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# The Use of Xylanases from Different Microbial Origin in Bread Baking and Their Effects on Bread Qualities

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**Abstract:** Effects of xylanases on bread quality were examined. Enzymes used were endo-xylanase (EC 3.2.1.8) from different sources of microorganisms. Baked loaves were assessed for Loaves volume, colour and staling rate. Xylanases produced from rumen microorganisms M6 had clearly positive effects on loaf volume of bread as well as anti-firming potential. M3 (produced from *Trichoderma longibrachiatum*) improved crumb softness. The use of xylanase for breadmaking lowered firmness of bread crumb effectively compared with control loaf. It can be summarized that xylanases had significant positive effects on bread characteristics. In particular, they had advantage in retarding the staling rate of bread. It is recommended that the optimum dosage of enzymes, method of application in industrial scale especially with xylanase should be studied further in order to gain the great advantages of enzyme addition in breadmaking.

**Key words:** Xylanase, bread making quality, anti-staling, bread colour, bread volume

## INTRODUCTION

The use of enzymes during the manufacture of baking products is a primitive process. In fact, our ancestors already used these enzymes without their being aware of them because flour naturally contains enzymes. During recent decades, enzymes have been used on purpose and the application of enzymes in the bakery has become widespread (Poldermans and Schoppink, 1999; Rani *et al*., 2001; Gámbaro *et al*., 2006; Caballero *et al*., 2007).

Xylanase has been introduced recently as it can improve the handling properties of dough, the ovenspring and the bread volume. Moreover, it has the potential to retard staling thus increases the shelf life of the bread (Hilhorst *et al*., 1999). Xylanases are enzymes that specifically hydrolyze xylans which are the most widely occurring polysaccharides (Uhlig, 1998). In wheat flour, xylans are mainly present as arabinoxylans which are the cell wall components. Arabinoxylans can be in both water-soluble and water-insoluble forms. Water-soluble pentosans will hold water about 10 times of their weight in water (Mannie, 2000). In order to increase the amount of water-soluble pentosans, xylanases are added to bread dough. During the process of enzymatic hydrolysis, Xylanases can break glycosidic linkages in arabinoxylans, leading to a smaller fragments of carbohydrates and therefore water is released in the dough. As a consequence, the dough becomes softer; the handling properties of dough, the ovenspring and the bread volume are improved. It also increases the shelf life of bread (Hilhorst *et al*., 1999).

Over recent years, the role of xylanases in breadmaking has been investigated intensively (Hilhorst *et al*., 2002; Jiang *et al*., 2005; Collins *et al*., 2006; De Schryver *et al*., 2007). Girhammar (1993) reported that addition of xylanase increased loaf volume of standard wheat flour breads significantly. The application of *Aspergillus aculeatus* xylanase in bread and bakery products has been introduced by Qi-Si (1995). The effects of purified endo-beta-xylanase on the structure and baking characteristics of rye doughs have been investigated by Autio *et al*. (1996). Red winter wheat flour, which was treated with beta-xylanase before the addition to bread formula, resulted in slightly improved crumb grain (Lin-Wang *et al*., 1998). Hilhorst *et al*. (1999) claimed that the use of peroxidase in combination with xylanase improved the handling properties of the doughs and the final baked product. The combination of xylanase and lipase decreased fermentation time and increased dough extensibility (Collar *et al*., 2000). There have been several studies concerned with anti-staling potential of xylanases incorporated with other enzymes such as amylase, lipase and protease (Martinez-Anaya *et al*., 1998, 1999; Gil *et al*., 1998, 1999).

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Hence, the main purpose of the present study was to investigate the effect of xylanase from different sources on bread quality and bread staling.

## MATERIALS AND METHODS

**Materials:** Super Bakers Flour (Goodman Fielder) (Moisture, 11.9%; Protein, 11.7%; Ash, 0.66%), Lowan Instant Dry Yeast, Saxa Iodised Cooking Salt, White Sugar (CSR), xylanases (EC3.2.1.8) (Megazyme International Ireland Ltd.), they were identified as M1 (from *Trichoderma viride*, 205 U $mg^{-1}$); M2 and M3 (from *Trichoderma longibrachiatum*, 64 and 132 U $mg^{-1}$, respectively); M4 (from *Aspergillus niger*, 79.3 U $mg^{-1}$); M5 (from *Humicola insolens*, 200 U $mg^{-1}$) and M6 (from rumen microorganism, 405 U $mg^{-1}$), they were suspended in 3.2M ammonium sulphate solution and kept under refrigerated temperature before use.

**Bread making formula:** Four hundred and fifty grams strong breadmaking flour, 9 g sugar, 8 g instant yeast, 7.5 g salt, 9 g vegetable oil and 283.5 mL water. The mixture was processed in an automatic breadmaker (Panasonic SD-253, Matsushita Electric Ind. Co. Ltd., using a rapid cycle of 1 h and 55 min. Test loaves were baked from each formula. Baked loaves were allowed to cool for 1 h at 25°C before storage and stored in sealed polyethylene bags at room temperature for periods of up to 5 days, breads were treated with 10 μL of the xylanase per dough.

**Bread firmness:** Bread firmness measurements were made with a Texture Analyser (TA-XT2, Stable Micro Systems, England). Slices (25 mm thickness) were compressed to 40% (6 mm) using a 35 mm diameter aluminium plunger with a 5 kg load cell. The rate of compression was 1.7 mm $sec^{-1}$. The compression curves of the bread crumb (distance vs. force) were plotted and the force readings (in Newton) at 25% compression were taken as a measure of firmness in accordance with AACC method 74-09 (AACC, 2001). Two slices were analyzed from each loaf.

**Loaf volume:** The values of bread loaf volume samples were determined by the RACI standard procedure (RACI, 1995). For this, bread loaf volume was estimated from the sum of two circumference values of the loaf. The second measurement was taken perpendicular to the first. All measurements were taken after 1 h of cooling at room temperature and the sum expressed in centimeters.

**Colour measurement:** The colour of bread was measured by the Minolta Chroma Meter (CR-300). The results were recorded by the L*, a* and b* values at three different points on crust and crumb of bread (RACI, 1995).

**Assessment and scoring of baked loaves:** This assessment was scored out of a total of ten points. One meant the poorest and 10 the best. The factors to be considered were overall symmetry, smoothness, stickiness, uniformity of crumb cells, aroma and taste.

**Data analysis:** Experimental data were analyzed using analysis of variance (ANOVA) (SPSS v.11, SPSS Inc., Chicago, IL), a value of ($p<0.05$) was considered significant difference.

## RESULTS AND DISCUSSION

In consideration of loaf volume, the control loaf without any of xylanases was smaller than the other loaves Fig. 1. Loaf using M6 (from rumen microorganism) clearly was the largest loaf with the highest loaf volume. Loaf using M2 and M3 was also larger than control, M1 and M3 in volume.

These findings confirm data that obtained with the other xylanases reported (McCleary, 1986; Maat *et al*., 1992; Martinez-Anaya and Jimenez, 1997; Norma and Guillermo, 2003). Courtin *et al.* (1999) found the use of endoxylanases impacted significantly on final loaf volume and Jiang *et al*. (2005) who fond that the specific volume of bread was increase 30% by using xylanase.

Crumb firmness of control and treated loaves was measured and the results are shown in Fig. 2. During the first day, loaves with M1, M2 and M6 were significantly softer than the control loaves and other loaves ($p<0.05$). Second and third day, the loaves treated by enzyme had significantly lower firmness than the control loaf ($p<0.05$). After that, the firmness of every loaf increased rapidly excluding the M3 loaf which the firmness was stable from the second day to the third day.

Firmness of crumb is one of the most evident changes observed during bread storage. The influence of xylanases on the process of bread staling is still being debated. Information on this aspect is confusing possibly because of the variety of xylanases exist (Jiang *et al*., 2005). Some results indicated that added xylanases (or hemicellulases or pentosanases) do not modify the crumb-firming rate, but decrease the initial crumb firmness, possibly by increasing the loaf volume (Rouau *et al*., 1994). However, the results in other studies show that the addition of xylanases can decrease the staling rate of bread (Martinez-Anaya and Jimenez, 1997; Laurikainen *et al*., 1998). It has also been reported that xylanases are among different carbohydrases that exerted

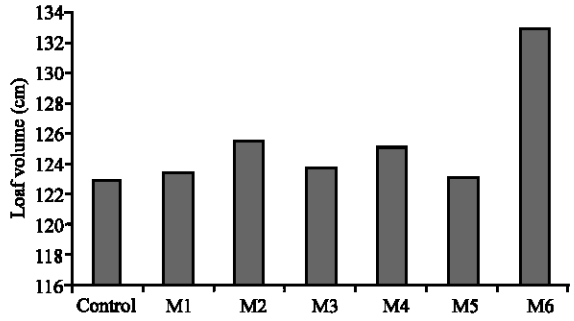


Fig. 1: The effect of xylanases on bread loaf volume

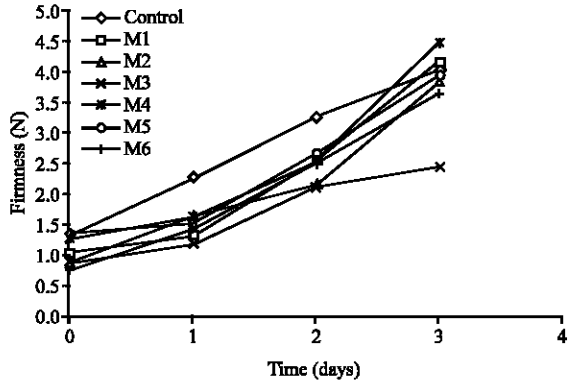


Fig. 2: The effect of addition xylanases on crumb firmness of loaf bread

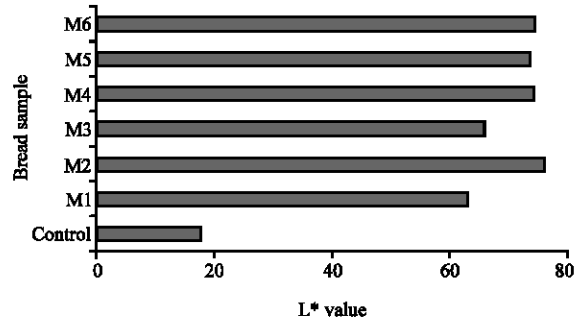


Fig. 3: The effect of addition xylanases on crust L* value of loaf bread

the greatest effect on the anti-staling during bread storage (Haros *et al*., 2002). All added xylanase retarded the staling rate of breads in this study. The staling rate was retarded possibly because of the breakdown of the polysaccharide network and the presence of more hygroscopic oligosaccharides. Hence, xylanase possibly induced retardation of the bread staling by reducing the initial crumb firmness and the firming process during storage (Jiang *et al*., 2005).

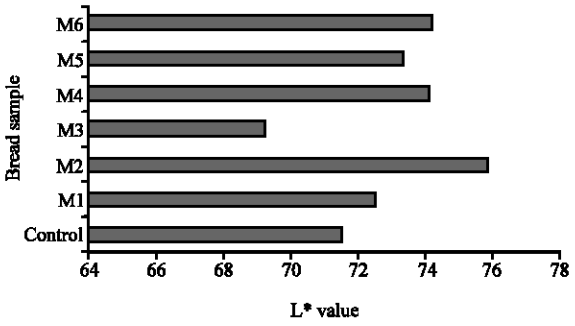


Fig. 4: The effect of addition xylanases on crumb L* value of loaf bread

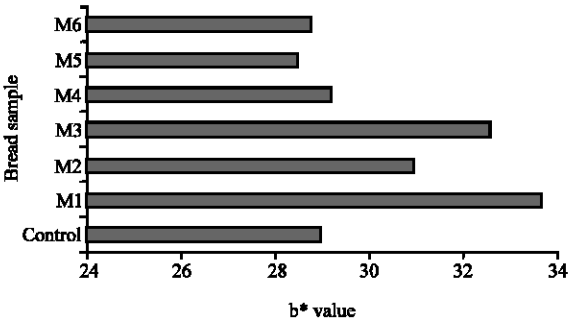


Fig. 5: The effect of addition xylanases on crust b* value of loaf bread

Colour is an important sensory attribute in bread. One criterion consumers used when selecting bread is its colour in terms of darkness (this usually has a low L* value) or lightness (this usually has a high L* value). Breads with xylanases from different sources were significantly lighter in colour (as indicated by their higher L* values) than the control loaves bread ($p<0.05$) (Fig. 3). The data reported in Fig. 4 indicate that the crumb were lighter than the control for all bread loaves except the sample with M3 (xylanase from *Trichoderma longibrachiatum*, 64 U $mg^{-1}$).

The positive b* values, which indicate yellow colour of crumb and crust bread sample Fig. 5 and 6 demonstrate that the crust bread samples with M3 and M1 were yellowier than the control and other samples, for the crumb colour (in term of b* values) the data did not show significantly effect by using xylanases.

There was a small difference of overall symmetry among the loaves (Table 1), Loaf with M3 had very smooth crust and loaf with M6 had many large holes, which resulted in the bad uniformity. The stickiness of every loaf was not good, as they were wet due to not enough resting time after baking. However, all of them had

Table 1: The effect of addition xylanases on sensory analysis of loaf bread

| Xylanase added | Control No. xylanase | M1 | M2 | M3 | M4 | M5 | M6 |
|---|---|---|---|---|---|---|---|
| | | ---------- | ---------- | ---------- | 10 µL | ---------- | ---------- |
| Overall symmetry | 8 | 7 | 9 | 8 | 8 | 7 | 9 |
| Smoothness | 8 | 6 | 8 | 9 | 7 | 6 | 8 |
| Stickiness | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
| Uniformity of cells | 7 | 6 | 8 | 7 | 6 | 7 | 6 |
| Flavour and aroma | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| Total | 38 | 34 | 40 | 39 | 36 | 35 | 38 |

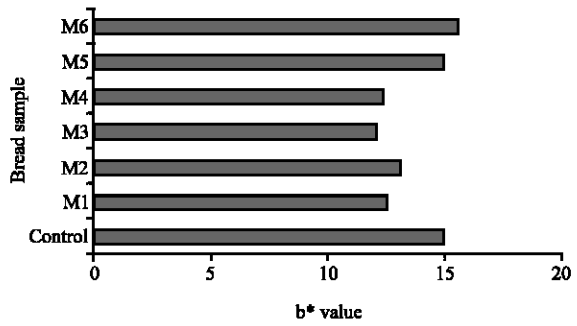


Fig. 6: The effect of addition xylanases on crumb b* value of loaf bread

good flavour and aroma of normal bread, M2 had the best performance with good shape of loaf, smooth surface of crust, best uniformity of crumb cells.

## CONCLUSION

The results obtained show that the addition of xylanases could lead to improved bread quality. In general, these enzymes significantly improved loaf volume, loaf colour and crumb texture and firmness. Xylanases produced from different types of microorganisms play various roles in baked product quality. In the current study, xylanases from six different sources (M1 (from *Trichoderma viride*, 205 U $mg^{-1}$); M2 and M3 (from *Trichoderma longibrachiatum*, 64 and 132 U $mg^{-1}$, respectively); M4 (from *Aspergillus niger*, 79.3 U $mg^{-1}$); M5 (from *Humicola insolens*, 200 U $mg^{-1}$); and M6 (from rumen microorganism, 405 U $mg^{-1}$) were studied and they showed different positive effects on bread characteristics, M6 have shown a great advantage particularly in loaf volume of bread. It is believed that xylanase plays a major role in converting the insoluble pentosan to soluble pentosan. The soluble pentosans will bind with water about 10 times of their weight (Mannie, 2000). Water is released in the dough through the partial hydrolysis of arabinoxylan by endoxylanase, as a consequence, the dough becomes softer which leads to better ovenspring and larger volume of bread with a softer, more delicate crumb (Poldermans and Schoppink, 1999). In relation to anti-staling potential, all enzymes appeared to show good tolerance on bread firmness compared with the controls. M3 could lower the firmness during 3 day storage. In comparison, each type of enzymes had different effects on bread quality. Furthermore, studies in the area of optimum levels of xylanases should be done in greater depth.

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