ISSN 1330-9862
(FTB-1838)

review

Xylanases and Their Applications in Baking Industry

Masood Sadiq Butt, Muhammad Tahir-Nadeem, Zulfiqar Ahmad and Muhammad Tauseef Sultan*

National Institute of Food Science and Technology, University of Agriculture, PK-38040 Faisalabad, Pakistan

Received: December 20, 2006
Accepted: October 1, 2007

Summary

Xylan is the second most abundant polysaccharide and a major component of plant cell wall. Cereal xylans contain large quantities of L-arabinose and are therefore, often referred to as arabinoxylans. Xylanases are hydrolytic enzymes, which randomly cleave the β-1,4 backbone of this complex plant cell wall polysaccharide. Different species of *Aspergillus* and *Trichoderma* produce these enzymes. Xylanases are of great value in baking as they have been found to improve the bread volume, crumb structure and reduce stickiness. When xylanases are used at optimum levels, they play a significant role in increasing shelf life of bread and reduce bread staling. There is an increasing trend in baking industry towards the application of xylanases in bread production. This review discusses the application of xylanase in the bakery industry, alone and in combination with other enzymes when it shows synergism in the action with them.

Key words: microorganisms, *Aspergillus niger*, *Trichoderma harzianum*, xylanases, bread, xylans, baking

Introduction

Bread is the most common and traditional food around the world. It has close links with enzymes. For years, enzymes such as malt and fungal α-amylases have been used in breadmaking. Due to the changes in baking industry and the ever-increasing demand for more natural products, enzymes have gained real importance in breadmaking, where they improve dough and bread quality leading to improved dough flexibility, machinability, stability, loaf volume and crumb structure (*1*,*2*). Enzymes such as proteases, xylanases, and cellulases directly or indirectly improve the strength of the gluten network and therefore, improve the quality of bread (*3*). Xylans have an important role in bread quality due to their water absorption capability and interaction with gluten (*4*).

The hydrolysis of pentosans using some enzymes like hemicellulase or pentosanase at the optimal level improves the dough properties, leading to a greater uniformity in quality characteristics (*5*). Xylanases are important enzymes for the degradation of plant cell wall material. Based on sequence similarities, xylan-degrading enzymes are classified into several families of glycosylhydrolases (*6*).

Fungi are the most common source of hemicellulases like glucanases and xylanases. Thermophillic fungi, because of the production of thermophillic enzymes, have a wide commercial importance. These thermophillic fungi can thrive at a temperature of 40–60 °C. They have higher kinetic rates and thermostability of enzymes, less chance of contamination and better storage capacity (*7*). Xylanases make the dough more tolerant of different flour quality and variations in processing parameters. They also make the dough soft, *i.e.* reduce the sheeting work requirements and significantly increase the volume of the baked bread (*8*,*9*).

Xylanases have gained much importance in biotechnology owing to their application in various industries like paper, feed, food and fermentation (*10*,*11*). The xyla-

*Corresponding author; Phone: ++92 321 6639 739; E-mail: tahir_nadeemm@yahoo.com

nolytic enzymes are also employed for clarifying juices and wines, for extracting coffee, plant oils and starches, for improving the nutritional properties of agricultural silage and grain feed (*12,13*). Sugars like xylose, xylobiose and xylooligomers can be prepared by the enzymatic hydrolysis of xylan. Bioconversion of lignocelluloses to fermentable sugars has a great economic prospect. The depolymerization action of endo-1,4-xylanase (EC 3.2.1.8) results in the conversion of the polymeric substance into xylooligosaccharides and xylosidases. Debranching enzymes and esterases allow the complete degradation of the xylooligosaccharides to their monomeric constituents (*12–15*).

Chemistry of Xylans

Xylan, the second most abundant polysaccharide and a major component in plant cell wall consists of β-1,4--linked xylopyranosyl residues (Fig. 1). The plant cell wall is a composite material in which cellulose, hemicellulose (mainly xylan) and lignin are closely joined together (*16*).

Lignin is bound to xylans by an ester linkage to 4--*O*-methyl-D-glucuronic acid residues. The structure of xylans found in cell walls of plants can differ greatly depending on their origin, but they always contain α-1,4--linked D-xylose backbone. Different structures attached to the xylan backbone can result in a large variety of xylan structures found in plants. Although most of the xylans are branched structures, some linear polysaccharides have been isolated (*17*).

The 'lignin barrier' in lignocellulose can be disrupted by using various pretreatment methods that expose most of the polysaccharide components to enzymatic hydrolysis (*18*). Selective hydrolysis of xylan has been observed when purified xylanases with an enriched xylanase preparation were used (*19*), and when crude enzyme was used under conditions in which cellulases were inhibited (*20*). In all of these cases, complete removal of the xylosyl residues from the fibers was not achieved. The residual xylosyl residues may be inaccessible to xylanolytic enzymes for several reasons: they may carry substituents; they may be substituents on various polysaccharides; they may have been modified during fiber synthesis; or they may be in the form of xylans, which are enclosed by other polysaccharides. There have been observations which suggest that cellulose is protected from cellulases by xylan and mannan (*21*). When xylan or mannan was selectively removed from delignified fiber, using enzymes, the residual cellulose was more accessible to hydrolysis by cellulases. However, a similar prehydrolysis of cellulose or mannan did not improve the accessibility of xylan to xylanases. Probably xylan is relatively more important to fiber cohesion so that its selective removal increases accessibility of the other polysaccharides by increasing fiber porosity. Fiber porosity has been shown to be positively correlated with cellulose hydrolysis in pretreated fibers (*22,23*).

In cereals, arabinoxylans form the major non-starch polysaccharide. They constitute 4–8 % of barley kernel and represent 25 and 70 % of the cell wall polysaccharides of endosperm and aleurone layer, respectively. The arabinoxylans are partly water-soluble and result in a highly viscous aqueous solution (*24*). Cereal xylans contain large quantities of L-arabinose and are therefore, often referred to as arabinoxylans, whereas hardwood xylans are often referred to as glucuronoxylans due to the large amount of D-glucoronic acid attached to the backbone. Arabinose is connected to the backbone of xylan *via* α--1,2 or α-1,3 linkage either as single residues or as short side chains (Fig. 2). These side chains can also contain xylose β-1,2-linked to arabinose, and galactose, which can be either β-1,5-linked to arabinose or β-1,4-linked to xylose (*17*).

The main component of non-starch polysaccharides in wheat flour are pentosans (mainly arabinoxylans, AX). Arabinoxylans occur as minor components of wheat flour (2–3 %, dry basis), and can be divided into soluble or water-extractable arabinoxylans (WE-AX) and insoluble or water-unextractable arabinoxylans (WU-AX). However, they play an important role in dough rheology and bread quality (*25,26*). Numerous studies on the functional role of pentosans in dough development have been performed studying their effect on bread properties in the last decades (*27–29*).

Xylanases

Xylanases are genetically single chain glycoproteins, ranging from 6–80 kDa, active between pH=4.5–6.5 and at temperature between 40 and 60 °C. Xylanases from different sources differ in their requirements for temperature, pH, *etc.* for optimum functioning (Table 1, *30–44*). The complete enzymatic hydrolysis of xylan into its constituent monosaccharides requires the synergistic action of a consortium of xylanolytic enzymes. This is due to the fact that xylans from different sources exhibit a significant variation in composition and structure (*7,45,46*). The most important enzyme is endo-1,4-xylanase (EC 3.2.1.8), which initiates the conversion of xylan into xylooligosaccharides. Xylosidase, debranching enzymes (L-arabinofuranosidase and glucuronidase) and esterases

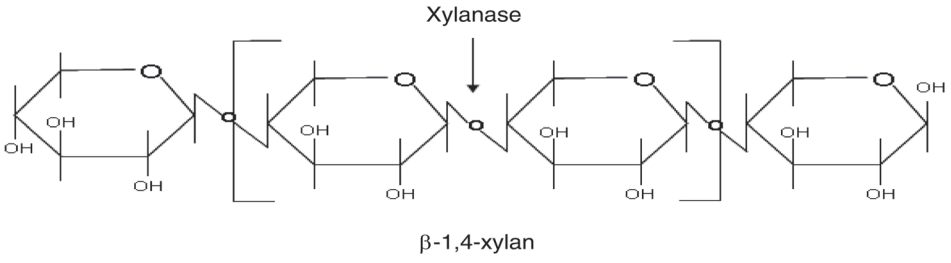

Fig. 1. Structure of xylan, xylopyranosyl residues linked through β-1,4 linkages; point of xylanase action is shown

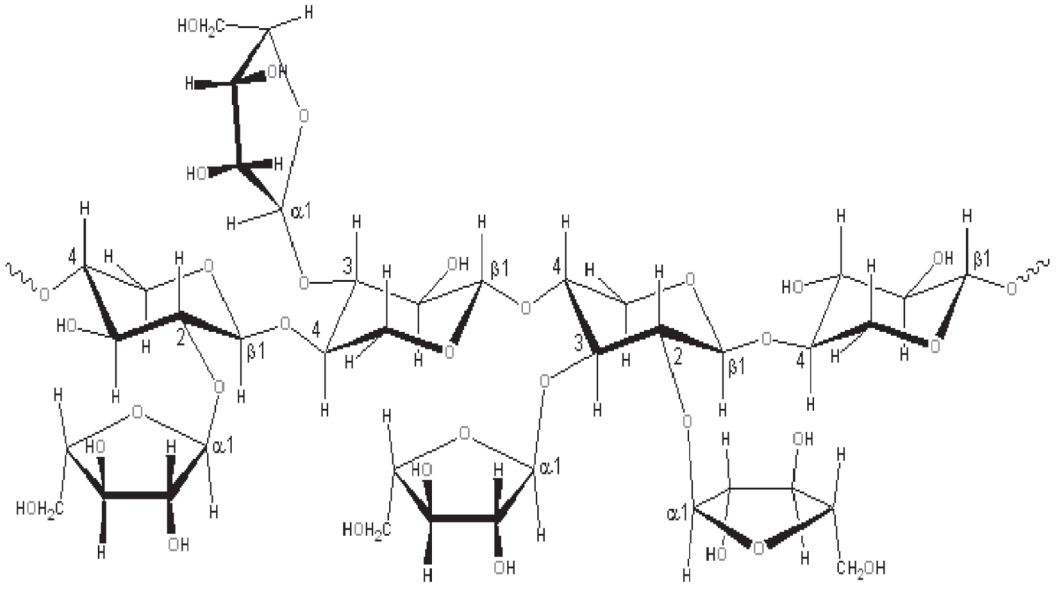

Fig. 2. Arabinoxylans; α-L-arabinofuranose residues attached as branch-points to β-(1→4)-linked D-xylopyranose polymeric backbone chains

Table 1. Characteristics of some xylanases produced by different microorganisms

Microorganism	Molecular mass/ kDa	Optimum pH	Optimum temperature/°C	Reference
Acrophialophora nainiana	22	7.0	55	(*30*)
Aspergillus awamori	39	5.5–6.0	40–55	(*31*)
Aspergillus nidulans	34	6.0	56	(*32*)
Aspergillus nidulans KK-99	nd	8.0	55	(*33*)
Aspergillus oryzae	35	5.0	60	(*34*)
Aspergillus sojae	32.7	5.0–5.5	50–60	(*35*)
Aspergillus terreus	nd	7.0	50	(*36*)
Aspergillus terreus	nd	4.5	45	(*37*)
Myceliophthora sp.	53	6.0	75	(*38*)
Penicillium capsulatum	22	3.48	48	(*39*)
Streptomyces sp.	24.5, 37.5, 38	6.0–8.0	55–60	(*40*)
Thermomyces lanuginosus	24.7	6.0–6.5	70	(*41*)
Trichoderma harzianum	20	5.0	50	(*42*)
Trichoderma longibrachiatum	37.7	5.0–6.0	45	(*43*)
Trichoderma viride	22	5.0	53	(*44*)

nd=not determined

(acetyl xylan esterase, feruloyl esterase) allow the complete degradation of the xylooligosaccharides to their monomeric constituents (*12,15,47*).

The heterogeneity and complexity of xylan has resulted in an abundance of diverse xylanases with varying specificities, primary sequences and folds, and hence has led to limitations with the classification of these enzymes by substrate specificity alone. Wong *et al*. (*48*) classified xylanases into two groups on the basis of their physicochemical properties: (*i*) having low molecular mass (<30 kDa) and basic pI, and (*ii*) having high molecular mass (>30 kDa) and acidic pI. However, many xylanases, in particular fungal xylanases, cannot be classified by this system.

A more complete classification system has been introduced which allows the classification of not only xylanases, but also glycosidases in general (EC 3.2.1.x). This system has now become the standard means for the classification of these enzymes. It is based on primary structure comparisons of the catalytic domains only and groups the enzymes in families of related sequences (*49*). As new glycosidase sequences are being identified after

each day, the number of families has reached 111 (*50*). About one-third of these families are polyspecific, *i.e.* contain enzymes with diverse substrate specificities. As the structure and molecular mechanism of an enzyme are related to its primary structure, this classification system reflects both structural and mechanistic features. Enzymes within a particular family have a similar three-dimensional structure and similar molecular mechanism (*51*). It has also been suggested that they may have a similar specificity of action on small, soluble, synthetic substrates (*52*). Furthermore, divergent evolution has resulted in some of the families having related three-dimensional structures and thus the grouping of families into higher hierarchical levels, known as clans, has been introduced. Untill now, 14 different clans have been proposed (GH-A to GH-N), with most clans encompassing two to three families, except clan GH-A, which currently encompasses 17 families (*53*).

Within this classification system, xylanases are normally reported as being confined to families 10 and 11 (*54,55*). A closer look at the available literature, however, shows that only those sequences classified in families 5, 7, 8, 10, 11 and 43 contain truly distinct catalytic domains with a demonstrated endo-1,4-β-xylanase (EC 3.2.1.8) activity. Those sequences reported for families 16, 52 and 62 appear, in fact, to be bifunctional enzymes containing two catalytic domains: family 10 or 11 of xylanase domain, as well as a glycosidase domain. In addition, those enzymes classified in family 26 do not appear to be endo-1,4-β-xylanases, but endo-1,3-β-xylanases. Thus, the enzymes with xylanase activity are included in families 10 and 11 as well as 5, 7, 8 and 43 (*56*).

Different enzymes may be more effective in the hydrolysis of xylobiose, substituted xylooligosaccharides, xylosyl substituents, or oligosaccharides containing xylosyl and other residues. The different forms may also have varying abilities to interact with xylanases in xylan hydrolysis (*57*).

Three apparent xylosidases have been classified as 'exoxylanases' because they have detectable activity on xylan. Two of these enzymes apparently lack transferase activity and one enzyme causes configuration inversion (to initially yield α-D-xylose during hydrolysis), which is the characteristic used to distinguish between β-glucosidases and exoglucanases (*57,58*). Furthermore, an exoglucanase from *Trichoderma viride* has been shown to attack xylan in an endwise fashion to initially yield xylobiose. Exoxylanases increase the rate of xylan hydrolysis by attacking large xylooligosaccharides, which are released by endoxylanases and are ineffectively hydrolyzed by β-xylosidases. This form of cooperation would not be expected to increase the extent of hydrolysis unless other factors are involved (*e.g.* accessibility of xylosidic linkages in short and/or branched xylooligosaccharides, reduction of product inhibitions, or amounts of extracellular β-xylosidases) (*59*).

Xylans are usually cleaved at unsubstituted regions to yield mixtures of unsubstituted xylooligomers, as well as short and long chain substituted xylooligomers (Fig. 1). Removal of the substituent groups by ancillary enzymes creates new substrates for endoxylanase (EC 3.2.1.8) action (*60–63*).

Application in Baking

The application of xylanolytic enzymes has increased for the last few decades owing to their potential effectiveness in breadmaking. Starch and non-starch carbohydrate-hydrolyzing enzymes are commonly used in the breadmaking industry as bread improvers (*64,65*). Enzymatic hydrolysis of non-starch polysaccharides leads to the improvement of rheological properties of dough, bread specific volume, and crumb firmness (*66*).

Xylanase transforms water-insoluble hemicellulose into soluble form, which binds water in the dough, therefore decreasing dough firmness, increasing volume and creating finer and more uniform crumbs. It significantly improves manufacturing conditions: dough is made more 'machine-friendly' as it does not stick to the machinery parts (*67*).

During gluten-starch separation process, gluten is formed first as a result of breakdown of the gliadin--glutelin structures during mixing, followed by their re--agglomeration. To study their effect, pentosans, enzymes, *etc.* can be added after the mixing step by simple modification of Glutomatic System. It has been observed that re-aggregation of gluten protein starts immediately after the first mixing step during the dough dilution phase. Addition of pentosans or xylanase during this phase can strongly affect gluten formation. The addition of xylanase prior to dough mixing can lead to overdose effects. This is not observed when xylanase is added later during the agglomeration phase. Pentosans and xylanase act mainly during the re-agglomeration of gluten, following the breakdown of gluten structures during mixing, which ultimately affects both gluten yield and gluten rheological properties. Effects of pentosans and xylanase on gluten are paralleled by effects on dough, especially on dough extensibility (*68*).

Xylan endohydrolases with differences in functionality have different abilities in the conversion of WU–AX into solubilized AX and in degradation of WE–AX. For instance, *Bacillus subtilis* (family GT11) endoxylanase and *Aspergillus niger* (family GT10) endoxylanase behave differently in baking applications. *B. subtilis* xylanases preferentially cleave the backbone in unsubstituted regions of AX, whereas *A. niger* xylanases can also act on branched regions, producing smaller units than *B. subtilis* xylanase (*12*). *A. niger* xylanase, which degrades WE–AX, decreases dough viscosity and the molecular mass of the WE–AX and solubilized AX, resulting in improved gluten agglomeration behaviour and the formation of larger gluten aggregates, whereas *B. subtilis* xylanase, which solubilizes WU–AX in particular, increases the viscosity of dough and has a negative effect on gluten agglomeration. The ability of this xylanase to solubilize WU–AX and its low activity on WE-AX and solubilized AX results in good performance during breadmaking (*14,69*).

Xylanases (hemicellulases) and enzymes that hydrolyze complex cell wall are used to improve dough handling properties, to enhance bread quality, extend shelf life by reducing the staling rate, and they appear to be particularly effective in straight dough process (*68,70,71*). The improved handling properties and stability of the dough are obtained by xylanase action on both soluble and insoluble pentosans in flour, whereby improving the elas-

ticity of the gluten network, crumb structure and bread volume. These can be used for all types of bread as an alternative to, or in combination with, emulsifiers (*72, 73*). These enzymes may also contribute to eliminating the use of chemical additives such as bromate (*74,75*).

The most important change associated with staling of bread is the gradual increase in crumb firmness (*3,76*). Xylanases have an antistaling action during bread storage and most of the added xylanases have been found to have a significant effect on softening of doughs. Because most of the xylanases used in breadmaking are specific for insoluble AX, the ones specific for soluble AX are reported to have a negative impact on bread quality. Softening has an indirect effect on the breakdown of arabinoxylans due to water release. In spite of the softening, dough tolerance, oven spring, as well as bread volume, shape and texture are all improved (*25,26*).

The addition of xylanase B (XynB) from *Thermotoga maritima* has been found to improve the specific volume. The highest specific volume increased up to 60.3 % when 120 ppm of XynB was present. The enzyme also improved cell structure of the crumb. The coarse and non-homogeneous crumb cell structure of the control sample became progressively finer and more homogeneous at the levels of 10–120 ppm. The xylanase acted on the dough mainly at the first stage (60 °C); however, it maintained activity even at the second stage (90 °C) due to its thermostability. The presence of XynB also modified the texture of the fresh breadcrumb. Positive effects of XynB were found on the crumb firmness of fresh bread. The enzyme reduced the firmness of the fresh bread (42.1–68.4 % as compared to the control) and made the crumb softer. The crumb firmness of the control was decreased to 57.9 % with the addition of 100 ppm of XynB (*77*).

Baking trials were performed with six types of wheat dough, prepared with xylanase, peroxidase, or glucose oxidase (GOX), and their combinations. Judging dough properties, baking performance, bread volume and crumb structure, the dough containing xylanase and peroxidase performed the best (*78*). Chemical analysis and high performance size-exclusion chromatography analysis of apparent molecular mass distribution indicated that xylanase acts on both cold-water-extractable arabinoxylans and those that can be solubilized from cell wall fragments by hot water extraction (*79*).

Xylanase along with protease, lipase and α-amylase are significantly effective for obtaining bread with higher specific volume in microwave oven, as compared to the bread with no enzyme added. The texture profile analysis was greatly modified by xylanases and the firmness of breadcrumb was reduced (*80–82*).

Laurikainen *et al.* (*83*) supplemented bread dough with partially purified xylanase (12 U/g of flour) from *Aspergillus foetidus* during mixing and observed remarkable difference in water absorption. As compared to control, less water was needed for xylanase-supplemented dough that had similar consistency without stickiness. Water absorption was reduced from 72 to 64 % as a result of xylanase addition. The added enzyme decreased dough stability and increased softening. Addition of enzyme caused significant differences in the stickiness of the wheat dough both with ($p<0.003$) and without ($p<0.001$) rye bran. The specific volume of the wheat bread was increased by almost 20 % by the addition of enzyme, both with and without rye bran. However, the lower volume than in other reported results (*77*) can be due to lower level used or the addition of rye bran that ultimately affected the rheological properties of the dough. The deleterious effects of strong over-dosage of endoxylanases on loaf volume are not representative of the effects obtained when endoxylanases are used at optimum concentrations (*84*).

The most important measure of bread quality is the final loaf volume. This is especially true of whole wheat bread, in which loaf volume tends to be lower than in bread made from white flour. The bran, when added to white flour, binds large volume of water and thus gluten is not properly hydrated. Poorly hydrated gluten results in lower loaf volume (*85,86*). Starch and non-starch hydrolyzing enzymes result in release of free water and change the soluble fraction of dough. These effects are apparent immediately after mixing and continue during resting, which changes viscoelastic properties of dough (*30,87*).

Xylanases of *Aspergillus niger* var. *awamori* and *Thermotoga maritima* have been found to improve specific volume of wheat bread (*60*). Moisture content of fresh bread is directly related to the softness of the baked product. Ideal moisture level for bread is between 35 and 40 % (*88*). Bread whose moisture content is reduced to less than 30 % cannot be refreshed even after heating (*89*). In wheat bread supplemented with xylanase from *Thermomyces lanuginosus* CAU44, the final moisture content of fresh bread has been observed to increase from 29.4 (control) to 34.5 % (*90*).

Xylanases (endoxylanases, EC 3.2.1.8) cleave the xylan backbones of water unextractable arabinoxylans (WU-AX), release water extractable arabinoxylans (WE-AX) and reduce their molecular mass. Extensive hydrolysis of AX causes water redistribution from AX to the gluten and starch phases, making the dough slacker, softer and more viscous. Solubilization of WU-AX increases the viscosity of the dough aqueous phase, whereas degradation of enzyme-solubilized AX and WE-AX results in a decrease in molecular mass of AX fragments, reducing their viscosity-forming properties. In addition to the redistribution of water from WU-AX to gluten, increased viscosity due to WU-AX solubilization is reported to correlate positively with bread quality (*25*). The increase in gluten volume gives more extensibility, which eventually results in better oven spring. Positive influence on dough as well as bread attributes has been observed by the addition of partially purified xylanase from *A. foetidus* MTCC 4898. The sensory evaluation of various bread attributes reflected the positive impact of the enzyme on the quality of whole wheat bread (*91*).

In another case, nine commercial enzyme preparations and two laboratory designed mixes containing amylase and/or pentosanase/xylanase activity were used to prepare bread samples and their effect on bread quality and keeping properties were determined. Enzyme addition significantly shortened fermentation time without affecting pH or machinability of the dough. Bread of improved volume, greater intensity of aroma and softer tex-

ture was obtained. All enzymes delayed bread firming, but rates varied with each preparation (*66*).

The effects of sourdough and enzyme mixture (α-amylase, xylanase and lipase) on the specific volume, staling and microstructure of white pan bread supplemented with wheat bran have been studied. The combination of bran, sourdough and enzyme mixture significantly improved the volume, texture and shelf-life of wheat bread supplemented with wheat bran (20 g bran/100 g of flour). The anti-staling effect of combined use of bran, sourdough and enzyme mixture was attributed to reduced starch retrogradation rate, slowed increase in rigidity of polymer structure and due to degradation of cell wall components leading to altered water distribution between starch-protein matrixes (*92*).

Gámbaro *et al*. (*93*) studied the effect of enzyme addition on brown pan bread texture. The addition of amylase or mixture of amylase and xylanase resulted in an improvement of the sensory characteristics of bread texture (smoothness, cohesiveness, soft center area and visual dryness), and an increase of overall acceptability. A synergistic effect was observed between amylase and xylanase, as the addition of the mixture resulted in a greater decrease of the staling rate, increased quality and consumer acceptability than the one achieved with each enzyme alone. Although xylanase has been generally described to have anti-staling effect, some of the authors (*93,94*) evaluated the effect of amylolytic-, non-amylolytic- and gluten-cross-linking enzymes on the viscoelastic properties of fresh and stored pan bread samples by using dynamic mechanical analysis and thermal mechanical analysis techniques. They also reported increased staling rate in bread, due to xylanase addition (*93,94*). Such contradictions in results might be due to the use of higher doses (~750 U/kg) of flour. However, more research is required in this regard because the mechanisms by which enzymes that degrade nonstarch polysaccharides affect bread staling are unclear, possibly because of the variety of xylanases (*77*).

Primo-Martín *et al.* (*95*) observed synergism between xylanase and glucose oxidase. Glucose oxidase was found not only to catalyse the formation of protein disulphide bonds, but also of AX-AX crosslinks. As the latter negatively affects bread quality, xylanase corrected this latter effect by cleaving arabinoxylan complexes and generating small ferulic acid-containing arabinoxylan fragments interfering with the cross linking of high molecular mass arabinoxylans. Therefore, the use of combination of different enzymes results in better performance due to their synergistic action on the formula ingredients, as reported in this case.

The endoxylanase from *Aspergillus niger* IBT-90 with the specific activity of 21.3 U/mg protein was used for improvement of wheat-rye and whole meal bread. The addition of this xylanase in the range of 500–1000 U/kg flour resulted in improvement of kneading and increase of loaf volume in the case of all kinds of bread. This dough supplementation caused better crumb porosity and higher moisture in bread and finally the shelf life was extended without increase in total acidity (*69*).

Baking trials using the crude filtrate of xylanase and purified enzyme have shown that addition of both preparations improves bread volume. Purified endoxylanase (EC 3.2.1.8) caused a 30 % increase in volume over the crude extract (Table 2). The too high doses of xylanases might give sticky dough and too low doses will not give the optimal functionality (*70,97*). The results of the application depend on the level of the enzyme used, its purification level and presence of other enzymes, *etc*. A more purified enzyme will definitely show better results as compared to the crude or partially purified enzymes, but it will add to the ultimate cost of production.

Addition of XYL22 (a xylanase having molecular mass of 22 kDa), from *Aspergillus* sp. FP 470, to bread dough increased loaf volume and improved crumb structure of the baked product. However, in cases with more than 150 or 50 ppm for crude culture filtrate and purified enzyme respectively, dough became sticky. As discussed earlier, the best results of the enzyme application are obtained when their optimum levels are used. The excessive doses result in deleterious effects on the quality of final product as evident from results reported for *T. viridae* and *Aspergillus niger* xylanases (*75,78,96*).

Xylanases, cellulase, and glucanase improve the properties of wheat bread and reduce staling during storage. The presence of the carbohydrases results in high specific volume (Table 2), reduces bread firmness and greatly improves texture profile (*98*).

Bread-making with a composite flour (CF) consisting of 60 % wheat flour (WF) and 40 % hull-less barley flour increased the total and soluble (1,3)-, (1,4)-β-D-glucan and total arabinoxylan (AX) contents of dough and bread samples, but decreased the specific bread loaf volume. Xylanase addition not only markedly improved loaf volume of CF bread, but also increased the soluble AX content of the wheat flour (WF) and composite flour (CF) dough and bread samples because of the conversion of water-unextractable AX into soluble AX. The combined

Table 2. Increase in final loaf volume by the addition of xylanases from different sources

Microorganism/Source	Increase/%	Dosage	Reference
Aspergillus foetidus	53	12000 U/kg flour	(*91*)
Aspergillus niger IBT-90	13	500 U/kg flour	(*69*)
Aspergillus sp. FP-470	30	25 ppm	(*95*)
Commercial preparation	29	14600 U/kg kernel (used during tempering)	(*96*)
Thermomyces lanuginosus	41.1	40 ppm	(*84*)
Thermotoga maritima	60.3	120 ppm	(*77*)

use of CF and xylanase resulted in bread with much higher total and soluble AX, (1,3)- and (1,4)-β-D-glucan contents, which implies a positive contribution to daily recommended dietary fibre levels (*99*). AX and (1,3)- and (1,4)-β-D-glucan act as cholesterol and blood glucose lowering agents. The former action reduces the risk of coronary heart diseases, while the latter is beneficial for diabetics. Increasing the levels of these dietary fiber constituents in food would be beneficial for human health (*100,101*).

The increasing levels of eight commercial enzymes representing four types of fungal hydrolytic enzymes (amylases, proteases, xylanases, and cellulases) on Canadian short process (CSP) bread quality and processing characteristics showed that addition of all enzymes at optimum levels can result in increased loaf volume, bread score and produce softer crumb. All four types of enzymes appeared to be equally effective in improving bread properties compared to the controls (*102*).

Rheological behaviour of dough made with different commercial enzyme preparations consisting of amylases, xylanases, lipases, and glucose oxidase, singly and in mixed combinations has been investigated. It was found that enzyme supplementation results in softening and weakening of the dough immediately after mixing and further during resting. Xylanases caused the main changes, when compared to the dough without the supplement (*66*).

Studies with the enzymes such as α-amylase, protease and xylanase, using primarily straight dough processes, have demonstrated their ability to increase loaf volume (*26,63,71,103–108*). Effects of endogenous flour enzymes supplemented with enzymes of microbial origin, *e.g.* α-amylase, xylanase and proteases, have been examined. Xylanase, through the modification of wheat flour arabinoxylans, gave up to a 10 % increase in loaf volume (*62*). The enzyme preparations containing bacterial α-amylase, laccases, lipase, xylanase, mixture of two enzymes (xylanase and laccase 2) or a mixture of three enzymes (α-amylase, lipase and xylanase) added to the dough have been found to increase the specific volume of bread baked from pre-fermented frozen dough. The mixtures of enzymes were most effective in improving the loaf volume and retaining the softness of bread (*109*).

Xylanases are also being investigated in flat bread. Prabhasankar *et al*. (*110*) studied the role of enzymes in baking process of unleavened Indian flat bread, namely South Indian parotta. Comparisons were made on the effects of fungal amylase (FA), glucose oxidase (GO), proteinase and xylanase on rheological characteristics and microstructure of parotta dough. The addition of proteinase and xylanase resulted in increased quality score of 92 and 82, respectively, as compared to 74 of the control.

Blends of fungal amylase, fungal xylanase/pentosanase and ascorbic acid are now being used to replace bromate in bread, buns, rolls or other yeast raised bakery products. Such preparations decrease the rate of crumb firming in bread during storage (*111*).

Conclusion

Xylanases have great potential for use in baking industry. There is strong evidence in literature that the application of xylanases in breadmaking results in increased volume, reduced stickiness and staling, and increased shelf life. The enzyme can substitute the addition of different emulsifiers and other chemical additives used in bread production. However, for the best results, several enzymes should be used at optimum levels, as the overdosing has adverse effects on the final product. Moreover, it is a good strategy to use xylanase in combination with other enzymes because the synergistic effects of xylanase with other enzymes provide better results as compared to its sole use.

References

1. E. Baillet, G. Downey, M. Tuohy, Improvement of texture and volume in white bread rolls by incorporation of microbial hemicellulase preparations, *Recent Advances in Enzymes in Grain Processing, Proceedings of the 3rd European Symposium on Enzymes in Grain Processing (ESEGP-3)*, C.M. Courtin, W.S. Veraverbeke, J.A. Delcour (Eds.), Katholieke Universiteit Leuven, Leuven, Belgium (2003) pp. 255–259.
2. R.C.E. Guy, S.S. Sarabjit, Comparison of effects of xylanases with fungal amylases in five flour types, *Recent Advances in Enzymes in Grain Processing, Proceedings of the 3rd European Symposium on Enzymes in Grain Processing (ESEGP-3)*, C.M. Courtin, W.S. Veraverbeke, J.A. Delcour (Eds.), Katholieke Universiteit Leuven, Leuven, Belgium (2003) pp. 235–239.
3. J.A. Gray, J.N. BeMiller, Bread staling: Molecular basis and control, *Compr. Rev. Food Sci. Food Saf. 2* (2003) 1–21.
4. F.H. Nuyens, H. Verachtert, C. Michiels, Evaluation of a recombinant *Saccharomyces cerevisiae* strain secreting a *Bacillus pumilus* endo-beta-xylanase for use in bread-making, *Meeting of the Benelux Yeast Research Groups*, Leuven, Belgium (2001).
5. X. Rouau, M.L. EI-Hayek, D. Moreau, Effect of an enzyme preparation containing pentosanases on the bread-making quality of flours in relation to changes in pentosan properties, *J. Cereal Sci. 19* (1994) 259–272.
6. N.R. Gilkes, B. Henrissat, D.G. Kilburn, R.C. Miller, R.A.J. Warren, Domains in microbial β-1,4-glycanases: Sequence, conservation, function, and enzyme families, *Microbiol. Rev. 55* (1991) 303–315.
7. F. Latif, M. Asgher, R. Saleem, A. Akram, R. Legge, Purification and characterization of xylanase produced by *Chaetomium thermophile* NIBGE, *World J. Microbiol. Biotechnol. 22* (2006) 45–50.
8. G. Dervilly, C. Leclercq, D. Zimmermann, C.Roue, J.F. Thibault, L. Saulnier, Isolation and characterization of high molar mass water-soluble arabinoxylans from barley and barley malt, *Carbohydr. Polym. 47* (2002) 143–149.
9. L. Harbak, H.V. Thygesen, Safety evaluation of a xylanase expressed in *Bacillus subtilis*, *Food Chem. Toxicol. 40* (2002) 1–8.
10. R.C. Kuhad, A. Singh, Lignocellulosic biotechnology: Current and future prospects, *Crit. Rev. Biotechnol. 13* (1993) 151–172.
11. P. Bajpai, Application of enzymes in the pulp and paper industry, *Biotechnol. Progr. 15* (1999) 147–157.
12. P. Biely, M. Vrsanska, M. Tenkanen, D. Kluepfel, Endo-β-1,4-xylanase families: Differences in catalytic properties, *J. Biotechnol. 57* (1997) 151–166.
13. V. Malathi, G. Davegowda, *In vitro* evaluation of non-starch polysaccharide digestibility of feed ingredients by enzymes, *Poultry Sci. 80* (2001) 302–305.

14. C.M. Courtin, J.A. Delcour, Relative activity of endoxylanases towards water-extractable and water-unextractable arabinoxylan, *J. Cereal Sci. 33* (2001) 301–312.

15. S. Subramaniyan, P. Prema, Optimization of cultural parameters for the synthesis of endoxylanase from *Bacillus* SSP-34, *J. Sci. Ind. Res. 57* (1998) 611–616.

16. J. Puls, Chemistry and biochemistry of hemicelluloses: Relationship between hemicellulose structure and enzymes required for hydrolysis, *Macromol. Symp. 120* (1997) 183–196.

17. R.P. de Vries, J. Visser, *Aspergillus* enzymes involved in degradation of plant cell wall polysaccharides, *Microbiol. Mol. Biol. Rev. 65* (2001) 497–522.

18. M.M. Chang, T.Y.C. Chou, G.T. Tsao, Structure, pretreatment and hydrolysis of cellulose, *Adv. Biochem. Eng. 20* (1981) 15–42.

19. M.G. Paice, L. Jurasek, Removing hemicellulose from pulps by specific enzymic hydrolysis, *J. Wood Chem. Technol. 4* (1984) 187–198.

20. F. Mora, J. Comtat, F. Barnoud, F. Pla, P. Noe, Action of xylanases on chemical pulp fibers. Part I: Investigations on cell-wall modifications, *J. Wood Chem. Technol. 6* (1986) 147–165.

21. M. Sinner, N. Parameswaran, H.H. Dietrichs: Degradation of Delignified Spruce Wood by Purified Manganese, Xylanase, and Cellulases. In: *Hydrolysis of Cellulose; Mechanism of Enzymatic and Acid Catalysis*, *Advances in Chemistry Series No. 181*, R.D. Brown Jr, L. Jurassek (Eds.), American Chemical Society, Washington DC, USA (1979) pp. 303–329.

22. H.E. Grethlein, The effect of pore size distribution on the rate of enzymatic hydrolysis of cellulosic substrates, *Bio/Technology, 3* (1985) 155–160.

23. K.K.Y. Wong, K.F. Deverell, K.L. Mackie, T.A. Clark, L.A. Donaldson, The relationship between fiber porosity and cellulose digestibility in steam-exploded *Pinus radiata*, *Biotechnol. Bioeng. 31* (1988) 447–456.

24. G. Dervilly-Pinel, J.F. Thibault, L. Saulnier, Experimental evidence for a semi flexible conformation for arabinoxylans, *Carbohydr. Res. 330* (2001) 365–372.

25. C.W. Courtin, J.A. Delcour, Arabinoxylans and endoxylanases in wheat flour bread-making, *J. Cereal Sci. 35* (2002) 225–243.

26. B.V. McCleary, Enzymatic modification of plant polysaccharides, *Int. J. Biol. Macromol. 8* (1986) 349–354.

27. C.G. Biliaderis, M.S. Izydorczyk, O. Rattan, Effect of arabinoxylans on bread-making quality of wheat flours, *Food Chem. 53* (1995) 165–171.

28. E. Denli, R. Ercan, Effect of added pentosans isolated from wheat and rye grain on some properties of bread, *Eur. Food Res. Technol. 212* (2001) 374–376.

29. T. Maeda, N. Morita, Flour quality and pentosan prepared by polishing wheat grain on breadmaking, *Food Res. Int. 36* (2003) 603–610.

30. M.A. Martínez-Anaya, T. Jimenez, Physical properties of enzyme supplemented doughs and relationship with bread quality parameters, *Z. Lebensm. Unters. Forsch A*, *206* (1998) 134–142.

31. R.J. Ridgwell, J.H. de Michieli, M. Fischer, S. Reymond, P. Nicolas, D. Sievert, Xylanase induced changes to water- and alkali-extractable arabinoxylans in wheat flour: Their role in lowering batter viscosity, *J. Cereal Sci. 33* (2001) 83–96.

32. B.C. Salles, R.B. Cunha, W. Fontes, M.V. Sousa, E.X.F. Filho, Purification and characterization of a new xylanase from *Acrophialophora nainiana*, *J. Biotechnol. 81* (2000) 199–204.

33. F.J.M. Kormelink, M.J.F. Searle-van Leeuwen, T.M. Wood, A.G. J. Voragen, Purification and characterization of three endo-(1,4)-β-xylanases and one β-xylosidase from *Aspergillus awamori*, *J. Biotechnol. 27* (1993) 249–265.

34. M.T. Fernández-Espinar, F. Piñaga, L.H. de Graaff, J. Visser, D. Ramón, S. Vallés, Purification, characterization and regulation of the synthesis of an *Aspergillus nidulans* acidic xylanase, *Appl. Microbiol. Biotechnol. 42* (1994) 555–562.

35. K. Taneja, S. Gupta, R.C. Kuhad, Properties and application of a partially purified alkaline xylanase from an alkalophilic fungus *Aspergillus nidulans* KK-99, *Bioresour. Technol. 85* (2002) 39–42.

36. N. Kitamoto, S. Yoshino, K. Ohmiya, N. Tsukagoshi, Purification and characterization of the overexpressed *Aspergillus oryzae* xylanase, XynF1, *Biosci. Biotechnol. Biochem. 63* (1999) 1791–1794.

37. I. Kimura, H. Sasahara, S. Tajima, Purification and characterization of two xylanases and an arabinofuranosidase from *Aspergillus sojae*, *J. Ferment. Bioeng. 80* (1995) 334–339.

38. N.B. Ghanen, H.H. Yusef, H.K. Mahrouse, Production of *Aspergillus terreus* xylanase in solid-state cultures: Application of the Plackett-Burman experimental design to evaluate nutritional requirements, *Bioresour. Technol. 73* (2000) 113–121.

39. M. Ghareib, M.M.N. El Dein, Purification and general properties of xylanase from *Aspergillus terreus*, *Zbl. Mikrobiol. 147* (1992) 569–576.

40. B.S. Chadha, B.K. Ajay, F. Mellon, M.K. Bhat, Two endoxylanases active and stable at alkaline pH from the newly isolated thermophilic fungus, *Myceliophthora* sp. IMI 387099, *J. Biotechnol. 109* (2004) 227–237.

41. S.E. Ryan, K. Nolan, R. Thompson, G.M. Gubitz, A.V. Savage, M.G. Tuohy, Purification and characterization of a new low molecular weight endoxylanase from *Penicillium capsularium*, *Enzyme Microb. Technol. 33* (2003) 775–785.

42. J. Georis, F. Giannotta, E. de Buyl, B. Granier, J.M. Frère, Purification and properties of three endo-β-1,4-xylanases produced by *Streptomyces* sp. strain S38 which differ in their ability to enhance the bleaching of kraft pulp, *Enyme Microb. Technol. 26* (2000) 178–186.

43. S. Singh, P. Reddy, J. Haarhoff, P. Biely, B. Janse, B. Pillay, D. Pillay, B.A. Prior, Relatedness of *Thermomyces lanuginosus* strains producing a thermostable xylanase, *J. Biotechnol. 81* (2000) 119–128.

44. L.U.L. Tan, K.K.Y. Wong, E.K.C. Yu, J.N. Saddler, Purification and characterization of two D-xylanases from *Trichoderma harzianum*, *Enzyme Microb. Technol. 7* (1985) 425–430.

45. T. Cesar, V. Mrsa, Purification and properties of xylanase produced by *Thermomyces lanuginosus*, *Enzyme Microb. Technol. 19* (1996) 289–296.

46. G.P. Hazlewood, H.J. Gilbert: Molecular Biology of Hemicellulases. In: *Hemicelluloses and Hemicellulases*, M.P. Coughlan, G.P. Hazlewood (Eds.), Portland Press, London, UK (1993) pp. 103–126.

47. T.W. Jeffries, Biochemistry and genetics of microbial xylanases, *Curr. Opin. Biotechnol. 7* (1996) 337–342.

48. K.K.Y. Wong, L.U.L. Tan, J.N. Saddler, Multiplicity of β-1,4 xylanase in microorganisms: Functions and applications, *Microbiol Rev. 52* (1988) 305–317.

49. B. Henrissat, P.M. Coutinho, Classification of glycoside hydrolases and glycosyltransferases from hyperthermophiles, *Methods Enzymol. 330* (2001) 183–201.

50. CAZy, Carbohydrate-active enzymes (*http://afmb.cnrs-mrs.fr/CAZY/fam/acc_fam.html*).

51. J. Gebler, N.R. Gilkes, M. Claeyssens, D.B.Wilson, P. Beguin, W.W. Wakarchuk, D.G. Kilburn, R.C. Miller Jr, R.A. Warren, S.G. Withers, Stereoselective hydrolysis catalyzed by related β-1,4-glucanases and β-1,4-xylanases, *J. Biol. Chem. 267* (1992) 12559–12561.

52. M. Claeyssens, B. Henrissat, Specificity mapping of cellulolytic enzymes: Classification into families of structurally

related proteins confirmed by biochemical analysis, *Protein Sci. 1* (1992) 1293–1297.

53. Y. Bourne, B. Henrissat, Glycoside hydrolases and glycosyltransferases: Families and functional modules, *Curr. Opin. Struct. Biol. 11* (2001) 593–600.

54. P.L. Bergquist, M.D. Gibbs, D.D. Morris, D.R. Thompson, A.M. Uhl, R.M. Daniel, Hyperthermophilic xylanases, *Methods Enzymol. 330* (2001) 301–319.

55. A. Torronen, J. Rouvinen, Structural and functional properties of low molecular weight endo-1,4-β-xylanases, *J. Biotechnol. 57* (1997) 137–149.

56. T. Collins, C. Gerday, G. Feller, Xylanases, xylanase families and extremophilic xylanases, *FEMS Microbiol. Rev. 29* (2005) 3–23.

57. P.J. Reilly: Xylanases Structure and Function. In: *Trends in the Biology of Fermentation for Fuels and Chemicals*, A. Hollaender (Ed.), Plenum Press, New York, USA (1981) pp. 111–129.

58. K.E. Eriksson, T.M. Wood: Biodegradation of Cellulose. In: *Biosynthesis and Biodegradation of Wood Components*, T. Higuchi (Ed.), Academic Press, Orlando, FL, USA (1985) pp. 469–503.

59. S. Shikata, K. Nisizawa, Purification and properties of an exo-cellulase component of novel type from *Trichoderma viride*, *J. Biochem. 78* (1975) 499–512.

60. R. Maheshwari, G. Bahadradwaj, M.K. Bhat, Thermophilic fungi: Their physiology and enzymes, *Microbiol Mol. Biol. Rev. 64* (2000) 461–488.

61. K.B. Bastawde, Xylan structure, microbial xylanases, and their mode of action, *World J. Microbiol. Biotechnol. 8* (1992) 353–368.

62. R.L. Uffen, Xylan degradation: A glimpse at microbial diversity, *J. Ind. Microbiol. Biotechnol. 19* (1997) 1–6.

63. J.Q. Si, Synergistic effect of enzymes for bread baking, *Cereal Food World, 42* (1997) 802–807.

64. M.L.T.M. Polizeli, A.C.S. Rizzatti, R. Monti, H.F. Terenzi, J.A. Jorge, D.S. Amorim, Xylanases from fungi: Properties and industrial applications, *Appl. Microbiol. Biotechnol. 67* (2005) 577–591.

65. P.F.I. Javier, G. Óscar, J. Sanz-Aparicio, P. Díaz: Xylanases: Molecular Properties and Applications. In: *Industrial Enzymes: Structure, Function and Applications*, J. Polaina, A.P. MacCabe (Eds.), Springer, Dordrecht, The Netherlands (2007) pp. 65–82.

66. M. Martínez-Anaya, A. Jimenez, Functionality of enzymes that hydrolyse starch and non-starch polysaccharide in breadmaking, *Z. Lebensm. Unters. Forsch. A, 205* (1997) 209–214.

67. X. Rouau, Investigations into the effects of an enzyme preparation for baking on wheat flour dough pentosans, *J. Cereal Sci. 18* (1993) 145–157.

68. M. Wang, T. van Vliet, R.J. Hamer, Evidence that pentosans/xylanase affects the re-agglomeration of the gluten network, *J. Cereal Sci. 39* (2004) 341–349.

69. I. Romanowska, J. Polak, K. Janowska, S. Bieleckim, The application of fungal endoxylanase in breadmaking, *Comm. Agr. Appl. Biol. Sci. 68* (2003) 317–320.

70. J.F. Sorensen, O. Sibbesen, C.H. Poulsen, Degree of inhibition by the endogenous wheat xylanase inhibitor controls the functionality of microbial xylanases 'in Dough', *AACC Annual Meeting, Enzymes and Baking – 213AB*, Charlotte, NC, USA (2001).

71. A. Monfort, A. Blasco, J.A. Prieto, P. Sanz, Construction of baker's yeast strains that secrete different xylanolytic enzymes and their use in breadmaking, *J. Cereal Sci. 26* (1997) 195–199.

72. H.S. Olse: Use of Enzymes in Food Processing. In: *Enzymes, Biomass, Food and Feed Biotechnology*, G. Reed, T. Nagodawithana (Eds.), Wiley-VCH, Weinheim, Germany (1995) pp. 663–736.

73. B.G. Sprössler, Xylanases in baking, *Proceedings of the 1st European Symposium on Enzymes and Grain Processing (ESEGP-1)*, S.A.G.F. Angelino, R.J. Hamer, W. van Hartingsveldt, F. Heidekamp, J.P. van der Lugt (Eds.), TNO Nutrition and Food Research Institute, Zeist, The Netherlands (1997) pp. 177–187.

74. N. Kulkarni, A. Shendye, M. Rao, Molecular and biotechnological aspects of xylanases, *FEMS Microbiol. Rev. 23* (1999) 411–456.

75. J. Maat, M. Roza, J. Verbakel, H. Stam, M.J. Santos Da Silva, M. Bosse, M.R. Egmond, M.L.D. Hagemans, R.F.M. Gorcom, J.G.M. Hessing, C.A.M.J.J. Van den Hondel, C. Van Rotterdam: Xylanases and Their Applications in Bakery. In: *Xylan and Xylanases, Progress in Biotechnology No. 7*, J. Visser, M.A. Kusters van Someren, G. Beldman, A.G.J. Voragen, (Eds.), Elsevier Science Publishers, Amsterdam, The Netherlands (1992) pp. 349–360.

76. M.G. Scanlon, M.C. Zghal, Bread properties and crumb structure, *Food Res. Int. 34* (2001) 841–864.

77. Z. Jiang, X. Li, S Yang, L. Li, S. Tan, Improvement of the breadmaking quality of wheat flour by the hyperthermophilic xylanase B from *Thermotoga maritima*, *Food Res. Int. 38* (2005) 37–43.

78. R. Hilhorst, B. Dunnewind, R. Orsel, P. Stegeman, T. Van Vliet, H. Gruppen, H.A. Schols, Baking performance, rheology, and chemical composition of wheat dough and gluten affected by xylanase and oxidative enzymes, *J. Food Sci. 64* (1999) 808–813.

79. R. Hilhorst, H. Gruppen, R. Orsel, C. Laane, H. A. Schols, A.G.J. Voragen, Effects of xylanase and peroxidase on soluble and insoluble arabinoxylans in wheat bread dough, *J. Food Sci. 67* (2002) 497–506.

80. P.R. Mathewson, Enzymatic activity during bread baking, *Cereal Food World, 45* (2000) 98–101.

81. O. Ozmutlu, G. Sumnu, S. Sahin, Effects of different formulations on the quality of microwave baked breads, *Eur. Food Res. Technol. 213* (2001) 38–42.

82. S.O. Keskin, G. Sumnu, S. Sahin, Usage of enzymes in a novel baking process, *Nahrung/Food, 48* (2004) 156–160.

83. T. Laurikainen, H. Härkönen, K. Autio, K. Poutanen, Effects of enzymes in fibre-enriched baking, *J. Sci. Food Agric. 76* (1998) 239–249.

84. C.W. Courtin, G.G. Gelders, J.A. Delcour, Use of two endoxylanases with different substrate selectivity for understanding arabinoxylans functionality in wheat flour breadmaking, *Cereal Chem. 78* (2001) 564–571.

85. P.L. Bruckner, D. Habernicht, G.R. Carlson, D.M. Wichman, L.E. Talbert, Comparative bread quality of white flour and whole grain flour for hard red spring and winter wheat, *Crop Sci. 41* (2001) 1917–1920.

86. C.S. Lai, R.C. Hoseney, A.B. Davis, Effects of wheat bran in breadmaking, *Cereal Chem. 66* (1989) 217–219.

87. R.J. Ridgwell, J.H. de Michieli, M. Fischer, S. Reymond, P. Nicholas, D. Sievert, Xylanase induced changes to water and alkali-extractable arabinoxylans in wheat flour: Their role in lowering batter viscosity, *J. Cereal Sci. 33* (2001) 83–96.

88. M.K. Kamaliya, K.B. Kamaliya: *Baking Science and Industry, Vols. I and II*, M.K. Kamaliya (Ed.), Anand Publisher, Calcutta, India (2001).

89. E.J. Pyler: Keeping Properties of Bread. In: *Baking Science and Technology, Vol. 2*, E.J. Pyler (Ed.), Sosland Publisher, Kansas, USA (1988) pp. 815–849.

90. Z.Q. Jiang, S.Q. Yang, S.S. Tan, L.T. Li, X.T. Li, Characterization of a xylanase from the newly isolated thermophilic *Thermomyces lanuginosus* CAU44 and its application in breadmaking, *Lett. Appl. Microbiol. 41* (2005) 69–76.

91. A.R. Shah, R.K. Shah, D. Madamwar, Improvement of the quality of whole wheat bread by supplementation of xylanases from *Aspergillus foetidus*, *Bioresour. Technol. 97* (2006) 2047–2053.

92. K. Katina, M. Salmenkallio-Marttila, R. Partanen, P. Forssell, K. Autio, Effects of sourdough and enzymes on staling of high-fibre wheat bread, *Lebensm. Wiss. Technol. 39* (2006) 479–491.

93. A. Gámbaro, A. Giménez, G. Ares, V. Gilardi, Influence of enzymes on the texture of brown pan bread, *J. Texture Stud. 37* (2006) 300–314.

94. C. Bollaín, A. Angioloni, C. Collar, Bread staling assessment of enzyme-supplemented pan breads by dynamic and static deformation measurements, *Eur. Food. Res. Technol. 220* (2005) 83–89.

95. C. Primo-Martín, M. Wang, W.J. Lichtendonk, J.J. Plijter, R.J. Hamer, An explanation for the combined effect of xylanase-glucose oxidase in dough systems, *J. Sci. Food Agric. 85* (2005) 1186–1196.

96. X. Rouau, S. Daviet, T. Tahir, B. Cherel, L. Saulnier, Effect of the proteinaceous wheat xylanase inhibitor XIP-I on the performances of an *Aspergillus niger* xylanase in breadmaking, *J. Sci. Food Agric. 86* (2006) 1571–1740.

97. N.A. Camacho, G.O. Aguilar, Production, Purification and characterization of low molecular mass xylanase from *Aspergillus* sp. and its application in baking, *Appl. Biochem. Biotechnol. 104* (2003) 159–172.

98. M. Haros, C.M. Rosell, C. Benedito, Effect of different carbohydrases on fresh bread texture and bread staling, *Eur. Food Res. Technol. 215* (2002) 425–430.

99. I. Trogh, C.M. Courtin, A.A.M. Andersson, P. Aman, J.F. Sørensen, J.A. Delcour, The combined use of hull-less barley flour and xylanase as a strategy for wheat/hull-less barley flour breads with increased arabinoxylan and (1-3, 1-4)-β-D-glucan levels, *J. Cereal Sci. 40* (2004) 257–267.

100. R.S. Bhatty, The potential of hull-less barley: Review, *Cereal Chem. 76* (1999) 589–599.

101. Z.X. Lu, K.Z. Walker, J.G. Muir, T. Mascara, K. O'Dea, Arabinoxylan fiber, a byproduct of wheat flour processing, reduces the postprandial glucose response in normoglycemic subjects, *Am. J. Clin. Nutr. 71* (2000) 1123–1128.

102. E. Harada, D. Lysenko, K.R. Preston, Effects of commercial hydrolytic enzyme additives on canadian short process bread properties and processing characteristics, *Cereal Chem. 77* (2000) 70–76.

103. E.G. Bayfield, W.E. Young, Flour brew studies VII: The use of malt syrup and enzymes, *Baker's Dig. 38* (1964) 52–56.

104. K. Kulp, Pentosans of wheat endosperm, *Cereal Sci. Today, 13* (1968) 414–417.

105. E.C. McDdonald, Proteolytic enzymes of wheat and their relation to baking quality, *Baker's Dig. 43* (1969) 22.

106. S.P. Cauvain, N. Chamberlain, The bread improving effect of fungal amylase, *J. Cereal Sci. 8* (1988) 239–248.

107. U. Bajwa, Effects of glyceryl monostearate and α-amylase supplements on rheological and breadmaking properties of medium protein wheat flour, *J. Sci. Food Agric. 52* (1990) 97–105.

108. P. Ranum, V.A. De Stefanis, Use of fungal α-amylase in milling and baking, *Cereal Food World*, *35* (1990) 931–933.

109. L. Flander, M. Salmenkallio-Martilla, M. Tenkanen, K. Autio, K. Pountanen, Enzymes as stabilizers of bread structure, *Proceedings of the 2nd European Symposium on Enzymes in Grain Processing (ESEGP-2)*, T. Simoinen, M. Tenkanen (Eds.), Julkaisija-Utgivare Publisher, Helsinki, Finland (1999) pp. 305–308.

110. P. Prabhasankar, D. Indrani, R. Jyotsna, G.V. Rao, Influence of enzymes on rheological, microstructure and quality characteristics of parotta – An unleavened Indian flat bread, *J. Sci. Food Agric. 84* (2004) 1989–2144.

111. D. Indrani, P. Prabhasankar, J. Rajiv, G.V. Rao, Synergistic improver mixture. *US patent 20040191362* (2004).