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Enzymes Action on Wheat–Soy Dough Properties and Bread Quality

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Abstract High levels of soy flour added to wheat bread produce negative effects on gluten network formation, dough properties, and on bread final quality. The objective of this study was to assess the influence of three enzymes, transglutaminase (TG), glucose oxidase (GOX), and endoxylanase (XYL), on dough properties and final quality of high-protein breads. The addition of TG and GOX increased the mixing stability and maximum resistance of dough, decreased its extensibility, and produced stronger and more consistent dough samples. XYL incorporation produced opposite results. XYL addition and the lowest GOX dose increased bread volume significantly and decreased initial crumb firmness, while high doses of TG (0.3%) produced detrimental effects on bread volume and crumb firmness. In conclusion, XYL and GOX 0.001% addition improved the final quality of soy-fortified breads, but XYL was the best additive to improve dough properties, bread volume, and quality.

Keywords Dough · Bread · Wheat · Soy · Enzymes

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Introduction

In the last years, consumers' needs have led to a food production with high nutritional quality and health benefits properties. Soy flour has long been recognized as an excellent means of fortifying cereal products, such as bread, cookies, crackers, and pasta. Its significant levels of high-quality proteins rich in essential amino acids have high digestibility and provide health benefits effect, such as reduced risk of coronary heart disease, osteoporosis, menopausal and postmenopausal women symptoms, and cancer prevention (Friedman and Brandon 2001; Brouns 2002). However, the unique viscoelastic properties of gluten–water mixture capable of retaining carbon dioxide during the proofing and baking stages are affected by soy products' incorporation. The addition of high levels of soy protein depresses loaf volume, gives poor crumb characteristics, and decreases acceptability (Fleming and Sosulski 1978; Ribotta et al. 2005).

There are several additives used in bakery to compensate flour quality deficiencies which could be used to improve the functionality of wheat flour mixed with legume products. Transglutaminase (TG) catalyzes acyl-transfer reaction, producing covalent crosslinks in proteins between glutamine and lysine residues without affecting the nutritional availability of lysine (Seguro et al. 1996). Basman et al. (2002a) reported that TG could polymerize proteins from one or more sources through the formation of intermolecular crosslinks and that soy proteins were the best substrates of TG. Among wheat proteins, gliadins and high molecular weight glutenins were reported as substrates for TG (Larré et al. 2000). Thus, TG promotes the formation of high molecular polymers improving the elasticity and strength of the dough (Gerrard et al. 1998).

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Glucose oxidase (GOX) catalyses the oxidation of β-D-glucose to δ-D-gluconolactone and hydrogen peroxide in the presence of oxygen. The gluconolactone is hydrolyzed to gluconic acid by a non-enzymatic mechanism. Hydrogen peroxide produced during GOX reaction causes the oxidation of the free sulfhydryl units from gluten protein producing disulfide linkages (Haarasilta and Pullinen 1992) which result in a stronger dough. (Vemulapalli et al. 1998) reported that hydrogen peroxide may also lead to the formation of arabinoxylan crosslinkings or even of a gel from the water-soluble arabinoxylan that increment dough consistency.

Endoxylanases (XYL) are hydrolytic enzymes that can randomly attack arabinoxylan (AX) backbone to cause a decrease in polymerization degree, hence having a strong impact on AX structure and functionality. The addition of xylanases to wheat flour has been reported to improve dough-handling properties, loaf volume, and bread final quality (Courtin and Delcour 2002).

Despite the widely available literature about these additives utilized in breadmaking, there is no information about their use in soy–wheat bread. The objective of this study was to evaluate the influence of three enzymes, endoxylanase (XYL), glucose oxidase (GOX), and transglutaminase (TG), on dough properties and quality of high-protein bread.

Materials and Methods

Materials

Commercial bread wheat flour and defatted soy flour were provided by local milling companies (Tiranti SA, Argentina and Complementos Proteicos SA, Argentina, respectively). Wheat flour had 30.27% wet gluten, 518 s falling number, and 11.88% moisture content. Alveograph parameters were dough strength (W)=4.33×10^{-4} J, tenacity (P)=139.9 mm, extensibility (L)=77 mm, and P/L ratio=1.82 (AACC 1995). The proximate composition of flours (moisture, crude protein, dietary fiber, crude fat, and ash) in dry basis was determined according to AACC methods 44-19, 46-12, 32-05, 30-26, and 08-01, respectively. Compressed yeast (Calsa SA, Argentina) and other dough ingredients (food grade) were purchased at a local market. Enzymes used as additives in this work were: transglutaminase (TG) (Transglutaminase active WM, Ajinomoto, Japan) activity 100 U/g; glucose oxidase (GOX) (Gluzyme Mono 10000 BG, Novozymes, Denmark) activity 10,000 U/g; and 1.4-endoxylanase (XYL), (Pentopan Mono BG, Novozymes, Denmark) activity 2,500 U/g.

Flour–enzyme blends were prepared using 85:15 wheat: soy flours proportion based in previous studies (Sabanis and Tzia 2009), and enzymes were added in the following doses, according to manufacturer recommendation levels: TG 0.05% and 0.3%; GOX 0.001% and 0.01%; XYL 0.006% and 0.012% (flour basis). A control sample was prepared without additives incorporation.

Dough Properties' Evaluation

Dough samples were elaborated using flour–enzyme blends, water based on farinograph absorption (500 BU line), and 2% NaCl (flour basis). Ingredients were mixed in a Philips HR 1495 mixer (Philips, Argentina) at low speed for 2 min.

Dough properties were evaluated as follows:

Farinograph Procedure

Determination of dough-mixing behavior of the different flour–enzyme blends was made with a Brabender farinograph (Brabender, Duisburg, Germany). The following parameters were determined according to the approved method 54-21 (AACC 1995): water absorption (WA), dough development time (DT), stability (S), and softening degree (DS).

Uniaxial Extension

Extensibility measurements were made with a TA.XT2i texture analyzer (Stable Micro Systems, Godalming, Surrey, UK) using the SMS/Kieffer rig for dough extensibility measurements. Dough samples (~20 g) were pressed into strip form and allowed to relax for 40 min. Ten dough strips by sample were extended at 3.3 $mm\,s^{-1}$. Resistance to extension (Rm, maximum resistance) and extensibility (E, maximum extensibility) were automatically calculated using Texture Expert 1.22 software (Stable Micro Systems).

Dynamic Rheological Measurements

The dynamic rheological properties of dough samples were characterized with a rheometer (RS600, Haake, Karlsruhe, Germany) at 25°C. A serrated plate–plate geometry (PP30) with a gap of 1.5 mm was used. Silicone was added around the plate edges to prevent dough dehydration. Storage modulus (G'), loss modulus (G"), complex modulus (G*), and loss tangent (tan $\delta=G"/G'$) were obtained. Frequency sweeps were performed at 0.005–100 Hz within the linear viscoelasticity range at a fixed strain of 0.017%.

Dough Expansion Capacity

Evolution of dough volume through the whole fermentation period was determined following the method described by IRAM (Instituto Argentino de Racionalización de Materiales)

norms (method number 15865, IRAM 1991). Dough samples were prepared using 200 g flour–enzyme blends, water (based on farinograph absorption), 3% yeast, and 2% NaCl on a flour basis. Ingredients were mixed in a Philips HR 1495 mixer (Philips, Argentina) at low speed for 2 min. The resulting dough was allowed to rest for 5 min, and the bulk dough was subsequently sheeted by hand. The dough was then divided into 100 g pieces and rounded. To determine gas production, two pieces of dough were immediately put into 1,000 mL calibrated graduated cylinders. Dough samples were pressed to achieve a smooth surface, and the cylinders were left for 150 min in a water bath at 30±0.5°C. Dough rising was measured every 15 min.

Bread Quality Evaluation

Bread-making in this study followed the process described in IRAM norms (method number 15858-1, IRAM 1996) with minimal modification in the baking time. The basic bread-dough formulation consisted of 100% wheat/soy flour (85:15 ratio), 3% compressed yeast, 1% sodium chloride, 2.5% sugar, 0.5% sodium stearoyl-2-lactylate (SSL, Alpha Emulsionantes, Argentina), 0.003% α-amylase (Bel' Ase A75, 75,000 SKB/g), and water based on a farinograph test (500 BU line). Enzymes were added as described before. The ingredients were mixed in an Argental L-20 mixer (Argental, Argentina). Yeast suspension and salt–sugar solution were incorporated separately to the rest of solids. The resulting dough (~27°C) was taken to a first proof for 80 min at 30°C and 70% RH with two intermediate punches (partial lost of gas to the atmosphere) at 45 and at 60 min. The bulk dough was then sheeted in a Mi-Pan vf roller (Mi-Pan, Córdoba, Argentina) and divided into 150 g pieces, molded, panned, and returned to the fermentation cabinet for 75 min (second proof). After this time, dough was baked at 215°C for 24 min.

Bread quality was determined by the following procedures:

Specific Loaf Volume

Loaves were weighed, and bread loaf volume was determined by rapeseed displacement 2 h after baking. The specific loaf volume (SLV) was expressed as the volume/mass ratio (cubic centimeter per gram) of bread according to AACC method 10-05 (AACC 1995).

Bread Crumb Structure

Digital images of bread crumb were obtained through a HP Photosmart Premier 6.5 scanner. For each bread loaf, four slices were obtained and analyzed with an image analyzer (Image J 1.38n, National Institute of Health, USA). Images were converted to 8-bit image (256 gray level). A field of view (FOV) (size: 4×4 cm) was selected to exclude the crust and 0.8–1 cm of the crumb placed next to the crust. A threshold method was used for conversion to a binary image. Crumb images were considered to contain gray level information from pixels of which the darkest individuals belong to the cell and the brightest individuals belong to the cell wall. The following crumb grain features were determined from FOV: total number of cells (TNC) and grain uniformity (U). U was determined as the ratio of small to large cell counts (i.e., the ratio of the number of cells smaller than 4 mm^2 to the number of cells larger than 4 mm^2); higher values indicate greater uniformity of crumb grain (Zghal et al. 2001).

Bread Crust Color

Bread crust color was measured from the top position of each bread loaf with a Minolta 508d spectrophotometer. At least four breads from each test point were taken and at least three readings from each bread were recorded as CIE-LAB, L^* (lightness), a^* (redness–greenness), and b^* (yellowness–blueness) values.

Bread Staling Evaluation

Bread Crumb Texture

Texture profile analysis (TPA) was determined using a TA.XT2i texturometer (Stable Microsystems, Surrey, UK) equipped with a 25-kg load cell. A cylindrical probe, 3.6 cm in diameter, was attached to a moving crosshead. At different storage times, two bread loaves were cut into three slices (2.5 cm thick) each, and the ends were discarded. Each slice was subjected to a double compression cycle under the following conditions: crosshead speed 1 mm/s and maximum deformation, 40%. The texture profile parameters were determined using Texture Expert 1.22 software (Stable Microsystems, Surrey, UK). The firmness, F (the force in grams required to compress the sample) and the chewiness, CH (the amount of energy required to disintegrate solid food to a state ready for swallowing) of the crumb were calculated from a force–distance graph. F and CH parameters were measured at time intervals of 0, 2, and 7 days and noted as follows: F_0, F_2, F_7, and CH_0, CH_2, and CH_7 for the respective days of storage. Six slices were analyzed per point, and average values were reported.

Differential Scanning Calorimeter

Analyses of starch retrogradation of amylopectin were performed using a Calorimeter Mettler Toledo DSC 823^e (Switzerland) with a STARe Default DB V9.00 software

(Mettler Toledo, Switzerland). Dough samples were prepared using the same recipe as in the breadmaking procedure. Ingredients were mixed for 2.5 min and proofed for 1 h at 30°C and 80% RH. Small dough pieces (30±3 mg) were taken from the center, weighed in DSC pans, and hermetically closed. Dough samples were gelatinized in the DSC following a temperature profile to simulate the temperature in the center of the crumb during baking (León et al. 1997) and stored at 25°C. The onset temperature (T_0g), gelatinization temperature range (ΔTg) and enthalpy of starch gelatinization (ΔHg) (expressed as millijoules per milligram of dry sample) were determined. At 0 (2 h), 2, and 7 days, pans were heated again in the calorimeter as follows: samples were kept at 25°C for 2 min, heated from 25°C to 120°C, at 10°C/min. Onset temperature and retrogradation temperature range, as well as the enthalpy of starch retrogradation (ΔHr) (expressed as millijoules per milligram of dry sample) were determined from the thermograms.

Statistical Analysis

Determinations were realized at least in duplicate, and the values were informed as means±the standard deviation. ANOVA, Pearson's test, and Fisher LSD test at a significance level of 0.05 were performed using INFOSTAT statistical software (InfoStat 2002).

Results and Discussion

Soy flour has been recognized as an excellent means of fortifying cereal products because of its high lysine content and its own health benefits. Table 1 shows the composition of defatted soy and wheat flours used in this study. The protein content of wheat breads and wheat–soy bread (85:15 wheat:soy flour) were 12.7±0.0% and 20.2±0.0%, respectively. These results showed that 15% substitution of wheat flour by defatted soy flour increased the protein content of breads in 63% approximately. In Table 1, results show the high dietary fiber and mineral content supplied by soy flour that adds extra nutritional value to wheat–soy breads. Determination of dietary fiber in wheat bread and wheat–soy bread showed values of 5.3±0.0% and 8.1±0.3%, respectively, which means an increment of 53% approximately.

Effect of Enzymes on Dough Properties

The study of the rheological characteristics of dough prepared with enzymes (Table 2) provides information about the quality of the end product. Enzyme incorporation increased water absorption (WA), except TG 0.3% that did not change it. The highest WA was produced by XYL. This result is in contrast with those informed by other authors for wheat dough (Courtin et al. 2001; Wang et al. 2003; Jiang et al. 2005).

Since a higher level of pentosans (determined in our previous study, Roccia et al. 2009) and a higher influence of soy flour hydrophilic components on gluten formation in soy–wheat dough than in wheat dough, are expected, the positive influence of xylanase on gluten formation should be more affected by AX degradation in wheat–soy dough, producing higher WA levels compared to wheat dough.

Also, XYL produced less stable dough samples at over-mixing, and the high dose of XYL led to the greatest value of softening degree (DS). In contrast, the highest level of GOX increased the stability (*S*) over 20 min and led to the lowest DS value (11 UF), showing that this enzyme produced highly stable dough. These results are consistent with the effects observed in over-oxidized dough (Hamer and Hosseney 1998).

The extensibility parameters of dough were modified by enzyme incorporation (Table 2). Results showed that in general, enzymes increased resistance to extension (Rm) and produced a less extensible dough. However, XYL 0.012% did not significantly change Rm, and it increased extensibility (*E*) with respect to the control. In previous studies, it was shown that the incorporation of different levels of soy flour to wheat dough decreased free water, suggesting that there was a great competition for water between soy proteins and gluten proteins (Roccia et al. 2009; Ribotta et al. 2010). Dough having a higher percentage of bound water should be somewhat firmer because there is not much lubricating action within the dough. This fact would lead to an increase in the dough resistance and a decrease in extensibility. In our previous studies on gluten–soy protein dough, the increment of water levels decreased dough resistance (Roccia et al. 2009). The beneficial effects of xylanases addition to wheat dough were attributed to the redistribution of water from AX to flour components such as gluten and the removal of the physical barrier created by AX on gluten matrix formation

Table 1 Proximate composition of wheat and soy flours

Values were informed as means± the standard deviation

	Moisture (%)	Protein (%)	Dietary fiber (%)	Fat (%)	Ash (%)
Wheat flour	12.4±0.1	11.6±0.2	5.7±0.4	0.9±0.2	0.5±0.0
Soy flour	7.8±0.0	53.1±0.8	19.9±0.1	0.7±0.0	7.1±0.0

Table 2 Effect of enzymes on rheological parameters of wheat–soy dough

Sample	WA (%)	DT (min)	*S* (min)	DS (UF)	Rm (g)	*E* (mm)	*G*' (Pa ×10^3)	*G*" (Pa ×10^3)	*G** (Pa ×10^3)
Control	66.9	10.0	13.5	41	39.7^{a}	36.0^{c}	36.1^{b}	10.2^{cd}	37.5^{b}
TG 0.05%	67.1	9.8	13.5	36	54.0^{b}	28.5^{ab}	52.1^{d}	15.1^{e}	54.3 ^{a}
TG 0.3%	66.9	9.7	15.1	32	75.9^{d}	24.0^{a}	53.0^{d}	12.0^{d}	54.3 ^{a}
GOX 0.001%	67.6	10.0	14.0	34	66.7^{c}	27.9^{ab}	44.9^{c}	12.1^{d}	39.6 ^{b}
GOX 0.01%	67.8	9.5	>20.0	11	54.4^{b}	28.8^{ab}	35.5^{b}	9.2^{bc}	36.6 ^{b}
XYL 0.006%	68.5	8.8	9.6	39	53.8^{b}	32.9^{bc}	24.6^{a}	7.4^{ab}	25.7^{c}
XYL 0.012%	68.6	9.7	9.3	50	34.2^{a}	49.1^{d}	23.3^{a}	6.6^{a}	24.3^{c}

Values were informed as means. Values followed by the same letter within a column are not significantly different ($p>0.05$)

G', *G''*, and *G** were determined at 1 Hz from frequency sweep

WA water absorption, *DT* development time, *S* stability, *DS* degree of softening, *Rm* resistance, *E* extensibility, *G*' storage modulus, *G*" loss modulus, *G** complex modulus

TG transglutaminase, *GOX* glucose oxidase, *XYL* xylanase

(Matt et al. 1992). In the present study, in addition to the effect on wheat dough described, the increment of WA decreased the detrimental effects produced by soy flour addition on gluten network formation, improving dough extensibility.

The dynamical rheological properties of dough are shown in Table 2. Dough samples presented higher storage modulus (*G*') compared with loss modulus (*G*") in the entire frequency range, following the typical viscoelastic behavior of dough. The addition of enzymes modified both moduli. The incorporation of TG and the lowest dose of GOX increased either *G*' or *G*". These results indicated a firmer and more consistent dough than the control, probably due to higher crosslinking of gluten and soy proteins produced by TG and to the low levels of GOX addition (Miller and Hoseney 1999; Basman et al. 2002b). This change in dough consistency was reflected in the higher *G** values, related to the global viscoelastic behavior of the system. On the other hand, there was a significant ($p<0.05$) decrease in *G*' and *G*" and so in *G** values due to XYL addition. XYL influence on *G*', *G*", and *G** suggested that there was a significant reduction of dough consistency and firmness, probably due to the effect of XYL on water redistribution, which leads to dough softening. Loss tangent ranged between 0.23 and 0.29; however, no significant differences were found as a consequence of enzyme addition, which indicated that the contribution of both elastic and viscous moduli to dough behavior remains the same in all cases.

From our results, it was evident that there is not a clear correlation between the parameters obtained from the large deformation rheological assays (Rm, *E*) and the rheometric assays, performed at low deformations within the linear viscoleastic range (i.e., without rupture of the structure). However, large deformation assays seem more sensitive to detect than the effect of enzyme dose on dough behavior. For practical breadmaking purposes, assays at large deformations should be taken into account since they can be related to the large strains undergone during fermentation and baking processes.

The dough volume through fermentation process depends on gas production, gas retention capacity, and dough expansion capacity under CO_2 pressure. In the present study, the first parameter was kept constant, and both gas retention and dough expansion capacity changed with dough formulation. Figure 1 shows the effects of enzyme addition on dough volume during fermentation process. Significant correlations were obtained between the dough volume and rheological parameters WA, Rm, *E* and *G*' (Pearson coefficient $r=0.8$; −0.8; 0.82; and −0.81, respectively, $p<0.05$). XYL addition increased while TG 0.3% addition decreased dough volume after 30 min of fermentation, and GOX incorporation did not change it. It is known that soy protein incorporation to wheat dough increments dough resistance drastically (Maforimbo et al. 2006; Roccia et al. 2009) and decreases the gas retention capacity (Ribotta et al. 2005). Formation of new covalent bonds in the gluten network, as a consequence of TG addition changes a very weak gluten into a very strong one (Larré et al. 2000). The treatment of wheat–soy dough with high doses of TG produced an over-reinforced dough that could explain the negative effect on dough expansion capacity. On the other hand, the improvement on rheological parameters by XYL addition, that is the increment of dough extensibility, was reflected on the increment of dough expansion during the fermentation process.

Effect of Enzymes on Bread Quality

The addition of enzymes to wheat–soy dough had a different, negative and positive, influence on specific loaf volume (SLV) (Table 3). The SLV showed a similar

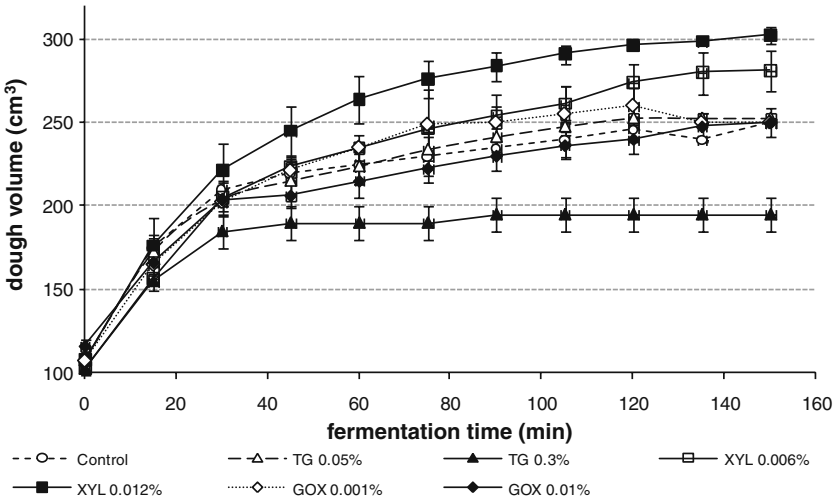

Fig. 1 Effect of enzymes on evolution of dough expansion capacity through the fermentation periods of wheat–soy dough. *TG* transglutaminase, *GOX* glucose oxidase, *XYL* xylanase

tendency to the dough expansion capacity (Pearson coefficient $r=0.85$, $p<0.05$). The major improving effect on SLV was provided by XYL. Similar results were found in wheat–soy bread by Ribotta et al. (2010) and Jiang et al. (2005) who reported that the specific volume in wheat bread increased by raising enzyme concentration up to 120 ppm. This positive effect of XYL on SLV is probably due to the influence of this enzyme on water absorption, increase in dough viscosity, redistribution of bound water over the flour components such as gluten, and consequently on the viscoelastic properties of dough.

The addition of low GOX concentration (0.001%) yielded loaves with a significant high specific volume, while the highest dose of GOX (0.01%) only showed a slight, but not significant, improvement of the SLV. Similar effects were observed by Bonet et al. (2006) on wheat bread. These authors reported that the over-reinforcement of gluten network produced by high amounts of glucose oxidase will retain gas poorly. In agreement, Vemulapalli and Hoseney (1998) established that the negative effect of high doses of GOX might be due to an over-oxidizing effect on the proteins and an intense gelation of water-soluble pentosans by hydrogen peroxide action.

A detrimental effect on SLV was observed as the level of TG was increased, following the trends of dough volume after fermentation. Again, the increment in crosslinks induced by TG among wheat proteins themselves and among soy and wheat proteins themselves, strengthens the dough in such a way that the gas produced by yeast is not capable of extending the dough. These results agreed with those of Basman et al. (2003), who reported the negative effect of transglutaminase on bread with several levels of soy substitution.

The image analysis enabled us to better understand the changes produced in the crumb structure by enzyme incorporation (Table 3). Higher values of cell uniformity (*U*) indicated a greater homogeneity of crumb grain. Low levels of TG incremented *U*. This is in accordance with a previous study on 10% soy flour substitution breads: TG addition produced a poor bread volume but improved the

Table 3 Effect of enzymes on bread quality

Sample	SLV (cm^3/g)	TNC	*U*	*L**	*a**	*b**
Control	3.4^{bc}	226^{ab}	6^{ab}	40.1^{b}	17.7^{ab}	25.8^{bc}
TG 0.05%	3.1^{ab}	229^{abc}	11^{c}	41.0^{b}	18.0^{b}	26.3^{c}
TG 0.3%	2.7^{a}	202^{a}	6^{ab}	48.8^{d}	16.9^{ab}	29.3^{d}
GOX 0.001%	4.1^{d}	252^{abc}	6^{ab}	40.5^{b}	18.1^{b}	25.8^{bc}
GOX 0.01%	3.7^{c}	270^{bc}	7^{b}	44.8^{c}	16.9^{ab}	27.6^{cd}
XYL 0.006%	4.3^{d}	340^{d}	6^{ab}	35.2^{a}	16.8^{a}	17.4^{a}
XYL 0.012%	4.4^{d}	281^{c}	5^{a}	39.9^{b}	17.6^{ab}	23.5^{b}

Values were informed as means. Values followed by the same letter within a column are not significantly different ($p>0.05$)

SLV specific loaf volume, *TNC* total number of cells, *U* grain uniformity, *L**, *a**, and *b** color parameters (CIE-LAB)

TG transglutaminase, *GOX* glucose oxidase, *XYL* xylanase

cell uniformity of crumb (Ribotta et al. 2010). At the same way, Basman et al. (2003) observed that TG addition produced a better crumb cell structure in wheat bread with different levels of soy flour substitution.

No effect on crumb uniformity was observed with XYL and GOX incorporation. However, high SLV values produced by XYL were reflected in an increment of total number of cells (TNC).

In this study, incorporation of enzymes in wheat–soy breads influenced lightness (L^*), yellowness–blueness (b^*), and redness–greenness (a^*) parameters (Table 3). High doses of TG increased L^*, producing a lighter crust color. Similar results were reported by Basman et al. (2003) who informed that wheat–soy bread treated with TG decreased crust darkening if compared with non-treated samples. The TG mechanism of action on lightening of crust color on wheat–soy breads has not yet been established, but it is possible that, since lysine is a substrate of the enzyme, a proportion of this amino acid is blocked by the high TG doses used, and thus becomes unavailable for Maillard reaction.

On the other hand, a darker crust color was obtained by XYL incorporation on bread (Table 3). L^*, b^*, and a^* values showed a tendency to decrease at the lowest dose of XYL addition, and this effect was significant for L^* and b^* parameters.

Effect of Enzymes on Bread Storage

Bread staling is a complex process that is still not completely understood. The most accepted theory postulates that bread firming is mainly caused by recrystallization of starch fraction, involving amylopectin chains (Schoch and French 1947; Zobel and Kulp 1996). Other authors have suggested that the main reason for bread firming is the formation of hydrogen bonds between gluten and starch granules (Martin et al. 1991). It is known that with storage time, the outer crumb loses moisture to the crust, thereby setting up a moisture gradient in the crumb. Rogers et al. (1988) indicated that rate of firming was a function of bread moisture content; as bread moisture decreased, rate of firming increased. Higher values of firmness (F) and chewiness (CH) are associated with poorer bread quality. A strong relationship between SLV and both F_0 and CH_0 TPA parameters, was observed (Pearson coefficient $r=-0.99$ and $r=-0.79$, respectively, $p<0.05$). It has previously been shown that the negative correlation between bread loaf volume and firmness was probably due to more entanglements and interactions that occurred between the more densely packed polymers in samples derived from low-volume breads (Eliasson 1986; Every et al. 1998). Figure 2 shows the influence of enzymes on crumb firmness during bread staling. The initial breadcrumb firmness (F_0) was affected by enzymes incorporation. The low SLV produced by TG may explain the high F_0, if compared to the low F_0 obtained in breads with the greatest volume (XYL and GOX). TG addition incremented F during all days of storage, with respect to the control bread showing a negative effect of this enzyme on storage of wheat–soy breads. This contribution of TG on bread staling was observed by Basman et al. (2003) from wheat–soy breads at different levels of soy substitution. Despite GOX, treated samples decreased the F_0, with respect to the control; however, an increment of F was observed on the second day of storage. Crumb firmness results (Fig. 2) showed the lowest firmness increment over the storage period reached by XYL-bread, suggesting an anti-staling effect of this enzyme. This was more evident as the level of XYL was increased. Similar results were obtained previously in wheat–soy bread (90:10 ratio) (Ribotta et al. 2010). The influence of xylanases on the process of wheat bread staling, especially on the anti-staling action, has been widely reported, but the mechanism of action is not clear (1997; Haros et al. 2002; Jiang et al. 2005). Several author concluded that the presence of AX interferes with the intermolecular associations of both amylopectin and amylose (Kim and D'Appolonia 1977; Courtin and Delcour 2002). In the present study, the positive correlation obtained for loaf volume and crumb firmness can

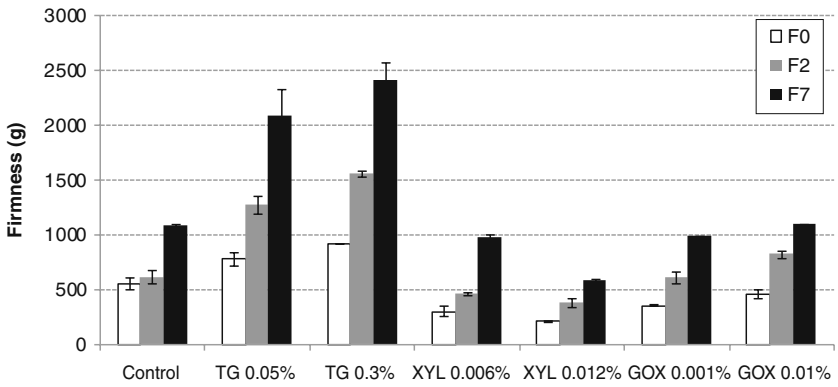

Fig. 2 Influence of enzymes on firmness evolution of wheat–soy breads during storage time. *F*0, *F*2, and *F*7: firmness at the initial, second, and seventh days of storage. *TG* transglutaminase, *GOX* glucose oxidase, *XYL* xylanase

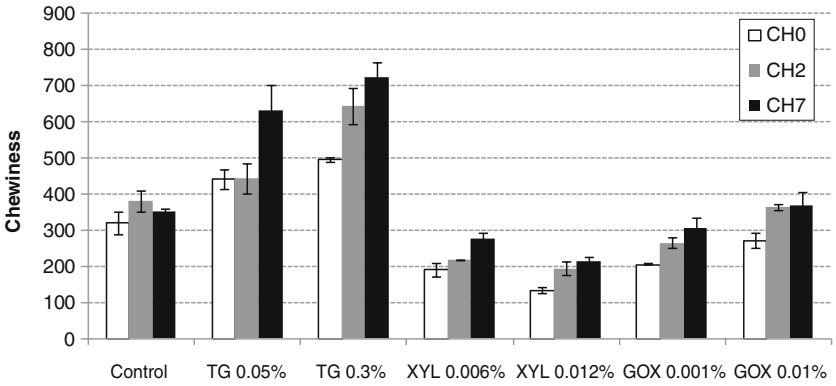

Fig. 3 Influence of enzymes on chewiness evolution of wheat–soy breads during storage time. CH0, CH2, and CH7: chewiness at the initial, second, and seventh days of storage. *TG* transglutaminase, *GOX* glucose oxidase, *XYL* xylanase

explain the decrease of firmness by XYL incorporation according to the theory by Eliasson (1986) and Every et al. (1998) previously described. On the other hand, Matt et al. (1992) postulated that the positive impact of xylanase on bread texture is due to water redistribution from the pentosan phase to the gluten phase. The increase in gluten volume gives more extensibility, which eventually results in a better oven spring.

The chewiness (CH) values (Fig. 3) did not show significant differences for the control sample during all days of storage. In contrast, the enzyme incorporation changed the CH values. In general, CH results showed a similar tendency to the *F* results when control and additive-treated breads were compared. However, differences were observed for the lowest doses of TG and GOX. In spite of TG 0.05% incrementing the CH with respect to the control, the value of CH was kept constant up to the second day of storage; and GOX 0.001% showed a decrease in CH_0 and CH_2 compared to the control bread. These results were probably due to the high association between SLV and CH values.

When dough was heated in the calorimeter, simulating the conditions of baking, two different endotherms were obtained (Ribotta et al. 2003). The first peak is known as the starch gelatinization endotherm, and the second one is defined as the energy required to melt the most stable crystalline structures resulting from the low water level in the sample. The average onset temperature (T_0g) and the gelatinization temperature range (ΔTg) for dough samples were 64.86±0.41°C and 31.42±2.63°C, respectively. The enthalpy of starch gelatinization (ΔHg) ranged between 4.33 and 4.65 mJ/mg, but these results did not presented significant differences ($p>0.05$).

In order to analyze starch retrogradation, DSC-baked dough sample were submitted to a second DSC scan after storage. The experiment conditions did not permit us to see the typical endotherm corresponding to the melting of the amylose–lipid complex (Ribotta et al. 2003). Amylopectin retrogradation was evaluated according to the endotherm at a temperature (57.18±2.15°C) lower than that of gelatinization. Figure 4 shows the influence of enzymes on amylopectin retrogradation (ΔH_r) during storage time. A highly significant correlation (Pearson coefficient $r=0.76$, $p<0.05$) was obtained between ΔH_r and firmness at the second day of storage. Ghiasi et al. (1984) reported that firmness increased linearly between days 0 and 5 when bread was stored at room temperature, but recrystallization of amylopectin was linear between days 0 and 3, and then the rate was slower.

On the second day of storage, XYL addition resulted in a lower firmness value with respect to the control sample and

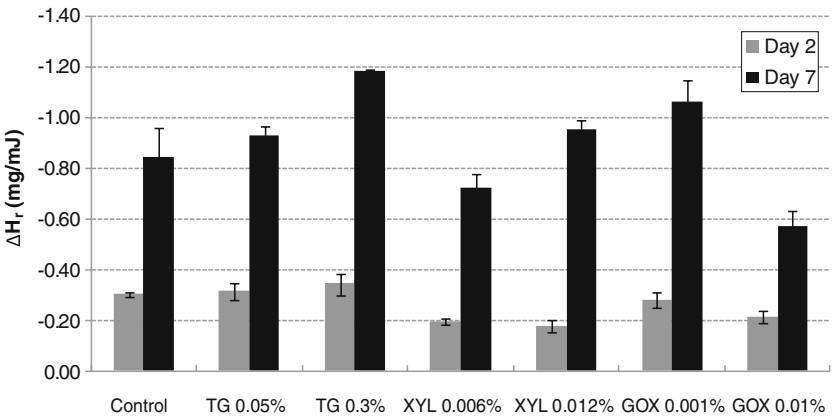

Fig. 4 Effect of enzymes on amylopectin retrogradation (ΔH_r) of wheat–soy breads during storage time. *TG* transglutaminase, *GOX* glucose oxidase, *XYL* xylanase

a lower amylopectin retrogradation, suggesting that the anti-staling effect of this enzyme is not only the result of high SLV values (high correlation between SLV and *F*), but probably also of a more complex mechanism conducting to a less recrystallization of amylopectin (Fig. 4).

On the second and seventh days of storage, incorporation of high doses of GOX produced lower ΔHr values when compared to the control sample. Because of this, the detrimental effect of GOX on firmness is probably related to the low SLV obtained by high levels of GOX addition.

XYL and GOX 0.001% could be incorporated to wheat–soy bread not only for improving loaf volume but also for maintaining bread freshness for 7 days of storage.

Conclusion

The study of the rheological parameters of dough allowed us to understand the mechanism of enzyme action affecting bread quality. The over-reinforcement of dough produced by high doses of TG and GOX addition was the principal factor that influenced on the negative effect of both enzymes on bread volume. However, low doses of TG improved crumb uniformity, and low levels of GOX incremented the SLV significantly.

XYL addition increased dough extensibility, which was reflected on the increment of dough gas retention capacity and expansion capacity and, consequently, on SLV increment. The addition of XYL not only improved dough properties and incremented the specific volume of wheat–soy bread but kept bread fresh for a longer storage time.

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