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ORIGINAL ARTICLE

Xylanolytic Modification in Wheat Flour and its Effect on Dough Rheological Characteristics and Bread Quality Attributes

Zulfiqar Ahmad · Masood Sadiq Butt · Anwaar Ahmed · Nauman Khalid

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Abstract Effects of various xylanase treatments applied at different stages of bread making process on dough rheological characteristics and bread quality attributes were investigated. Different doses (200, 400, 600, 800, and 1000 IU) of purified enzyme were applied at two stages (tempering and mixing). In milling and dough making processes, both types of flour (subjected to enzyme treatment during tempering and flour mixing) exhibited decreasing trend in water absorption, dough development time, dough stability, softening of dough, dough mixing time, viscosity peak, set back, and increasing tendency in peak height and pasting temperature. Treatments during tempering resulted in more significant effects as compared to applications during flour mixing. The dough rising during proofing resulted in enhancement from 137±3.21% (control) to maximum value (192.33±2.90%), when 600 IU of xylanases were applied to 1 kg of wheat grains during tempering. The bread sensory attributes also exhibited significant improvement in response to various doses of purified enzymes.

Z. Ahmad (✉)
University College of Agriculture and Environmental Sciences, The Islamia University of Bahawalpur, Bahawalpur, Pakistan
E-mail: zulfiqar2233@gmail.com

M. S. Butt
National Institute of Food Science and Technology, University of Agriculture, Faisalabad, 38040, Pakistan

A. Ahmed
Department of Food Technology, PMAS-Arid Agriculture University, Rawalpindi, 36400 Pakistan

N. Khalid (✉)
Department of Food Technology, PMAS-Arid Agriculture University, Rawalpindi 36400, Pakistan and Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan
E-mail: nauman_khalid120@yahoo.com

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Introduction

Wheat bread is one of the major food products across the world. Several additives such as vital gluten, emulsifiers, oxidants, and preservatives are being used to improve the quality of bread and to increase its shelf life (Wang et al., 2004; Selinheimo et al., 2006). Although wheat grains contain a small fraction (1.37–2.06%) of arabinoxylan, it plays an important role in bread making process (Izydorczyk and Biliaderis, 2007). Wheat arabinoxylan are classified into water-extractable (WE-AX) and water un-extractable (WU-AX). WU-AX is detrimental to bread making, whereas WE-AX with medium to high molecular weight have a positive impact on loaf volume (Courtin et al., 2001). The most important property of arabinoxylan is its water-binding potential. One gram of arabinoxylan can bind up to 15 g of water (Bushuk, 1966). Xylanase is able to hydrolyze WU-AX (Bushuk, 1966; Sprössler, 1995; Poutanen, 1997). It hydrolyses highly polymerized β-1,4 D-xylosidic linkages and substituted β-1,4 linked D-xylobiose, xylotriose and glucuronosyl residues. causing them to lose their strong water-holding capacity (Martínez-Anaya and Jiménez, 1997; Courtin and Delcour, 2002). Enzymatic hydrolysis of non-starch polysaccharides leads to the improvement of rheological properties of dough, bread specific volume, and crumb firmness (Martínez-Anaya and Jiménez, 1997). Endo-1,4-xylanase (EC 3.2.1.8) randomly attacks the arabinoxylan backbone to cause a decrease in the degree of polymerization, hence leaving a strong impact on arabinoxylan structure and functionality (Courtin and Delcour, 2002; Si and Lustenberger, 2002). At optimum dosage, monomers and oligomers resulting from enzymatic activity affect

the water balance, modify protein-starch interaction thus improving dough machinability, dough stability, oven spring, loaf volume, crumb structure, and shelf life as well as reducing the staling rate (Poutanen, 1997; Sorensen et al., 2001). Addition of enzyme significantly shortens fermentation time without affecting pH or machinability of dough. It improves bread volume and enhances aroma, which results in softer texture (Martínez-Anaya and Jiménez, 1997). The hydrolytic action of xylanases releases free sugars such as pentoses, which could be used by microorganisms for fermentation. Moreover, xylooligosaccharides released by endoxylanases could form healthier food. The health effects of xylooligosaccharides are mainly related to their effects on the gastrointestinal flora (Vázquez et al., 2000). Xylanases also liberate nutrients by hydrolyzing the non-degradable hemicellulose fibers thus making them available for use in bread-making process (Leisola et al., 2002). On account of these multiple beneficial roles of enzymes in baking industry with special reference to xylanase, the present study was designed to investigate effect xylanase on dough rheological parameters and bread sensory characteristics.

Materials and Methods

Materials for dough making. A popular wheat variety, *Inqulab 91*, used for the production of straight grade flour was obtained from the Post-Graduate Agricultural Research Station, University of Agriculture, Faisalabad, Pakistan. The xylanase (optimum temperature 60°C and pH 7.5) produced from an indigenous stain of *Aspergillus niger* in the Microbiology and Biotechnology Laboratory, National Institute of Food Science and Technology, Faisalabad, Pakistan was used in dough preparation (Ahmad, 2009).

Proximate analysis. Proximate analyses of flour (straight grade flour) were carried out according to their respective methods described in AACC (2000) *i.e.* moisture (Method 44-15 A), ash (Method 08-01), crude fat (Method 30-10), crude protein (Method 46-10), and crude fiber (Method 32-10).

Enzyme addition during tempering. Initially, different doses (200, 400, 600, 800, and 1000 IU in T_1, T_2, T_3, T_4, and T_5 respectively) of purified enzyme (in tempering water) along with the control (T_0) were applied to 1 kg of wheat grains during tempering (a preliminary step before milling in which grains were moistened up to 15.5%) and stored for 14 h at room temperature. One international unit (IU) is the quantity of enzyme required for consuming 1 μmol of substrate for producing 1 μmol of product per minute. The samples were agitated at different intervals for uniformity in tempering. The amount of water required for tempering was computed by following the expression given in eq. (1) outlined in AACC (2000) method No. 26–95.

$$\text{Amount of water (mL)} = \frac{100-\text{original moisture (\%)}}{100-\text{desired moisture (\%)}} \times \text{weight of sample (g)}$$

The tempered wheat was milled through Brabender Quadrumate Senior Mill (capacity: 5 kg/h; C.W. Brabender Instruments, Inc. Germany) and straight grade flour was obtained by mixing break and reduction flour fractions.

Enzyme addition during mixing. In a parallel trial, same doses (200, 400, 600, 800, and 1000 IU) of purified xylanase as those used in tempering process were applied to 1 kg of wheat flour (straight grade flour, obtained by milling of wheat kernels at 15.5% moisture level using Quadrumate Senior Mill) during dough mixing stage.

Rheological studies. Xylanase-treated flour samples were subjected to farinographic, mixographic, and amylographic studies. Both types of flour samples (enzyme-treated during tempering and mixing) along with control sample (untreated) were analyzed for farinographic characteristics such as water absorption (the amount of water required to reach the curve at 500 Brabender Unit (BU) line of the graph; the water absorption capacity of flour was directly measured using the microburette attached with the commercially available rheological instruments including farinograph, mixograph and amylograph and expressed in percentage of dough development time, which indicates the time interval in minutes from first addition of water to the point of maximum consistency just before the indication of weakening, dough stability (time difference between the point where top of curve first intersected 500 BU line (arrival time) and point where top of curve left 500 BU line (departure time) and softening of the dough (the difference in BU from center of curve at peak and center of curve 12 min after peak) were derived from the farinograms obtained as a result of sample running in farinograph (Brabender GmbH & Co. KG, Germany) following the procedure of AACC (2000) method 54-21.

Flour samples were also run through mixograph equipped with 10 g capacity bowl (National Mfg. Co, USA) to assess the mixing properties of dough following the procedure described in AACC (2000) Method 54-40. The characteristics such as mixing time and peak height were calculated.

The amylographic studies were performed using amylograph (Brabender, Germany) for the untreated (control) as well as enzyme-treated flour samples (tempering and mixing) for characteristics including pasting temperature, viscosity peak, and setback.

Bread production. Bread samples from both enzyme-treated flours along with untreated flour (control) were prepared using straight-dough method (AACC, 2000). All food grade ingredients (flour 200 g, sugar 6 g, salt 2 g, shortening 10 g, yeast 4 g, water 120 mL purchased from local market) were mixed for 5 min in a Hobart mixer (Model N-50, Hobart Corp. Troy, Chio, USA) to form dough and fermented at 30°C and 75% relative humidity. The dough was punched after 120 and 150 min. Subsequently, the dough samples were divided, molded, panned (100 g capacity), and kept for final proofing for 45 min, at 35°C and 85% relative humidity. The breads were baked in commercial bread baking unit at 230°C for 25 min. Each sample was prepared in triplicate.

Determination of dough volume. The increase in dough volume during proofing was determined using graduated beakers and dough rising was calculated as the ratio (Driss et al., 2012).

$$\text{Dough volume} = \frac{\text{increase in dough height}}{\text{initial height of the dough}} \times 100$$

Sensory evaluation. Sensory evaluation of xylanase-supplemented and control bread was carried out by an expert panel of five judges according to the 100-point evaluation scheme given by Pyler (1988). The maximum scores of bread characteristics were: volume 15; color and nature of crust 5; symmetry of form 5; uniformity of baking 5; texture 15; color of crumb 10; grain 10; aroma 15; taste 20.

Statistical analysis. The data obtained from different parameters were analyzed by Complete Randomized Design and level of significance was determined by analysis of variance technique at $p < 0.05$ as described by Steel et al. (1997). The data were processed using the software package Statistix 8.1 (Analytical Software, USA).

Results and Discussion

Proximate analysis of flour. Because the dough rheological characteristics depend upon wheat used, thus its proximate analysis was performed, and different components were found: moisture, 11.71±0.02%; total ash, 0.58±0.01%; crude fat, 0.93±0.02%; crude protein, 11.31±0.03%; crude fiber, 0.35±0.01%; nitrogen-free extract (NFE), 86.83±2.34%.

Farinographic characteristics. The rheological characteristics facilitate the understanding of dough handling properties and these rheological parameters can be improved by the use of xylanase. Mean of water absorption in response to xylanase treatments during tempering of wheat kernels and flour mixing in parallel were expressed in Fig. 1A. It is obvious from mean values that upon applying various doses of xylanase, water absorption decreased significantly ($p < 0.05$). The untreated flour (control) showed maximum water absorption of 57.6% followed by 56.40 and 55.77% in response to doses T_1 (200 IU/kg) and T_2 (400 IU/kg), respectively when they were applied during mixing of flour. Further increase in enzyme concentration to 600 IU/kg (T_3), water absorption decreased to 54.47 and 54.93% for flour samples receiving xylanase treatments during tempering and mixing processes, respectively. Minimum values were noted in the case of T_5 (1000 IU/kg): 51.93% (tempering) and 52.80% (mixing). In general, enzyme applications in both cases resulted in decreased water absorption.

Xylanase breaks down larger molecules of carbohydrates into smaller ones which have low water-binding capacities as compared to their parent molecules that result in reduced water absorption. Thus, the findings of present study are supported by the results of Haros et al. (2002); they described a decrease in water absorption with xylanase addition during wheat tempering. Earlier, Laurikainen et al. (1998) prepared bread using purified xylanase from *Aspergillus foetidus* during mixing and observed significant differences in water absorption that reduced from 72 to 64%. However, the findings of present study slightly differ from those of Girhammar (1993) who described a slight change in water absorption when xylanase was directly added to flour in the farinographic assay.

Fig. 1B illustrates the effect of enzyme treatments on dough development time (DDT). It was obvious that increased enzyme level decreases DDT in both cases (tempering and mixing). However, xylanase addition during tempering showed more pronounced effect on this characteristic. In the case of control sample, maximum DDT (4.50 min) was decreased to 4.00 and 3.60 min in response to T_1 (200 IU/kg) and T_2 (400 IU/kg) respectively when applied during mixing process. Enzyme concentration at 1000 IU/kg (T_5), resulted in decreased dough development time to 2.00 min (tempering) and 1.80 min during mixing; the lowest value calculated among treatments. These results reveal that enzyme application exhibited notable impact on this trait with more pronounced effect in the case of tempering; (except T_5 where the results are mutually non-significant) possibly due to the fact that enzyme received more time for its activity. The diffusion of xylanase through kernel into endosperm during tempering process makes the dough soft, which results in decreased DDT. The results are in close association with the findings of Haros et al. (2002); they described a decrease in DDT in response to xylanase treatments when supplemented during tempering and dough mixing. However, Al-Suaidy et al. (1973) reported a deviating trend and described a slight variation in farinographic data when wheat was treated with different doses of cellulase and hemicellulase.

The mean values in Fig. 1C depict differences in dough stability as a function of xylanase treatments. The enzymatic treatments showed marked effect on this trait and there was a significant ($p < 0.05$) negative correlation between enzyme concentration and dough stability. Dough stability was gradually decreased with the progressive increased in enzyme concentration in both cases (tempering as well as mixing). Maximum dough stability 11.10 min was recorded in the case of control sample followed by T_1 with 10.80 (mixing) and 10.40 min (tempering), whereas minimum dough stability time was observed in the case of T_5 i.e. 6.50 and 7.50 min during tempering and mixing, respectively. All xylanase applications decreased dough stability indicating dough strength. Similar results were found by Laurikainen et al. (1998); they supplemented bread dough with xylanase from *Aspergillus foetidus* and observed a decrease in dough stability. The results of present study are also in agreement with those of Jiang et al. (2005); they reported a decrease in dough stability time in response to xylanase applications. The mean values (Fig. 1D) indicate that enzyme addition had an inverse correlation with dough softening BU values (lower mean values of BU suggest more soft dough); however, dough remained tough with highest value of 70.00 BU in control (T_o). Treatment T_5 exhibited minimum values for softening, 45.67 and 48.00 BU when enzyme was

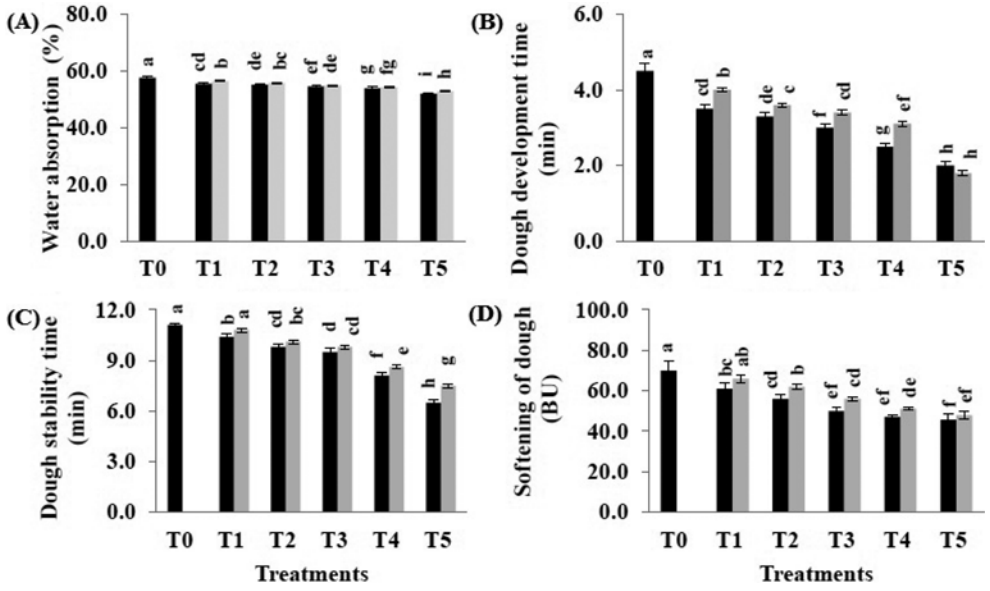

Fig. 1 Effect of xylanase treatments during tempering of wheat kernels and mixing of wheat flour on dough farinographic characteristics. ■ indicates tempering and □ indicates mixing (T_0=Control, T_1=200, T_2=400, T_3=600, T_4=800, T_5=1000 IU/Kg). (A) effect of xylanase treatment on water absorption (B) effect of xylanase treatment on dough development time (C) effect of xylanase treatment on dough stability time (D) effect of xylanase treatment on dough softening.

applied during tempering and mixing stage, respectively. Xylanase breaks down the soluble pentosans present in flour, which results in softening of dough. The present results are supported by Courtin and Delcour (2002), Jiang et al. (2005), and Laurikainen et al. (1998); they described similar results regarding xylanase applications and reported an increasing trend in softening (smaller values suggest softness) of dough in response to enzyme treatments.

Mixographic characteristics. Fig. 2A indicates that xylanase treatments at varying levels exhibited a momentous effect on mixing time. Enzyme addition had inverse relationship with this mixographic characteristic, with increase in xylanase doses, mixing time decreased in both samples (receiving xylanase treatment during tempering and mixing). However, the effect was more pronounced in the case of tempering. The means expound that untreated flour sample (control) showed maximum time (3.5 min) for dough mixing followed by 3.40 and 3.30 min in response to T_1 and T_2 respectively, when xylanase was applied during mixing of dough, whereas minimum mixing time (2.70 min) was noted in response to T_5 when enzyme was used during tempering. The mean values in Fig. 2B elaborate the variations in peak height (%) of samples in response to xylanase treatments during wheat tempering and dough mixing stage. Peak height exhibited an increasing trend with increase in xylanase concentration. The control sample showed minimum value (62%) for this attribute. The enzyme level of 200 IU/kg as in the case of T_1 increased the peak height to 62.8 and 62.6% during tempering and mixing, respectively. Maximum peak height (64.9%) was observed when enzyme was used at 1000 IU/kg (T_5) during tempering. As xylanase splits macro-molecules into micro-molecules in wheat flour, which makes the mixing easy and mixing time is reduced. The studies conducted by Nadeem et al. (2009) and Su et al. (2005) also reported that application of hydrolytic enzymes to wheat flour resulted in decreased mixing time and increased peak height.

Amylographic characteristics. Polysaccharides are mainly responsible for wheat hydration and final viscosity. Therefore, impact of xylanase on pasting behavior of flour samples was also studied. Fig. 3A represents the effect of various xylanase treatments on pasting temperature of dough samples. Pasting temperature exhibited an increasing trend with progressive increase in xylanase concentrations in both cases, tempering and mixing; however, enzyme supplementation during tempering exhibited more significant effect compared to application during mixing process. In the case of enzyme applications during wheat tempering, the dough samples were found to have maximum pasting temperature (84.60°C) as in T_5 followed by 84.33°C in response to T_5 (mixing) and T_4 (tempering), whereas the control sample showed minimum value (82.80°C) for this attribute. The addition of xylanase splits large hemicelluloses into oligosaccharides and monosaccharides, which may interfere with gelatinization by hindering the swelling of starch granules that increases the temperature of pasting. Haros et al. (2002) also reached the same conclusion and described that the addition of different doses of enzymes to tempering solution changed the pasting properties; dough samples with enzyme treatment exhibited higher pasting temperature as compared to the control sample. Likewise, Cristophersen et al. (1997) also documented similar effect of xylanase on wheat slurry.

Means for viscosity peak of control as well as enzyme-treated flour samples are presented in Fig. 3B. It is clear from the graphical illustration that viscosity showed a negative correlation

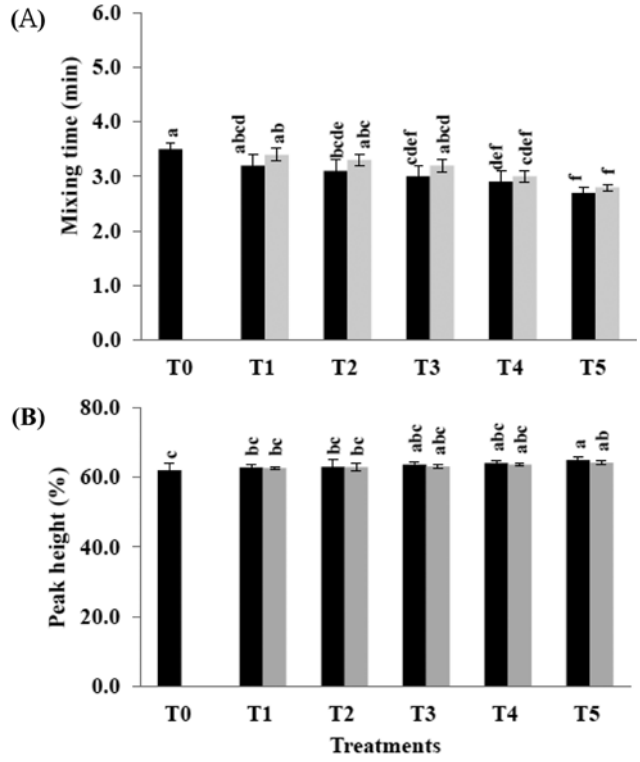

Fig. 2 Effect of xylanase treatments during tempering of wheat kernels and mixing of wheat flour on dough mixographic characteristics. ■ indicates tempering, □ indicates mixing (T_0=Control, T_1=200, T_2=400, T_3=600, T_4=800, T_5=1000 IU/Kg). (A) effect of xylanase treatment on dough mixing time (B) effect of xylanase treatment and peak height of dough mixing.

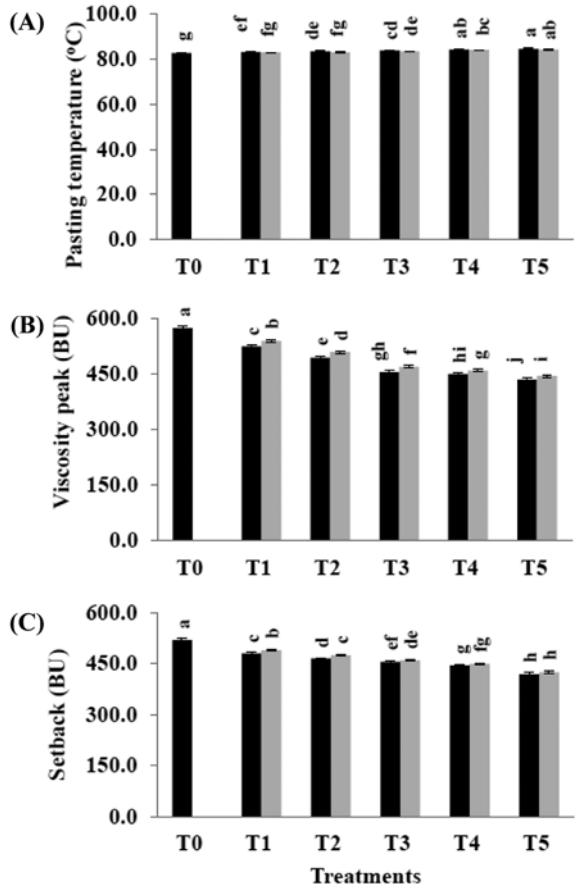

Fig. 3 Effect of xylanase treatments during tempering of wheat kernels and mixing of wheat flour on dough amylographic characteristics. ■ indicates tempering and □ indicates mixing (T_0=Control, T_1=200, T_2=400, T_3=600, T_4=800, T_5=1000 IU/Kg). (A) effect of xylanase treatment on pasting temperature of dough (B) viscosity peak and xylanase effect (C) **e**ffect of xylanase treatment on setting back of dough.

with increase in xylanase concentrations in both cases of tempering and mixing; nonetheless, the impact was more pronounced during tempering. Maximum value (575 BU) for this parameter was found for control sample (T_0) that decreased to 540 BU in the case of mixing and 525 BU in the case of tempering in response to T_1 (200 IU/Kg),whereas minimum viscosity peak was observed as 435 BU in T_5 (1000 IU/Kg) during tempering. The viscosity peak reduction as a function of xylanase treatments shows hydrolytic action of enzyme, resulting in water release and a decrease in viscosity peak. In addition, Haros et al. (2002) reported the effect of xylanase in dough viscosity reduction as compared to cellulase and glucanase. The presence of non-starch polysaccharides is associated with increase in viscosity peak, and their breakdown by enzymes causes reduction in viscosity and increase in dough elasticity, extensibility, and coherency (Ahmad et al., 2012).

There is a negative correlation of setback with enzyme applications during tempering and mixing (Fig. 3C). The dough without enzyme treatment (T_o) exhibited maximum value for setback as 520 BU, whereas xylanase addition up to 200 IU/kg (T_1) during tempering showed decreased value for this attribute, 480 BU. On the other hand, minimum mean value 420 BU was observed in response to T_5 (1000 IU/kg) when xylanase was applied during tempering. Similarly, Haros et al. (2002) reported a declining trend in setback with the addition of xylanase. Setback describes the tendency of amylose to retrograde after leaching out the starch granules during baking stage (Rojas et al., 1999). Increase in the retrogradation rate will result in higher bread staling; xylanase prevents the rearrangement of amylose molecules and slows down the retrogradation process; thus application of xylanase during tempering can be considered as useful tool to obtain flours with anti-staling properties in the bread-making process.

Fig. 4 shows the effect of different doses of xylanase on volume increase (%) of various dough samples during the same period (45 min) of proofing. The graph elaborates that T_3 (600 IU) in the case of tempering exhibited maximum (192.33 %) increase in dough volume followed by 189.00 % when T_4 (800 IU) was applied in the case of dough mixing, whereas T_o was found to have minimum volume increase of 137.00 % during proofing. This increase in dough volume may be the result of higher concentration of

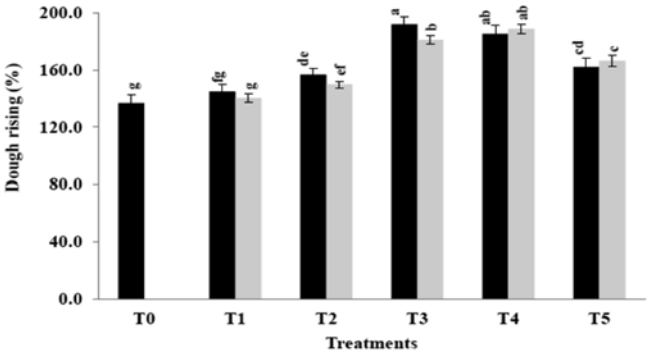

Fig. 4 Effect of xylanase treatments during tempering of wheat kernels and mixing of wheat flour on dough rising. ■ indicates tempering and □ indicates mixing (T_0=Control, T_1=200, T_2=400, T_3=600, T_4=800, T_5=1000 IU/Kg).

fermentable sugars released in xylanase-supplemented dough samples, allowing more carbon dioxide to be produced by yeast. The results of present study regarding increase in dough volume are supported by the earlier findings of Shah et al. (2006), who reported an increase of 185.00% in the volume of enzyme-treated dough samples. Poutanen (1997) explicated that optimum dose of xylanase improves dough machinability, stability, oven spring, loaf volume, crumb structure, and shelf life. Similarly, the most important impact of xylanase treatment is that in shorter time it causes maximum increase in dough volume that shortens the time of bread processing, reduces energy inputs, increases capacity and efficiency of process, and makes the business more profitable.

Bread quality characteristics. The effects of various xylanase treatments at different stages (tempering and mixing) on bread quality attributes are given in Tables 1 and 2. It is clear from mean values that enzyme treatments affected the quality attributes significantly and T_3 (600 IU), in the case of tempering, gave best results than any other treatment applied in the present study. Rouau et al. (1994) and Krishnarau and Hoseney (1994) also reported that application of hemicellulases resulted in reduced bread firmness and increased loaf volume, whereas Gil et al. (1999) described that addition of pentosanase had no detectable effect on firmness. However, in the present study, the improvement of bread quality attributes indicates the useful role of xylanase in bread making process.

Xylanases break glycosidic linkages in arabinoxylans in an endo- or exo-fashion, creating smaller fragments. This action removes the insoluble arabinoxylan, which interferes with the formation of gluten network in dough. The increase in gluten volume gives better flexibility and extensibility, which eventually results in improved dough handling properties, machinability, stability, better oven spring, larger loaf volume, improved crumb structure, and also retards staling. This impact of xylanase is due to redistribution of water from the pentosan phase to the gluten phase. Due to multidimensional role of xylanase in baking, its importance is likely to increase as consumers demand increase for more natural products free of chemical additives.

The present study gives insight of the comparative effect of various xylanase treatments on dough rheological characteristics and sensory attributes of bread quality when different doses of xylanase were applied in parallel during tempering of wheat kernels and dough making process. The results showed that xylanase exhibited better performance and modified the dough

Table 1 Effects of xylanase treatments on bread quality characteristics

	Volume		Colour and nature of Crust		Symmetry of form		Uniformity of baking		Texture	
	Tempering	Mixing	Tempering	Mixing	Tempering	Mixing	Tempering	Mixing	Tempering	Mixing
T_0	7.20 ± 0.25^{g}	7.20 ± 0.25^{g}	2.52 ± 0.14^{f}	2.52 ± 0.14^{f}	2.72 ± 0.13^{d}	2.72 ± 0.13^{d}	3.08 ± 0.07^{d}	3.08 ± 0.07^{d}	5.92 ± 0.19^{e}	5.92 ± 0.19^{e}
T_1	8.40 ± 0.24^{f}	7.52 ± 0.22^{g}	2.94 ± 0.13^{def}	2.70 ± 0.23^{ef}	3.42 ± 0.13^{bc}	3.12 ± 0.12^{cd}	3.52 ± 0.20^{bcd}	3.20 ± 0.10^{cd}	6.50 ± 0.20^{e}	6.32 ± 0.31^{e}
T_2	10.60 ± 0.21^{d}	9.70 ± 0.33^{e}	3.50 ± 0.15^{abc}	3.14 ± 0.09^{cde}	3.90 ± 0.13^{ab}	3.42 ± 0.19^{bc}	3.82 ± 0.26^{ab}	3.30 ± 0.13^{cd}	8.22 ± 0.29^{d}	7.64 ± 0.28^{d}
T_3	12.94 ± 0.35^{a}	11.32 ± 0.21^{cd}	3.92 ± 0.16^{a}	3.22 ± 0.10^{bcd}	4.10 ± 0.09^{a}	3.72 ± 0.30^{ab}	4.10 ± 0.13^{a}	3.82 ± 0.27^{ab}	10.70 ± 0.20^{a}	9.92 ± 0.40^{abc}
T_4	12.60 ± 0.41^{ab}	12.32 ± 0.16^{ab}	3.70 ± 0.27^{ab}	3.52 ± 0.22^{abc}	3.80 ± 0.29^{ab}	3.92 ± 0.13^{ab}	4.00 ± 0.15^{ab}	4.00 ± 0.13^{ab}	9.72 ± 0.29^{bc}	10.32 ± 0.21^{ab}
T_5	11.92 ± 0.34^{bc}	12.10 ± 0.33^{bc}	3.30 ± 0.13^{bcd}	3.20 ± 0.14^{cd}	3.50 ± 0.20^{bc}	3.60 ± 0.23^{abc}	3.70 ± 0.25^{abc}	3.84 ± 0.14^{ab}	9.22 ± 0.22^{c}	9.80 ± 0.34^{bc}

Values with different superscript letters in a column are statistically significant (p <0.05) and (n =5).

Table 2 Effects of xylanase treatments on color of crumb and grain, as well as aroma and taste

	Color of crumb		Grain		Aroma		Taste	
	Tempering	Mixing	Tempering	Mixing	Tempering	Mixing	Tempering	Mixing
T_0	5.10 ± 0.11^{h}	5.10 ± 0.11^{h}	3.62 ± 0.10^{g}	3.62 ± 0.10^{g}	6.52 ± 0.15^{h}	6.52 ± 0.15^{h}	8.12 ± 0.16^{h}	8.12 ± 0.16^{h}
T_1	5.90 ± 0.24^{fg}	5.40 ± 0.18^{gh}	4.63 ± 0.09^{f}	4.30 ± 0.22^{f}	7.80 ± 0.34^{fg}	7.20 ± 0.14^{gh}	9.82 ± 0.28^{fg}	9.14 ± 0.26^{g}
T_2	6.80 ± 0.17^{bcd}	6.20 ± 0.13^{ef}	6.42 ± 0.16^{cde}	5.92 ± 0.33^{e}	9.12 ± 0.19^{e}	8.30 ± 0.17^{f}	13.46 ± 0.25^{c}	11.84 ± 0.23^{d}
T_3	7.60 ± 0.17^{a}	6.80 ± 0.34^{bcd}	7.30 ± 0.15^{b}	6.50 ± 0.22^{cde}	11.82 ± 0.27^{a}	10.88 ± 0.38^{bc}	15.90 ± 0.28^{a}	14.80 ± 0.34^{b}
T_4	7.32 ± 0.21^{ab}	7.20 ± 0.13^{abc}	6.90 ± 0.24^{bc}	6.70 ± 0.29^{bcd}	11.38 ± 0.19^{ab}	11.16 ± 0.23^{ab}	14.80 ± 0.48^{b}	15.32 ± 0.33^{ab}
T_5	6.42 ± 0.21^{def}	6.72 ± 0.30^{cde}	8.40 ± 0.30^{a}	6.20 ± 0.13^{de}	9.80 ± 0.34^{de}	10.40 ± 0.21^{cd}	10.86 ± 0.37^{e}	10.68 ± 0.29^{ef}

Values with different superscript letters in a column are statistically significant (p <0.05), and (n =5).

rheological attributes and bread quality more significantly when it was applied to wheat grains during tempering. Therefore, for better performance, xylanase should be applied during tempering than mixing.

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