THE QUALITY OF FEED GRAIN: ENDOXYLANASE AND ENDOXYLANASE INHIBITION ACTIVITY LEVELS IN TRITICALE

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Summary. This is the first report in Lithuania of endoxylanase and endoxylanase inhibition activity levels in triticale. Six winter triticale varieties grown in 2006 and 2007 were tested for their variation in apparent endoxylanase and endoxylanase inhibition activities against glycoside hydrolase family 11 endoxylanases of *Trichoderma reesei* and *Thermomyces lanuginosus,* and a family 10 endoxylanase of *Aspergillus aculeatus*. The levels of apparent endoxylanase activity in the triticale varieties were largely affected by grain growing conditions. Contrary, the endoxylanase inhibition activity levels were mainly influenced by genetics. The *A. aculeatus* enzyme was not inhibited by the triticale proteins, whereas the inhibition activities against the *T. lanuginosus* varied between 43.6-56.8 and 30.1-38.2 IU/100 mg, and against *T. reesei* between 19.8-35.4 and 13.8-24.0 IU/100 mg, respectively for triticale samples of 2006 and 2007. The isolated triticale protein fractions indicating inhibition activity contain components with molecular weights of about 11, 18.4, 30.1, 29.8 and 39.9 kDa. The different functionalities of commercial endoxylanases can be explained by the obtained results and allow screening for endoxylanases suitable for processes, in which triticale is involved.
Keywords: triticale, albumins, endoxylanase, endoxylanase inhibitors.

PAŠARINIŲ GRŪDŲ KOKYBĖ: ENDOKSILANAZIŲ IR ENDOKSILANAZIŲ INHIBITORIŲ AKTYVUMAS KVIETRUGIUOSE

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Santrauka. Atlikti pirmieji tyrimai įvertinant endoksilanazių ir endoksilanazių inhibitorių aktyvumą kvietrugiuose. Analizuota šešių žieminių kvietrugių veislių, išaugintų 2006 ir 2007 m., endoksilanazių aktyvumas ir juose galimai esančių inhibitorių poveikis glikozidhidrolazių 11 šeimos *Trichoderma reesei* ir *Thermomyces lanuginosus* bei glikozidhidrolazių 10 šeimos *Aspergillus aculeatus* endoksilanazių aktyvumui. Endoksilanazių aktyvumui kvietrugiuose didžiausią įtaką turėjo grūdų auginimo sąlygos, o endoksilanazių inhibitorių kiekiui įtaką darė veislės ypatumai. *A. aculeatus* endoksilanazė nebuvo slopinama kvietrugiuose esančių inhibitorių, tuo tarpu 2006 ir 2007 metų kvietrugių mėginiuose nustatytas inhibicinis aktyvumas prieš *T. lanuginosus* endoksilanazę atitinkamai 43,6–56,8 ir 30,1–38,2 IU/100 mg, prieš *T. reesei* – atitinkamai 19,8–35,4 ir 13,8–24,0 IU/100 mg. Inhibiciniu aktyvumu pasižymėjusios kvietrugių albuminų frakcijos, sudarytos iš 11; 18,4; 30,1; 29,8 ir 39,9 kDa molekulinės masės baltymų. Gauti rezultatai gali būti panaudoti išaiškinant skirtingo pramoninių ksilanazių funkcionalumo priežastis, taip pat parenkant pramoninius fermentus, reikalingus įvairiuose kvietrugių perdirbimo procesuose.
Raktažodžiai: kvietrugiai, albuminai, endoksilanazė, endoksilanazių inhibitoriai.

Introduction. Triticale (x *Triticosecale* Wittmack) is a new cereal that offers considerable promile because of its potential for greater yield compared to established crops in certain areas and because it may have greater nutritive value. A main advantage of triticale use for food and feed is its unique nutritional quality. Triticale is characterized by a high content of cell-wall polysaccharides (Varughes *et al*., 1996; Seghal *et al*., 2004), higher protein content than in its parental species ranging from 10-16% (Igne *et al*., 2007). The levels of lysine and threonine are 10-25% higher than that for wheat grains, so the feed value of triticale protein is higher (Haydon *et al*., 2010). High proportions of albumins and globulins, and simultaneously a lower proportion of prolamin protein (gliadins) than wheat and rye enhance digestibility of triticale-based products (Coffey and Gerrits, 1988; Siriamornpun *et al*., 2004; Salmanowicz and Nowak, 2009). Hegher *et al*. (1990, 1991) indicated triticale protein digestibility comparable to wheat and higher than for rye ranging between 86.4 and 90.6%. This fact is very important since many proteins in the water-soluble fraction exhibit biological activity such as enzymes and exogenous enzyme inhibitors (McLauchlan *et al*., 1999; Payan, 2004; Svensson *et al*., 2004).

The use of triticale in poultry feeds is limited by the presence of soluble non-starch polysaccharides (NSP), specially xylans and arabinoxylans (Antoniou and Marquardt, 1981). Consequently, on purpose to intensify the hydrolysis of NSP the xylanolytic enzyme preparations are of great relevance. The last decade, the interest in carbohydrate-active enzymes has increased due to the potential application for these enzymes in the food and feed industries to improve the process ability and the quality of the end product by changing the structure and physicochemical properties of arabinoxylans (Beg *et al.*, 2001; Polizeli *et al*., 2005). Pettersson and Aman (1988)

reported significant improvement in growth and feed conversion for broilers when diets containing triticale groups were supplemented with an enzyme source containing a high level of β-glucanase and pentosanase activity. Hydrolysis of xylan due to supplemental xylanase and also β-glucan destruction by added β-glucanase have been reported by several researchers (Classen, 1996; Pourreza *et al*., 2004).

The efficiency of added commercial xylanases depends on cereal variety, growing conditions and harvest year (Dornez *et al*., 2006a; Dornez *et al*., 2008a). It may be due to the level of kernel-associated endoxylanases (Schryver *et al*., 2006; Dornez *et al*., 2008b) and endoxylanase inhibitors (Gebruers *et al*., 2005). The latter components may reduce the level of xylanase actvity or alter certain properties of these enzymes.

The optimization of enzymatic hydrolysis of triticale cause the problem due to the lack of studies related to the endoxylanase and endoxylanase inhibition activity in triticale and to the influence of the growth and varietal factors on the levels of these components. The problem requires also selection of the microbial endoxylanases possibly resistant to inhibition.

Two structurally different cereal endoxylanase inhibitors TAXI (*Triticum aestivum* xylanase inhibitor) and XIP (xylanase inhibiting protein) in wheat, rye, barley and rice have been reported (McLauchlan *et al*., 1999; Goesaert *et al*., 2001; Goesaert *et al*., 2002; Goesaert *et al*., 2005), which affect the functionality both of kernel-associated endoxylanases and the industrial enzymes in cereal processing. XIP-type inhibitors were not detected in sorghum and maize (Elliot *et al*., 2003). A third type of xylanase inhibitor, TL-XI (thaumatin-like xylanase inhibitor) has been identified in wheat as a basic (pI>9.3) protein with a molecular mass of approximately 18 kDa which occur 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 (Gebruers *et al*., 2004; 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 (Flatman *et al*., 2002; Juge *et al*., 2004).

The aim of this study was to measure the apparent endoxylanase activity in the different winter triticale cultivars and to investigate the effect of the endoxylanase inhibitors possibly present in the triticale grain on the activity of different microbial endoxylanases usually applied to the cereal-based processes.

Materials and Methods

Triticale samples, enzymes and chemicals. Six winter triticale varieties (Talentro, Triticon, Trimester, Mungis, Cultivo and Falmoro) were supplied by the Plant Research Center (PC) located in the central part of the Lithuania after 2006 and 2007 years harvest. Microbial *Trichoderma reesei* and *Thermomyces lanuginosus* glycoside hydrolase family 11 *endo*-1,4-*β*-D-xylanases (EC 3.2.1.8) were from Biosinteze (Vilnius, Lithuania) and Novozymes (Bagsvaerd, Denmark), respectively. *Aspergillus aculeatus* family 10 endoxylanase (EC 3.2.1.8.) was supplied by Puratos (Groot-Bijgaarden, Belgium). All chemicals and reagents were purchased from Sigma-Aldrich (Taufkirchen, Germany) and were of analytical grade. Birchwood xylan was from Carl Roth (Karlsruhe, Germany). The electrophoresis media and markers were from Pharmacia Biotech (Uppsala, Sweden).

Determination of apparent endoxylanase activity. The apparent endoxylanase activity was measured by an assay based on 3,5-dinitrosalicylic acid procedure according to Miller (1959) and description by Rassmussen *et al*. (2001) with some modifications. Triticale crude extract was prepared by suspending 5 g of wholemeal in 50 ml of sodium acetate buffer (10 mM, pH 4.5) and under stirring 1 h and centrifuged (10000 *g*, 20 min, 10°C). The substrate solution (0.5% w/v) was prepared by adding 0.5 g of powdered substrate to boiling and vigorously stirring water on a hot-plate stirrer until the polysaccharide is completely dissolved. Stop solution (DNS reagent) was made by mixing 3,5-dinitrosalicylic acid (1 g) and sodium potassium tartrate (30 g) dissolved in 100 mL of 0.4 M sodium hydroxide.

The reaction mixture containing triticale crude extract (200 µl) and substrate (50 µl) in sodium acetate buffer (10 mM, pH 4.5, 750 µl) was incubated for 1 h at 40°C. The reaction was stopped by the addition of 1 ml DNS reagent. Tubes were boiled for 5 min and diluted with 10 ml of distilled water. The absorbance of the solution was measured at 540 nm against two controls (the extract and substrate alone in buffer) using an Ultrospec 4000 UV/Visible spectrophotometer (Pharmacia Biotech, England). To assess the endoxylanase activity in terms of reducing sugars formation, a xylose standard curve was prepared with D-xylose solutions (0-450 mg/ml) instead of substrate. All analyses were at least in triplicate. Activities were expressed in enzyme units (U/g grain). One unit is the amount of enzyme needed to releases 1 µmol of xylose equivalents per minute from the birchwood xylan under the assay conditions used. Endoxylanase measurements in triticale wholemeal extracts may yield apparent activities because of the possibly presented endoxylanase inhibitors (Gys *et al*., 2004).

Protein separation by cation exchange chromatography (CEC). The triticale extract for protein separation was prepared by suspending 10 g of wholemeal in 100 ml of sodium acetate buffer (10 mM, pH 4.5). The suspension was shaken for 1 h at room temperature, centrifuged (10000 *g*, 20 min, 10°C) and subjected for protein content determination. The supernatant was applied to SP-Sepharose Fast Flow Column (XK16/20; 6% agarose, cation-exchange group-sulphopropyl) for protein separation. The column was equilibrated with a buffer A (10 mM sodium acetate; pH 4.5). Gradient elution was performed with the buffers A and B (10 mM sodium phosphate; pH 8.3; flow rate 2 ml/min; UV-detection 280 nm). The elution was continued with the buffers B and C (10 mM sodium phosphate and 500 mM NaCl; pH 8.3). After the purification step the eluted CEC-fractions (size 8 ml) containing protein were collected, dialyzed against distilled water (10°C; 48 h) and lyophilized.

Endoxylanase inhibition assay procedure. The enzyme solutions (25 U/ml) were prepared in sodium acetate buffer (0.1 M, pH 4.5) containing bovine serum albumin (0.5 mg/ml). The reaction mixture (0.3 ml) containing the 250 µl of CEC-fraction (1.4 mg/ml 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 mixture was incubated 1 h at 30°C. The reaction was stopped by adding the DNS reagent (0.3 ml). After boiling, cooling and dilution procedure, the A_{540} values were measured against a control, prepared by incubating the enzyme with buffer instead of protein solution. The endoxylanase inhibition activity (XIA) 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 100 mg of protein (XIA_P) or dry weight (XIA_{DW}). All analyses were performed at least in triplicate.

Protein electrophoresis. Protein fractions indicating the inhibition activity were analyzed by 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; 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). All gels were silver stained according to the manufacturer's instructions.

Analysis. Total nitrogen was determined by the Kjeldahl method according to AOAC 960.52 (1999) approved method. Crude protein content was estimated using a conversion factor of 5.7. The measurements of protein concentrations in CEC-fractions were based on direct UV spectroscopy at 280 nm. Hagberg falling numbers (HFNs) were determined according ISO 3093:2004 Method. Total starch content was determined using AOAC Method 996.11. (1998).

All chemical determinations were conducted in duplicate, endoxylanase and endoxylanase inhibition activity assays were carried out in triplicate. The variability of the endoxylanase activity was analysed 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 analyses were also performed with the same software.

Results and discussion

Apparent endoxylanase activities in different triticale cultivars. Total protein, albumin, starch contents and HFN values of triticale samples are given in Table 1.

Table 1. **HFNs, albumin and protein contents of the triticale varieties from harvest years 2006-2007**

Triticale	2006				2007			
	HFN (s)	Starch (%)	Protein (%)	Albumin (%)	HFN (s)	Starch (%)	Protein (%)	Albumin (%)
Cultivo	63	63.9	15.0	2.48	188	72.3	11.1	1.89
Falmoro	61	64.6	15.3	2.92	222	68.5	11.2	2.12
Mungis	68	65.1	15.1	2.65	183	72.7	9.9	1.78
Talentro	61	66.9	17.7	3.54	149	73.3	10.3	2.16
Trimester	62	66.3	14.0	2.27	83	71.2	10.2	1.63
Triticon	61	65.8	14.6	2.81	102	72.1	11.6	2.09

Table 2. **Average temperature and rainfall data for triticale growing seasons of 2005-2006 and 2006-2007**

Month	Temperature (°C)			Rainfall (mm)		
	2005–2006	2006–2007	1961–1990	2005–2006	2006–2007	1961–1990
Sep	14.2	14.7	11.9	28	67	68
Oct	8.0	9.8	7.2	31	64	60
Nov	3.0	4.6	2.0	40	67	60
Dec	-1.6	4.4	-2.4	53	50	55
Jan	-7.0	1.2	-5.1	17	111	42
Feb	-6.2	-6.7	-4.6	27	36	33
Mar	-3.1	5.0	-0.7	26	32	36
Apr	6.1	6.4	5.4	32	22	43
May	12.0	13.2	11.9	53	72	51
Jun	16.2	17.4	15.5	32	79	66
Jul	20.6	16.9	16.7	31	194	79
Aug	17.9	18.5	16.2	141	62	77

The climatic conditions in the summer of 2006 and 2007 were completely different (Table 2). The summer of 2006 was very hot and dry, the observed HFN values for the triticale varieties ranged from 61 to 68 s with an average of 64 s. In 2007, humid weather prevailed, and strong precipitations occured in July Higher HFNs for the different triticale varieties were observed, ranging from 83 to 222 s with an average of 155 s (Table 1).

Large differences ($p<0.05$) were found in apparent endoxylanase activities between the harvest years: in 2007 the endoxylanase activity values were much more higher than in 2006. This was evidenced by the average values, which were 0.35 and 0.91 U/g respectively (Table 3). This could be related to the different temperature and precipitations and their influence on triticale development and microbial contamination. Statistically significant differences ($p<0.05$) in apparent endoxylanase activity between triticale varieties were found. The triticale-associated endoxylanase activity values in 2006 varied from 0.08 to 0.58 U/g, and in 2007 – from 0.38 to 1.57 U/g. Among the six varieties analyzed, the lowest apparent endoxylanase activities were found in the Cultivo and Mungis, the highest – in the Falmoro and Trimester samples (Table 3).

Results show that apparent endoxylanase activity in triticale is partially genetically determined. Considering that the microorganisms on the cereal kernels can produce endoxylanases and the most part of the endoxylanase activity is located in the outer layer of the kernels, the susceptibility of triticale varieties to microbial infection could play a role (Corder and Henry 1989; Gys *et al*., 2004; Dornez *et al*., 2006b). Hereby, triticales Mungis and Cultivo having the lowest apparent endoxylanase activity can be characterized as more resistant to microbial contamination than the other varieties.

The endoxylanase activity values in triticale seem to be relatively higher than those measured in rye and lower than in wheat. Autio *et al.* (1998) determined the endoxylanase activity of 0.06 and 0.3 U/g in ungerminated and germinated rye kernels, respectively. The measured apparent endoxylanase activity values in the eight winter rye varieties from two harvest years ranged between 0.31 and 1.42 U/g grain (Vidmantiene and Juodeikiene, 2010). Rasmussen *et al*. (2001) found the activity of endoxylanase in extracts from ungerminated rye grain at low level (0.00066 U/g). The results of Dornez *et al*. (2006a) showed the apparent endoxylanase activity variation from 0.14 to 0.64 U/g and from 0.34 to 2.24 U/g grain in the ten wheat varieties grown on different climatic conditions. The higher endoxylanase activity of triticale compared to rye may be likely due to their inherited genetic information from wheat.

Endoxylanase inhibition activities in triticale cultivars. The protein fractions obtained after separation by CEC were assayed for their ability to inhibit the different endoxylanases. Values of inhibition activity of different triticale are given in Table 3. Under the experiment conditions used, the endoxylanase of *A. aculeatus* was not inhibited by any of the triticale samples. The activity of *T. lanuginosus* and *T. reesei* endoxylanase were both inhibited. The *T. lanuginosus* endoxylanse was found at least 2 times more sensitive to triticale inhibitors than *T. reesei* endoxylanase.

Table 3: **Endoxylanase** (U/g) **and endoxylanase inhibition activities** (IU/100 mg) **against *T. lanuginosus* and *T. reesei* endoxylanases, expressed as 100 mg of protein** (XIA_P) **or dry weight** (XIA_{DW}) **of triticale samples from 2006 and 2007 harvest years**

Variety	2006					2007				
	T. reesei endoxylanase		*T. lanuginosus* endoxylanase		Triticale endoxylanase	*T. reesei* endoxylanase		*T. lanuginosus* endoxylanase		Triticale endoxylanase
	XIA_P	XIA_{DW}	XIA_P	XIA_{DW}		XIA_P	XIA_{DW}	XIA_P	XIA_{DW}	
Cultivo	113±2[a]	19.8±0.2	253±3[a]	44.0±0.3	0.18±0.01[b]	107±2[a]	13.8±0.2	242±2[a]	31.3±0.2	0.38±0.01[a]
Falmoro	nd*	nd	nd	nd	0.42±0.01[d]	nd	nd	nd	nd	1.57±0.01[f]
Mungis	136±4[b]	23.9±0.1	249±2[a]	43.6±0.2	0.08±0.01[a]	144±4[b]	16.6±0.1	262±4[b]	30.1±0.1	0.44±0.02[b]
Talento	172±3[d]	35.4±0.1	276±4[b]	56.8±0.2	0.58±0.01[f]	167±3[c]	19.9±0.1	303±4[d]	36.3±0.2	0.86±0.01[c]
Trimester	nd	nd	nd	nd	0.49±0.02[e]	nd	nd	nd	nd	1.22±0.01[e]
Triticon	165±4[cd]	27.9±0.2	269±2[b]	45.7±0.1	0.35±0.01[c]	178±4[d]	24.0±0.1	283±2[c]	38.2±0.1	0.98±0.01[d]
Average	146	27	262	48	0.35	149	19	272	34	0.91

Data are the mean±SD of three analyses

[a-f]Means within a column with different superscript letters are significantly different ($p < 0.05$)

*not detected

The inhibition activities in the different triticale samples against the *T. lanuginosus* varied between 43.6–56.8 and 30.1–38.2 IU/100 mg, and against *T. reesei* between 19.8–35.4 and 13.8–24.0 IU/100 mg of dry wholemeal, respectively for 2006 and 2007. For these enzymes, the highest inhibition activities were measured in the Talentro samples, while the lowest inhibition activities were obtained for the Cultivo and Mungis samples. No inhibition activity was found in Trimester and Falmoro samples, in which the latter albumin fraction was not detected. The $XIA_{DW}^{T.reesei}$ and $XIA_{DW}^{T.lanuginosus}$ values of the different triticale samples were linearly related ($R^2 = 0.829$). The *T. lanuginosus* and *T. reesei* endoxylanase were inhibited to a different extent, the former more than the latter. This

finding may indicate that the levels of endoxylanase inhibtors are also linearly related, e.g. one of these inhibitors dominate or has higher specific activity, causing almost all inhibition activity measured. Xylanase inhibitors are believed to occur in multi-isoform families, and different isoforms have different specificities towards xylans, as reported in wheat for TAXI-I and TAXI-II (Goesaert *et al*., 2002) or XIP-I and XIP-II (Elliot *et al*., 2003).

The significant relations (R^2 = 0.913 and R^2 = 0.816, respectively for *T. reesei* and *T. lanuginosus* enzymes) were found between the XIA_{TP} in 2006 and 2007. This would seem that the inhibition activities in triticale are independent of harvest year. Introductory experiments show that the endoxylanase inhibition activity levels in triticale are at least partially influenced by genotype as well as was reported in wheat and rye (Goesaert *et al.*, 2003a; Dornez *et al.*, 2008a).

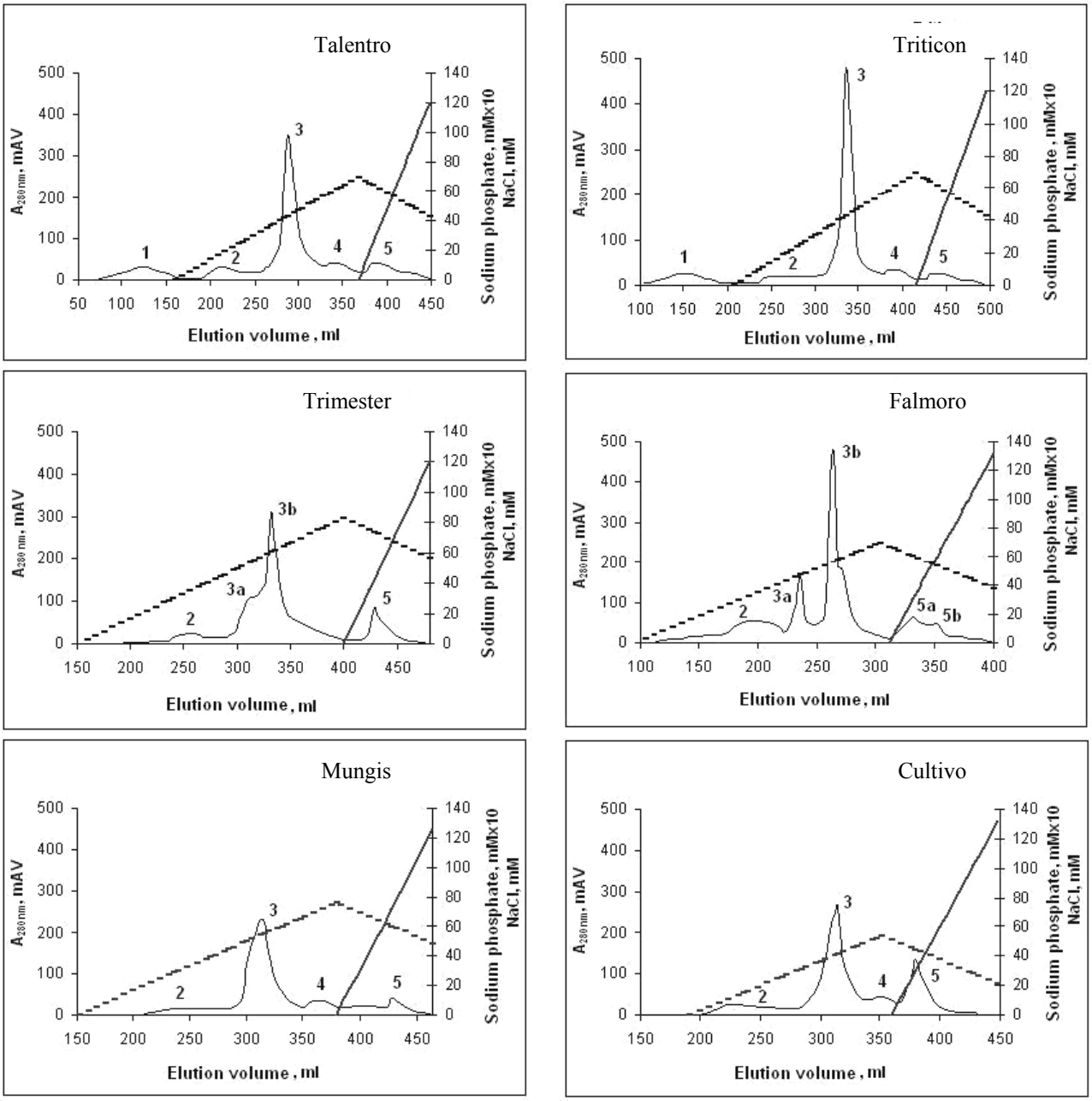

Figure 1. **The example of triticale protein chromatographic profiles of CEC protein fractions containing inhibition activity. Lines: sodium phosphate buffer** (—)**, 0.5 M NaCl solution** (---)

The different intensity of protein bands was found in SDS–PAGE profiles of the different triticale varieties (Fig. 2). 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. 2a) and pI's between 8.15 and 9.3 (Fig. 2b).

Whereas, the microbial endoxylanases sensitive to inhibition are widely used in biotechnological applications, therefore the efficiency of enzymes may be directly related to the level of inhibitors presented in cereal raw material (Gebruers *et al*., 2005). The different functionalities of commercial endoxylanases can be explained by the obtained results and allow screening for endoxylanases suitable for processes, in which triticale is involved, i.e. hydrolysis of non-starch polysaccharides.

Characterization of proteins with inhibition activity. Following a selective extraction and separation by CEC on Sepharose Fast Flow column, a single fraction (No. 5) enriched in the endoxylanase inhibition activity was obtained (Fig. 1). SDS-PAGE and IEF procedures were used for detection of molecular masses and pI's of proteins. The protein separation showed that this fraction were a mixture of low molecular weight proteins.

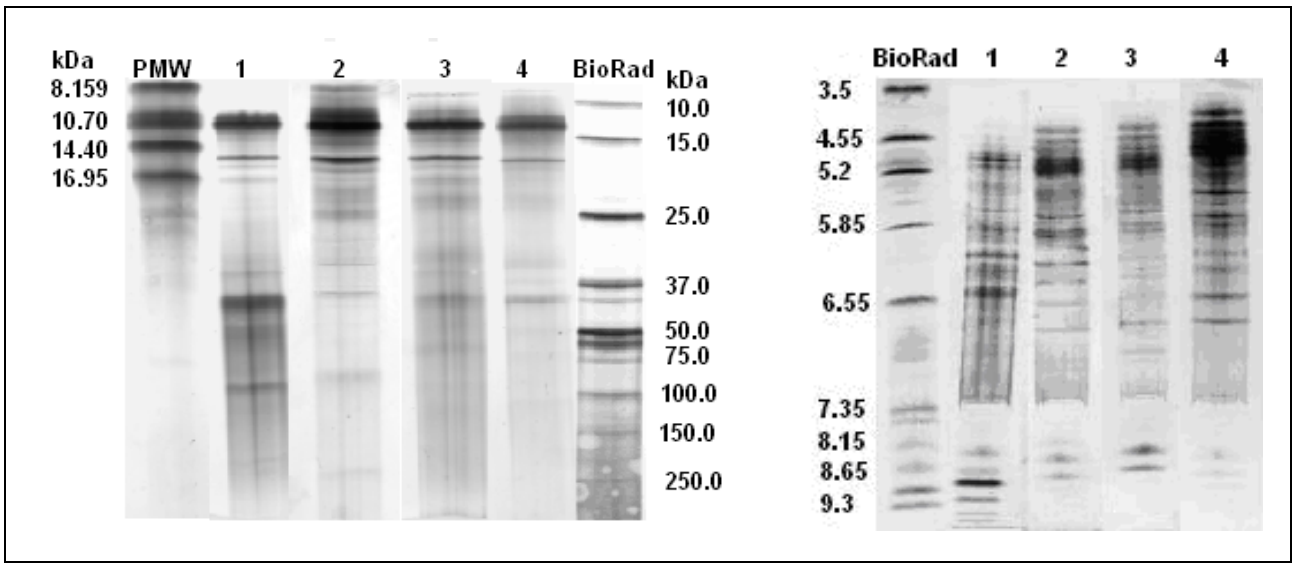

Figure 2. **Detection of proteins in the fractions containing inhibition activity by: a) SDS-PAGE and b) IEF. Gel** (a): **PMW – low molecular weight marker; BioRad – molecular mass protein standard; gel** (b): **BioRad – pI standard; CEC-fractions no.5 of: 1 – Talentro, 2 – Triticon, 3 – Mungis, 4 – Cultivo**

Over the last decade, studies have revealed that wheat and rye are particularly rich in TAXI-type and XIP-type inhibitors with the latter inhibitors being more abundant. Wheat contains two isoforms of TAXI-type family inhibitors, TAXI-I and TAXI-II, showing different activities towards endoxylanases (Gebruers *et al*., 2001). The estimated inhibitor levels of rye are found to be similar to those of wheat. A TAXI-II iso-inhibitor is a predominant isoform in wheat, meanwhile at least four TAXI-I-type isoforms with the similar structures and specificities exist in rye (Goesaert *et al*., 2002; Goesaert *et al*., 2003b). The presence of multiple isoforms of XIP-type inhibitors naturally occurred in wheat and rye grains were detected (Elliot *et al*., 2003).

All known TAXI-type xylanase inhibitors are high-pI proteins and occur in two molecular forms (form A, with a molecular mass 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*., 2003b). XIP-type inhibitors are proteins with a molecular mass of 29 kDa and pI values of 8.7–8.9 (Goesaert *et al*., 2001; Goesaert *et al*., 2003a). Considering to the results, we could expect that the mentioned types of the endoxylanase inhibitors occur in triticale, considering that endoxylanase inhibitors with the similar characteristics were found in parental species.

Whereas triticale is the product of an artificial cross between wheat and rye genomes, the determination of inhibition activity could be useful to differentiate triticale, and can also to a certain extent provide information about the level of genetic influence received from each of its parents.

Conclusions. Analytical results showed that apparent endoxylanase activities in wholemeal triticale samples were in part dependent on genetic background. Weather conditions also had a large impact on the apparent endoxylanase activities. The isolated triticale protein fractions indicating inhibition activity contain components with molecular weights of about 11, 18.4, 30.1, 29.8 and 39.9 kDa. The levels of endoxylanase inhibitors, which are only synthesized by the triticale plant, were less dependent on climatic conditions and were to a large extent genetically determined. Investigation of the biochemical and molecular properties of separated albumins with inhibitory activity are essential for the further specification of endoxylanase inhibitors occurring in local triticale varieties.

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