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Synergistic effects of bacterial xylanase and glucose oxidase for substitution of chemical oxidant in baking wheat flour

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ABSTRACT

Individual and combined effects of commercial enzymes were studied on the rheological properties of dough wheat flour, in order to substitute the chemical oxidant Azodicarbonamide (ADA). A bacterial xylanase (XYL-B) and a glucose oxidase (GOX) were tested in a standard strong flour, destined to industrial baking. Falling Number (FN), farinography and alveography analysis were evaluated. The best individual concentrations identified to XYL-B and GOX were 25 U.kg^{-1}. After, XYL-B and GOX were tested in combination to find the best mixture. The final combination of enzymes was AMY-B (166 U.kg^{-1}), AMY-M (133 U.kg^{-1}) – bacterial and maltogenic α-amylases – , XYL-B (150 U.kg^{-1}) and GOX (8 U.kg^{-1}). It was identified a synergistic effect between XYL-B and GOX to improvement of W value (10^{-4} J) and the P/L ratio, prejudiced by the FN adjustment. The final enzyme mixture was compared with ADA, and, the enzyme mixture was more effective to flour rheology improvement.

Keywords: Enzymes; rheology; azodicarbonamide; Falling Number; farinography; alveography.

INTRODUCTION

Wheat flours used to baking generally require a correction of α-amylase activity for adjustment to baking process, performed mainly by the addition of commercial α-amylase. However, this adjustment often results in negative distortion on other rheological parameters that imply in the quality of flour and its bread making potential (BARRERA et al., 2015). In order to minimize these side effects, the wheat milling industry often employ chemical oxidants, as azodicarbonamide (ADA), and enzymes preparations.

ADA is a synthetic oxidizing agent that quickly oxidizes the S-H groups of glutenin by the formation of S-S groups. However, ADA is linked to a high-level of allergies and diseases, and its use have been banned in many countries and subject to heavy penalties for the non-compliance (BECALSKI et al., 2006; YE et al., 2011).

On the other hand, treatments with enzymes have been preferred to the addition of chemical improvers since they are natural, highly specific and completely safe to health. Enzymes as amylases, xylanases and glucose oxidases are known by its beneficial effects on rheology, mainly by improvement of machinability parameters and strengthening of dough (BARRERA et al., 2015). Commercial enzymatic preparations usually have a mixture of these enzymes that acts in different fractions of flour, according to their specific action mode. However, when used together, they do not have always the same standard behavior as for their individual performance. (PRIMO-MARTÍN et al., 2005; CABALLERO et al., 2007).

Therefore, based in this lack of knowledge of combined effects of enzymes on dough rheology, the aim of this work was to evaluate the effects of xylanase and glucose oxidase

when used in combination, on rheology parameters of a strong baking flour in comparison with the effects promoted by the chemical oxidant agent azodicarbonamide.

MATERIAL AND METHODS

The following enzymes were tested: Bacterial α-amylase (AMY-B) Spring Alfa Bac 7500 from *Bacillus amyloliquefaciens* (Granolab); maltogenic α-amylase (AMY-M) Veron Mac from *Bacillus stearothermophilus* (AB Enzymes); Bacterial xylanase (XYL-B) Veron RL from *Bacillus subtilis* (AB Enzymes) and Glucose oxidase (GOX) Spring Gluz from *Bacillus subtilis* (Granolab). For comparative purposes, enzymatic activities were determined and expressed as $U.g^{-1}$ of commercial preparation: AMY-B (1800 $U.g^{-1}$); AMY-M (2079 $U.g^{-1}$); XYL-B (2077 $U.g^{-1}$) and GOX (2376 $U.g^{-1}$).

The chemical oxidant used was ADA 100% (Granotec), suggested dosage 5 – 40 ppm.

The flour sample were provided by Tondo S.A. (Forqueta, RS) without previous bleaching and chemical or enzyme additives, and it was composed by a mix of the Brazilian (50 %), American (10 %) and Argentinian (40 %) wheat; with an extraction of 70 % and classified as Type 1, commercially designed for baking. The flour specifications was: moisture 13.30 %; Ash – dry weight 0.48 %; Gluten – dry weight 8.5 %.

The rheological analysis were executed according official (AACC, 2009).

The enzymes were added to the wheat flour individually, varying their concentrations from 0 to 833 $U.kg^{-1}$ of flour, and all rheological parameters were measured. Based in these results, XYL-B and GOX were evaluated in combination for identification of synergistic interaction to improvement of dough rheology. The statistical analysis of results was carried out using Statistica 12.0 (Statsoft, USA), by analysis of variance (ANOVA) and Tukey's test ($p \leq 0.05$).

Enzymatic treatments were compared to the chemical oxidant azodicarbonamide (ADA). The FN was adjusted by the addition of α-amylase (previously identified) and ADA was tested in the concentrations 5 and 10 ppm.

RESULTS AND DISCUSSION

Table 1 presents the effects of XYL-B and GOX when used individually in the wheat flour. As can be seen on Table 1, XYL-B presented a small influence on FN ($p \leq 0.05$) in comparison to the control. One possible explanation for that is the fact that the FN is a viscosimetric method, which has a reduction on the viscosity of the analyzed suspension when the xylanases break the arabinoxylans (AX), decreasing the water-binding capacity, and resulting in a false effect on the FN.

Additionally, the lower water absorption caused by the AX depolymerization by xylanases activities, also affected the reduction of the DT and ST by softening dough structure. XYL-B promoted beneficial effects in the most of the rheological parameters at the lowest studied concentration of 25 $U.kg^{-1}$ (12 ppm).

GOX presented positive effects on water absorption at 250, 583 and 833 $U.kg^{-1}$, without statistical difference ($p \geq 0.05$) among them. At these concentrations, GOX increased the WA from 57.1% (control) to around 58.1 %, while at 333 $U.kg^{-1}$ caused an undesirable decrease in this parameter. It also increased the DT until 583 $U.kg^{-1}$ followed by a good improvement on stability time from 150 to 333 $U.kg^{-1}$.

Table 1: Concentration effects of individual enzymes on rheological parameters of flour.

Enzyme	Enzyme Concentration	FN	Farinographic parameters*			Alveographic parameters*	
	(U.kg^{-1} flour)	(s)	WA (%)	DT (min)	ST (min)	W (10^{-4} J)	P/L
Ideal Ranges	-	250 - 320	> 56	4 – 10	> 15	200 - 300	0.88-1.2
XYL-B	0	420 a	57.1 b	12.1 b	22.8 a	221 a	1.31 ab
	25	420 a	57.2 b	13.4 a	18.3 d	207 b	1.06 bc
	150	394 d	57.5 a	12.1 b	22.0 b	162 e	1.01 c
	250	399 c	57.3 ab	12.1 b	17.5 e	192 c	0.89 c
	333	413 b	57.1 b	12.2 b	20.4 c	141 g	1.47 a
	583	390 e	56.4 c	11.5 c	16.5 f	172 d	1.07 bc
	833	400 c	55.7 d	8.5 d	15.3 g	156 f	1.0 c
GOX	0	420 a	57.1 a	12.1 cd	22.8 c	221 c	1.31 f
	25	407 d	57.2 a	13.8 bc	20.1 d	255 a	1.32 f
	150	411 c	57.7 a	17.1 a	27.0 a	207 e	1.94 e
	250	407 d	58.1 a	16.8 a	23.2 bc	216 d	2.33 d
	333	415 b	56.8 a	17.1 a	25.1 ab	189 f	2.85 c
	583	408 d	58.1 a	14.6 b	20.1 d	187 f	5.0 a
	833	409 cd	58.3 a	11.0 d	15.6 e	224 b	4.44 b

* Same letters in the same column, for each enzyme, are statistically equal by Tukey's test ($p < 0.05$).

The most important effect for GOX was on P/L ratio, which increased with the enzyme concentration. This indicates a strengthening of the dough by increasing the tenacity in detriment of the extensibility, which has been related by the formation of additional protein crosslinks via disulfide linkages (PRIMO-MARTÍN et al., 2005). This strengthening of the dough was also observed by the W value, where with the increase in the enzyme concentration, it was reduced, except at 25 U.kg^{-1} (10 ppm), when the concentration of GOX was not enough to rigidify the gluten network.

Based in these results, XYL-B and GOX were tested in combination and the results are presented in Table 2.

Table 2: Effects of combination of XYL-B and GOX on dough rheology.

Assay*	XYL-B	GOX	FN	WA	DT	ST	W	P/L
Ideal ranges**	-	-	250 - 320	> 56	4 – 10	> 15	200 - 300	> 0.88
Control	-	-	420	57.1	12.1	22.8	221	1.31
1	150	25	300	56.2	15.1	22.4	181	1.36
2	200	25	276	56.1	14.6	21.0	181	1.25
3	150	16	265	55.6	12.1	20.8	194	1.06
4	200	16	296	55.9	14.5	21.0	144	1.81
5	200	0	275	55.7	14.9	20.4	138	2.24
6	150	8	303	55.7	13.1	21.2	239	1.17

* All assays contained AMY-B (166 U.kg^{-1}) and AMY-M (133 U.kg^{-1}).**(PIZZINATTO et al., 2004; CAUVAIN e YOUNG, 2006).

Initial tests showed than XYL-B concentration should range from 150 – 200 U.kg^{-1}, while GOX from 0 – 25 U.kg^{-1}. Initially, GOX concentration was 25 U.kg^{-1} and tested with two concentrations of XYL-B (150 and 200 U.kg^{-1}). However, the W was not improved and the P/L ratio remained at high values, indicating that there is still a high strength of the dough caused by excess of GOX dosage. Using 16 U.kg^{-1} of GOX, at 150 U.kg^{-1} of XYL-B, the DT and P/L ratio were desirably reduced and W was improved. Increasing the concentration of XYL-B to 200 U.kg^{-1}, the results were out of the standard range for DT, W and P/L ratio. When GOX was eliminated, it was observed the worst result for W and P/L ratio, confirming its importance in the enzyme mixture. Therefore, after the FN adjust by AMY-B (166 U.kg^{-1}) and AMY-M (133 U.kg^{-1}), it was possible to obtain ideal dough rheological characteristics by the combined addition of XYL-B (150 U.kg^{-1}) and GOX (8 U.kg^{-1}).

The comparison of enzymatic and chemical treatment is presented on Table 3.

Table 3: Comparison of chemical and enzymatic treatment for flour.

Assay	AMY-B ($U.kg^{-1}$)	AMY-M ($U.kg^{-1}$)	ADA (ppm)	FN	WA	DT	ST	W	P/L
Ideal ranges	-	-	-	250 - 320	> 56	4 – 10	> 15	200 - 300	> 0.88
Control	-	-	-	420	57.1	12.1	22.8	221	1.31
Optimal condition	-	-	-	303	55.7	13.1	21.2	239	1.17
1	166	133	0	289	55.7	17.0	22.5	166	2.34
2	166	133	5	289	54.9	14.8	23.5	200	1.81
3	166	133	10	287	55.1	14.4	21	183	2.63

As could be seen on Table 3, even using 10 ppm of ADA, most of the rheological parameters could be adjusted to the ideal ranges, but W value reduced and P/L ratio increased, showing an excessive strengthening of the dough.

CONCLUSIONS

It can be concluded that only the FN adjustment by amylases, and the addition of a chemical oxidant, it is not enough to improve and frame the rheology of wheat flours aimed to industrial baking. However, the reduction on development time and P/L ratio (suitable for baking) promoted by XYL-B, and the strengthening effect promoted by GOX on W (10^{-4} J) value and stability time, showed that their use, individually or in combination, is a more efficient alternative to flour correction. Moreover, by the combination of these enzymes, it was identified a synergistic effect between them, allowing reduce the required concentrations to improve the flour rheology.

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