Research Paper

Effect of xylanase on the technological behaviour of wheat flours

ABSTRACT

The objective of this work is to investigate the effect of enzyme xylanase on the technological behaviour of wheat flours, particularly in alveograms and bread making, using two flour qualities and two bread making methods. The enzyme used was xylanase from *Bacillus subtilis* which is sold for mainly for baking applications. Breads were made applying both the French and the pan bread methods. Experts scored the external and internal characteristics of breads. Increase in enzyme concentration produced a decrease in maximum pressure (P) and tensile strength/extensibility (P/L) but deformation energy (W) remained almost constant in the alveogram. This means that the water released by the hydrolysis of insoluble pentosans has reduced the tenacity of the dough. The higher the enzyme concentration, the lower the dough consistency during kneading. Besides, the greatest improvement of quality was produced when xylanase was added to low quality flour and when the French type bread making method was applied.

Key words: Xylanase, wheat flour, bread, baking, dough.

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INTRODUCTION

Cereals are staple foods for human nutrition and their incorporation into a wide range of products is of great economic importance, wheat being one of the most significant raw materials for food products around the world. It is used in all kinds of applications, breadmaking as one of the most important. In this regard, the assessment of wheat flour quality becomes significantly relevant for the wheat industry. The production of baking products is considered the most accurate method in bread quality evaluation (Colombo et al., 2008). Bread manufacturing process is based on conversions of sugars using microbial or plant-produced enzymes such as amylases, proteases, hemicellulases and xylanases. Bread making relies essentially on yeast fermentation and enzymes are added to enhance fermentation performance (Collins et al., 2006; De Schryver et al., 2008). The main components of flour are starch (70–80%), proteins (10–15%) and nonstarch polysaccharides derived from the cell walls, only accounting for about 3–8%. These components, however, may determine to what extent cereal grains are used (in milling, baking, and animal feed) not only due to their viscosity in aqueous solution but also to their hydration properties (Saulnier et al., 2007). Xylans are the principal type of hemicelluloses, which are main components of plant cell wall polysaccharides (Sun et al., 2007). The role of arabinoxylan (AX) in the formation and properties of wheat flour dough has been the subject of many investigations. Wheat AXs have stimulated much research interest since they have been shown to have significant influence on the water distribution and rheological properties of flour dough, starch retrogradation, and bread quality (Labat et al., 2002). Enzymes are widely used as baking aids since different quality aspects, e.g. flavor, bread volume, crumb structure, and shelf life can be improved with them.

Depending on the type of enzyme used, either hydrolysis or cross-linking of flour biopolymers occurs during the enzymatic treatment. Xylanase can substitute the addition of different emulsifiers and other chemical additives used in bread production. However, for the best results, several xylanases should be used at optimum levels, as the overdosing has adverse effects on the final product (Butt et al., 2008). Xylanases are routinely used in biotechnological processes such as breadmaking and gluten-starch separation. They hydrolyze internal linkages in the backbone of arabinoxylan (AX), a nonstarch polysaccharide that constitutes the major component of wheat grain cell walls and that is either water-extractable (WE-AX) or water-unextractable (WU-AX). This latter AX has strong water-holding capacity, whereas the former forms highly viscous aqueous solutions. WU-AX has a negative effect on bread characteristics, whereas WE-AX and solubilized AX (S-AX) are positive for breadmaking (Trogh et al., 2004; Skendi et al., 2011; Damen et al., 2012). It is clear that xylanases strongly affect AX structure and functionality (Wang et al., 2003; Trogh et al., 2004; Selinheimo et al., 2006; Benejam et al., 2009; Jiang et al., 2010). Schoenlechner et al. (2013) found that xylanase transforms water-insoluble hemicelluloses into soluble forms, which binds water in the dough, therefore decreasing dough firmness, increasing volume and creating finer and more uniform crumbs. Xylanases improve the elasticity of the gluten network, dough tolerance, oven spring, shape and texture. The level of xylanases used in wheat flour depends on both the xylanase activity level of the wheat sample and its different tissues and, hence, on the milling process. Indeed, both endogenous and microbial xylanase activity levels in wheat vary strongly as a function of genetic, climatic, and agronomic factors and a strong correlation has been found between apparent xylanase activity levels and ash contents in flour milling fractions (Dornez et al., 2008). Taking into account nutritional considerations, the increasing content of soluble arabinoxylan in bread improves postprandial glucose and insulin responses in a dose-dependent manner (Lappi et al., 2010). The aim of this study was to research the impact of the enzyme xylanase on the technological behaviour of wheat flours, particularly in alveograms and breadmaking, using two flour qualities and two breadmaking methods.

MATERIALS AND METHODS

Materials

Samples of two wheat flours, suitable for industrial breadmaking, were used for the experience. Sample A, from Molinos Matilde S. A. (Santa Fe – Argentina), had the following chemical characteristics: moisture (wet basis) 13.1 g/100 g, protein 10.7 g/100 g (Nx5.7), damaged starch 5.6 g/100 g, and wet gluten 25 g/100 g. Physical properties at Brabender faringraph were: water absorption, 61.3 g/100 g; development time, 2 min; dough stability, 4.3 min, and dough softening, 50 Brabender units (BU); and physical properties at Chopin alveograph were: deformation energy (W) = 275 Jx10^{-4} and tensile strength/extensibility (P/L) ratio = 1.42. The chemical characteristics of sample B, from cultivars obtained by INTA Pergamino (Buenos Aires – Argentina), were as follows: moisture (wet basis), 13.5 g/100 g; protein, 9.6 g/100 g (Nx5.7); damaged starch, 6.4 g/100 g and wet gluten, 20 g/100 g. The physical properties tested with Brabender faringraph were: water absorption, 61.5 g/100 g; development time, 2 min; dough stability, 4.0 min, and dough softening, 70 Brabender units (BU); while the physical properties tested with Chopin alveograph were: deformation energy (W) = 200 Jx10^{-4} and tensile strength/extensibility (P/L) ratio = 1.30. According to Osella et al. (2008), values of chemical and physical characteristics indicate that deficient breadmaking behaviour of sample B would be expected. The enzyme used in this study was a xylanase from *Bacillus subtilis* which is sold for breadmaking under the name Estabilase XX 6000 and manufactured by Guarner Argentina.

Methods

Physical and chemical analyses were made according to AACC (1994), as follows: moisture 44-19, protein 46-11A, damaged starch 76-30A and gluten-hand washing method 38-10. A Brabender faringraph with 300 g flour sample was used to determine water absorption, development time, degree of dough stability, and degree of dough softening (drop off). A Chopin alveograph with 250 g flour sample was used according to Faridi and Rasper (1987) W (deformation energy), P (maximum pressure), L (rupture length) and G (inflation index) were measured, and P/L (tensile strength/extensibility) was then calculated. Xylanase activity was measured according to the Nelson-Somogyi method at 30ºC in 0.1 M sodium acetate buffer at pH 4.5 (Nelson, 1944; Teleman et al., 2002). One international unit (IU) is defined as the enzyme required obtaining 1 micromole of sugar in a minute.

French type breadmaking

Breads were made as in the baking test for French type bread proposed by Sánchez et al. (1983). The formula contained 300 g flour (14% moisture basis), 6 g yeast, 6 g NaCl and 100 mg/kg ascorbic acid. To study the effect of xylanase, 0, 75, 100 and 125 mg/kg of flour were added to the mixture that mean 0, 9, 22 and 26 IU xylanase/kg of flour. Water was added according to the farinograph water absorption of the control samples (61.3% for sample A and

61.5% for sample B), and was tempered so as to obtain, at the end of kneading, a dough temperature between 24 and 26ºC. Kneading was done in the faringraph at 60 rpm for 15 min, adding NaCl 5 min before the end of kneading. After mixing, the dough was rounded and bulk fermented at 27ºC and 80 % relative humidity, controlling the rising with a push-meter. This device consists of a glass cylinder (75 mm high, 45 mm i.d.) with a tight-fitting plastic piston that records the rising during proofing of 25 g of dough, from 12 to 25 mm, for 50-60 min. After proofing, the dough was divided into 100 and 200 g pieces, then shaped and proofed (final fermentation) at 27ºC and 80% relative humidity, controlling the rising from 15 to 45 mm height with the push-meter, during 70-80 min. Pieces were baked at 210ºC for 30 min using an electric steam oven (Ojalvo S.A.,Santa Fe, Argentina).

Pan bread making

Pan bread manufacturing tests were performed according to the following formulation: 300 g flour (14% moisture basis), 15 g yeast, 6 g NaCl, 18 g sucrose, 9 g shortening (melting point 32ºC), 6 g deffated dry milk and 100 mg/Kg ascorbic acid. To study the effect of xylanase, 0, 75, 100 and 125 mg/kg of flour were added to the mixture that mean 0, 9, 22 and 26 IU xylanase/kg of flour. Water was added according to the faringraph water absorption of the control samples (61.3% for sample A and 61.5% for sample B), and was tempered so as to obtain, at the end of kneading, a dough temperature between 24 and 26ºC. All the ingredients were kneaded together in the faringraph during ten minutes at 60 rpm. After mixing, the dough was rounded and bulk fermented at 27ºC and 80% relative humidity, controlling the rising with a push-meter. The first fermentation ended when the dough doubled its volume (30-40 min). Then, 240 g of dough portions were laminated, rolled up and put in molds for a second fermentation. The proofing time finished when the dough quadruplicated the initial volume, which meant a 15 to 60 mm push-meter displacement, during 55-65 min. Baking molds were 5.5 cm high, 7 x 17.5 cm bottom sides and 9 x 18 cm top sides. Baking was carried out in the electric oven at 215ºC during 28 min.

Bread evaluation

One hour after baking, the cooled breads were evaluated. Specific volume was determined in triplicate by the method of rapeseed displacement. Experts in a number of five, scored the individual characteristics of the loaf which were related to those of a hypothetical standard loaf (total score). As recommended by Pyler (1973) for standard white bread and modified by Sánchez et al. (1983), attributes evaluated and maximum scores were: specific volume, 15 (5 ml/g or higher corresponded to the maximum); crust, 15 (color and thickness); crumb texture, 15 (elasticity and stickiness); crumb color, 10 (cream white being the highest score); crumb structure, 10 (grain); aroma, 15 (fresh-bread like); and taste, 20 (flavor and mouth feeling). Bread score was qualified as follows: excellent (90–100), very good (80–89), good (70–79), acceptable (60–69), poor (50–59), very poor (40–49), and extremely poor (30–39) (Tosi et al., 2002).

Statistical analysis

Results were expressed as the mean of three replicates. Data were analyzed in a software package Statgraphics, trough ANOVA, and the results were compared by Duncan test at a 0.05 significance level.

RESULTS AND DISCUSSION

Alveographic results

Table 1 shows the results obtained with flours A and B with the addition of xylanase enzyme. As the enzyme concentration increases, the overpressure (P) and the ratio P/L decreases, though the energy W remains almost constant. This could be due to the rupture of pentosans chains, which increase the viscosity of aqueous phase but they reduce the dough tenacity. According to Courtin and Delcour (2001), the ability of this enzyme to solubilise insoluble pentosans and its low activity on soluble and solubilised pentosans produce a good performance in breadmaking. Wang et al. (2004) working on the physical characterization of gluten and using the Kieffer extensibility rig, found that Rmax does not change with the addition of xylanase, but results in a larger E at gluten Rmax. Besides, it is necessary to take into account Osella et al. (2008) results, who found a high association between dough properties measured in alveograph and bread quality parameters like specific volume and total score.

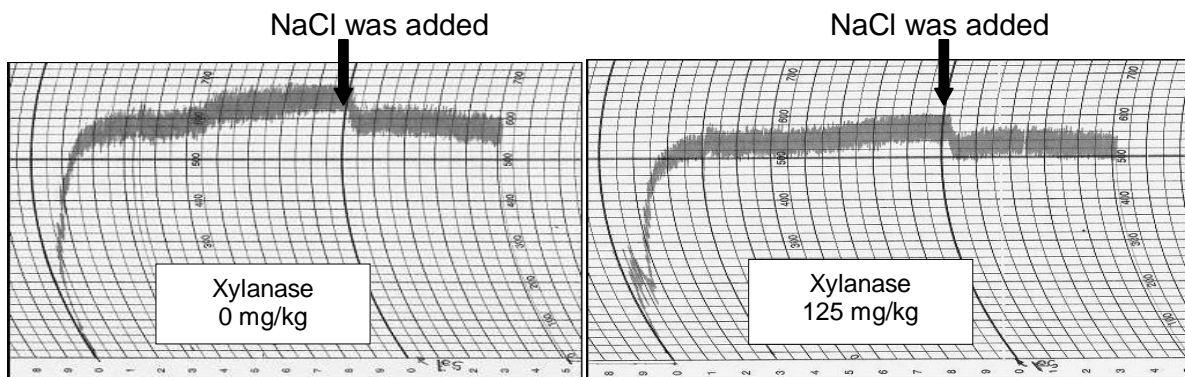### Dough consistency during kneading

Figures 1 and 2 show the behaviour of flours A and B for French type bread and for pan bread, respectively, with regard to dough consistency during kneading in the faringraph, without and with the addition of xylanase (125 mg/kg). The differences observed when comparing the figures are attributed to the different formulations used for French and for pan bread. In all cases, as the amount of enzyme increases, there is a reduction in dough consistency at the end of kneading, though without a significant decrease in consistency during the kneading time. It has

**Table 1.** Alveographic values for flours A and B with the addition of xylanase.

Xylanase (mg/kg of flour)	Flour A				Flour B			
	P	G	P/L	W	P	G	P/L	W
0	115	20	1.42	275	95	19	1.30	200
75	110	20	1.36	270	92	19	1.26	205
100	106	21	1.20	270	91	19	1.25	200
125	104	20	1.18	260	87	18	1.20	195

The error on the physical properties measurement using Alveograph is approximately 3%.
P: maximum pressure, G: inflation index, P/L: tensile strength/extensibility, W: deformation energy
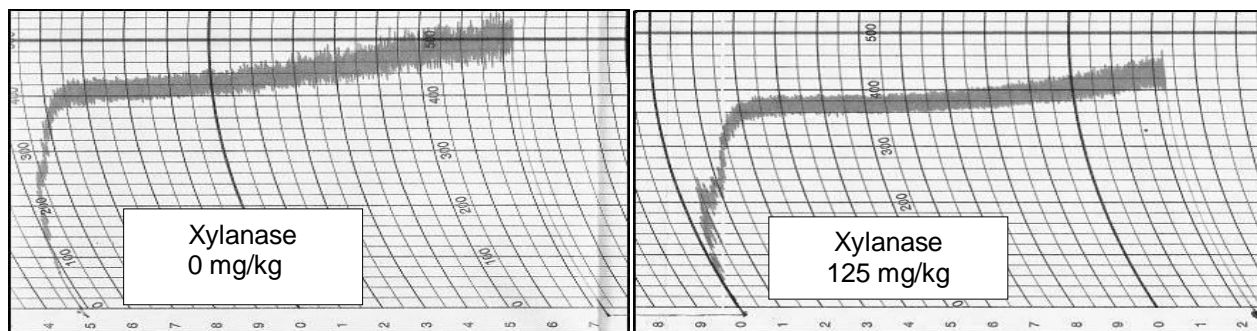
Flour A

Flour B
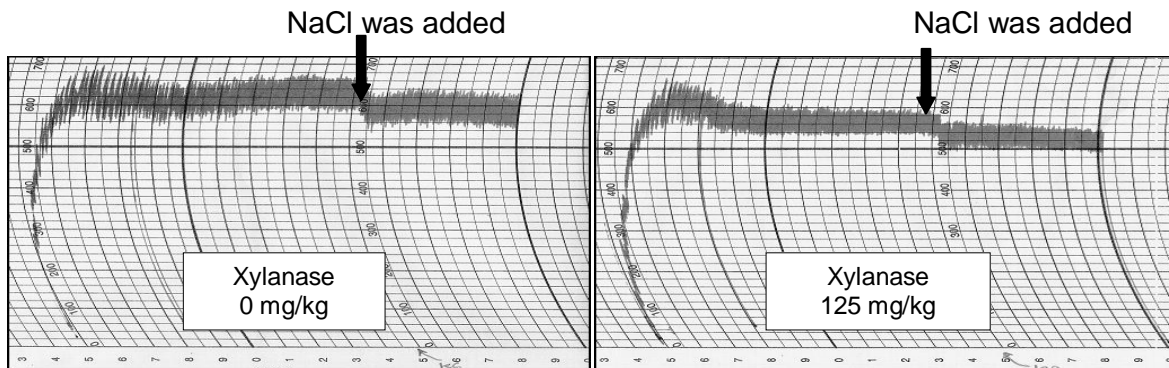
Figure 1. Dough consistency during kneading of French type breadmaking for flours A and B with xylanase addition.

been proved that high dosages of xylanase can lead to a loss of dough water holding capacity (Trogh et al., 2004). The decrease in consistency obtained with the addition of xylanase produces no stickiness in hand, a fact that should be considered very positive since it results in a great improvement in dough machinability.
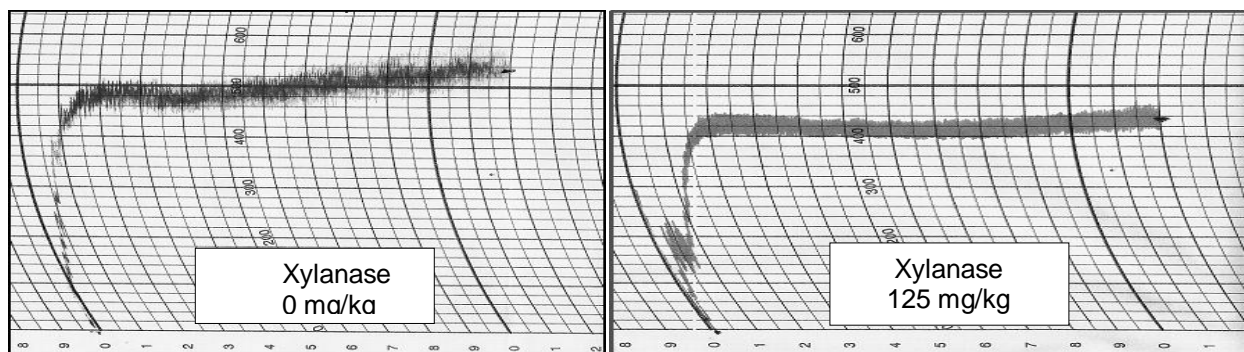
Bread specific volume

Tables 2 and 3 show values of specific volume with the addition of xylanase for French type and for pan type bread, respectively. As regards French type bread, the specific volume increases in both flours with increasing levels of

Flour A

Flour B

Figure 2. Dough consistency during kneading of pan bread making for flours A and B with xylanase addition.

Table 2. Specific volume of French type bread for flours A and B with xylanase addition

Xylanase (mg/kg of flour)	Flour A		Flour B	
	Specific volume (ml/g)	Increase compared to control (%)	Specific volume (ml/g)	Increase compared to control (%)
0	4.0[c]	--	3.5[c]	--
75	4.3[b]	7.5	4.1[b]	17.1
100	4.7[a]	17.5	4.8[a]	37.1
125	4.8[a]	20.0	4.9[a]	40.0

The error on the specific volume determination using rapeseed displacement method is approximately 2%.
Different letters mean significant differences at $p \leq 0.05$

xylanase, the increment being particularly evident when the flour used is of low quality, such as flour B. In the case of pan bread (Table 3), although no increase of specific volume is detected in flour A at increasing levels of xylanase, a moderate increase is obtained with flour B. The improvement in specific volume of bread is caused by a favourable condition of the dough for gas retention. This condition may be a consequence of the hydrolysis of insoluble pentosans, which also leads to an increase in the medium viscosity, thus facilitating the achievement of a greater volume of bread. According to Trogh et al. (2004), xylanases can hydrolyze insoluble compounds, resulting in

Table 3. Specific volume of pan bread for flours A and B with xylanase addition

Xylanase (mg/kg of flour)	Flour A		Flour B	
	Specific volume (ml/g)	Increase compared to control (%)	Specific volume (ml/g)	Increase compared to control (%)
0	5.1[a]	--	4.0[c]	--
75	5.1[a]	--	4.4[b]	10.0
100	5.1[a]	--	4.8[a]	20.0
125	5.2[a]	2.0	4.7[a]	17.5

The error on the specific volume determination using rapeseed displacement method is approximately 2%.
Different letters mean significant differences at p ≤ 0.05
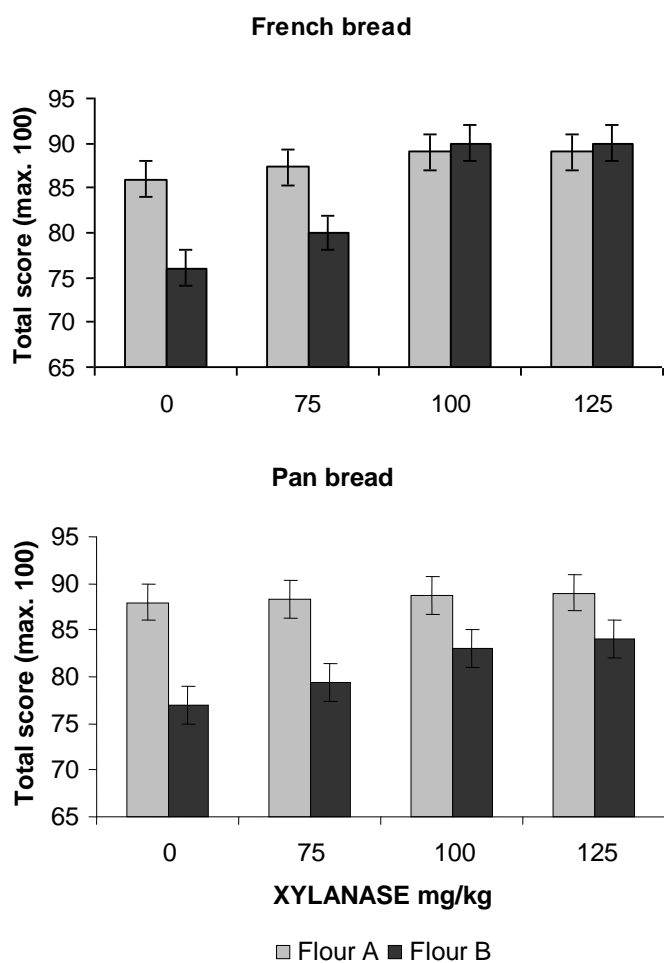
Figure 3. Total score for French bread and pan bread made with flours A and B with xylanase addition.

by counteracting excessive aggregation of gluten (Primo-Martin et al., 2003). In the case of French type bread, the fermentation time is longer than that in pan bread, which can be the cause of the greater effect of the enzyme. This fact leads to the use of French breadmaking as a technological preference when trying to evaluate the effect of a xylanase.

Bread subjective evaluation

Figure 3 shows the increasing total score with xylanase addition. The greatest improvement was produced when xylanase was added to flour B, using any of the proposed technological processes. For French type bread, the total score increases by 4.7, 17.3 and 17.8% when xylanase was added at 75, 100 and 125 mg/Kg, respectively. With regard to pan bread, the magnitude of the total score increases for flour B is 8.7, 11.6 and 13.1% for 75, 100 and 125 mg/kg of xylanase, respectively.

In sum, this total score could be corroborating that addition of xylanase has a greater effect on weak versus strong flours.

Conclusions

Regarding physical properties of dough, xylanase is seen to produce a softening effect on the dough tenacity, which is evidenced through the alveogram values P and P/L. This action of xylanase improves the technological performance of flours, particularly for those flours which have a low technological behaviour using the process of French type breadmaking.

the release of soluble components and, consequently, in an increased viscosity of the aqueous phase. Pentosans associated with gluten could influence dough handling properties, decreasing the gluten tenacity due to steric hindrance. Thus, the use of xylanase would improve quality

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