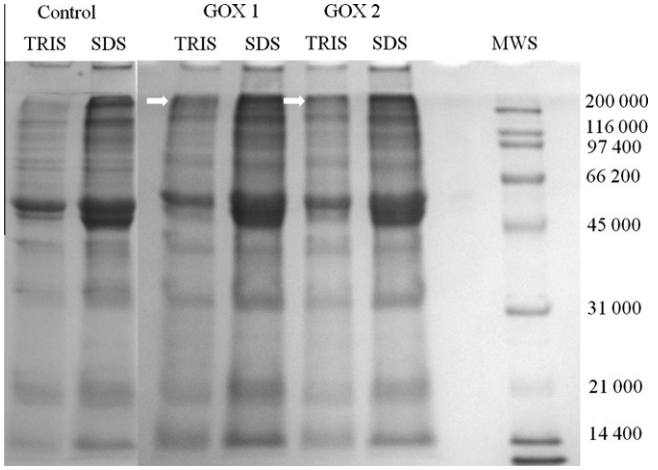

Fig. 3. Non-reducing SDS–PAGE gel of TRIS/HCl (TRIS) and TRIS/HCl + SDS (SDS) extracted proteins. MWS, molecular weight standard.

be observed. Gujral and Rosell (2004a) have also informed about the modification of protein fraction from rice flour after GOX incorporation, obtaining higher molecular weight polymers while they observed a reduction in free sulfhydryl groups, associated to disulfide bond formation. Besides, sulfhydryl groups are also present in soy proteins (Kinsella, 1979) which may potentially be modified by H_2O_2. No differences were found in electrophoretic pattern of protein extracted from doughs with hydrocolloids, emulsifiers or α-amylase (data not shown).

3.1.3. Calorimetric behavior

As shown in Table 3, gelatinization enthalpy was reduced by emulsifier addition, and this effect was more evident for DATEM which, besides, shifted the gelatinization peak toward higher temperatures. Eliasson (1986) reported that the inclusion of emulsifiers such as SSL delayed starch gelatinization; they also found a decrease in ΔH and attributed this finding to the simultaneous occurrence of exothermic phenomena, such as the amylose-emulsifier complex formation. Ghiasi et al. (1982a,b); observed a restriction in wheat starch swelling in the presence of SSL, which partially explains the higher transition temperatures observed. It is widely accepted that the addition of emulsifier (mainly SSL and DATEM) leads to a decrease in starch retrogradation (Batres and White, 1986; Krog, 1981; Eliasson and Ljunger, 1988; Biliaderis and Tonogai, 1991; Gudmundsson, 1992). Nevertheless, in this work an increase in ΔH_{ret} was observed. It has been previously reported (Sciarini et al., 2012) that soy proteins diminish cassava starch retrogradation. Thus, the possible interaction of emulsifiers with proteins and/or starch may disrupt soy proteins and cassava starch interaction. Retrogradation peak width was reduced, suggesting the formation of crystallites with similar stability.

Starch gelatinization was not modified by enzymes addition; only the presence of Am increased To. Durán et al. (2001) evaluated the incorporation of maltodextrins with different degrees of polymerization (3–7 DP) into different starches; they found an increase in gelatinization temperature and ascribed this behavior to a stabilizing effect of oligosaccharides on starch amorphous regions, while they found no effect on ΔH. Am did not affect the retrogradation process, as compared to the control sample. Goesaert et al. (2009) have questioned the impact of α-amylase on starch recrystallization, considering that it has little effect on the outer, crystallizable branches of amylopectin. In this sense, these authors suggested the use of maltogenic amylase (exoamylase) which degrades amylopectin chains producing α-maltose almost exclusively. GOX addition augmented amylopectin retrogradation. Again, the possible modification of soy proteins by GOX action may negatively affect the interaction between soy proteins and starch.

Hydrocolloid presence did not affect starch gelatinization behavior; no significant differences were observed in To or ΔH. Hydrocolloids are expected to compete with starch for water uptake (thus modifying gelatinization process) due to their high hydrophilic nature, but since doughs with hydrocolloids were prepared with higher water amount (75% vs. 65% for the control sample), it was difficult to draw a direct comparison. Ferrero et al. (1996) assessed the effect of different hydrocolloids incorporation on corn starch gelatinization and retrogradation; they found that at low hydrocolloid:starch ratios (1:10), in excess water, gelatinization parameters were not modified, while higher ratios (1:2 and 1:1) produced a shift toward higher temperatures, while peak width was also increased. Regarding the retrogradation process, ΔH was increased by hydrocolloid addition, indicating higher amylopectin recrystallization during storage. It has been reported that ΔH_{ret} presents a bell-shaped response as a function of moisture content, with minimum values at extreme moisture concentrations (higher than 90% and lower than 20%) and a maximum value at approximately 50% of water amount (Zeleznak and Hoseney, 1986). Breadcrumb presents moisture contents between 35% and 45% (Rogers et al., 1988; He and Hoseney, 1990; Baik and Chinachoti, 2000; Ribotta and Le Bail, 2007); under such conditions, higher water amount leads to higher amylopectin retrogradation. Thus, the higher water content of

Table 3
DSC parameters of gelatinized and retrograded samples.

Additive	Baking			Retrogradation		
	ΔH (J/g solids)	To (°C)	Peak width (°C)	ΔH (J/g solids)	To (°C)	Peak width (°C)
None	−7.01 ± 0.72c[a]	66.23 ± 0.29a	22.10 ± 1.88a	−1.71 ± 0.22b	42.88 ± 0.97a	16.58 ± 1.28b
DATEM	−4.98 ± 0.39a	67.82 ± 0.53b	22.59 ± 0.67a	−2.89 ± 0.42a	44.19 ± 0.68a	12.21 ± 1.14a
SSL	−5.86 ± 0.37b	66.46 ± 0.49a	21.79 ± 1.49a	−2.09 ± 0.39b	43.25 ± 1.73a	15.59 ± 1.71b
None	−7.01 ± 0.72a	66.23 ± 0.29a	22.10 ± 1.88a	−1.71 ± 0.22bc	42.88 ± 0.97a	16.58 ± 1.28b
GOX 1	−7.27 ± 0.58a	66.43 ± 0.35a	23.09 ± 0.96a	−2.13 ± 0.29ab	43.79 ± 0.37b	15.89 ± 0.67b
GOX 2	−6.98 ± 0.73a	66.67 ± 0.84ab	22.54 ± 0.74a	−2.42 ± 0.24a	44.32 ± 0.41b	14.99 ± 0.42a
Am 1	−7.19 ± 0.69a	67.33 ± 0.47c	21.59 ± 0.45a	−1.68 ± 0.18c	43.15 ± 0.17a	16.54 ± 0.62b
Am 2	−7.49 ± 0.76a	67.23 ± 0.20bc	21.21 ± 0.67a	−2.11 ± 0.24abc	44.03 ± 0.14b	14.83 ± 0.49a
None	−7.01 ± 0.72a	66.23 ± 0.29a	22.10 ± 1.88c	−1.71 ± 0.22c	42.88 ± 0.97a	16.58 ± 1.28b
X	−7.16 ± 0.69a	67.07 ± 1.15a	18.94 ± 0.29ab	−2.09 ± 0.26b	43.17 ± 0.75a	15.14 ± 0.91a
CMC	−7.09 ± 0.62a	66.28 ± 0.54a	19.35 ± 1.09b	−2.52 ± 0.22a	43.30 ± 0.36a	14.30 ± 0.45a
C	−6.23 ± 0.62a	66.45 ± 0.29a	19.67 ± 0.68a	−2.62 ± 0.32a	43.07 ± 0.20a	14.39 ± 0.23a
Al	−6.55 ± 0.67a	67.14 ± 0.58a	17.96 ± 0.59a	−1.92 ± 0.14bc	43.05 ± 0.38a	15.12 ± 0.57a

ΔH: enthalpy value; To: onset temperature; PW: peak width.
[a] Different letters within a column and within an additive group are significantly different ($p < 0.05$).

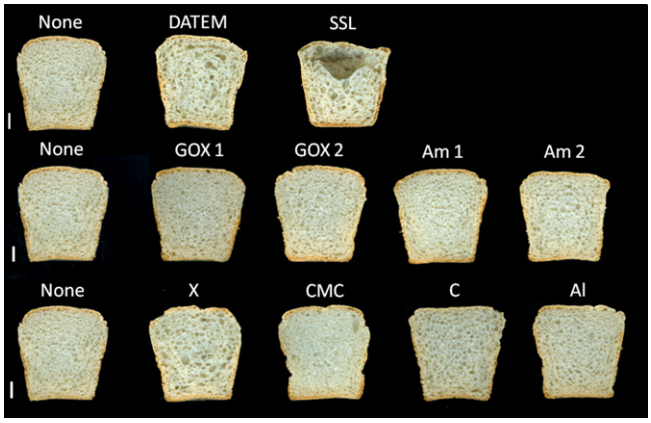

Fig. 4. Images of gluten-free breads. None: control. Bar: 1 cm.

samples with hydrocolloids may explain the higher ΔH_{ret} found in DSC studies.

3.2. Bread quality

3.2.1. Specific bread volume (SBV)

The addition of emulsifiers did not lead to an increase in SBV; in fact, SSL addition decreased SBV when compared to the control bread (Table 4). A negative correlation ($r = -0.80$, $p < 0.05$) was found between dough resistance and SBV. In previous works, Sciarini et al. (2010a,b) observed an opposite trend in gluten free systems with high water amount (~150%, flour basis), where an increase in batter/dough resistance led to better quality breads, with increased SBV; this effect was then ascribed to the higher capacity to retain the gases formed during fermentation. In this work, a significantly lower water amount was used (65–75%, flour basis). It is natural then that systems with a higher resistance to certain values will have more difficulty to expand during proofing and baking.

The lowest Am dose produced an increase in SBV. This effect is mainly due to the hydrolysis of the starch fraction leached as a result of gelatinization during baking, reducing dough resistance with a positive effect on SBV; and, besides, to the production of fermentable sugars. On the other hand, the highest dose did not increase SBV; it produced a higher reduction in dough resistance as compared to the lowest dose, and this could lead to a decreased gas holding capacity. The presence of GOX produced breads with a SBV similar to the control bread, although protein polymerization has been observed. Breads with hydrocolloid addition showed poor technological parameters – such as very low specific volume, high firmness and dense crumb structure – when 65% of water was used. Consequently, different water amounts from 65 to 95 g were evaluated, obtaining the best result (concerning bread volume, crumb firmness and structure) when using 75%. For this reason, breads with hydrocolloid addition were made with 75% of water incorporation. C addition led to the highest SBV among samples with hydrocolloids, followed by CMC. Breads with X and Al addition showed the same SBV than the control bread ($p > 0.05$).

3.2.2. Crumb firmness

From Table 4 it is observed that crumbs with emulsifier were harder than the control, and the same trend was observed for firming rate (related to staling). Considering breads with enzymes, crumb firmness was reduced by the presence of GOX, in agreement with Gujral and Rosell (2004a) who also found a diminished crumb firmness when adding GOX. But the firming rate increased with respect to the control bread. As it was already explained, the possible disruption of soy protein/starch interaction for GOX action may negatively affect crumb behavior during storage (Sciarini et al., 2012). The presence of Am led to reduced initial crumb firmness and did not modify the firming rate when compared to the control. Initial crumb firmness and firming rate were reduced with hydrocolloids incorporation. This effect would not be related to a decrease in amylopectin retrogradation (ΔH_{ret} was higher for samples with hydrocolloids), but more likely to a reduction in moisture loss during storage, which retards staling phenomena (Rosell et al., 2007). These results are in agreement with Rogers et al. (1988), who found higher crumb firmness and firming rate in breads with lower moisture contents (between 22% and 37%) and this effect was not associated to an increase in amylopectin retrogradation, which was lower at lower water contents.

3.2.3. Crumb structure

Fig. 4 shows representative images of the gluten-free breads obtained. Table 4 presents crumb structure parameters of all the samples studied. DATEM samples showed a lower cell number. Breads with SSL systematically presented a big cell near the surface, this effect being characteristic of systems with a rapid water loss; the vapor thus formed exerts certain pressure on the forming crumb, producing the collapse of the structure. These breads had lower air area fractions. GOX crumb presented a higher cell number of small size, while the air area fraction was lower.

Table 4
Gluten-free bread quality parameters.

Additive group	Additive	SBV (cm^3/g)	Hardness		Crumb structure			
			Initial Firmness (g)	Staling rate(g/day)	N° cells/mm^2	Cell size (mm^2)	% Cell area	Uniformity
Emulsifiers	None	1.98 ± 0.05b[b]	249 ± 39a	208.8 ± 2.3a (0.998)[a]	1.30 ± 0.12b	4.26 ± 0.6a	54.9 ± 2.7b	2.07 ± 0.21b
	DATEM	1.99 ± 0.09b	280 ± 11a	361.4 ± 77.9b (0.991)	1.12 ± 0.05a	4.96 ± 0.5a	54.9 ± 1.3b	1.31 ± 0.12a
	SSL	1.71 ± 0.00a	833 ± 46b	380.0 ± 24.8b (0.997)	1.04 ± 0.05a	4.48 ± 0.3a	45.9 ± 0.9a	2.18 ± 0.17b
Enzymes	None	1.99 ± 0.05a	249 ± 39b	208.8 ± 2.3a (0.998)	1.30 ± 0.12a	4.26 ± 0.6b	54.9 ± 2.7c	2.07 ± 0.21a
	GOX 1	2.05 ± 0.04ab	169 ± 11a	329.9 ± 16.3b (0.995)	1.60 ± 0.15b	3.17 ± 0.3a	50.8 ± 2.7ab	2.54 ± 0.22bc
	GOX 2	2.01 ± 0.04a	169 ± 19b	304.4 ± 37.7ab (0.999)	1.92 ± 0.17b	3.34 ± 0.5a	49.0 ± 4.9a	2.62 ± 0.20c
	Am 1	2.15 ± 0.03b	171 ± 15ab	287.3 ± 32.1ab (0.999)	1.34 ± 0.15a	4.05 ± 0.5b	53.2 ± 2.3bc	2.58 ± 0.28bc
	Am 2	2.04 ± 0.04ab	229 ± 22ab	316.2 ± 81.8ab (0.999)	1.27 ± 0.05a	4.15 ± 0.2b	53.7 ± 1.9bc	2.32 ± 0.21ab
Hydrocolloids	None	1.98 ± 0.05a	249 ± 39c	208.8 ± 2.3c (0.998)	1.30 ± 0.12c	4.26 ± 0.6c	54.9 ± 2.7b	2.07 ± 0.21c
	X	1.86 ± 0.04a	162 ± 9b	172.7 ± 13.6b (0.991)	1.07 ± 0.12b	5.38 ± 0.6d	55.8 ± 1.5b	1.68 ± 0.12ab
	CMC	2.14 ± 0.02b	113 ± 7a	136.5 ± 4.6a (0.999)	1.67 ± 0.19d	2.73 ± 0.3a	49.0 ± 1.7a	4.32 ± 0.47d
	C	2.38 ± 0.09c	132 ± 1ab	170.9 ± 13.0b (0.996)	0.89 ± 0.08a	8.06 ± 0.7e	58.8 ± 2.0c	1.42 ± 0.06a
	Al	1.99 ± 0.02a	141 ± 3ab	160.3 ± 14.3ab (0.994)	1.58 ± 0.14d	3.38 ± 0.3b	51.4 ± 2.8a	2.01 ± 0.19bc

[a] Values between parentheses correspond to the determination coefficient of the regression straight-line.
[b] Different letter within a column and within an additive group are significantly different ($p < 0.05$). SBV: specific bread volume.

Breads with Am presented a crumb structure qualitatively similar to that of the control. As observed in samples with hydrocolloids, breads with C – as well as breads with X – showed a more open structure. Cell number/mm^2 was lower in these samples and, accordingly, cells were bigger. In breads with C, the cell area fraction was the highest of all, while CMC and Al had the lowest values. Uniformity showed the highest values in breads with CMC, and it corresponds with a greater number of smaller cells. On the other hand, C and X had the lowest uniformity values, associated to a more open structure. Typically, gluten free breads, present a dense crumb structure with thick cell walls; so the presence of bigger cells leads to the formation of a spongier crumb not easily found in this type of breads.

4. Conclusions

These results show that additive incorporation modified dough behavior, evidenced by the different calorimetric and rheological (at small and large deformations) properties. Besides, the electrophoretic pattern of dough extracted proteins changed after the addition of glucose oxidase. As a whole, these modifications resulted in breads with different specific volumes, firmness and firming rates, and crumb structures. Nonetheless, the breads obtained did not necessarily show better quality parameters than the control bread. Control dough produced gluten free breads of acceptable volume, crumb structure and, principally, with lower hardening rate during storage. In a previous work it was observed that there was a specific interaction between soy proteins and cassava starch, which the results reported herein seem to support, as this interaction is the one that leads dough and bread behavior. Under such conditions, high quality bread was obtained. From this viewpoint, additive incorporation did not improve final bread technological quality, as they may disrupt and/or impede the interaction between both polymers (soy proteins and cassava starch). Contrary to widespread opinion, this work shows that the presence of additives is not essential for gluten free bread production. This fact provides new perspectives at the moment of selecting raw materials and technological parameters, considering that a careful selection of such simple variables may notably diminish production costs and facilitate gluten free products development.

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