APPARENT ENDOXYLANASE ACTIVITY IN RYE CULTIVARS FROM LITHUANIA

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Abstract

The efficiency of cereal-based biotechnological processes closely linked to both their chemical composition and selection of cell-wall degrading enzymes. Degradation of cell-wall polysaccharides during processing, by means of added or naturally occurring endogenous xylanases, may also affect the quality of the end-product. The aim of the present study was determination of the apparent activity of endogenous xylanases in four winter rye varieties and in two newly developed rye hybrids. The influence of variety and climatic conditions on apparent endoxylanase activity was investigated. Rye cultivars were grown on different soils at three Plant Breeding Stations of Lithuania in 2003 and 2004. The apparent endoxylanase activity was studied in the albumin type extracts from meal of the rye kernels (pH 4.5; 40 °C) by an assay based on dinitrosalicylic acid. The arabinoxylan used as substrate was isolated from wheat bran, and the reducing sugars were purified by cation-exchange chromatography. The results showed the significant influence of the genotype and agro-climatic conditions on the endoxylanase activity varying between 5.1±0.6 and 23.6±0.9 nkat/g grain. No significant correlations were found between Falling number (FN) and apparent endoxylanase activity in rye.

Key words: rye, endoxylanase, activity, genotype.

Introduction

The efficiency of cereal-based biotechnological processes and the quality of end- products are closely linked to the chemical composition of the fermented cereal raw material. Degradation of cell-wall polysaccharides during processing, by means of added or naturally occurring endogenous xylanases may also affect the quality of the end-product.

Rye is the second important crop in Lithuania next to wheat because of its production extent and is widely used in various fermentation processes. Rye is characterized as to have a higher amount (~16%) of the dietary fiber polysaccharides the most important constituent of which are arabinoxylans (AX) (Vinkx and Delcour, 1996). AX-fraction is of considerable importance for rye processing, bread-making quality and nutritional properties of food and feed (Vinkx *et al*, 1993; Åman *et al.*, 1997). Although, arabinoxylans together with β-glucans cause the low extract yields, high wort viscosity and decreased the rate of filtration or haze formation in beer (Antoniuou *et al.*, 1981; Izydorczyk and Biliaderis, 1995; Schwarz and Han, 1995). The presence of water-extractable AX constituting approximately 25–30 % of the total AX increases dough viscosity, bread volume, gas retention, crumb texture, colour and taste (Bengtsson and Åman, 1990; Courtin and Delcour, 2002).

In recent years, the interest in carbohydrate-active enzymes such as xylanases has increased due to their potential application in the food and feed industry. Endoxylanases are primarily responsible for the degradation of AX increasing the level of water soluble AX and affect the fermentation process, herewith the quality of the end-product (Poulsen *et al.*, 2002). However, efficiency of added commercial endoxylanases can vary depending on rye variety and growing location making the optimal dosage of enzyme difficult to determine. It may be due to the levels of endoxylanases and endoxylanase inhibitors in rye cultivars (Sørensen *et al.*, 2001; Dornez *et al.*, 2006). Similarly, evaluation of the action of endogenous xylanases is of a growing importance in case of increasing the efficiency of fermentation process of rye-derived stock in different applications. The optimization of enzymatic hydrolysis of rye causes the problems due to the lack of studies related to the endoxylanase activity and the influence of the growth conditions and genotype on the endoxylanase level.

The present work has the objective of determination the apparent activity of endogenous xylanase in winter rye crude extracts, which serve as a model system of fermentable raw material.

Materials and Methods

Rye samples. The samples of four rye varieties 'Joniai', 'Fernando', 'Matador', 'Picasso' and new developed hybrids LVA 426 and LVA 391 were obtained from Plant Breeding Stations (PBS) located in different parts of Lithuania after two growing seasons (2002–2003 and 2003–2004). The weather conditions in the summer of 2003 and 2004 were completely different as well as the precipitation levels at PBS I and PBS II locations (Figure 1). The summer of 2003 was warm and dry. Higher falling numbers (FN) for the rye varieties grown in 2003 were observed with an average of 266 s. In contrary, the summer of 2004 was cool and wet. Heavy rainfall before harvest increased the risk of sprouting and the microbial contamination of the rye kernels. This was evidenced by relatively low FN values with an average of 169 s.

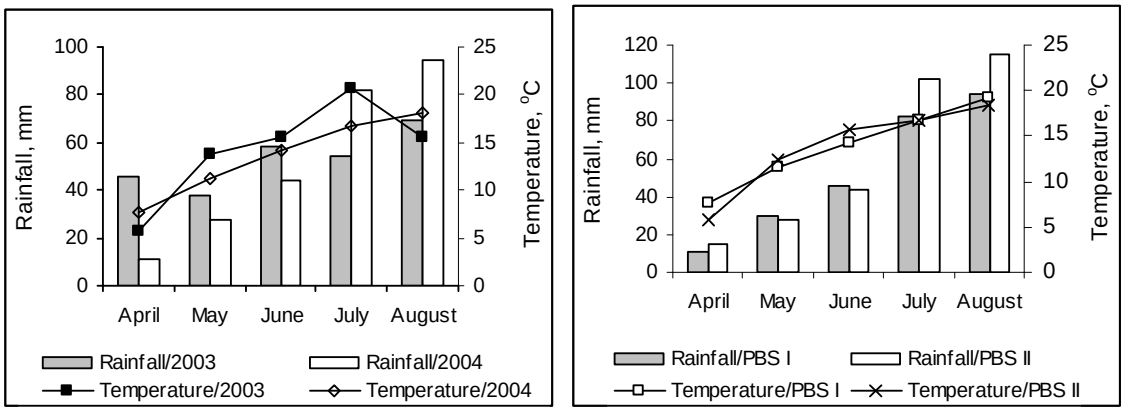

Figure 1. Average temperature (curve) and total rainfall (bars) in 2003 and 2004 (a) and at PBS I and PBS II locations (b)

Sample preparation

Rye crude extracts. The rye wholemeal (5±0.01 g) was homogenized in 40 ml of sodium acetate buffer (0.1 M; pH 4.5) on ice-bath for 10 min. The homogenate was centrifuged (10000 g; 4 °C; 25 min) and filtered.

Substrate solution. The wheat arabinoxylan (3.2 mg/ml) solution was prepared from wheat bran at the same manner as rye crude extracts. After homogenization and centrifugation procedures the solution was boiled for 15 min and cooled to 4 °C.

Stop solution (DNS reagent). 3.5-dinitrosalicylic acid (1±0.01 g g) and sodium potassium tartrate (30±0.01 g g) dissolved in 100 ml of 0.4 M NaOH.

Determination of falling number. Falling number were determined according Hagberg-Perten by ISO 3093:2004 method.

Endoxylanase activity assay. The endoxylanase activity in rye extracts was measured by an assay based on dinitrosalicylic acid according Miller (1959) with some modifications. One unit of enzyme activity is defined as the amount of enzyme required to releases 1 μmol of xylose equivalents per minute from the arabinoxylans under the assay conditions used (pH 4.5; 40 °C).

The reaction mixture (1 ml) containing cereal extract (200 μl)) and substrate (100 μl) in acetate buffer (0.1 M; pH 4.5) was incubated at 40 °C for 60 min. The reaction mixture prior to treatment by dinitrosalicylic acid was subjected to carbohydrate purification by CEC according to the principles described by Sørensen *et al*., (1999). The equal amounts of

carbohydrate solution and DNS reagent were mixed and incubated in a boiling water bath for exactly 5 min. After cooling, reaction solution was diluted (1:10) and the absorbance of the solutions was measured at 540 nm. The mean absorbance values of the triple determinations and the two blanks were calculated. The D-xylose standards (0–0.25 mM) were made up in acetate buffer (0.1 M; pH 4.5) and allowed the construction of a calibration curve. For the calculation of the endoxylanase activity (nkat/g grain) the slope from the xylose curve was used.

Carbohydrate purification by cation-exchange chromatography (CEC). CEC columns contained the strongly acidic cation-exchanger (Dowex 50WX8 H^+ 200–400 mesh) were washed with water to neutral pH. The reaction mixture (1 mL) was added, and the aqueous effluent contains neutral compounds including carbohydrates was washed with 10 mL of water.

Statistical analysis. The variations in the endoxylanase activity were analysed with the Statistical software Analyse-it for comparison of the means by one-way analysis of variance (ANOVA). The significant of the results from the data analysis was considered by $p<0.05$.

Results and Discussion

Endoxylanase variability. Table 1 presents the apparent endoxylanase activities in different rye varieties of 2003 and 2004. The measured endoxylanase activity values in the rye grain extracts after 1 hour of incubation varied from 5.1±0.6 to 23.6±0.9 nkat/g. The statistically significant differences between the apparent activities of rye varieties from PBS I and PBS II same as between varieties of 2003 and 2004 (PBS III) harvest were found. This verifies that the growing location influence ($p<0.05$) on the formation of the endoxylanase in rye cultivars. Also a strong correlations between apparent endoxylanase activities within mentioned groups was found ($R^2=0.74$ and $R^2=0.76$, respectively; $p<0.05$). The apparent endoxylanase activities in the rye varieties of 2004 were found to be higher on an average by 46% comparing to the activities of analogous cultivars of 2003. The results show the significant influence ($R^2=0.99$; $p<<0.05$) of the precipitation levels on the apparent endoxylanase activity.

The lowered endoxylanase activity values determined after 6 hours (Table 1) of incubation indicated the decrease in activity which can be due to the action of rye inhibitors which are able to inhibit microbial kernel-associated endoxylanases. The apparent endoxylanase activity of rye variety ‘Fernando’, ‘Picasso’ and LVA hybrids show the lowest changes during incubation. They may have the lowest activity of microbial endoxylanases and can be characterized as more resistant to microbial contamination than the other varieties.

Table 1

Apparent enoxylanase activity levels in winter rye cultivars

Rye variety	FN, s	Activity, nkat/g		$lnA=-k_Dx+lnA_o$	$T_{1/2}$, h
		after 1 h	after 6 h		
PBS I (2004)					
‘Joniai’	202	16.65±0.88	7.42±0.16	y=0.173x+3.049	4
‘Matador’	208	14.50±0.82	6.85±0.19	y=-0.156x+2.862	4
‘Fernando’	297	8.90±0.42	3.57±0.18	y=-0.170x+2.417	4
‘Picasso’	253	5.06±0.37	2.87±0.19	y=-0.138x+2.010	5
PBS II (2004)					
‘Joniai’	203	19.07±0.57	7.72±0.17	y=-0.157x+3.006	4
‘Matador’	248	23.55±0.92	9.22±0.28	y=-0.176x+3.193	4
‘Fernando’	232	10.59±0.39	6.21±0.22	y=-0.103x+2.461	7
‘Picasso’	262	8.29±0.50	5.73±0.19	y = -0.076x + 2.142	9

Rye variety	FN, s	Activity, nkat/g		$lnA=-k_Dx+lnA_o$	$T_{1/2}$, h
		after 1 h	after 6 h		
PBS III (2003/2004)					
'Joniai'/2003	244	8.54±0.35	5.28±0.16	y=-0.107x+2.327	6
'Joniai'/2004	184	16.72±0.96	8.41±0.18	y=-0.143x+2.944	5
LVA 426/2003	263	7.62±0.26	5.08±0.18	y=-0.082x+2.131	8
LVA 426/2004	164	14.50±0.92	7.97±0.29	y=-0.120x+2.794	6
LVA 391/2003	292	7.10±0.28	4.78±0.18	y=-0.061x+1.924	11
LVA 391/2004	160	12.26±0.82	7.60±0.29	y=-0.099x+2.623	7

The obtained activity values were found higher than those reported in literature by Autio *et al*. (1998). They measured endoxylanase activity of 1 and 5 nkat/g·grain in ungerminated and germinated rye kernels, respectively, using birchwood xylan as substrate. Either, the activities of endogenous β-D-xylanase were quantified in extracts from ungerminated rye grain by Rasmussen *et al*. (2001). The obtained activity of the endoxylanase against RBB-xylan was 11 pkat/g·grain. The higher activity of endoxylanase in our assay might to some extent be explained by the increased susceptibility of the soluble wheat AX to enzyme attack.
No significant correlations were found between FN and endoxylanase activities in rye (R^2=0.232). However, low reverse correlation (R^2=0.414) between FN and endoxylanase activity after 6 hours of incubation was detected. This suggests that the sources of endoxylanase and amylase activities in rye are quite different. Also the susceptibility of rye varieties to microbial infection could play a role.

Conclusions

The apparent endoxylanase activities are at least partially genetically determined, but the levels of endoxylanase largely depended on the climatic conditions prior to harvesting. The fact that not only sprouting kernels but also microorganisms on the cereal can produce endoxylanases may explain why no significant correlation could be found between FN and endoxylanase activity.
The functionality of commercial xylanases in different applications involving cereal processing may be influenced to different degrees by the relative quantities of cereal endoxylanase and endoxylanase inhibitors presented in cereal raw material and by the sensitivity of the endoxylanase to the inhibition. As endoxylanases strongly influenced on rye functionality, endoxylanase activity could be used as one of additional criterion for the selection of rye varieties suitable for applications required.

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References

1. Åman, P., Nilsson, N., Andersson, R. (1997) Positive health effects of rye. *Cereal Foods World*, 42, pp. 684–688;
2. Antoniuou, T., Marquardt, R., Cansfield. P. (1981) Isolation, partial characterization and antrinutritional activity of a factor (pentosans) in rye grain. *J. Agric Food Chem*, 29, pp. 1240–1247;
3. Autio, K., Fabritius, M., Kinnunen, A. (1998) Effect of germination and water content on the microstructure and rheological properties of two rye doughs. *Cereal Chem*, 75, pp.10–14;
4. Bengtsson, S., Åman, P. (1990) Isolation and chemical characterization of the water-soluble arabinoxylans in rye grain. *Carbohydr Polym*, 12, pp. 267–277;
5. Courtin, C.M. and Delcour, J.A. (2002) Arabinoxylans and endoxylanases in wheat flour bread-making. *J. Cereal Sci*, 35, pp. 225–243;

6. Courtin, C.M., Gelders, G.G., Delcour, J.A. (2001) Use of two endoxylanases with different substrate selectivity for understanding arabinoxylan functionality in wheat flour breadmaking. *Cereal Chem*, 78, pp. 564–571;
7. Dornez E., Joye I.J., Gebruers K., Lenartz J., massaux C., Bodson B., Delcour J.A. and Courtin C.M. (2006a) Insight into variability of apparent endoxylanase and endoxylanase inhibitor levels in wheat kernels. *J. Sci Food Agric*, 86, pp. 1610–1617;
8. Dornez E., Joye I.J., Gebruers K., Delcour J.A. and Courtin C.M. (2006b) Wheat-Kernel-associated endoxylanases consis of a majority of microbial and minority of wheat endogenous endoxylanases. *J. Agric Food Chem*, 54, pp. 4028–4034;
9. Izydorczyk, M.S., Biliaderis, C.G. (1995) Cereal arabinoxylans: Advances in structure and physicochemical properties. *Carbohydr Polym*, 28, pp. 33–48;
10. Miller, G. L. (1959) Use the dinitrosalicylic acid reagent for determination of reducing sugar. *Analyt Chem*, 31, pp. 426–428;
11. Poulsen, C., Figueroa-Espinoza, M.C., Borch Soe, J., Zargahi, M.R., Rouau, X. (2002) Enzymatic solubilization of arabinoxylans from isolated rye pentosans and rye flour by different endo-xylanases and other hydrolyzing enzymes. *Agric Food Chem*, 50, pp. 6473–6484;
12. Rasmussen, C. V., Boskov Hansen, H., Hansen, A., Melchior Larsen, L. (2001) pH-, temperature- and time-dependent activities of endogenous endo-β-D-xylanase, β-D-xylosidase and α-L-arabinofuranosidase in extracts from ungerminated rye (Secale cereale L.) grain. J. *Cereal Sci*, 34, pp. 49–60;
13. Schwarz, P.B., Han, J-Y. (1995) Arabinoxylan content of commercial beers. *J. Am Soc Brew Chem*, 53(4), pp. 157–159;
14. Sørensen J.F., Sibbesen O., Poulsen C.H. (2001) Degree of inhibition by the endogenous wheat xylanase inhibitor controls the functionality of microbial xylanases in dough. In: *Abstracts of the AACC Annual Meeting*, Charlotte, NC;
15. Sørensen, H., Sørensen, S., Bjergegaard, C., Michaelsen, S. *Chromatography and capillary electrophoresis in food analysis*. (1999) Denmark: The Royal Society of Chemistry, pp. 93–240;
16. Vinkx, C.J.A., Reynaert, H.R., Goirbet P.J., Delcour J.A. (1993) Physicochemical and functional properties of rye nonstarch polysaccharides. Variability in the structure of water-soluble arabinoxylans. *Cereal Chem*, 70, pp. 311–317;
17. Vinkx, C.J.A., Delcour, J.A. (1996) Rye (Secale cereale L.) arabinoxylans: A critical review. *J. Cereal Sci*, 24, pp. 1–14.