



## Review Article

# Xylanases and its Application in Food Industry: A Review

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**ABSTRACT:** Xylan is the most abundant and principal type of hemicellulose. It is a linear polymer of  $\beta$ -D xylopyranosyl units linked by (1–4) glycosidic bonds. Xylanases are most predominantly present in plant cell walls and are produced by different kinds of microorganisms like bacteria, fungi, protozoans and some yeast. Recently there is an increasing demand for cost effective microbial xylanolytic enzyme which benefits the industrial applications and are produced commercially. Xylanases has a wide range of applications in pulp and paper, food, animal feed, textiles and pharmaceuticals. This review discusses some of the properties of xylanases and their application in food industry.

**Key words:** Xylanases, Xylan, Food, Hemicellulose

## Introduction

Enzymes are distinct biological polymers that catalyze the chemical reactions and convert substrates to particular products. They are specific in function and speed up reactions by providing alternative pathways of lower activation energy without being consumed. These are the fundamental elements for biochemical processes and utilized in a number of food processing industries (Haq *et al.*, 2006). The demand for the production of different enzymes from microorganisms in a large amount has been increased. There has been growing interest in xylanase production and its application because xylanase is important in the bioconversion of hemicellulose, which is a significant component of lignocellulosic material.

Xylanase is a class of enzymes produced by microorganisms to break down a component of plant cell walls known as hemicellulose. Xylan is a polymer of glucose molecules and a major component of hemicellulose, helping to hold the cell walls together. Thus, the action of a xylanase enzyme helps to break down plant cell walls. This activity has applications in the food and paper-making industries, along with uses in agriculture and for human health. Xylanase (endo-1, 4- $\beta$ -D-xylanohydrolase; EC 3.2.1.8) is a hydrolytic enzyme involved in depolymerization of xylan, the major renewable hemicellulosic polysaccharide of plant cell wall. It is produced by bacteria (Gilbert, H.J., *et al.*, 1993, Kiddinamoorthy, J *et al.*, 2008, Sanghi, A *et al.*, 2007 and Sunna, A *et al.*, 1997), fungi (Nair, S.G *et al.*, 2008, Sunna, A *et al.*, 1997), actinomycetes (Ninawe, S. *et al.*, 2007) and yeast (Liu, W. *et al.*, 1998). Recently, interest in xylanase has markedly increased due its wide variety of biotechnological applications such as pre-bleaching of pulp, improving the digestibility of animal feed stocks, modification of cereal-based stuffs, bioconversion of lignocellulosic material and agro-wastes to fermentable products, clarification of fruit juices and degumming of plant fibers (Kapoor, M *et al.*, 2001, Kuhad, R.C. *et al.*, 1993, Virupakshi, K. *et al.*, 2005) etc. Cellulase-free xylanases active at high temperature and pH are gaining importance in pulp and paper industry as they reduce the need for toxic chlorinated compounds making the bleaching process environment-friendly (Srinivasan, M.D. *et al.*, 1999, Viikari, L *et al.*, 1994).

For the development of suitable xylanase as a pre-bleaching agent, the stability of enzyme at higher optimum pH and temperature is desirable (Bajpai B *et al.*, 1994). Apart from its use in the pulp and

paper industry, xylanases are also used as food additives to poultry (Bedford and Classen 1992), in wheat flour for improving dough handling and quality of baked products (Maat *et al.* 1992), for the extraction of coffee, plant oils, and starch (Wong and Saddler 1992), in the improvement of nutritional properties of agricultural silage and grain feed (Kuhad and Singh 1993), and in combination with pectinase and cellulase for clarification of fruit juices (Biely 1985) and degumming of plant fiber sources such as flax, hemp, jute, and ramie (Kapoor *et al.* 2001; Puchart *et al.* 1999; Sharma 1987). In this review, industrial applications of microbial xylanases are discussed with the main emphasis on food industrial applications.

## Xylan structure

Hemicelluloses include xylan, mannan, galactan, and arabinan as the main heteropolymers. The classification of these hemicellulose fractions depends on the types of sugar moieties present. The principal monomers present in most of the hemicelluloses are D-xylose, D-mannose, D-galactose, and L-arabinose. Xylan is a complex polysaccharide comprising a backbone of xylose residues linked by  $\beta$ -1, 4-glycosidic bonds (Fig. 1). The main chain of xylan is composed of  $\beta$ -xylopyranose residues (Whistler and Richards 1970). Xylan is the most common hemicellulosic polysaccharide in cell walls of land plants, representing up to 30%–35% of the total dry weight (Joseleau *et al.* 1992). Xylan is the major hemicellulose in hardwood from angiosperms, but is less abundant in softwood from gymnosperms; it accounts for approximately 15%–30% and 7%–12% of the total dry weight, respectively (Whistler and Richards 1970). Most xylans occur as heteropolysaccharides, containing different substituent groups in the backbone chain and in the side chain (Biely 1985). The common substituents found on the backbone of xylan are acetyl, arabinosyl, and glucuronosyl residues (Whistler and Richards 1970). Homoxylans, on the other hand, consists exclusively of xylosyl residues. This type of xylan is not widespread in nature and has been isolated from tobacco stalks (Eda *et al.* 1976), and guar seed husk (Montgomery *et al.* 1956). Arabinoxylans have been identified in wheat, rye, barley, oat, rice, sorghum, as well as in some other plants like pangola grass, bamboo shoots and rye grass. Although these polysaccharides are minor components of entire cereal grains, they constitute an important part of plant cell walls (Izidorczyk and Biliaderis 1995).

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Fig.1. Structure of xylan

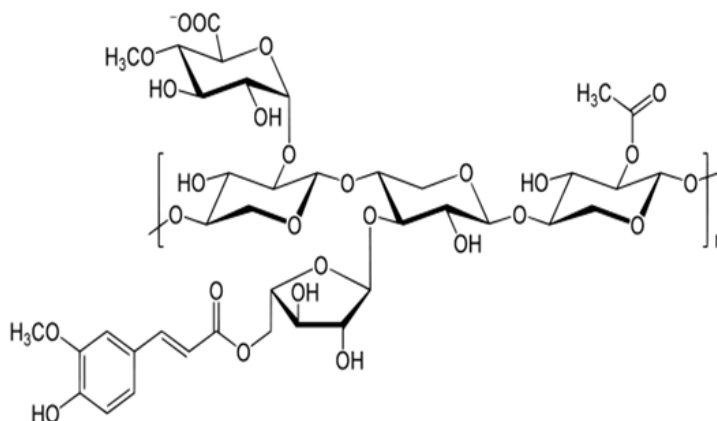
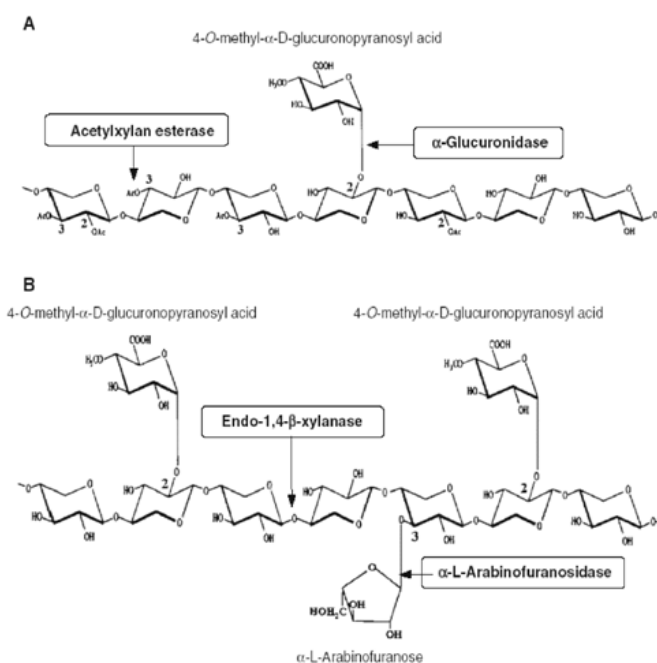


Fig 1(a): Structure of the O-acetyl-4-O-methylglucuronoxylan (a), of hardwood and of the arabino-4-O-methylglucuronoxylan (b), of soft wood. Xylanolytic enzymes involved in the degradation of the xylan: acetylxyylan esterase, α-glucuronidase, endoxylanase and α-L-arabinofuranosidase. Hydrolysis realized by β-xylosidase (c). The numbers indicate carbon atoms to which group substitutions are bound. Ac Acetyl group (M.L.M.Polizeli et al., 2005)



### Xylanolytic enzymes

Xylanases catalyze the hydrolysis of xylans. These enzymes are produced mainly by microorganisms and take part in the breakdown of plant cell walls, along with other enzymes that hydrolyze polysaccharides, and also digest xylan during the germination of some seeds (e.g. in the malting of barley grain). Xylanases also can be found in marine algae, protozoans, crustaceans, insects, snails and seeds of land plants (Sunna and Antranikian 1997).

### Endo -1-4-β-xylanases

Endo-1, 4-β-xylanase (1,4-β-D-xylan xylanohydrolase; EC 3.2.1.8) cleaves the glycosidic bonds in the xylan backbone, bringing about a reduction in the degree of polymerization of the substrate (Fig. 1a). Xylan is not attacked randomly, but the bonds selected for hydrolysis depend on the nature of the substrate molecule, i.e. on the chain length, the degree of branching, and the presence of substituents (Reilly 1981, Puls and Poutanen 1989, Li et al. 2000). Endoxylanases have been differentiated according to the end products they release from the hydrolysis of xylan (e.g. xylose, xylobiose and xylotriose and arabinose). Thus, xylanases may be classified as non-debranching (arabinose non-liberating) or debranching (arabinose liberating) enzymes. Many organisms are able to produce both types of xylanases, resulting in the maximum

efficiency of xylan hydrolysis. In view of the synergistic interactions between endoxylanases and arabinofuranosidases, resolution of this dichotomy may be possible by determining whether cloned enzymes retain the ability to free arabinose as well as to hydrolyse main chain linkage, this would apply for enzymes cloned from different sources (Wu S.C. et al., 2006, Okazaki F et al., 2005, Liu J.R et al., 2005). In general, the endoxylanases show peak activity between 40 and 80°C, and between pH 4.0 and 6.5, but optimal conditions have been found outside these ranges (Tables 1). Individual fungi and bacteria can exhibit a multiplicity of endoxylanases, in some cases three or more enzyme activities have been separated from a single culture (Rizzatti et al. 2004). Fungal and bacterial endoxylanases are almost exclusively single subunit proteins with molecular weight values ranging from 8.5 to 85 kDa and isoelectric point (pI) values between 4.0 and 10.3, most of them are glycosylated (Coughlan M.P et al., 1993, Polizeli M.L et al., 2005). The physicochemical property of fungal and bacterial endoxylanases is the apparent strong relationship between their molecular weight and pI, noted that with some exceptions endoxylanases fall in two main classes, those with molecular weight of less than 30kDa are usually basic proteins and those with molecular weight values in excess of 30kDa are acidic (Octavio Loera Corral et al., 2006). Some properties of endoxylanases are summarized in Table 1.

**β- Xylosidases**

β-D- Xylosidases (1, 4-β-D-xylan xylohydrolase; EC 3.2.1.37) can be classified according to their relative affinities for xylobiose and larger xylooligosaccharides. It may be monomeric, dimeric or tetrameric with molecular weight ranging from 26 to 360 kDa. They are produced by a variety of bacteria and fungi and may be found in the culture fluid associated with the cell or both (Octavio Loera Corral et al., 2006). Purified β-xylosidases usually do not hydrolyze xylan, their best substrate is xylobiose and their affinity for xylooligosaccharides is inversely proportional to its degree of polymerization. They are able to cleave artificial substrates such as p-nitrophenyl- and o-nitrophenyl-β-D-xylopyranoside (Polizeli M. L. T. M. et al., 2005). An important role attributed to β- xylosidases comes into play after the xylan has suffered a number of successive hydrolyses by xylanase. This reaction leads to the accumulation of short oligomers of β-D-xylopyranosyl, which may inhibit the endoxylanase. β- Xylosidase then hydrolyzes these products, removing the cause of inhibition, and increasing the efficiency of xylan hydrolysis (Andrade et al., 2004). The optimum temperature can vary from 40 to 80°C, but most β-xylosidases gives best assay results at 60°C. Their thermo stability is highly variable and depends on the organism in question. A good example of a stable enzyme is that from *Aspergillus phoenicis*, which retained 100% of its activity after 4 h at 60°C or 21 days at room temperature (Rizzatti et al., 2001).

**α -Arabinofuranosidases**

Arabinofuranosidases removes L-arabinose residues substituted at positions 2 and 3 of the β-D-xylopyranosyl. There are two types with distinct modes of action, exo-α-L-arabinofuranosidase (EC 3.2.1.55) which degrades p-nitrophenyl-α- L-arabinofuranosides and branched arabinans (Fig. 1a), and endo-1, 5-α-L-arabinase (EC 3.2.1.99) which only hydrolyzes linear arabinans (Kaneko et al. 1993, De Vries et al. 2000). While the arabinose is released, there will be no degradation in the xylan backbone as there is no production of xylooligosaccharides.

**Acetylxylan esterase**

Acetylxylan esterase (EC 3.1.1.6) removes the O-acetyl substituents at the 2 and 3 positions of xylose residues in acetylated xylans. Some xylans are acetylated in their native state, although most of the xylans used to study xylanolytic enzymes are deacetylated after alkali extraction (Tenkanen M and Poutanen K, 1992, Sunna A and Antranikian G, 1997). Acetylxylan plays an important role in the hydrolysis of xylan, since the acetyl side-groups can interfere with the approach of enzymes that cleaves the backbone by steric hindrance and their elimination thus facilitates the action of endoxylanases (Octavio Loera Corral et al., 2006).

**α – Glucuronidases**

α- Glucuronidase (EC 3.2.1.131) hydrolyzes the α-1, 2 bonds between the glucuronic acid residues and β-D-xylopyranosyl backbone units found in glucuronoxylan. The substrate of α-Glucuronidases differs according to enzyme sources. However, the substrate specificity varies with the microbial source and some glucuronidases are able to hydrolyze the intact polymer (Puls and Schuseil 1993, Tenkanen and Siika-aho 2000). It has also been noted that acetyl groups close to the glucuronosyl substituents can partially hinder the α-glucuronidase activity.

**The xylanosome concept**

Xylanosomes are discrete, multifunctional, multienzyme complexes found on the surface of several microorganisms (Sunna and Antranikian 1997). These complexes play an important role in the degradation of hemicelluloses. The extra cellular xylanosome complex B (CB) from *Butyvirbio fibrisolvens* H17c (Lin and Thomson 1991) exists as a multisubunit protein aggregate. The complex has a molecular weight >669 kilo Daltons (kDa) and is composed of 11 protein bands with xylanase activity and 3 bands showing endoglucanase activity. *Clostridium papyrosolvens* C7 possesses a multicomplex cellulase- xylanase system, which is responsible for hydrolysis of cellulose and xylan (Pohlschroder et al. 1994). This multiplex system consists of seven protein complexes whose molecular weight ranges from 500 to 660 kDa. Recently, Jiang et al., 2005, have described a xylanosome with a molecular weight of 1200 kDa.

Table 1: Characteristics of xylanases from different microorganisms (kDa-kiloDaltons) (Beg et al 2001)

Microorganism	Molecular weight (kDa)	Optimum pH	Opt.tempe- rature (°C)	pI	K <sub>m</sub> (Mg/ml)	Vmax (µM/ mine per mg)	References
<b>Bacteria</b>							
<i>Acidobacterium capsulatum</i>	41	5	65	7.3	3.5	403	Inagaki et al.1998
<i>Bacillus sp.W-1</i>	21.5	6	5	8.5	4.5		Okazaki et al.1985 Esteban et al.1985
<i>Bacillus circulans</i> WL-12	15	5.5-7	-	9.1	4	-	Khasin et al.1993
<i>B.stearothermop-hilus</i> T-6							Blanco et al.1995
<i>Bacillus sp.strain</i> BP-23	43	6.5	55	7,9	1.63	-	Lopez et al.1998
<i>Bacillus sp.strain</i> BP-7	32	5.5	50	9.3	-		Morales et al.1995
<i>Bacillus polymyxa</i> CECT 153						288	
<i>Bacillus sp.strain</i> K-1	22-120	6	55	7-9	-		Ratannakanokchai et al.1999 Gupta et al.1992
<i>Bacillus sp.NG-27</i>	61	6.5	50	4.7	17.1	-	Bataillon et al.1998
<i>Bacillus sp.SPS-0</i>							Gessesse 1998
<i>Bacillus sp.strain</i> AR-009	23	5.5	60	-	-	-	Dey et al.1992
<i>Bacillus sp.NCIM</i> 59							Khanna & Gauri1993
<i>Cellulomonas fimi</i>	-	7,8,4	70	-	-		Chaudary & Deobagkar 1997
<i>Cellulomonas sp.N.C.I.M2353</i>	-	6	75	-	-	112	Gessesse & Mamo1998
<i>Micrococcus sp.AR-135</i>							
<i>Staphylococcus sp.</i> SG-13	23,48	9-10	60-75	-	-		Gupta et al.2000

<i>Thermoanaeroba-cterium</i> <i>sp.3W/SL-YS 485</i>	15,8,35	6	50-60	4,8	1.58, 3.50 1.25-1.72	-	Shao et al.1995
<i>Thermotoga maritime</i> MSB8	14-150	5-6.5	40-45	4.5-8.5	1.7, 1.5	-	Winterhalter & Liebel 1995
	22,33,53	6.5	55	8	-	-	
	56	7.5-9	55	-	4	-	
	60	7.5,9.2	50	-	3	-	
	24-180	6.2	80	4.37	1.1, 0.29	0.017, 0.742	
	40,120	5.4,6.2	92-105	5.6	-	380, 690	
						90	
					374, 4760		
<b>Fungi</b>							
<i>Acrophialophora nainiana</i>	17	6	50	-	0.731, 0.343	-	Ximenes et al.1999
<i>Aspergillus niger</i>	13.5-14.0	5.5	45	9	-	-	Frederick et al.1985
<i>Aspergillus kawachii</i> IFO 4308	26-35	2-5.5	50-60	3.5-6.7	-	-	Ito et al.1992
<i>Aspergillus nidulans</i>	22-34	5.4	55	-	-	-	Fernandez –Epsinar et al.1992
<i>Aspergillus fischeri</i> Fxn1		5.4	55	-	-	-	Raj & Chandra 1996 Kimura et al.1995
<i>Aspergillus sojae</i>	31	6	60	-	4.88	5.88	Ghosh & Nanda1994
<i>Aspergillus sydowii</i> MG 49	32.7, 35.5	5, 5.5	60,50	3.5, 3.75	-	-	Fujimoto et al.1995
<i>Aspergillus aculeatus</i>	30	5.5	60	-	-	-	Kormelink et al.1993
<i>Aspergillus awamori</i>	18, 26,52	5.5	60	-	-	-	Silva et al.1999
<i>Aspergillus fumigatus</i>	39,23, 26	4.0, 5.0	50,50, 70	-	-	-	Kitamoto et al.1999
<i>Aspergillus oryzae</i>	19,8.5	4.0-5.5	45-55	-	-	-	Bansod et al.1993
<i>Cephalosporium sp.</i>							Christakopo-lous et al.1996
<i>Fusarium oxysporum</i>	35	5.5	55	-	-	-	Radionova et al.2000 Kelly et al.1989
<i>Geotrichum candidum</i>	30,70	5.0	60	-	-	-	Belancic et al.1995
<i>Paecilomyces varioti</i>	20.8, 23.5	8	40	-	0.15	-	Cesar & Mrsa 1996
<i>Penicillium purpurogenum</i>	60-67	6	60,55	-	9.5, 8.45, 8.7	0.41, 0.37	Lin et al 1999
<i>Thermomyces lanuginosus</i> DSM5826	20	6	60,55	-	-	-	Tan et al 1985
<i>Thermomyces lanuginosus</i> - SSBP	33,23	4	50	3.4	-	-	Tenkanen et al.1992

<i>Trichoderma harzianum</i>					49.5	-	
<i>Trichoderma reesei</i>	25.5	4	50	5.2	-	-	
		7,3.5	60,50	8.6, 5.9	-	-	
	23.6	7	60-70	4.1	7.3	-	
	20	6.5	70-75	3.8	3.26	6300	
	20,19	5	50	-	0.58	0.106	
		5-5.5, 4-4.5	45,40	9,5.5	3-6.8, 14.8-22.3	-	
<b>Yeast</b>							
<i>Aureobasidium pullulans</i> Y-2311-1	25	4.4	54	9.4	7.6	2650	Li et al.1993
<i>Cryptococcus albidus</i>	48	5	25	-	5.7, 5.3	-	Morosoli et al.1986
<i>Trichosporon cutaneum</i> SL409	-	6.5	50	-	-	-	Liu et al.1998
<b>Actinomycetes</b>							
<i>Streptomyces sp.</i> EC 10							Lumba & Pennickx 1992
<i>Streptomyces sp.</i> B-12-2	32	7-8	60	6.8	3	-	Elegir et al.1994
<i>Streptomyces T7</i>	23.8-40.5	6-7	55-60	4.8-8.3	0.8-5.8	-	Kesker 1992
<i>Streptomyces thermoviolaceus</i> OPC-520	20	4.5-5.5	60	7.8	10	-	Tsujibo et al.1992
<i>Streptomyces chattanoogensis</i> CECT 3336	33,54	7	60-70	4.2, 8	-	-	Lopez-Fernandez et al.1998
<i>Streptomyces viridisporus</i> T7A	48	6	50	9	4,0.3	7610	Magnuson & Crawford 1997
<i>Streptomyces sp.</i> QG-11-3	59	7-8	65-70	10.2-10.5	-	-	Beg et al.2000a
<i>Thermomonospora curvata</i>	-	8.6	60	-	1.2	-	Stutzenberger & Bodine1992
	15-36	6.8-7.8	75	4.2-8.4	1.4-2.5	78.2, 19.1	
						-	
						158.85	
						-	

### Xylanase production

From the industrial point of view, xylanases are important enzyme in the bioconversion of hemicellulose, which is a significant component of lignocellulosic material. Filamentous fungi are particularly interesting producers of xylanases from an industrial point of view,

due to the fact that they excrete xylan degrading enzymes into the medium, eliminating the need for cell disruption prior to purification (Sunna and Antranikian, 1997, Polizeli M.L et al., 2005). The various biotechnological techniques like submerged and solid state fermentation are employed for xylanase biosynthesis (Cai et al.,

1998; Gawande and Kamat, 1999, Kansoh and Gammel, 2001). The submerged fermentation is most beneficial as compared to other techniques due to more nutrients availability, sufficient oxygen supply and less time required for the fermentation (Hoq et al., 1994, Gomes et al., 1994, Veluz et al., 1999, Bim and Franco, 2000 and Gouda, 2000). The production of microbial xylanases is preferred over plant and animal sources because of their availability, structural stability and easy genetic manipulation (Bilgrami and Pandey, 1992). Most xylanase manufacturers produce these enzymes using submerged fermentation. The carbon source plays another major role in the economics of xylanase production. In order to replace the cost of the xylan, cost effective natural lignocellulosic substrates like wheat bran, sugarcane bagasse, rice straw, corn cobs etc., are used for the production of xylanase. In cultures on solid substrate, wheat bran and rice are regarded as inducers. Alternative substrates for enzyme production have also been reported, such as sugarcane bagasse, rice husks and wood pulp (Kadowaki et al. 1995, Damaso et al. 2000, Medeiros et al. 2000, Pandey et al. 2000, Singh et al., 2000, Anthony et al., 2003). In liquid culture, xylanase is produced in response to xylians from various sources (Gomes et al. 1994, Liu et al. 1999, Rani and Nand 2000). A number of studies have been done on lignocellulosic wastes mainly wheat bran (Gwande P.V et al., 1999), sugarcane bagasse (Gutierrez-correa M et al.,1998) and wheat straw. *Thermoascus aurantiacus* ATCC 204492 is able to produce a high level of thermostable xylanase when sugar cane bagasse is used as a substrate (A.M.F.Milagres et al., 2004). In solid substrate fermentation using wheat bran and eucalyptus kraft pulp as the primary solid substrates, *Streptomyces* sp. QG-11-3 (Beg et al. 2000b) produces maximum xylanase yield at substrate-to-moisture ratio of 1:2.5 and 1:3, respectively. However, on increasing or decreasing the moisture level, the xylanase yield marginally decreased. In contrast, a lower solid substrate to- moisture level of 1:1 has been reported for maximum xylanase production by *Bacillus* sp. A-009 (Gessesse and Mamo 1999). An improvement in xylanase production by fungal mixed culture (*Trichoderma reesei*/LM-UC4 E 1, *Aspergillus niger* ATCC 10864, and *A. phoenicis* QM 329) using solid substrate fermentation has also been reported (Gutierrez-Correa and Tengerdy 1998). A higher xylanase yield using solid substrate fermentation compared with submerged fermentation using wheat straw and sugarcane bagasse has been reported from thermophilic *Melanocarpus albomyces* IIS-68 (Jain 1995). Biswas et al. (1990) produced xylanase from *Aspergillus ochraceus* employing both fermentation methods i.e. liquid broth and solid state fermentation.

The enzyme was purified using ammonium sulphate precipitation and gel filtration. The optimum pH for the enzyme was found to be 6.0. Chen et al. (1990) screened a strain of *Aspergillus niger* C-2 from the soil and treated with UV and EMS to obtain mutant colonies and the conditions for submerged fermentation were studied. The produced enzyme had weak thermal stability and when incubated at 55°C for one hour, it lost 60% of its stability. Xiong et al. (2005) studied the effect of L-arabinose-rich plant hydrolysate for the synthesis of xylanase by *T. reesei* C-30. The researchers reported higher activities of xylanase in cultures containing oat husk and sugar beet pulp hydrolysate than on lactose. The xylanase activity was about 9 times higher with oat husk (510 IU/ml) than in lactose (60 IU/ml). In the case of batch cultivations on sugar beet pulp hydrolysate and lactose even higher xylanase activity (630 IU/ml) was obtained. Park et al. (2002) optimized conditions in solid state fermentation for xylanase synthesis. The activity of xylanase obtained after 5 days of fermentation was 50171 IU/ml. Senthilkumar et al. (2005) used *A. fischeri* to produce alkali-stable xylanase at pH 9.0 using wheat bran as carbon source in solid state fermentation. Enhanced production of xylanase is obtained from a local soil isolate *Trichoderma viride*, using various lignocellulosic substrates like maize straw, bajra straw, jowar straw, wheat straw, oat hay and barseem hay in submerged culture fermentation (Meenakshi Goyal et al.,2008). The production of extracellular xylanase,  $\beta$ -xylosidase and  $\alpha$ -L-arabinofuranosidase by the mesophilic fungus *Penicillium janczewskii* under submerged cultivation was investigated with different carbon sources like sugarcane bagasse, oat bran, wheat bran, corncobs, rice straw, orange waste and cassava peel ( Cesar Rafael et al., 2010). Two xylanases, MFX I and MFX II, from the thermophilic fungus *Malbranchea flava* MTCC 4889 with molecular masses of 25.2 and 30 kDa and pIs of 4.5 and 3.7, respectively were purified to homogeneity. The xylanases were optimally active at pH 9.0 and at 60 °C, exhibited a half-life of 4 h at 60 °C, and showed distinct mode of action and product profiles when applied to birchwood, oat spelt, and larchwood xylan, and to wheat and rye arabinoxylan (Manju Sharma et al.,2010). Two xylanases were purified to electrophoretic homogeneity from the thermophilic fungus *Sporotrichum thermophile* grown in submerged liquid culture using wheat straw as carbon source. The enzymes, StXyn1 and StXyn2, have molecular masses of 24 kDa and 48 kDa, respectively, and are optimally active at pH 5 and at 60 °C (Christina Vafiadi et al.,2010).

Table 2: Commercial preparations of Xylanases (Beg et al., 2001, Haltrich et al., 1996 and Octavia Loera et al., 2006) SSF: Solid stated fermentation, SmF: Submerged fermentation, N.c: Not cited.

Company	Product	Strain and mode of fermentation	Applications
Alltech ,Inc,(USA)	"Allzym PT"	<i>Aspergillus niger</i> (SmF)	Upgrading animal feed.
Alltech ,Inc,(USA)	" Fibrozyme "	<i>Aspergillus niger</i> & <i>Trichoderma viride</i> (SSF)	Upgrading animal feed.
Amano Pharmaceutical Co,Ltd(Japan)	"Amano 90"	<i>Aspergillus niger</i> (SSF)	Pharmaceutical, food and feed industry.
A/S	"Resinase"	N.c	Cellulose and paper industry
Biocon ,(India)	"Bleachzyme F"	N.c	Pulp bleaching
Biotec	"Ecosane"	<i>Trichoderma reesei</i> (SmF)	Animal feed
Clariant(UK)	"Cartazyme"	<i>Termomonospora fusca</i>	Pulp bleaching
Ciba –Geiby Ltd(Switzerland)	"Irgazyme40"	<i>Trichoderma longibrachiatum</i> (SmF)	Pulp and paper industry and animal feed
Danisco Ingredients (Denmark)	"Grindazym PF" & "Grindazym GP 5000"	<i>Aspergillus niger</i> (SmF)	Supplementation of poultry and piglet food
Gamma Chemie GmbH(Germany)	"Gammafeed X" & "Gammazym X4000L"	<i>Trichoderma longibrachiatum</i> (SmF) & <i>Trichoderma reesei</i> (SSF)	Production of wheat starch, baking and brewing industry. Feed and brewing industry
Genecor International Europe Ltd(Finland)	"Multifect XL"	<i>Trichoderma longibrachiatum</i> (SmF)	Food industry
Hankyo Bioindustry Co.Ltd(Japan)	"Xylanase250" & "Hemicellulase 100"	<i>Trichoderma viride</i> (SSF) & <i>Aspergillus niger</i> (SSF)	Baking industry & for macerating vegetables and fruits. Improving the filtration speed of saccharified cereal solutions and fruit juices
Iogen Corp(Canada)	"Xylanase GS35"	<i>Trichoderma reesei</i> (SmF)	Pulp bleaching,pulp cleaning and animal feed processing.



Novozymes (Denmark)	"Bio-feed-plus"	<i>Humicola insolens</i> (SmF)	Animal feed
	"Novozym 431"	<i>Trichoderma longibrachiatum</i> (SmF)	Animal feed
	"Pulzyme"	<i>Bacillus sp.</i>	Cellulose and paper industry
Primalco Ltd Biotec(Finland)	"Ecopulp X-200"	<i>Trichoderma reesei</i> (SmF)	Improve the bleachability of softwood & hardwood kraft pulps
Quest International Ireland(Ireland)	"Bioxylanase"	<i>Trichoderma reesei</i> (SmF)	Brewing and animal feed industry
Rohm GmbH(Germany)	"Rohalasa 7118"	<i>Aspergillus sp. &amp; Trichoderma sp.</i> (SmF)	Reduction of viscosity in starch processing.
	"Vernon 191"	<i>Aspergillus sp. &amp; Trichoderma sp.</i> (SmF)	Baking industry
Seikagaku Corporation(Japan)	No commercial name	<i>Trichoderma sp.</i> (SmF)	Structure studies of carbohydrates
Shin Nihon Chemical (Japan)	"Sumizyme X"	<i>Trichoderma koningii</i> (SSF)	Manufacturing of mushroom and vegetable extracts,enzymatic peeling of cereals and baking industry.
Solvay Enzymes GmbH& Co.(Germany)	"Solvay pentosanasa"	<i>Trichoderma reesei</i> (SmF)	Starch and baking industry
Stern –Enzym GmbH & Co(Germany)	"Sternzym HC46" " Sternzym HC40"	<i>Trichoderma reesei</i> (SmF) <i>Aspergillus niger</i> (SSF/SmF)	Bakery industry Animal feed, hydrolysis of plant raw materials.

### Applications of xylanases

Xylanases have aroused great interest recently due to their potential application in many industrial processes. In recent years, the biotechnological use of xylans and xylanases has grown remarkably (Bhat 2000, Aristidou and Pentilla 2000, Subramaniyan and Prema 2000, 2002, Beg et al., 2000, 2001, Techapun et al., 2003). Xylanase began to be used in the 1980s, initially in the preparation of animal feed and later in the food, textile and paper industries. Currently, xylanase and cellulase, together with pectinases, account for 20% of the world enzyme market (Polizeli M.L et al., 2005). In the food industry, xylanase enzymes are used to accelerate the baking of cookies, cakes, crackers, and other foods by helping to break down polysaccharides in the dough (Godfrey T et al., 1996). In animal feeds, xylanase aids in the digestibility of wheat by poultry and swine, by decreasing the viscosity of the feed (Godfrey T et al., 1996). Most commercial xylanases are produced by *Trichoderma*, *Bacillus*, *Aspergillus*, *Penicillium*, *Aureobasidium*, and *Talaromyces* sp (Godfrey T et al., 1996). In this review the main emphasis will be focused on xylanase application in food industries.

### Xylanases in baking and brewing industry

The application of xylanolytic enzymes has increased for the last few decades owing to their potential effectiveness in breadmaking (M.S.Butt et al., 2008). Starch and non-starch carbohydrate hydrolyzing enzymes are commonly used in the bread making industry as bread improvers (Polizeli M.L et al., 2005, P.F.I.Javier et al., 2007). Enzymatic hydrolysis of non-starch polysaccharides leads to the improvement of Rheological properties of dough, bread specific volume and crumb firmness (M.Martinez-Anaya et al., 1997). The xylanases, like the other hemicellulases, break down the hemicellulose in wheat-flour, helping in the redistribution of water and leaving the dough softer and easier to knead. During the bread-baking process, they delay crumb formation, allowing the dough to grow (Polizeli M.L et al., 2005). With the use of xylanases, there has been an increase in bread volumes, greater absorption of water and improved resistance to fermentation (Maat et al. 1992; Harbak and Thygesen 2002; Camacho and Aguilar 2003). Also, a larger amount of arabinoxylooligosaccharides in bread would be beneficial to health (Polizeli M.L et al., 2005).

Xylanase transforms water insoluble hemicellulose into soluble form, which binds water in the dough, therefore decreasing the dough firmness, increasing volume and creating finer and more uniform crumbs (M.S. Butt et al., 2008).xylanases 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(M.Wang et al., 2004,J.F.Sorensen et al.,2001 and A.Monfort et al., 1997).

Xylanases improve dough characteristics and bread quality leading to improved dough flexibility, machinability, stability, loaf volume and crumb structure (Baillet, 2003; Guy and Sarabjit, 2003). Many enzymes such as proteases, xylanase and cellulases improve the strength of the gluten network and therefore, improve the quality of bakery products (Gray and BeMiller, 2003).The enzymatic hydrolysis of pentosans by hemicellulases or pentosanases at the optimal level improves the dough properties resulting in greater uniformity in quality characteristics (Rouau et al., 1994). Xylanases make the dough more tolerant to different flour quality parameters and variations in processing methods. They also make the dough soft, reduce the sheeting work requirements and significantly increase the volume of the leavened pan bread (Dervilly et al., 2002, Harbak and Thygesen, 2002). Xylanase along with protease, lipase and  $\alpha$ -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 bread crumb was reduced (P.R.Mathewson 2000,O. Ozmutlu et al.,2001,S.O.Keskin et al.,2004).The positive effect of xylanase on bread volume is due to the redistribution of water from the pentosan phase to the gluten phase. The increase in the volume of the gluten fraction increases its extensibility, which will result in better oven spring (Maat et al., 1992). The improving effect of pentosanases on bread volume may be associated with a better gas retention during proofing, probably due to the action of enzyme in reducing the viscosity of the gelling starch and allowing greater and longer expansion in the oven before enzyme inhibition and protein denaturation (Martinez and Jimenez, 1997).

In biscuit-making, xylanase is recommended for making cream crackers lighter and improving the texture, palatability and uniformity of the wafers (Polizeli M.L et al., 2005). Xylanases, in conjunction with cellulases, amylases and pectinases, lead to an improved yield of juice by means of liquefaction of fruit and vegetables; stabilization of the fruit pulp; increased recovery of aromas, essential oils, vitamins, mineral salts, edible dyes, pigments etc., reduction of viscosity, hydrolysis of substances that hinder the physical or chemical clearing of the juice, or that may cause cloudiness in the concentrate (Polizeli M.L et al., 2005). Xylanase, in combination with endoglucanase, takes part in the hydrolysis of arabinoxylan and starch, separating and isolating the gluten from the starch in the wheat flour. This enzyme is also used in coffee-bean mucilage (Wong et al. 1988; Wong and Saddler 1993). The main desirable properties for xylanases for use in the food industry are high stability and optimum activity at an acid pH. With the advances in the techniques of molecular biology, other uses of xylanases are being discovered (Polizeli M.L et al., 2005).Recently, a recombinant yeast of wine was constructed with the gene for xylanase of *Aspergillus nidulans*,xlnA, resulting in a wine with a

more pronounced aroma than is conventional (Ganga et al. 1999). During the manufacture of beer, the cellular wall of the barley is hydrolyzed releasing long chains of arabinoxylans which increase the beer's viscosity rendering it "muddy" in appearance. Thus, xylanases are used to hydrolyze arabinoxylans to lower oligosaccharides diminishing the beer's viscosity and consequently eliminating its muddy aspect (Debyser et al. 1997; Dervilly et al. 2002).  $\alpha$ -L-Arabinofuranosidase and  $\beta$ -D-glucopyranosidase have been employed in food processing for aromatizing musts, wines, and fruit juices (Spagna et al. 1998).

#### Xylanase in animal feed

Xylanase is used in the pretreatment of forage crops to improve the digestibility of ruminant feeds and to facilitate composting (Gilbert and Hazlewood 1993). Xylanases are used in animal feed along with glucanases, pectinases, cellulases, proteases, amylases, phytase, galactosidases and lipases. These enzymes break down arabinoxylans in the ingredients of the feed, reducing the viscosity of the raw material (Twomey et al. 2003). If xylanase is added to feed containing maize and sorghum, both of which are low viscosity foods, it may improve the digestion of nutrients in the initial part of the digestive tract, resulting in a better use of energy. Young fowl and swine produce endogenous enzymes in smaller quantities than adults, so that food supplements containing exogenous enzymes should improve their performance as livestock. Moreover, this kind of diet is found to reduce unwanted residues in the excreta (phosphorus, nitrogen, copper and zinc), an effect that could have a role in reducing environmental contamination (Polizeli M.L.M et al., 2005).

Café et al. (2006) gave nutritionally rich diets, with or without the addition of 0.1% Avizyme 1500 (xylanase, protease, and amylase) to the poultry birds. Birds fed on the diets supplemented with Avizyme exhibited significantly higher body weights, less mortality and greater amount of net energy from their diets as compared to the control group. Babalola et al. (2006) observed improved apparent nitrogen and fiber absorption as well as feed transit time by the application of xylanase in poultry feed. Moreover the enzyme addition in boiled castor seed meal (up to 150g/kg) was found to be acceptable and showed no adverse effect on growth performance or blood constituents.

#### Conclusion

Xylanases of microbial origin have great potential and highly benefits industrial application. Xylanase enzyme should be promoted in the food processing industry to replace the chemical emulsifiers and additives. Xylanase enzyme in combination with other enzyme can provide better results.

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