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Endoxylanase and endoxylanase inhibition activities in the grain of winter rye cultivars

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Abstract

The study was designed to investigate the effect of variety, cultivation site and crop year on the endoxylanase activity in the winter rye varieties ‘Joniai’, ‘Matador’, ‘Recrut’, ‘Fernando’ and ‘Picasso’, and the newly developed hybrids LIA 426, LIA 391 and LIA 463. Partial purified soluble rye proteins were tested for their inhibition activity against commercial glycoside hydrolase (GH) family 11 endoxylanases of *Trichoderma reesei* and *Thermomyces lanuginosus*. The apparent endoxylanase activity values in the different rye samples varied between 0.31 and 1.42 U g^{-1} grain. The endoxylanase activity levels largely depended on the variety and weather conditions before harvest. The inhibition activities in the different rye samples against *T. lanuginosus* and *T. reesei* enzymes varied between 19.0 and 33.4, and 11.4 and 24.8 IU 100 mg^{-1} of dry wholemeal, respectively, and were linearly related. The endoxylanase inhibition activity was mainly dependent on genetic background and was less affected by the growing conditions and precipitation levels. The newly developed rye hybrids seem to have nearly related endoxylanase inhibition activity irrespective of the environmental conditions and genetics. The *T. lanuginosus* enzyme was found to be much more inhibited by the rye inhibitors. The rye protein fractions with inhibition activity contain the components with the molecular masses of about 10–11, 14 and 29–31 kDa. The differences in functionality of commercial enzymes and in screening these enzymes for the application to the rye-based processes can be explained according to the obtained results.

Key words: rye, extractable protein, endoxylanase, inhibition activity, growing conditions.

Introduction

The quality of end products of a fermentation process in which cereal-based raw materials are used is closely linked to the chemical composition of the cereals. The optimization of enzymatic hydrolysis of cereal raw material requires the complex evaluation of endogenous enzyme levels and also selection of the commercial enzyme preparations more resistant to cereal inhibitors.

Rye is the second important crop next to wheat in Lithuania and is widely used in the manufacturing of bread, other food products and bio-ethanol in which fermentation processes play a crucial role. Rye is characterized by the high non-starch polysaccharide content of which the most important part is arabinoxylans (AX) (Vinkx, Delcour, 1996). The conformation and physicochemical properties of AX affect their functionality (Bengtsson, Åman, 1990; Vinkx et al., 1993; Izydorczyk, Biliaderis, 1995) and that of cereals used in breadmaking, for animal feeding and gluten-starch separation (Gudmunsson et al., 1991; Härkönen et al., 1996; Denli, Ercan, 2001; Courtin, Delcour, 2002; Frederix et al., 2004). In recent years, the interest in carbohydrate-active enzymes especially endoxylanases (EC 3.2.1.8) of microbial origin has increased due to their application in food and feed technologies to degrade or modify the AX in order to improve processing and/or the quality of the end product (Jiang et al., 2005; Collins et al., 2006; Selinheimo et al., 2006; Sterk et al., 2007). Xylanase functionality depends on the biochemical properties of the enzyme, the substrate

specificity of the hydrolysis pattern and the relative activity towards the water-unextractable and water-extractable AX fractions (Courtin, Delcour, 2001; Courtin et al., 2001).

Information about the functionality of rye endoxylanases and their contribution to rye quality variation is very limited (Rasmussen et al., 2001; Simpson et al., 2003; Salmenkallio-Marttila, Hovinen, 2005). It is known that endogenous xylanases are initially synthesized during germination, and accumulated in the aleurone cells. The release of the endoxylanase from the aleurone layer is accompanied by the proteolytic cleavage of the precursor molecule, which activates the enzyme. This active endoxylanase then contributes to the breakdown of aleurone and endosperm cell walls (Caspers et al., 2001).

As reported in literature, the endoxylanase associated with cereal kernel consists of cereal endogenous endoxylanases produced during germination which are not inhibited by the known inhibitors, and of microbial endoxylanases which are populating the outer layers of the kernel and are sensitive to inhibition (Gys et al., 2004; Dornez et al., 2006). However, the efficiency of added microbial endoxylanases can vary depending on harvest year, cereal variety and growing site, making the dosage of the enzyme needed to obtain the optimal effect difficult to determine. That may be due to the levels of cereal endogenous xylanases and their inhibitors.

The structurally different endoxylanase inhibitors TAXI (*Triticum aestivum* xylanase inhibitor) and XIP (xylanase inhibiting protein) have been purified from wheat (McLauchlan et al., 1999; Goesaert et al., 2003), barley and rye (Goesaert et al., 2002; Elliot et al., 2003). The third TL-XI-type (thaumatin-like xylanase inhibitor) xylanase inhi-bitor has been identified as a basic protein occurring in wheat as the multiple isoforms (Fierens et al., 2007).

Consequently, the optimization of enzymatic hydrolysis of rye causes the problem due to the lack of studies related to the influence of the growth and genetic factors on the levels of these components. The present work has the objective to determine the apparent activities of endogenous xylanases in winter rye crude extracts, which serve as a model system of fermentable raw material. The sensitivity of two commercial endoxylanases of *Trichoderma reesei* and *Thermomyces lanuginosus* to the inhibition was studied.

Materials and methods

Rye samples. The rye varieties 'Joniai', 'Matador', 'Recrut', 'Fernando' and 'Picasso', grown at Kaunas and Vilnius Plant Variety Testing Centers (PVTC) in 2004 were analyzed to ascertain the effect of variety and cultivation site on endoxylanase and endoxylanase inhibition activity. The rye variety 'Joniai' and the newly developed hybrids LIA 426, LIA 391 and LIA 463, grown at the Lithuanian Institute of Agriculture (Dotnuva) in two consecutive years (2003–2004) were used to assess the influence of the crop year on the above-mentioned biological activities.

Chemicals. Water-soluble wheat AX (medium viscosity) was purchased from Megazyme (Bray, Ireland). Commercial GH 11 family *endo*-1.4-β-D-xylanases (EC 3.2.1.8) of *T. reesei* (activity 17800 U g^{-1}) and *T. lanuginosus* (activity 17100 U g^{-1}) were obtained from Biosinteze (Vilnius, Lithuania) and Novozymes (Bagsvaerd, Denmark), respectively. All chemicals and reagents were purchased from Sigma-Aldrich (Taufkirchen, Germany) and were of analytical grade.

Methods. The endoxylanase activity in rye extracts was measured by an assay based on dinitrosalicylic acid according to Miller (1959) with some modifications. The rye wholemeal (5 g) was homogenized (10 min, +4°C) in 40 ml of the sodium acetate buffer (10 mM, pH 4.5). The homogenate was centrifuged (10000 g, +4°C, 25 min) and diluted (1:5). The wheat arabinoxylan solution (5 g l^{-1}) was prepared in the same buffer and was used as a substrate. Stop solution (DNS reagent) was prepared by mixing 1 g of 3.5-dinitrosalicylic acid and 30 g of sodium potassium tartrate, dissolved in 100 ml of the 0.4 M NaOH. The reaction mixture (1 ml) containing cereal extract (200 µl) and substrate (50 µl) in sodium acetate buffer (10 mM, pH 4.5, 750 µl) was incubated at +40°C for 1 h. The reaction was stopped by the addition of the DNS reagent (1 ml). The mixture was boiled for 5 min, cooled at room temperature and centrifuged (10000 g, 5 min). Finally, the reaction solution was diluted (1:10) with distilled water and the absorbance was measured at 540 nm by the spectrophotometer. The mean absorbance values of the triple determinations were measured against two blanks including substrate or rye crude extract. Activities were expressed in enzyme units. One unit of enzyme activity (1 U) is defined as the amount of enzyme required to release 1 µmol of xylose equivalents per minute from the soluble

wheat arabinoxylan under the assay conditions used. The D-xylose standards (0–450 g l^{-1}) were made up to construct a calibration curve. For the calculation of the endoxylanase activity the slope from xylose standard curve was used.

Protein separation by cation exchange chromatography (CEC). The rye extract for protein separation was prepared by suspending wholemeal in l of distilled water (1:10) and shaking for 30 min at room temperature. The suspension was centrifuged (10000 g, 20 min, +10°C) and after adjusting pH to 3.0 the supernatant was applied to SP-Sepharose Fast Flow Column (XK16/20, 6% agarose, cation-exchange group-sulphopropyl). Protein separation was performed with sodium acetate (10 mM sodium acetate, pH 3.0), sodium phosphate (10 mM, pH 8.3) buffers and NaCl solution (500 mM, pH 8.3). After the purification step the eluted protein fractions (CEC fractions) were collected, dialyzed against distilled water (+10°C, 48 h) and lyophilized.

Endoxylanase inhibition assay procedure. The measurement of endoxylanase inhibition activities was thus based on the specificity of added endoxylanase solutions. The enzyme solutions (25 U ml^{-1}) were prepared in sodium acetate buffer (0.1 M, pH 4.5) containing bovine serum albumin (0.5 g l^{-1}). The reaction mixture (0.3 ml) containing the 250 µl of CEC-fraction (1.7 g l^{-1} protein) and the enzyme solution (25 µl) in sodium acetate buffer (100 mM, pH 4.5) was pre-incubated for 30 min at +30°C in order to achieve interaction between enzyme and the inhibitor possibly present. After addition of the substrate (0.25% w/v, 25 µl), the time of incubation was 1 h at +30°C. The reaction was stopped by adding the DNS reagent (0.3 ml). After boiling, cooling and dilution procedure, the absorbance at 540 nm was measured against a control, prepared by incubating the enzyme with buffer instead of protein solution. The endoxylanase inhibition activity was expressed as a number of inhibition units (IU), defined as the amount of inhibitor resulting in 50% decrease of endoxylanase activity under the experimental conditions of dry weight (XIA_{DW}).

Protein electrophoresis. Protein fractions indicating the inhibition activity were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and iso-electric focusing (IEF). Desalted and denatured (0.002 M TRIS, heating at +100°C, 5 min) proteins were electrophoresed in the presence of SDS using 8–25% polyacrylamide gels with a PhastSystem unit (Amersham Biosciences, Sweden). Molecular weights were calculated from a plot of migration distances versus $\log_{10}$ of the molecular weight of a series of protein markers (BioRad: 250–10 kDa and PMW: 6.21–16.95 kDa). The pI was determined with the same instrument using polyacrylamide gels containing ampholytes (pH 3.0–9.0) and appropriate standards (Pharmacia Biotech calibration kit, pI 3.5–9.3).

Analysis. Protein contents were determined by the Coomassie Brilliant Blue method of Bradford (1976) using bovine serum albumin as a standard. The measurements of protein concentrations in CEC-fractions were based on direct UV spectroscopy at 280 nm. The statistical analysis was performed by the *Analyse-it Software* using the one-way analysis of variance (*Anova*). A Tukey multiple comparison procedure was used with a 5% significance level. Pearson's correlation coefficient analysis was also performed with the same software.

Results and discussion

Description of the rye growing conditions. The growing conditions in the summer of different harvest years in Dotnuva (Fig. 1 A) were completely different as well as the precipitation levels at Kaunas and Vilnius PVTCs (Fig. 1 B). The summer of harvest year 2003 was warm and dry. Higher falling number (FN) values for the rye varieties were observed with an average of 266 s (Table 1). Conversely, the summer of 2004 was cool and wet. Heavy rainfall before the harvest increased the risk of sprouting and the microbial contamination of rye kernels (Mares, 1993). This was evidenced by relatively low FN values with an average of 182 s (in Dotnuva), 239 s (at Vilnius PVTC) and 236 s (at Kaunas PVTC) (Table 1).

Because the precipitation levels during the last two months (July and August) prior to harvesting were different at Kaunas and Vilnius PVTCs (Fig. 1 B), the comparison between rye samples from these two groups created the possibility to determine the impact of the growing conditions on the apparent levels of the endoxylanase activity.

Variation in the apparent endoxylanase activity. The apparent endoxylanase activity was studied in the albumin type of extracts from the meal of rye kernels. Table 2 presents the apparent endoxylanase activities in different rye samples of two harvest years and different locations.

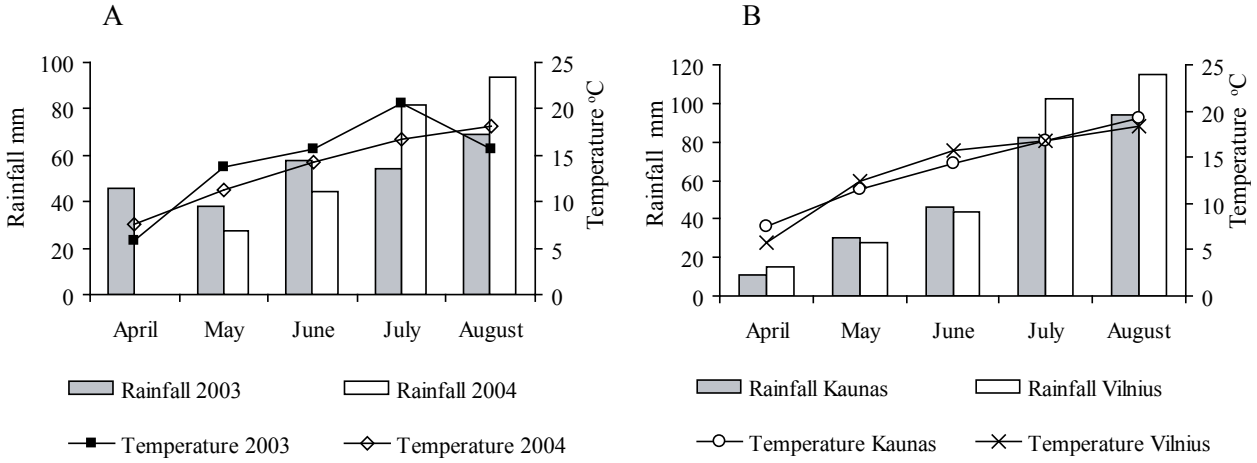

Figure 1. An average temperature (curve) and monthly rainfall (bars): A – in Dotnuva (2003–2004), B – at Kaunas and Vilnius PVTCs (2004)

Table 1. The quality parameters of the rye varieties grown at different PVTCs during 2003–2004

Variety	Location, harvest year	1000 grain weight *g*	FN *Kritimo* s	Protein %	Albumin mg g^{-1}
'Joniai'	Kaunas, 2004	37.2	202	9.3	29.4 ± 2.4
'Matador'		32.6	208	8.6	26.2 ± 2.1
'Recrut'		33.3	219	8.5	27.2 ± 1.9
'Fernando'		33.9	297	7.6	26.1 ± 2.2
'Picasso'		33.9	253	7.5	24.2 ± 2.3
'Joniai'	Vilnius, 2004	37.1	203	9.6	31.0 ± 2.3
'Matador'		37.4	238	10.1	27.3 ± 1.9
'Recrut'		32.4	261	9.1	28.3 ± 1.9
'Fernando'		33.7	232	9.2	26.6 ± 2.1
'Picasso'		33.5	262	8.6	25.1 ± 2.4
'Joniai'	Dotnuva, 2004	–	184	12.8	31.8 ± 2.1
LIA 426		–	164	12.2	28.4 ± 2.4
LIA 391		–	160	11.7	29.4 ± 2.0
LIA 463		–	218	10.8	30.2 ± 1.8
'Joniai'	Dotnuva, 2003	38.3	244	9.9	32.7 ± 1.9
LIA 426		37.9	263	10.2	30.1 ± 2.3
LIA 391		36.7	292	10.1	30.6 ± 2.0
LIA 463		37.2	263	11.2	31.4 ± 1.9

The statistically significant differences in apparent endoxylanase activities between varieties from different locations were found: in rye varieties from Kaunas PVTC the apparent endoxylanase activity varied between 0.3 and 1.0 U g^{-1}, and in the same varieties from Vilnius PVTC – between 0.5 and 1.42 U g^{-1} (Table 2). Similarly, the significant variation in the endoxylanase activity was found in the rye samples of different harvest years in Dotnuva. In 2003, the endoxylanase activity values varied between 0.74 and 1.04 U g^{-1}, in 2004 between 0.42 and 0.68 U g^{-1}. The strong relations between the apparent endoxylanase activities within mentioned groups was found ($R^2 = 0.57$ and $R^2 = 0.76$, respectively). These differences could be explained by the composition of the soil and different precipitation levels or storage conditions after harvest.

The results show that the levels of plant endoxylanases are genetically determined, but the susceptibility of rye varieties to microbial infection also possibly plays a role. Because of the fact that endoxylanases from micro-organisms are populating the outer layers of the cereal grain and can account for over 80% of the total endoxylanase activity (Dornez et al., 2006), the rye varieties 'Picasso' and LIA 391, showing the lowest endoxylanase activity could be characterized as more resistant to microbial contamination than the others. The endoxylanase activity could be used as an additional criterion for the evaluation of rye varieties.

Table 2. Apparent endoxylanase (XA) activity (U g^{-1}) and endoxylanase inhibition activity (IU 100 mg^{-1}) against *T. lanuginosus* and *T. reesei* endoxylanases, expressed as 100 mg of protein (XIA_P) or dry weight (XIA_{DW}) in rye

Variety	Location, harvest year	XA activity	*T. reesei*		*T. lanuginosus*	
			XIA_P	XIA_{DW}	XIA_P	XIA_{DW}
‘Joniai’	Kaunas, 2004	1.00 ± 0.04	106 ± 2	11.4 ± 0.2	176 ± 2	19.1 ± 0.3
‘Matador’		0.87 ± 0.04	126 ± 3	12.6 ± 0.2	201 ± 2	20.1 ± 0.2
‘Recrut’		0.62 ± 0.03	142 ± 2	15.4 ± 0.1	192 ± 4	20.8 ± 0.5
‘Fernando’		0.53 ± 0.03	178 ± 5	19.2 ± 0.1	264 ± 3	28.6 ± 0.4
‘Picasso’		0.31 ± 0.04	250 ± 6	21.8 ± 0.2	322 ± 6	28.1 ± 0.5
‘Joniai’	Vilnius, 2004	1.14 ± 0.05	150 ± 5	16.7 ± 0.5	223 ± 4	24.8 ± 0.4
‘Matador’		1.42 ± 0.06	124 ± 4	14.5 ± 0.2	199 ± 2	23.4 ± 0.4
‘Recrut’		0.75 ± 0.03	136 ± 2	14.7 ± 0.2	184 ± 5	19.0 ± 0.4
‘Fernando’		0.64 ± 0.03	169 ± 2	18.3 ± 0.5	270 ± 4	29.2 ± 0.5
‘Picasso’		0.50 ± 0.04	248 ± 7	24.8 ± 0.3	334 ± 8	33.4 ± 0.2
‘Joniai’	Dotnuva, 2004	0.98 ± 0.04	131 ± 3	19.4 ± 0.5	217 ± 4	32.3 ± 0.1
LIA 426		0.86 ± 0.03	153 ± 4	21.8 ± 0.4	228 ± 3	32.1 ± 0.1
LIA 391		0.74 ± 0.03	158 ± 2	21.3 ± 0.4	226 ± 5	30.8 ± 0.5
LIA 463		1.04 ± 0.03	167 ± 5	19.6 ± 0.5	227 ± 4	25.9 ± 0.3
‘Joniai’	Dotnuva, 2003	0.51 ± 0.02	156 ± 2	17.9 ± 0.3	234 ± 4	26.9 ± 0.3
LIA 426		0.46 ± 0.02	155 ± 3	18.4 ± 0.3	225 ± 3	26.7 ± 0.4
LIA 391		0.42 ± 0.02	152 ± 4	17.8 ± 0.2	219 ± 2	25.6 ± 0.5
LIA 463		0.68 ± 0.03	161 ± 6	18.9 ± 0.2	229 ± 5	26.9 ± 0.5

The results obtained by analysis of rye varieties verify that the weather conditions prior to harvesting have significant influence on the formation of the endoxylanase levels in the rye grain. For the 2004 year crop, the endoxylanase activities in the rye varieties from Dotnuva were higher on average by 43% in comparison with the activities of analogous cultivars of the 2003 year crop. The endoxylanase activity was also affected by precipitation level before harvest at different growing sites: the average apparent endoxylanase activity of rye samples from Vilnius PVTC was higher by 17% than that of samples from Kaunas PVTC.

No significant correlations were found between the FN and endoxylanase activity ($R^2 = 0.28$). This suggests that the causes of endoxylanase and amylase activities in rye are different. The fact that micro-organisms on the surface of the cereal grain can produce endoxylanases may explain why no significant correlation could be found (Gys et al., 2004). No relation was found between endoxylanase activity and total protein and albumin contents.

Endoxylanase inhibition activity in rye. For testing of the inhibition sensitivity of commercial endoxylanases commonly used in rye processing, the CEC protein fractions of different rye varieties were used as inhibiting agents. The experiment shows relatively different sensitivities of commercial xylanolytic enzyme preparations to inhibition. Following a selective extraction and separation, a single fraction enriched with endoxylanase inhibition activity was obtained.

The *T. lanuginosus* endoxylanase was found to be more sensitive to inhibition than the *T. reesei* endoxylanase (Table 2). The inhibition activities in the different rye samples against the *T. lanuginosus* and *T. reesei* endoxylanases varied between 19.0 and 33.4, and 11.4 and 24.8 IU 100 mg^{-1} of dry wholemeal, respectively. For these enzymes, the highest average inhibition activity was measured in the ‘Picasso’, and the lowest in the ‘Matador’ sample.

The inhibition activities in the different rye varieties (Dotnuva) in 2003 and 2004 as well as in the varieties from different sites (Kaunas and Vilnius) significantly correlated ($R^2 = 0.73$ and $R^2 = 0.81$, respectively). The levels of endoxylanase inhibition activity in rye seem to be independent of the harvest year and cultivation site. Further experiments are needed to verify the present findings. However, the obtained results allow the preliminary statement that the inhibition activities in rye are genetically determined and their levels are not significantly influenced by the weather conditions or microbial contamination. This agrees with the results reported by Dornez et al. (2006), which showed the influence of genetic and climatic factors on the activity of endoxylanases and their inhibitors in wheat kernels. These findings may indicate the presence of the inhibitors with different endoxylanase specificity in the rye grain or that, some of them predominate and have higher specific activity, causing the inhibition activity measured.

The results of rye endoxylanase activity among the eight different rye varieties show the great relevance of the evaluation of the influence of the genotype on the technological characteristics of rye. In literature, the significant influence of the genotype on the chemical composition only of rye dietary fibre was reported (Saastamoinen et al., 1989; Boskov et al., 2003). Genotype and environment effect on feed grain was shown by O'Brien (1999). Salmenkallio-Marttila and Hovinen (2005) also reported large variation in the content of dietary fibre as influenced by the harvest year and variety in the nine winter rye cultivars. They noticed also that endoxylanase activity has a moderate positive correlation with total pentosan and soluble pentosan contents.

Protein electrophoresis. Albumin fractions indicating inhibition activity were applied to an SDS-PAGE and an IEF procedure for detection of molecular masses and pI of proteins. The protein separation showed that these fractions were a mixture of low molecular weight proteins (Fig. 2). The different intensity of protein bands was found in SDS-PAGE profiles of albumin fractions of different samples of the variety 'Joniai' (Fig. 2 A). The CEC protein fractions indicating inhibition activity contain components with molecular weights of about 11, 18.4, 30.1, 29.8 and 39.9 kDa (Fig. 2 A) and pI's between 8.15 and 9.3 (Fig. 2 B).

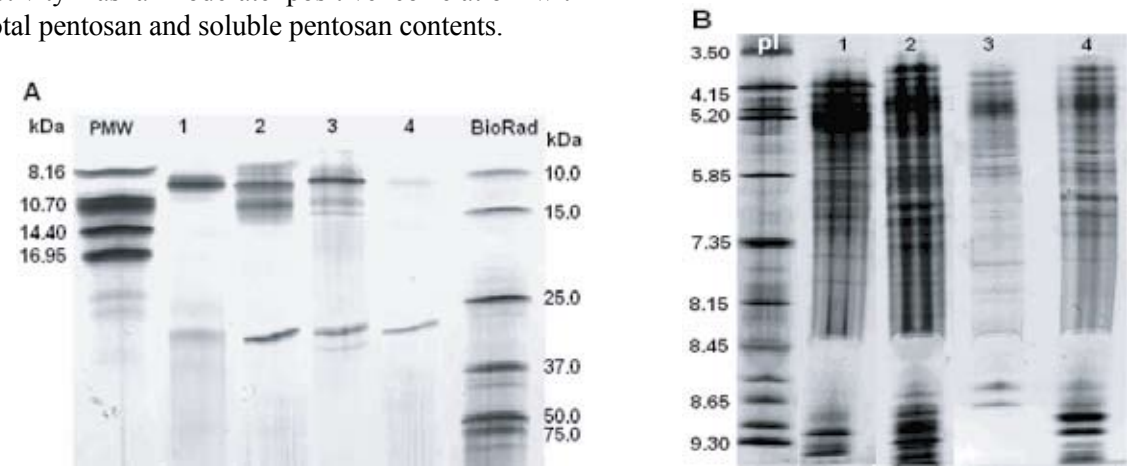

Figure 2. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (A) and iso-electrofocusing (B) of the albumin fractions indicating inhibition activity. BioRad and PMW – protein markers. 1 – 'Joniai' (Kaunas), 2 – 'Joniai' (Vilnius), 3 – 'Joniai' (Dotnuva, 2004), 4 – 'Joniai' (Dotnuva, 2003)

All known TAXI-type endoxylanase inhibitors are high-pI proteins and occur in two molecular forms (form A, with a molecular weight of approximately 40 kDa, and form B, made up of two subunits of approximately 30 and 10 kDa) and pI values of at least 8.9 (Gebruers et al., 2001; Goesaert et al., 2002). XIP-type inhibitors are glycosylated monomeric proteins with a molecular weight of 29 kDa and pI values 8.7–8.9 (Goesaert et al., 2001; Goesaert et al., 2003). A TL-XI has been identified in wheat as a basic (pI > 9.3) protein with a molecular weight of approximately 18 kDa which occurs in multiple isoforms (Fierens et al., 2007). These proteins have been detected and characterized by their ability to inhibit microbial xylanases. TAXI-type and TL-XI inhibits bacterial and fungal family 11 glycoside hydrolases (Fierens et al., 2007), whereas XIP-type has two independent enzyme-binding sites, allowing inhibition of two fungal endoxylanases, family 10 and family 11, but does not show activity against bacterial endoxylanases (Juge et al., 2004).

Conclusions

1. The apparent endoxylanase activity was at least partially dependent on the variety, but the weather conditions prior to harvesting had a large impact on the levels of endoxylanase in rye. As endoxylanases have a great impact on rye technological characteristics, the endoxylanase activity could be used for the selection of rye varieties suitable for the certain applications. Relatively small amounts of microbial enoxylanases in flour can exert a large impact on the functionality of rye flour. To improve rye processing, key attention must be paid to the development of rye genotypes with higher resistance to microbial contamination.

2. The endoxylanase inhibition activity in rye was mainly dependent on the genetic background and was less affected by the weather conditions. The testing of the commercial endoxylanases showed a relatively different sensitivity to inhibition. As the microbial endoxylanases are commonly used in cereal processing, the functionality of rye may be influenced to a different degree by the relative quantities of endoxylanase inhibitors present in rye kernels.

3. The biochemical properties of the tested proteins suggested that some isoforms of TAXI and TL-XI-type inhibitors occur in analyzed rye varieties. Investigation of the biochemical and molecular properties of purified albumins with inhibitory activity is essential for further specification of endoxylanase inhibitors occurring in local rye varieties.

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Endoksilanazių ir endoksilanazių inhibitorių aktyvumas žieminių rugių įvairių veislių grūduose

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Santrauka

Tirta veislės ir auginimo sąlygų įtaka žieminių rugių veislių 'Joniai', 'Matador', 'Recrut', 'Fernando' bei 'Picasso' ir naujai išvestų hibridų LIA 426, LIA 391 bei LIA 463 endoksilanazių aktyvumui. Taip pat analizuotas iš dalies išgrynintų tirpių rugių baltymų inhibicinis aktyvumas glikozidhidrolazių 11 šeimos *Trichoderma reesei* ir *Thermomyces lanuginosus* endoksilanazėms. Rugių veislių endoksilanazių aktyvumas svyravo nuo 0,31 iki 1,42 U g^{-1} grūdų. Reikšmingos įtakos endoksilanazių aktyvumo vertėms turėjo veislė ir kritulių kiekis prieš derliaus nuėmimą. Skirtingų rugių mėginių inhibicinio aktyvumo vertės *T. lanuginosus* ir *T. reesei* fermentams svyravo atitinkamai nuo 19,0 iki 33,4 ir nuo 11,4 iki 24,8 IU 100 mg^{-1} sausųjų medžiagų. Inhibicinis aktyvumas endoksilanazėms priklausė nuo veislės, o auginimo sąlygos turėjo mažesnę įtaką. Naujai išvesti rugių hibridai pasižymėjo panašiu inhibiciniu aktyvumu endoksilanazėms, nepriklausomai nuo auginimo sąlygų ir veislės. *T. lanuginosus* ksilanazė buvo jautresnė rugiuose esančių inhibitorių poveikiui. Rugių baltymų frakcijos, pasižyminčios inhibiciniu aktyvumu, yra sudarytos iš 10–11, 14 ir 29–31 kDa molekulinės masės komponentų. Remiantis gautais rezultatais, galima paaiškinti fermentinių preparatų veikimo skirtumus ir parinkti bei pritaikyti tam tikrus fermentus įvairiuose rugių perdirbimo procesuose.

Reikšminiai žodžiai: rugiai, tirpūs baltymai, endoksilanazė, inhibicinis aktyvumas, auginimo sąlygos.