CHAPTER 5

BIOTECHNOLOGICAL APPLICATIONS OF XYLANASES
5.1 INTRODUCTION

The use of enzymes in biotechnological processes is part of a long established tradition. Xylanases have great potential and find use in a number of industrially relevant processes such as bioconversion of agroresidues, preparation of improved animal feeds, baking, paper and pulp-making etc.

Plant based agro wastes have abundant amount of lignocelluloses, which are complex polymers of cellulose, hemicelluloses and lignin, and represent the most abundant renewable organic matter on earth (McCarthy, 1987). Large amount of different types of un-utilized agro residues and wastes (Table-5.1) are available for bioconversion on earth. Agro residues are a potential resource for bioconversion to glucose, ethanol and number of valuable products like xylooligosaccharides, furfural, xylitol etc (Kulkarni et al., 1999; Sun and Cheng, 2002). On an industrial scale xylitol is produced by the chemical reduction of the xylose derived from hemicellulose hydrolysates of birchwood or other xylose rich materials. As these raw materials contain polymers of other sugars, the process includes intensive purification and separation steps to remove these by products (Nigam and Singh, 1995). As the enzymatic reaction is a more specific process, enzymic saccharification of lignocellulosic material can avoid these problems. Considerable interest has been shown for hemicellulose rich agricultural crops for alcohol generation by Saccharomyces cerevisiae, Kluyveromyces marxianus and Zymomonas mobilis (Dumitriu and Chornet, 1998).
Table 5.1: The contents of cellulose, hemicellulose, and lignin in common agricultural residues and wastes.

<table>
<thead>
<tr>
<th>Lignocellulosic materials</th>
<th>Cellulose (%)</th>
<th>Hemicellulose (%)</th>
<th>Lignin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hardwoods stems</td>
<td>40–55</td>
<td>24–40</td>
<td>18–25</td>
</tr>
<tr>
<td>Corn cobs</td>
<td>45</td>
<td>35</td>
<td>15</td>
</tr>
<tr>
<td>Grasses</td>
<td>25–40</td>
<td>35–50</td>
<td>10–30</td>
</tr>
<tr>
<td>Paper</td>
<td>85–99</td>
<td>0</td>
<td>0–15</td>
</tr>
<tr>
<td>Wheat straw</td>
<td>30</td>
<td>50</td>
<td>15</td>
</tr>
<tr>
<td>Wheat bran *</td>
<td>30</td>
<td>27</td>
<td>21</td>
</tr>
<tr>
<td>Sorted refuse</td>
<td>60</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Leaves</td>
<td>15–20</td>
<td>80–85</td>
<td>0</td>
</tr>
<tr>
<td>Cotton seed hairs</td>
<td>80–95</td>
<td>5–20</td>
<td>0</td>
</tr>
<tr>
<td>Newspaper</td>
<td>40–55</td>
<td>25–40</td>
<td>18–30</td>
</tr>
<tr>
<td>Waste papers from chemical pulps</td>
<td>60–70</td>
<td>10–20</td>
<td>5–10</td>
</tr>
<tr>
<td>Primary wastewater solids</td>
<td>8–15</td>
<td>Na b</td>
<td>24–29</td>
</tr>
<tr>
<td>Swine waste</td>
<td>6.0</td>
<td>28</td>
<td>NA b</td>
</tr>
<tr>
<td>Solid cattle manure</td>
<td>1.6–4.7</td>
<td>1.4–3.3</td>
<td>2.7–5.7</td>
</tr>
<tr>
<td>Coastal Bermuda grass</td>
<td>25</td>
<td>35.7</td>
<td>6.4</td>
</tr>
<tr>
<td>Switch grass</td>
<td>45</td>
<td>31.4</td>
<td>12.0</td>
</tr>
</tbody>
</table>


b NA – not available.
Selective removal of hemicellulose by xylanases enhances the susceptibility of cellulose to cellulase action for saccharification. A mixture of hemicellulases or pectinases with cellulases, exhibited a significant increase in the extent of cellulose conversion (Ghose and Bisaria, 1979; Beldman et al., 1984). But still complete utilization of lignocellulosic residues continues to remain a stubborn problem (Bisaria, 1998). Moreover, the enzymatic cleavage of xylan to smaller oligosaccharides is itself a reversible reaction and most of the enzymes show transglycosylating activity and synthesis of higher oligosaccharides under certain reaction conditions. Hence a specific process for the enzymatic cleavage of xylan needs to be designed (Kulkarni et al., 1999).

Effective chemical processes exist which can fractionate and hydrolyse lignocellulose into its essential building blocks. However, the chemical processes, besides being energy intensive, require expensive corrosion resistant equipment, extensive washing and disposal of chemical wastes. Severe conditions and chemical treatments may also generate toxic by-products like furfural in acid hydrolysis. Enzymatic treatment of such residues, on the other hand, results in specific release of end products and can be achieved at moderate conditions of pH and temperature. However, at present it is not a competitive process because of low catalytic efficiencies of enzymes especially towards crystalline cellulosic substrates. Moreover enzymatic hydrolysis is greatly influenced by thermal stability, adsorption, end product inhibition and shear inactivation (Bisaria, 1998).

In the present investigation Aspergillus foetidus MTCC 4898 crude xylanase preparation produced under SSF was attempted for saccharification of corn cobs, wheat bran and birchwood xylan.
For application of microbial enzymes in food processing, care should be taken for consumer safety. The isolate *A. foetidus* is a member of *A. niger* group which is considered as GRAS (Generally Regarded As Safe) organism. Moreover it does not have potential to produce ochratoxin A (Schuster *et al.*, 2002). Therefore, the enzyme preparations obtained from this isolate may be safe to use in bread making.

In today's modern society, consumer's food needs as well as preferences have changed substantially. In order to have more wholesome and fibre rich foods, the baking industry is shifting towards producing whole wheat bread with limited use of chemicals. Production of this kind of breads also poses technological difficulties. In order to improve the consumers' acceptance to whole wheat bread with improved body and texture as well as flavour and other desirable properties, the technology is also required to be changed. Microbial enzymes can be of great help in over coming these problems and they can be added as processing aids/additives. The added enzymes can be easily inactivated by the baking time-temperature treatment (Hammer, 1995). Endoxylanases have strong impact on arabinoxylans of wheat flour and amylases on starch of wheat flour. Synergistic action of both have been shown to be beneficial for improvement of important properties of whole wheat bread. In the present study, the xylanase alone and in combination of commercial amylase was tried for whole wheat bread making (Si and Lustenberger, 2002).

In the present study, the partially purified xylanase of *A. foetidus* xylanase alone and in combination of commercial amylase was tried for whole wheat bread making. Further, evaluation of various dough and bread attributes,
sensory qualities and rheological properties was also carried out to analyse the influence of xylanases on bread making.

5.2 MATERIALS AND METHODS

5.2.1 Xylanase Production by SSF:

Xylanase production under SSF was done using modified MS medium as described in 3.2.4, which was partially purified by ammonium sulphate fractionation as described in 4.2.5.

5.2.2 Enzyme Assay:

Xylanase (EC 3.2.1.8) activity was measured using 1% birchwood xylan (4-O-methyl glucuronoxylan) solution as a substrate as described in 2.2.6. Filter paper cellulase activity was measured according to IUPAC recommendations employing filter paper (Whatman No.1) as substrate as described in 2.2.6.

5.2.3 Protein Estimation:

Soluble protein from filtered fermentation broths was determined by following Lowry's method (Lowry et al., 1951).

5.2.4 Saccharification:

Saccharification of Birchwood xylan, wheat bran and corn cobs was carried out at 50 °C in 0.05 M citrate buffer (pH 5.3), using 500 U crude xylanase per gram of substrate. The substrate was used at a concentration of 10.0 g/l. The hydrolysis was carried out for 24h in a water bath under mild shaking conditions (30 rpm). At regular intervals, the samples were withdrawn and immediately kept in boiling water bath for inactivation of xylanase followed by centrifugation at 7000 rpm for
10 min. The supernatant was than used for estimation of reducing sugars by DNS method (Miller, 1959).

Saccharification was also done in a similar way at 45 °C. Influence of different levels of enzymes: 150, 500 and 1000 U/g of substrate, was also studied on the three substrates. Saccharification of wheat flour was carried out in a similar way using 500U xylanase/g of wheat flour at 50 °C.

5.2.5 Alkali Treatment of Corn Cobs:
Corn cobs powder (5.0%) was pretreated for 24 h with 1.0 N of NaOH. After the treatment, corn cobs were thoroughly washed with distilled water and dried at 50 °C in hot-air oven.

5.2.6 Preparation of Whole Wheat Bread:

5.2.6.1 Ingredients:

The whole wheat bread was prepared from whole wheat flour (Annapurna Brand, Hindustan Lever make, India).

The formulation for bread was: wheat flour - 100 g, sugar - 3.0g, edible salt -2.0 g, wet compressed yeast - 1.5 g and fat (pure ghee) 1.25 g. The water requirement for dough preparation varied, depending upon the various types as well as levels of enzymes added to the bread dough.

Diastase (α-amylase) was procured from Loba Chemie, India.

5.2.6.2 Bread making process:

Preparation of flying ferment is the first step in mixing process. To one-tenth part of lukewarm water (37 °C); one-fifth part of the total sugar and crumbled yeast were
added and the mixture was allowed to rest aside for 5 to 10 min until it disintegrated and floated on the surface. Little quantity of wheat flour was added to that suspension, beaten well and a thick paste was prepared. The paste was kept at 37 °C, till it rose. This paste was added to the dough. While flying ferment was getting ready, the required quantity of whole wheat flour was sieved and remaining salt and sugar are dissolved in water. Flying ferment along with sufficient water were added to wheat flour and kneaded well to prepare smooth dough. At the end of kneading, ghee was used for smearing. The dough was filled in the clean and sanitized glass beaker, covered with clean wet cloth and kept aside at 26 °C and 75% R.H. for bulk fermentation. After 30 min fermentation, the dough was knocked back and fermentation was continued for additional 15 min.

The fermented bulk dough was properly scaled (i.e. divided or cutting) and rounded. The rounded dough was moulded and placed in greased pan. The lid was covered and relaxed for 45 min at 35 to 36 °C and 80 to 85% R.H. This step is known as proofing. The dough was baked at 230 °C for 15 to 20 min. Bread was released from the mould, cooled sufficiently followed by slicing and packaging.

5.2.6.3 Study of dough and bread characteristics:

Dough rising capacity (Kamaliya, 2001) was measured as:

\[
\text{Dough rising capacity} = \frac{\text{Increase in dough height}}{\text{Height of the dough at the beginning}} \times 100
\]

Water absorption power (Kamaliya, 2001) was calculated as:

\[
\text{Water absorption power} = \frac{\text{Water absorbed by flour}}{\text{Weight of flour}} \times 100
\]
Volume of bread was measured by displacement of rapeseeds (Pyler 1988). Density and specific volume were calculated after weighing bread loaf. Final moisture of the bread was calculated by drying the preweighed bread slice in an oven at 130°C until constant weight (Pyler, 1988).

Sensory evaluation was done according to the 100-point evaluation scheme given by Pyler (1988).

The bread crumb (two slices having 19-21 mm height) was subjected to texture profile analysis using the mechanical device viz. Instron Universal Testing Machine (Instron Ltd., UK, Model-1000). Using a 0 to 50 kg load cell (area 100 cm²) operated at 0 to 50 kg chart recording range, at cross head speed of 20 mm/min, range 2/10 and 5-times chart magnification. From the two bite force distance compression curve, rheological properties such as hardness, cohesiveness, springiness, gumminess and chewiness were calculated. Interpretation of these properties was done according to Larmon (1976) and Breenan (1980) as shown below.

Hardness (kg): A force necessary to attain a given deformation i.e. the highest point of the peak in the first bite curve (H1) and 50% compression in the present study.

Cohesiveness: Extent to which the material can be deformed before it ruptures, i.e. the ratio of area A2 to A1.

Where,

\[ A1 = \text{area under the first bite curve before reversal of compression}; \]

\[ A2 = \text{area under the second bite curve before reversal of compression}. \]
Springiness: Springiness can be defined as the height the sample recovers between first and second compression on removal of deforming force.

\[ \text{Springiness} = \frac{L_2}{L_1} \]

Gumminess (kg): Energy required for disintegrating a semisolid food to a state ready for swallowing, a product of hardness and cohesiveness.

\[ \text{Gumminess} = H_1 \times \frac{A_2}{A_1} \]

Chewiness (kg): Energy required to masticate a food to a state ready for swallowing; a product of hardness, cohesiveness and springiness.

\[ \text{Chewiness} = H_1 \times \frac{A_2}{A_1} \times \frac{L_2}{L_1} \]

5.3 RESULTS AND DISCUSSION

5.3.1 Application of xylanases for saccharification of Hemicellulosic Substrates:

The saccharification of commercial birchwood xylan, and agro wastes viz. wheat bran and corn cobs was carried out at 45 and 50 °C as well as using different levels of crude xylanase preparation obtained from Aspergillus foetidus MTCC 4898 (Table-5.2 & 5.3). As shown in earlier chapter half-life of xylanases is quite higher at 45 °C (200 min) than at 50 °C (58 min) but reaction rate is higher at 50 °C. Hence, enzymatic hydrolysis was carried out at both the temperatures in the present investigation. Among the three substrates tested (Table-5.2), the birch wood xylan gave the highest yield (2745 mg/l in 18 h), followed by wheat bran (1569 mg/l in 18 h) and corn cobs (156 mg/l in 15 h) at 45 °C temperature.
The maximum yield of reducing sugars from birchwood xylan increased to 3067 mg/l in 18 h at 50 °C. In case of corn cobs and wheat bran, the yield was not enhanced but maximum reducing sugars were released in shorter period.

Generally enzymatic reaction rates are directly proportional to enzyme concentration, provided the substrate level exceeds the enzyme level (Tucker, 1995). The enzyme loading at which saturation of substrate occurs, appears to correlate with the optimum loading for hydrolysis (Singh et al., 1991). In the present study saccharification was done at three different levels of enzymes viz. 15, 50 and 100 U per 0.1g substrate (Table-5.3). Increase in the yield of reducing sugars was obtained by increasing the dose of the enzyme up to 100 U and maximum yield was 610 mg/l from corn cobs, 2575 mg/l from wheat bran and 5507 mg/l from birchwood xylan.

Using yeast cellulase free xylanase hydrolysis of three lignocellulosic materials viz. bagasse pulp, corn cobs and jute fibre was studied by Gokhale et al. (1998). Maximum reducing sugars were released at 220 U per 0.1g of substrates with 17.2% hydrolysis of bagasse pulp, 9.6% of corn cobs and 1.8% of jute fibre. Gawande and Kamat (1999) also used crude xylanase preparation from two Aspergillus sp. and obtained 8000 mg/l and 7200 mg/l reducing sugars from oat spelt xylan in 70 h at 45 °C temperature using 25 U per 0.1g substrate. From wheat bran maximum reducing sugar released were 3200 mg/l under the same conditions.
Table- 5.2: Saccharification at 45 °C and 50 °C using 50 U xylanase per 0.1 g substrate.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Amount of reducing sugars (mg/l) released from the substrate (Incubation period in h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Corn cob</td>
</tr>
<tr>
<td>45 °C</td>
<td>156 (15)</td>
</tr>
<tr>
<td>50 °C</td>
<td>149 (12)</td>
</tr>
</tbody>
</table>

Figures in bracket indicates incubation period (h)

Table-5.3: Saccharification at different levels of xylanase per 0.1 g substrate at 50 °C temperature.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Amount of reducing sugars (mg/l) released from the substrate at different dose of xylanase (U) per 0.1 g substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15</td>
</tr>
<tr>
<td>Corn cob</td>
<td>148 (18)</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>939 (18)</td>
</tr>
<tr>
<td>Xylan</td>
<td>2612 (21)</td>
</tr>
</tbody>
</table>

Figures in bracket indicates incubation period (h)
They found the soybean hulls more resistant to enzymatic saccharification as compared to wheat bran but this could be attributed to higher hemicellulose and lower lignin content of wheat bran compared with soybean hulls. In another study, results from the saccharification of various substrates showed that the commercial substrates like xylan were the most susceptible to the enzymatic hydrolysis. The greater resistance of agro wastes like corn cobs and rice hulls to enzymatic hydrolysis may be attributed chiefly to their lignin content (Okeke and Obe, 1995). The agro waste materials were significantly less susceptible to enzymatic hydrolysis than the commercial purified substrates. Among the crude lignocellulosic substrates, the highest degree of hydrolysis \( i.e. 15.1\% \) and \( 13.3\% \) were achieved using crude enzyme extracts having cellulases and hemicellulases of \textit{Sporotrichum pruinoseum} and \textit{Arthrographis} sp. respectively, from corn cobs at \( 40\, ^\circ\text{C} \) in 24 h. In this investigation, hemicellulose content of corn cobs is generally not lower than wheat bran and lignin is not higher than wheat bran, yet poor release of reducing sugars was observed from corn cobs, which shows higher complexity of corn cob heteroxylans and thereby poor hydrolysability. The thermostable xylanase produced by \textit{Melanocarpus albomyces} IIS-68 produced under SSF saccharified only 6.0% xylan of corn cobs (Jain \textit{et al.}, 1998). In the present study using 100 U xylanase per 0.1 g substrate, maximum hydrolysis was 6.1% for corncobs, 25.8% for wheat bran and 55.0% from xylan using crude fungal xylanase at \( 50\, ^\circ\text{C} \).

One of the important factors that limit the hydrolysis of lignocellulosic substrate by enzymatic method is the thermal stability of enzyme. Economics of the hydrolysis process can be improved if the enzyme could be stabilized. The crude xylanase
preparation used in this study was relatively thermolabile in nature and earlier characterization studies of the same had shown improvement in thermal stability in presence of CaCl₂ (Figure-5.1). By incorporating 50 mM CaCl₂ during saccharification at 50 °C, using 50 U per 0.1g substrate increased the maximum yield by 8.1% for birchwood xylan, 45.3% for wheat bran and 31.6% for corn cobs.

It was also observed that saccharification continued for longer period of time in presence of CaCl₂, which also indicates improvement in thermostability of the xylanase. Since CaCl₂ is a cheaper and non-toxic salt, it can also be used for preparing hydrolysates for food applications. Incorporation of CaCl₂ to improve heat stability of several enzymes has been reported (Wieille and Zeikus, 2001). However, its application for improving the thermostability of xylanase for its use in saccharification is not reported in the literature. Das and Nanda (1995) reported a profound effect of potassium and zinc salts on the rate of saccharification by Aspergillus ochraceus xylanases.

Pretreatment of lignocelluloses effectively enhance enzymatic hydrolysis (Durand, 1984). The results obtained from saccharification of alkali treated corn cobs in the present study confirms this. By using 1.0 N NaOH treated corn cobs, 6.1-fold increase in reducing sugar was obtained which resulted in 9.5% hydrolysis of corn cobs (Figure-5.2).
Figure 5.1: Effect of addition of calcium chloride on saccharification at 50 °C using xylanase (50 U)
Figure-5.2 : Effect of alkali treatment on saccharification of corn cobs by xylanase (U) at 50 °C temperature

Dilute alkali pretreatment caused swelling, leading to an increase in internal surface area, a decrease in the degree of polymerization, a decrease in crystallinity, separation of structural linkages between lignin and carbohydrates and disruption of the lignin structure (Fan et al., 1987). Matanguihan et al. (1988) used cellulase and xylanase produced by five selected molds were used for saccharifying corn cobs. The saccharification value using mold *Trichoderma reesei* NRRL 11460 cellulase increased by 6.0-fold i.e. 65.3% using pretreated (NaOH-ethanol) maize cobs. The hydrolysate contained 32.7 mg reducing sugars/ml consisting of xylose, glucose and cellobiose.
5.3.2. Saccharification of Wheat Flour:

A trial was conducted to study the extent of saccharification of wheat flour by the *A. foetidus* MTCC 4898 50U xylanase by adding it to 0.1g whole wheat flour (Figure-5.3). It was seen that the maximum reducing sugars (2397 mg/l) were released at the end of 18 h at 50 °C. Up to 15 h of incubation; the release of reducing sugar was relatively slow and steady. This study was conducted in order to check the hydrolysability of wheat flour by our xylanase preparation.
5.3.3 Application of Xylanases in Baking:

Whole wheat comprises of around 69.4 % total carbohydrates (Gopalan et al., 1982). The non-starch polysaccharide in the endosperm cell walls of wheat constitute up to 75 % of the cell wall dry matter weight (Mares and Stone, 1973). There are 3-4 % (w/w) pentosans in normal wheat flour, partially soluble and partially insoluble (Si and Lustenberger, 2002). Of these, the arabinoxylans (AX) are by far the most prominent group (Meuser and Suckow, 1986). Out of 1.5 to 2.5 % AX found in wheat flour endosperm, 20 to 30 % is water extractable. Water unextractable arabinoxylans, which make up 70 % of wheat endosperm cell walls (Mares and Stone, 1973) can be hydrolysed by endo-xylanases and causes them to lose their strong water holding capacity (Gruppen et al., 1993).

5.3.3.1 Preliminary studies:

In order to study the influence of xylanases of A. foetidus MTCC 4898 and commercial α-amylase, on whole wheat bread-making, preliminary trials were conducted (Figure-5.4) using different levels of partially purified xylanase (2.5 to 10.0 U/g wheat flour) and α-amylase (0.2 to 0.8 U/g wheat flour).

Based on subjective assessment of dough and bread characteristics, it was observed that the highest levels of α-amylase and xylanase selected in the present study gave the best results. Supplementation of xylanase at 5.0 U/g flour and above gave significantly better breads. Similarly, addition of α-amylase at 0.4 U/g flour and above gave distinctly better bread with respect to texture, volume and flavour. Overall, all the whole wheat breads prepared by addition of
enzymes had better body, texture and flavour characteristics than the control (without enzymes).

Synergistic effect of xylanases with α-amylase was also studied in another baking trial (Figure-5.5). As evident from the photograph, xylanase and α-amylase combinations as well as their individual additions were found to be beneficial with respect to bread volume. Based on subjective assessment of dough and bread characteristics by bakers bread prepared by addition of 5.0U/g xylanase and 0.8U/g amylase as well as the one with 15U/g xylanase were more acceptable. It was seen clearly that when xylanase was combined with α-amylase, lower dose of xylanase is required. Thus, it was evident from the study that xylanase acted synergistically with α-amylase. Similar observations have also been reported by Si and Lustenberger (2002).

Amylases are known to act on damaged starch; reducing its ability to immobilize water, thus increasing dough mobility. Dough handling is improved and enhanced production of fermentable sugars increases the yeast growth and thus the power to produce carbon dioxide. As a result, increase in loaf volume is obtained; colour, texture and flavour are improved along with extension of shelf-life by anti-staling effect (Si and Lustenberger, 2002).

To improve the handling properties of bread dough and final baked product, several ingredients are added to basic recipe. One of this is xylanase, which has a positive effect on the properties of dough, on the final loaf volume and on shelf-life (Rouau et al., 1994; Poutanen, 1997). The beneficial effect has been attributed to solubilization of insoluble pentosans, leading to a change in water distribution in the dough (Maat et al., 1992).
Figure-5.4 : Effect of addition of α-amylase and xylanase on whole wheat bread crumb characteristics.

Control

α-amylase 0.8 U/g

Xylanase 10 U/g

Figure-5.5 : Effect of addition of α-amylase, xylanase and their combination on whole wheat bread crumb characteristics.

Control

α-amylase 0.8 U/g

Xylanase -10 U/g

Xylanase -15 U/g

Xylanase -5 U/g

+ α-amylase - 0.8 U/g
The amount of soluble arabinoxylans and their total water holding capacity increases with decrease in water insoluble pentosans (Rouau and Moreau, 1993). Some as yet unidentified factors must be involved in explaining the role of high and low molecular weight arabinoxylans (Courtin and Delcour, 1998). The source and type of xylanase determines its pattern of action on arabinoxylans and whether it has a preference for soluble or insoluble arabinoxylans (Hilhorst et al., 2002). Different xylanase preparations have different effects on arabinoxylans in terms of their scission point and reaction products and therefore, they show different effects on bread making (Si, 1997). At the optimum dosage, they can improve dough machinability, dough stability, oven spring, larger loaf volume and improved crumb structure (Hammer, 1995).

5.3.2.2 Influence of xylanase addition on whole wheat bread preparation:

Partially purified xylanase (12 U/g) was supplemented to whole wheat bread dough during mixing. A comparative study of dough and bread properties of control and xylanase supplemented bread preparation indicated remarkable influence of xylanase on final product.

During mixing stage to obtain dough with similar consistency without problem of stickiness, lesser water was needed for xylanase-supplemented dough (Table- 5.4). Water absorption was reduced to 64% due to xylanase addition from 72% of the control. It has been reported that starch and nonstarch hydrolysing enzymes result in release of free water and changes the soluble fraction of dough. These effects are apparent immediately after mixing and continue during resting, which changes viscoelastic properties of dough (Martinez-Anaya and
Jimenez, 1998). Similar observation was also reported by Courtin et al., (2001). They observed decreased water absorption with increased levels of fungal and bacterial endoxylanase addition in wheat bread making.

Table-5.4 : Dough and Bread Attributes

<table>
<thead>
<tr>
<th>Attributes</th>
<th>Control</th>
<th>Xylanase</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dough</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water absorption (%)</td>
<td>72.0</td>
<td>64.0</td>
</tr>
<tr>
<td>Dough rising (%)</td>
<td>144.0</td>
<td>185.0</td>
</tr>
<tr>
<td><strong>Bread</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume of bread (ml)</td>
<td>300.0</td>
<td>460.0</td>
</tr>
<tr>
<td>Weight of bread loaf (g)</td>
<td>190.0</td>
<td>186.0</td>
</tr>
<tr>
<td>Density (g / ml)</td>
<td>0.635</td>
<td>0.404</td>
</tr>
<tr>
<td>Specific volume (ml / g)</td>
<td>1.58</td>
<td>2.47</td>
</tr>
<tr>
<td>Final moisture (%)</td>
<td>32.3</td>
<td>40.5</td>
</tr>
</tbody>
</table>

When both the dough was fermented for same time period (45 min), rising was remarkably higher (185%) as compared to control (144%) as shown in Table-5.4 and Figure-5.6. This can be explained on the basis of higher release of fermentable sugars in xylanase supplemented dough and thereby increased rate of carbon dioxide evolution by yeast. Specific volume of loaf or density was also greatly improved upon addition of xylanase (Table-5.4 and Fig.-5.7). Final moisture content was increased from 32.3 % (control) to 40.5% (added with xylanase).
Figure-5.6: Effect of addition of xylanase on dough rising capacity and dough characteristics (initial and at the end of fermentation) during whole wheat bread preparation.
Figure-5.7: Effect of addition of xylanase on whole wheat bread loaf volume and crumb characteristics.
In contrast to this observation, Courtin and Delcour (1998) reported decrease in moisture level in xylanase added wheat bread. Ideal moisture level for bread is between 35 to 40%. If lesser than dryness is felt and if higher than microbial spoilage is favoured (Kamaliya, 2001). Breads, whose moisture content is reduced to less than 30%, cannot be refreshed even after heating (Pyler, 1988).

A panel of five judges did sensory evaluation of both type of breads on 100 points. Average score was significantly higher (70) for xylanase supplemented bread than for control (Table-5.5). Whole wheat bread prepared without supplementation of xylanase exhibited compact loaf with a dark dense crumb and distinctly wheatty flavour, which yielded significantly low scores by the judges. While xylanase supplemented whole wheat bread was found much superior with respect to several attributes viz. volume, flavour, softness, grain etc. and was very well accepted by the judges.

Table-5.5 : Sensory Evaluation of Whole Wheat Bread

<table>
<thead>
<tr>
<th>Bread attributes</th>
<th>Max. Score</th>
<th>Control</th>
<th></th>
<th>Xylanase</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>Range</td>
<td>Mean</td>
<td>Range</td>
</tr>
<tr>
<td>Volume</td>
<td>15</td>
<td>6.8</td>
<td>5 - 9</td>
<td>12.2</td>
<td>11 - 13</td>
</tr>
<tr>
<td>Colour and Nature of crust</td>
<td>5</td>
<td>2.4</td>
<td>1 - 4</td>
<td>3.6</td>
<td>3 - 5</td>
</tr>
<tr>
<td>Symmetry of form</td>
<td>5</td>
<td>2.6</td>
<td>2 - 4</td>
<td>4.0</td>
<td>3 - 5</td>
</tr>
<tr>
<td>Uniformity of baking</td>
<td>5</td>
<td>3.0</td>
<td>2 - 4</td>
<td>4.0</td>
<td>3 - 5</td>
</tr>
<tr>
<td>Texture</td>
<td>15</td>
<td>5.6</td>
<td>4 - 7</td>
<td>10.4</td>
<td>7 - 12</td>
</tr>
<tr>
<td>Colour of crumb</td>
<td>10</td>
<td>4.8</td>
<td>2 - 7</td>
<td>7.0</td>
<td>6 - 8</td>
</tr>
<tr>
<td>Grain</td>
<td>10</td>
<td>3.4</td>
<td>2 - 6</td>
<td>6.8</td>
<td>6 - 8</td>
</tr>
<tr>
<td>Aroma</td>
<td>15</td>
<td>6.2</td>
<td>3 - 10</td>
<td>11.0</td>
<td>9 - 12</td>
</tr>
<tr>
<td>Taste</td>
<td>20</td>
<td>7.8</td>
<td>5 - 11</td>
<td>15.4</td>
<td>15 - 17</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>42.6</td>
<td>36 - 55</td>
<td>74.4</td>
<td>72 - 80</td>
</tr>
</tbody>
</table>
Texture of food products can be determined by two different ways: instrumental analysis and sensory evaluation. The use of instrumental analysis is more convenient than that of sensory evaluation. The determination of texture by instrumental tests is easy to perform, simple to reproduce and less time consuming (McCormick, 1988).

Studies on rheology of bread texture were also done for control as well as xylanase supplemented bread. As evident from texture profile analysis data (Table- 5.6, Figure-5.8), firmness or hardness was significantly reduced to more than four folds upon xylanase supplementation. Moreover, significant improvement in cohesiveness was also observed. Gumminess and chewiness decreased in xylanase treated bread, which is always desirable. But springiness was not much affected; rather marginal decrease was noted. Overall, significant improvement in textural properties was confirmed by rheological analysis.

Table-5.6 : Two Bite Texture profile analysis of whole wheat bread with and without supplementation of xylanase

<table>
<thead>
<tr>
<th>Rheological properties of bread crumb</th>
<th>Whole wheat bread without xylanase</th>
<th>Whole wheat bread supplemented with xylanase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hardness/ Firmness</td>
<td>44</td>
<td>10</td>
</tr>
<tr>
<td>Springiness</td>
<td>0.8448</td>
<td>0.7954</td>
</tr>
<tr>
<td>Cohesiveness</td>
<td>0.2121</td>
<td>0.6420</td>
</tr>
<tr>
<td>Gumminess</td>
<td>9.3324</td>
<td>6.42</td>
</tr>
<tr>
<td>Chewiness</td>
<td>7.884</td>
<td>5.1064</td>
</tr>
</tbody>
</table>
Figure-5.8 a: Two bite texture profile analysis of whole wheat bread without xylanase (control).

Figure-5.8 b: Two bite texture profile analysis of whole wheat bread added with xylanase
Haros et al. (2002) also studied influence of xylanase on bread texture and found comparable changes. According to hypothesis of Maat et al. (1992), the positive impact of xylanase on bread texture is due to the redistribution of water from the pentosans phase to the gluten phase.

It was evident from the above studies that *A. foetidus* low cost xylanase produced by SSF of corn cobs is suitable for applications like saccharification of hemicellulosic substrates and preparation of whole wheat bread.
REFERENCES


