

*Full Length Research Paper*

## Influence of enzymes and ascorbic acid on dough rheology and wheat bread quality

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The combined action of ascorbic acid and two commercial enzymatic complexes containing amylase and xylanase/amylase was analyzed to determine their effects on dough rheology and bread quality. Seven bread formulations containing different concentrations of these improvers were used in the analysis. The rheological properties of each dough formulation were determined by moisture, gluten and farinograph tests. The breads were also characterized in general aspect - especially shelf-life - based on the presence of fungi. The dough rheology results showed that the formulation developed in the presence of 0.01% xylanase/amylase and 200 ppm of ascorbic acid was more efficient. Improved shelf-life was obtained from the formulations containing xylanase. The results showed that some technological characteristics of dough rheology and gluten index produced from the combination of these improvers can indicate in order to obtain specific features of the bread.

**Key words:** Food biotechnology, xylanase, amylase, dough properties, bread improvers.

### INTRODUCTION

The development of baking technology is a phenomenon that has had a great impact on the food industry, and has increased the acceptance of food by the consumers (Asghar et al., 2011; Eddy et al., 2007). The use of different additives such as emulsifiers, oxidants and enzymes to improve the quality of bread is nowadays common practice (Nanditha and Prabhasankar, 2009; Asghar et al., 2011; Barrera et al., 2015). In Brazil the

consumption of bread reaches 30 kg per capita. Due to this high demand, the Brazilian market imports about 50% of the wheat for domestic consumption from Argentina and Canada. The Brazil is the second largest importer of wheat in the world, consuming 9.5 tons per year (Scheuer et al., 2011). Different imported wheat blended with the national cultivars make it difficult to maintain the rheological properties of flour, as a

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**Table 1.** Additives amount utilized in the breads formulations.

Additives	Form 1	Form 2	Form 3	Form 4	Form 5	Form 6	Form 7	Form 8
Life spring B ( $\alpha$ -amylase)	-	-	0.02%	0.02%	-	0.05%	-	0.02%
Spring 2002 (Xylanase/Amylase)	-	-	0.01%	-	0.01%	0.02%	0.01%	-
Ascorbic acid	-	200 ppm	200 ppm	200 ppm	200 ppm	500 ppm	-	-

Without additives (-); Form (formulation); Form1, standard formulation. Are indicated the amount of additives to 200g of flour.

consequence, dough and bread quality are affected (Scheuer et al., 2011). Similarly, there are many wheat varieties in the world, some of which are useful for bread making. Differences in baking quality of flours are affected by phenotypes and genotypes of wheat, and these factors determine the particular rheological properties of the bread (Gholamin and Khayatnezhad, 2011; Mirsaeedghazi et al., 2008).

Therefore, due to the variable technological quality of flour, the use of additives has become important to standardize the flour in terms of gluten strength, color and fermentability (Pecivová et al., 2010; Nanditha and Prabhasankar, 2009). Likewise, the formation of a gluten network is essential for the production of bread with organoleptic qualities especially dough formation and bread crumb texture. The gluten network is responsible also for dough elasticity, resistance and stability, while carbon dioxide production is due to the action of enzymes and yeast on sugar (Enriquez et al., 2003; Aamodt et al., 2003). Thus, to improve the gluten network formation, the baking industry has been using flour improvers, among the oxidizing agents, which act directly on the structure of the gluten proteins, reinforcing the gluten network by the formation of disulfide bonds (Elkassabany and Hosney, 1980).

Among the oxidizing agent quite studied is ascorbic acid. It was stated that it has influence on the fermented dough behavior and the correlation with the flour composition, such as the elucidation of the mechanism as improver on bread (Hrušková and Novotná, 2003; Every et al., 1999). Another type of wheat flour improvers are enzymes (Asghar et al., 2011). The enzymes most commonly used in baking are amylases, protease, glucose-oxidases and xylanases (Barrera et al., 2015; Moayedallaie et al., 2010; Kara et al., 2005; Martínez-Anaya and Jimenez, 1997). Thus, several studies have been conducted to elucidate the action of bread improvers and the combined effect of these food additives on bakery products (Stojceska and Ainsworth, 2008; Katina et al., 2006), but few have aimed at determining the effects of the combined action of ascorbic acid with xylanase and amylase on process. This study aimed to analyze the effects of ascorbic acid and enzymes on dough rheology properties and consequently bread quality. These formulations combine ascorbic acid and two commercial enzymatic complexes containing amylase and xylanase/amylose.

## MATERIALS AND METHODS

### Materials

The wheat bread was prepared using wheat flour Specht (Food Products Ltd., Joaçaba, Brazil; moisture content 10%, protein 14%, fat 2.2%, ash 1.8%, carbohydrates 72%), dry yeast (Levsaft - Lesaffre, Rio Janeiro, Brazil), edible salt, sugar (Alto Alegre, São Paulo, Brazil), vegetable fat (Qualy, Paraná Brazil) and ascorbic acid (L-AA; Sigma-Aldrich).

The commercial enzymes used in the experiments were suitable for baking and produced by Granotec SA - Nutrition and Biotechnology (Curitiba, Brazil) with the following specifications: xylanase, endo-1,4-beta-xylanase produced by *Aspergillus oryzae* (spring 2002 product brand; 4,260 U/g) and  $\alpha$ -amylase, a maltogenic alpha-amylase produced by *Bacillus stearothersophilus* (life spring B; 5,200 U/g).

### Determination of enzymatic activity

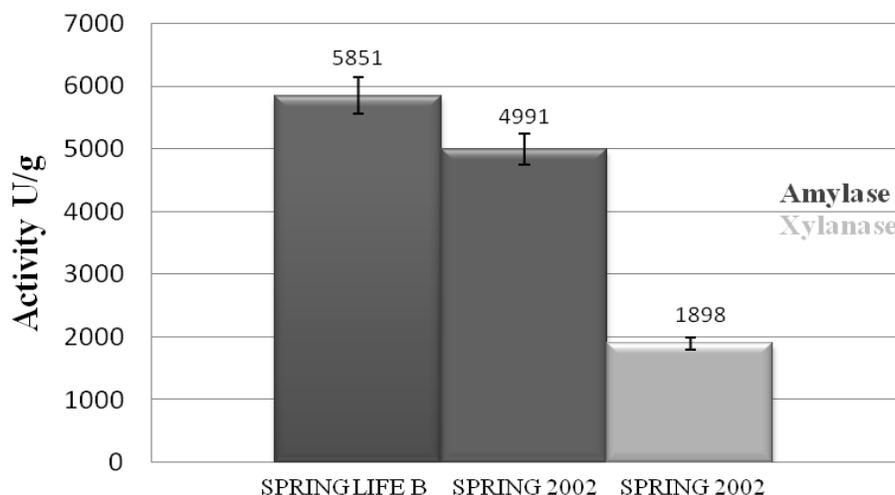
The xylanase activity (Endo-1,4-beta-xylanase; E.C. 3.2.1.8) was measured according to Shah et al. (2006) and the  $\alpha$ -amylase activity ( $\alpha$ -1,4-glucan glucanohydrolase, EC 3.2.1.1) was used the method of determining the starch saccharification activity, according Ammar et al. (2002). One unit of enzymes was defined as the amount of enzymes that produced 1  $\mu$ mol of reducing sugar per minute under standard conditions.

### Bread making process

The dough of the wheat bread was prepared using the following formulation: wheat flour, 200 g; sugar, 10 g; edible salt, 4 g; dry yeast, 0.15 g; fat, 20 g and water, 100 mL (Standard – without bakery additives). The test formulations were elaborated containing seven different combinations of enzymes and ascorbic acid (Table 1). The breads were produced in a standardized manner by the straight dough method, in which the ingredients were added to a planetary mixer (Arno, CL 390, São Paulo, Brazil). The dough was kneaded for 2 min at medium speed and then shaped by the cylinder (G Paniz, CS 390, São Paulo, Brazil). Each bread dough, obtained with different formulation (8 formulations drawn up in triplicate) was fermented for 2 h in a fermenter (Imeca, CS 390, Bauru, Brazil) at 35°C with 75% of humidity and baked for 25 min at 180°C at electrical cooker oven (Progás, Turbo Light intelligent PRP, Caxias do Sul, Brazil). Then breads were cooled for 30 min at room temperature, sliced and packaged for shelf-life analysis.

### Determination of dough rheological properties

The rheological properties of each dough formulation were determined by moisture, gluten and farinograph tests. Analyses of wet, dry and index gluten were performed according to the AACC International Approved Method (AACC, 2000) No. 38-12, using the



**Figure 1.** Enzymatic activity of commercial preparations life spring B and spring 2002.

Glutomatic device (Perten Instruments, Huddinge, SWEDEN). Dough moisture was measured according to AACC method 44-15. The farinograph properties were determined using AACC method 54-21 using Brabender® (Brabender OHG, Duisburg, Germany). The criteria assessed were water absorption, dough development time, dough stability, tolerance index, time to exhaustion and farinograph quality. The analyses were performed in triplicate.

#### Bread analysis

Breads obtained from each formulation were analyzed for shelf-life and microbiology parameters. Once chilled for 1 h at room temperature, the breads were sliced with 1.0 cm of thickness and 5 central slices were designated for the analysis of shelf-life estimative and visually structure. The slices were kept in the original container in a bacteriological incubator at 25°C and observed daily for two weeks for the presence of fungi on the surface, sides and bottoms of the samples. In addition, the breads were compared visually as to crumb structure, shell color and crumb color, and judged qualitatively by experienced bakers.

#### Statistical analysis

The results were subjected to statistical analysis of variance (ANOVA) and Tukey's multiple comparison test. The statistical differences in the samples were tested with  $p < 0.05$  using the BioStat 5.0 software (South America).

## RESULTS AND DISCUSSION

Firstly the enzymatic activities of the two commercial enzymes for the presence of xylanase (spring 2002) and amylase (spring life B) was determined. According to the results, the additive for baking spring life B presented an  $\alpha$ -amylase activity of 5,851 U/g, slightly higher than specified on the package (5,200 U/g). Moreover, the xylanase activity of the product spring 2002 was 1,898 U/g, significantly lower than the specified by the

enterprise (4,260 U/g), and also showed amylase activity of 4,991 U/g (Figure 1), not informed by the manufacturer. This data is important because the combined use of enzymes can lead to the over-amylase; this is an overdose of  $\alpha$ -amylase and in consequence leading to deleterious effects on bread (Van der Maarel et al., 2002).

In preliminary analysis (data not shown), the best concentration of each commercial improver was determined separately. The enzyme additives containing  $\alpha$ -amylase (life spring B) or xylanase/amylase (spring, 2002) showed better results of bread volume and properties of dough rheological in a concentration of 0.02 and 0.01%, respectively, an intermediate concentration according to manufacturer's indications. Based on these results the design of the formulations was elaborated. The results of the analysis of dough rheological properties (farinograph, moisture and gluten) for the seven formulations tested showed that, in comparison with the standard formulation (control without improvers) or with each other, some parameters improved while other parameters worsened as summarized in Tables 2 and 3.

An important test to check the action of improvers on dough rheology is the farinograph (Malomo et al., 2011). As regards the farinograph characteristics analyzed, water absorption was not significantly different ( $p > 0.05$ ) in the standard and test formulations (Table 2). However, dough development time was significantly lower for the formulation containing  $\alpha$ -amylase and ascorbic acid (formulation 4), and longer for the formulation containing xylanase/amylase and ascorbic acid (formulation 5). Although a shorter development time indicates that the gluten network formation takes less time, with less energy input on the dough, on the other hand this may reflect a weakened dough and low gluten quality (Dua et al., 2009). These data are evidenced by the tolerance index, breakdown time and stability parameters, in which

**Table 2.** Dough rheology of the formulations based on the farinograph analysis.

Parameters	Form 1	Form 2	Form 3	Form 4	Form 5	Form 6	Form 7	Form 8
Water absorption (%)	<b>61.9±2.0<sup>A</sup></b>	<b>59.2±2.2<sup>A</sup></b>	<b>58.8±2.1<sup>A</sup></b>	<b>58.2±2.0<sup>A</sup></b>	<b>59.1±2.2<sup>A</sup></b>	<b>59.1±2.3<sup>A</sup></b>	<b>60.7±3.0<sup>A</sup></b>	<b>61.5±2.0<sup>A</sup></b>
Development time (min)	1.7±0.3 <sup>A</sup>	1.8±0.3 <sup>A,B</sup>	1.7±0.2 <sup>A</sup>	1.5±0.1 <sup>B</sup>	3.0±0.3 <sup>C</sup>	1.9±0.2 <sup>D</sup>	1.9±0.1 <sup>D</sup>	1.7±0.3 <sup>A</sup>
Stability (min)	<b>8.0±0.3<sup>A</sup></b>	<b>3.3±0.5<sup>B</sup></b>	<b>1.6±0.4<sup>C</sup></b>	<b>1.6±0.5<sup>C</sup></b>	<b>8.8±0.2<sup>D</sup></b>	<b>2.4±0.5<sup>E</sup></b>	<b>3.4±0.5<sup>E</sup></b>	<b>6.7±0.6<sup>F</sup></b>
Tolerance index (BU)	46±2.5 <sup>A</sup>	79±2.3 <sup>B</sup>	123±5.0 <sup>C</sup>	123±4.0 <sup>C</sup>	38±3.0 <sup>D</sup>	112±6.0 <sup>E</sup>	67±5.0 <sup>F</sup>	55±5.0 <sup>F</sup>
Breakdown time (min)	<b>3.1±0.5<sup>A</sup></b>	<b>3.2±0.4<sup>A</sup></b>	<b>2.3±0.3<sup>B</sup></b>	<b>2.2±0.3<sup>B</sup></b>	<b>6.0±0.4<sup>C</sup></b>	<b>2.6±0.5<sup>B</sup></b>	<b>3.4±0.4<sup>E</sup></b>	<b>3.0±0.2<sup>A</sup></b>
Farinograph quality	31±2.0 <sup>A</sup>	32±2.0 <sup>A</sup>	23±3.0 <sup>B</sup>	22±2.0 <sup>B</sup>	60±5.0 <sup>C</sup>	26±3.0 <sup>B</sup>	34±2.0 <sup>A</sup>	30±3.0 <sup>A</sup>

Form (formulation). The form 1 comprises the standard formulation. Averages followed by the same letter in a row are not statistically different by Tukey test at 95% ( $p < 0.05$ ) probability level. Improved parameters as compared to control are in bold.

**Table 3.** Moisture content and gluten analysis of the formulations.

Parameters	Form 1	Form 2	Form 3	Form 4	Form 5	Form 6	Form 7	Form 8
Humidity (%)	14.6±1.0 <sup>A</sup>	13.7±1.0 <sup>A</sup>	13.9±2.0 <sup>A</sup>	13.8±2.0 <sup>A</sup>	14.1±1.5 <sup>A</sup>	13.8±1.4 <sup>A</sup>	14.3±1.0 <sup>A</sup>	14.4±2.0 <sup>A</sup>
Wet gluten (%)	29.7±0.8 <sup>A</sup>	27.6±1.0 <sup>A</sup>	27.3±1.3 <sup>A</sup>	27.2±1.2 <sup>A</sup>	<b>30.1±1.0<sup>A</sup></b>	29.0±0.5 <sup>A</sup>	29.4±0.9 <sup>A</sup>	27.7±1.1 <sup>A</sup>
Dry gluten (%)	9.6±0.3 <sup>A</sup>	9.0±0.2 <sup>B</sup>	8.2±0.5 <sup>B</sup>	8.6±0.5 <sup>B</sup>	9.6±0.4 <sup>A</sup>	9.5±0.5 <sup>A</sup>	9.4±0.3 <sup>A</sup>	9.6±0.3 <sup>A</sup>
Index gluten	90.2±2.0 <sup>A</sup>	<b>96.4±2.2<sup>B</sup></b>	<b>96.3±2.0<sup>B</sup></b>	<b>98.1±2.1<sup>B</sup></b>	82.8±2.0 <sup>C</sup>	89.4±2.5 <sup>A</sup>	85.0±1.9 <sup>C</sup>	81.2±3.4 <sup>C</sup>

Form (formulation). The form 1 comprises the standard formulation. Averages followed by the same letter in a row are not statistically different by Tukey test at 95% ( $p < 0.05$ ) probability level. Improved parameters as compared to control are in bold.

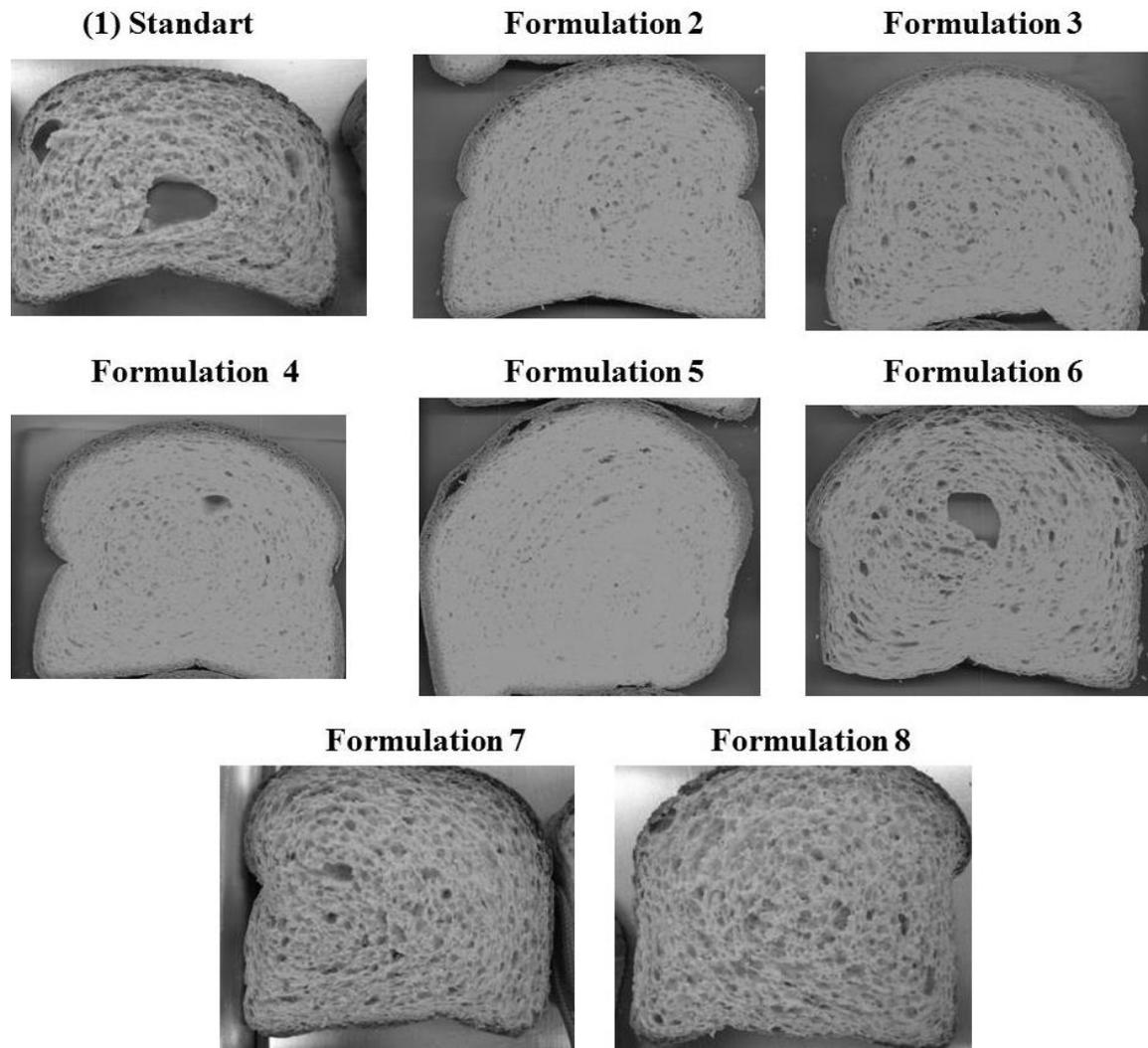
only formulation 5 (xylanase/amylase and ascorbic acid) showed a significant improvement (Table 2). The behavior of these parameters indicate that the flour is considered stronger (Dua et al., 2009), its power is improved as compared with control (formulation 1, without the additives) or with the other formulations.

Farinograph quality indicates the general quality of the mixture and water absorption in the dough of wheat flour. As might be expected, formulation 5 (Table 2) was improved, while formulations 3, 4 and 6 had the worst performance. The other formulations did not change significantly compared with the standard. The deterioration in bread quality can be explained by an excess of improvers or combinations of these, as had been expected for formulation 6, which contained excess of xylanase and especially of amylase (Van der Maarel et al., 2002). Although that bread formulations with different concentrations of xylanase does not present significant differences on bread specific volumes (Jaekel et al., 2012), and of ascorbic acid even in excessive levels are not deleterious in dough (up 200 ppm), perhaps because of the limited presence of oxygen necessary for its action (Hrušková and Novotná, 2003). Formulation 3 shows a possible excess of amylase too since the enzyme is present in both additives - life spring B (5,891 U/g) and spring 2002 (4,991 U/g). According to Van der Maarel et al. (2002), the overdose of amylase makes the dough stickier due to the production of maltodextrin, damaging dough quality. Formulations 4 and 5, on the other hand, showed the highest volume and best structure and color crumb, according to visual comparison of the

experienced bakers. In formulation 4, despite the worsening of the farinograph parameters (with the exception of development time), the action of amylase together with ascorbic acid possibly determined the final quality of the bread (Figure 2). At appropriate concentrations, this enzyme acts on the starch to increase fermentable sugars, interfering directly on bread volume (Katina et al., 2006), once the sugar released is one of the factors that affect the yeast cell growth and consecutively the production of CO<sub>2</sub> gas.

The presence of proteins is another important factor determining the quality of the flour or mixtures (Dua et al., 2009; Enriquez et al., 2003). This is because there is a direct correlation between protein percentage and gluten formation (Malomo et al., 2011), which finally affects bread quality (Ranhotra et al., 1992; Mirsaeedghazi et al., 2008). In the gluten analysis, formulation 5 (which showed better results in a previous analysis) showed no statistically significant difference compared with control, despite having a higher amount of wet gluten (Table 3). However, in the analysis of dry gluten, formulations 3 and 4 showed a decrease, whereas other formulations showed no significant differences. Finally, in the gluten index analysis, formulation 6 showed a value similar to control, while formulations 5, 7 and 8 showed a lower value and formulations 1, 2 and 3 showed a significant increase compared to control (highlighted in Table 3).

The results therefore indicate that there is no direct correlation between index and wet gluten, as suggested by Enriquez et al. (2003). Although the gluten index parameter is indicative of gluten quality and gluten



**Figure 2.** Effects of ascorbic acid and enzymes addition on characteristics of wheat bread.

strength, this is not always associated with a large bread volume (Ranhotra et al., 1992). This was evidenced by formulation 5, which produced a bread with higher volume and the results of gluten analysis showed a lower gluten index. Evidently, this occurs because the factors involved in the final quality of bread are diverse and quite complex. Therefore, rheological parameters such as gluten index are not always able to account for final quality. Rouillé et al. (2000) showed that, despite the interference of different formulations containing ascorbic acid and alpha-amylase with hemicellulase activity, the mixing conditions appeared to be the main factor affecting bread volume. Thus, in our results, among the tested formulations on manufacture condition the best overall results presented was the formulation 5 (Figure 2, Tables 2 and 3).

In general, the analysis of the structure of the breads obtained with different formulations indicated that amylase

(life spring B) was found to have a good impact on bread characteristics such as volume and visual structure, when compared to bread produced with standard formulation. As suggested by Ammar et al. (2002), the use of amylase on bread formulation to the obtainment of bread with a lower weight, higher volume and greater retention of softness, even after several days of conservation. Similarly, the bread made with the addition of xylanase/amylase (spring 2002) presented a smaller alveolar structure and larger volume in visual analysis of professional bakers (Figure 2). According to Shah et al. (2006), the use of partially purified xylanase from *Aspergillus foetidus* on dough has a positive influence on bread attributes such as crumb structure and loaf volume and promotes a significant improvement in textural properties, in accordance with the sensorial evaluation and the rheological properties. This improvement in texture is probably due to the redistribution of water, which increases the volume of

the gluten and increases extensibility, resulting in a better oven spring (Shah et al., 2006). Bread formulations made from whole grain wheat flour and added with xylanase also had specific volumes significantly higher than those of the control sample (Jaekel et al, 2012). Bread obtained from formulation containing only ascorbic acid as improver has the volume visually higher and a more homogeneous alveolar structure as compared with the standard. Ascorbic acid is one of the most commonly used baking improvers due to its properties, which lead to an increase in dough strength and consequently in bread volume (Aamodt et al., 2003), acting specifically on the final rise of dough (Hrušková and Novotná, 2003). This oxidizing agent acts directly on the structure of gluten proteins, enhancing the gluten network through the formation of disulfide bonds (Nakamura and Kurata, 1997).

Regarding the results of microbiological analysis performed the breads obtained with different formulations was not detected, the occurrence were filamentous fungi, yeasts, coliforms at 45°C or *Salmonella* sp. This is expected because after baking bread, microbiological levels decrease dramatically (Marín et al., 2002).

Another study performed was of the shelf-life determination. According to the results (data not showed) the bread control without the addition of improvers had a shelf-life of 7 days on average, showing high concentration of filaments fungi in this period. All formulations containing xylanase showed a statistically significant higher shelf-life, especially formulation 6 containing excess  $\alpha$ -amylase, xylanase and ascorbic acid, which in all tests had a shelf-life of 13 days on average. These data require further study, especially to evaluate the correlation between the use of xylanase with a longer shelf-life. This analysis suggests that some combinations of improvers can be recommended for specific bread characteristics or applications. Examples of these applications are to obtain bread with higher volume, retard the retrogradation of bread, bleach bread, extended shelf-life or improve other parameters to improve baking performance.

## Conclusion

The findings in this study have shown the potentiality of the combined use of improvers in bread production. Specific formulations in different combinations of ascorbic acid and xylanase and/or amylase were shown to produce particular effects on the rheological properties of dough. These combinations can be used to produce quality bread with specific characteristics. The formulation that showed the best dough rheology results was 0.01% xylanase/amylase (spring 2002) and 200 ppm of ascorbic acid. It produced good bread characteristics such as a large volume, as did the formulation combining  $\alpha$ -amylase (life spring B) and ascorbic acid. All formulations containing xylanases showed increased shelf-life,

most particularly the formulation containing excessive improvers which, despite an impaired dough rheology, showed the most extended shelf-life. A further study of the impact of these formulations on the sensory properties of bread is required, as well as additional studies on other rheological parameters, particularly to elucidate related biochemical processes or the ratio of the interactions between these improvers and the properties of different flours.

## Conflict of interests

The author(s) did not declare any conflict of interest.

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