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COMPOSITION AND MICROSTRUCTURE ALTERATION OF TRITICALE GRAIN SURFACE AFTER PROCESSING BY ENZYMES OF CELLULASE COMPLEX

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ABSTRACT

It is found that the pericarp tissue of grain have considerable strength and stiffness, that has an adverse effect on quality of whole-grain bread. Thereby, there exists the need for preliminary chemical and biochemical processing of durable cell walls before industrial use. Increasingly used in the production of bread finds an artificial hybrid of the traditional grain crops of wheat and rye – triticale, grain which has high nutritional value. The purpose of this research was to evaluate the influence of cellulose complex (*Penicillium canescens*) enzymes on composition and microstructure alteration of triticale grain surface, for grain used in baking. Triticale grain was processed by cellulolytic enzyme preparations with different composition (producer is *Penicillium canescens*). During experiment it is found that triticale grain processing by enzymes of cellulase complex leads to an increase in the content of water-soluble pentosans by 36.3 – 39.2%. The total amount of low molecular sugars increased by 3.8 – 10.5 %. Studies show that under the influence of enzymes the microstructure of the triticale grain surface is changing. Microphotographs characterizing grain surface structure alteration in dynamic (every 2 hours) during 10 hours of substrate hydrolysis are shown. It is found that the depth and direction of destruction process for non-starch polysaccharides of grain integument are determined by the composition of the enzyme complex preparation and duration of exposure. It is found, that xylanase involved in the modification of hemicelluloses fiber having both longitudinal and radial orientation. Hydrolysis of non-starch polysaccharides from grain shells led to increase of antioxidant activity. Ferulic acid was identified in alcoholic extract of triticale grain after enzymatic hydrolysis under the influence of complex preparation containing cellulase, xylanase and β-glucanase. Grain processing by independent enzymes containing in complex preparation (xylanase and β-glucanase) shows that more significant role in polysaccharide complex composition and grain surface microstructure alteration belongs to xylanase. Grain processing by independent of cellulolytic enzymes may decrease the strength of pericarp tissue of grain and improved sensory characteristics of the bread.

Keywords: triticale; grain; xylanase; microstructure; antioxidant activity

INTRODUCTION

Dietary fiber in cereals is presented by non-starch polysaccharides found in the cell walls and consisting mainly of arabinoxylan and β-glucan **(Jacobs et al., 1998; Gebruers et al., 2008)**. Wheat and rye, as a universal raw material used in baking, became the main objects in studies of the grain pentosans properties. Currently, however, an artificial hybrid of these grains named triticale get wider range of application **(Cauvain et al., 2007)**. Total dietary fiber content of triticale grain is 13 – 16 % depending on the sort. Triticale comprises 6.8% of arabinoxylan, 0.7% of β-glucan and 2.1% of cellulose on average **(Izydorczyk et al., 1995; Barron et al., 2007)**.

Studies show that the molecular structure and the structural organization of arabinoxylans and β-glucans of grain (pentosans) are important determinants of their physical properties, such as solubility in water, viscosity, digestibility. This determines the functionality of said polysaccharides and their physiological functions in the gastrointestinal tract of humans **(Vaikousi et al., 2004; Lazaridou et al., 2007)**. Inclusion of cereals products containing dietary fiber in diet helps reduce cholesterol concentration, that decrease the risk of coronary heart disease **(McIntosh et.al., 1991; Brown et al., 1999)**, improve the glycemic level control for people with type II diabetes **(Lu et al., 2004)**, increases intestinal peristalsis **(Cummings et al., 1992, 2009)**.

Arabinoxylans and β-glucans, along with providing benefits to human health, have the potential to improve the quality of bakery products **(Said et al., 2011)**. There are two types of arabinoxylans: water extractable (about 35% of the total) and non-extractable **(Leggio et al.,, 1999)**. These two fractions differ in physicochemical and functional properties, including water-binding and gel-forming ability **(Courtin et al., 2002)**. Most of the dietary fiber of rye and wheat bran is insoluble **(Grigelmo-Miguel et al., 1999; Van Craeyveld et al., 2009)**. However, water-extractable arabinoxylan is more effective compared to non-extractable in terms of quality improvement and shelf life extension of bread by reducing the effect of staling and starch retrogradation **(Courtin et al., 1999; Said et al., 2011)**.

The pericarp tissue of grain have considerable strength and stiffness, that has an adverse effect on quality of

whole-grain bread **(Antoine et al., 2003)**. There is a growing demand for the usage of sustainable processes of soft biotech processing of plant cell walls, which will replace the chemical treatment **(Ulvskov et al., 2011)**. Usage of a xylanase for the hydrolysis of water-insoluble non-starch polysaccharides of the cell walls leads to improvement in swelling, sensory performance and to deceleration of starch retrogradation process **(Gruppen et al., 1998; Andlaver et al., 2002; Charalampopoulos et al., 2002; Jiang et al., 2005)**.

The purpose of this research was to evaluate the influence of cellulose complex (*Penicillium canescens*) enzymes on composition and microstructure alteration of triticale grain surface, for grain used in baking.

MATERIAL AND METHODOLOGY

Two sorts of triticale grain from different genetic sources were studied. They are «Antaeus» and «Talva 100» (Russian Federation). Dry complex enzyme preparation comprising cellulase, β-glucanase and xylanase, as well as preparations containing individual enzymes (producer is *Penicillium canescens*, The Russian Academy of Sciences' Skryabin Institute of Biochemistry and Physiology of Microorganisms) were used during research. Enzymes had the following activity: cellulase 58711 nkat/g, xylanase 12135 nkat/g, β-glucanase 51317 nkat/g and were given by chemical faculty of Moscow State University **(Sinitsyna et al., 2003)**.

Enzyme preparation in powder was mixed by a magnetic stirrer with a citrate buffer (pH 4.5) for 0.5 hours at a concentration of 0.6 $g.L^{-1}$ before the analysis. This concentration corresponds with the optimum enzyme concentration for bread production from whole triticale grain **(Kuznetsova et al., 2010)**. Whole triticale grain was incubated in enzyme preparation solution with grain-solution ratio of 1:1.5 for 8 hours at 50 °C in thermostat. Duration of cereal substrate hydrolysis determined by the time during which the grain moisture was 40% or more that is required to get the cereal mass with ability to dispersion and allow to use grain raw material for bakery. To save material intact enzyme inactivation wasn't performed after incubation.

Determination of cellulose content, ratio of amorphous and crystalline cellulose and total amount of hemicellulose were carried out according to procedures described by **Ermakov (1972)**. To detect the soluble pentosans, grain sample was analyzed by orcinol-chloride method **(Hashimoto et al., 1987)**.

Concentration determination of low molecular carbohydrates in the grain samples was performed by a chromatographic method with electrochemical detection using liquid chromatograph Agilent 1100 with electrochemical detector ESA Coulochem III. Sugars mixture separation carried out using an anion exchange column with grafted amine phase followed by electrochemical detection.

Microstructural studies were conducted using an electron scanning microscope ZEISS EVO LS. Survey was carried out at an acceleration voltage of 15 kV.

Complex of phenolic compounds was determined by HPLC using MiLiChrome-5 device. Triticale grain ethanolic extract was used, eluent of composition is acetonitrile – water solution of trifluoroacetic acid (pH 2.5, in a ratio of 15:85); elution mode is isocratic, the analysis time is 12 – 25 min, the sample volume – 6.2 ml. Antioxidant activity was determined by spectrophotometric method in alcoholic extract described by **Silva et al., 2005**.

RESULTS AND DISCUSSION

Table 1 shows the research results of dietary fiber content in two sorts of triticale grain.

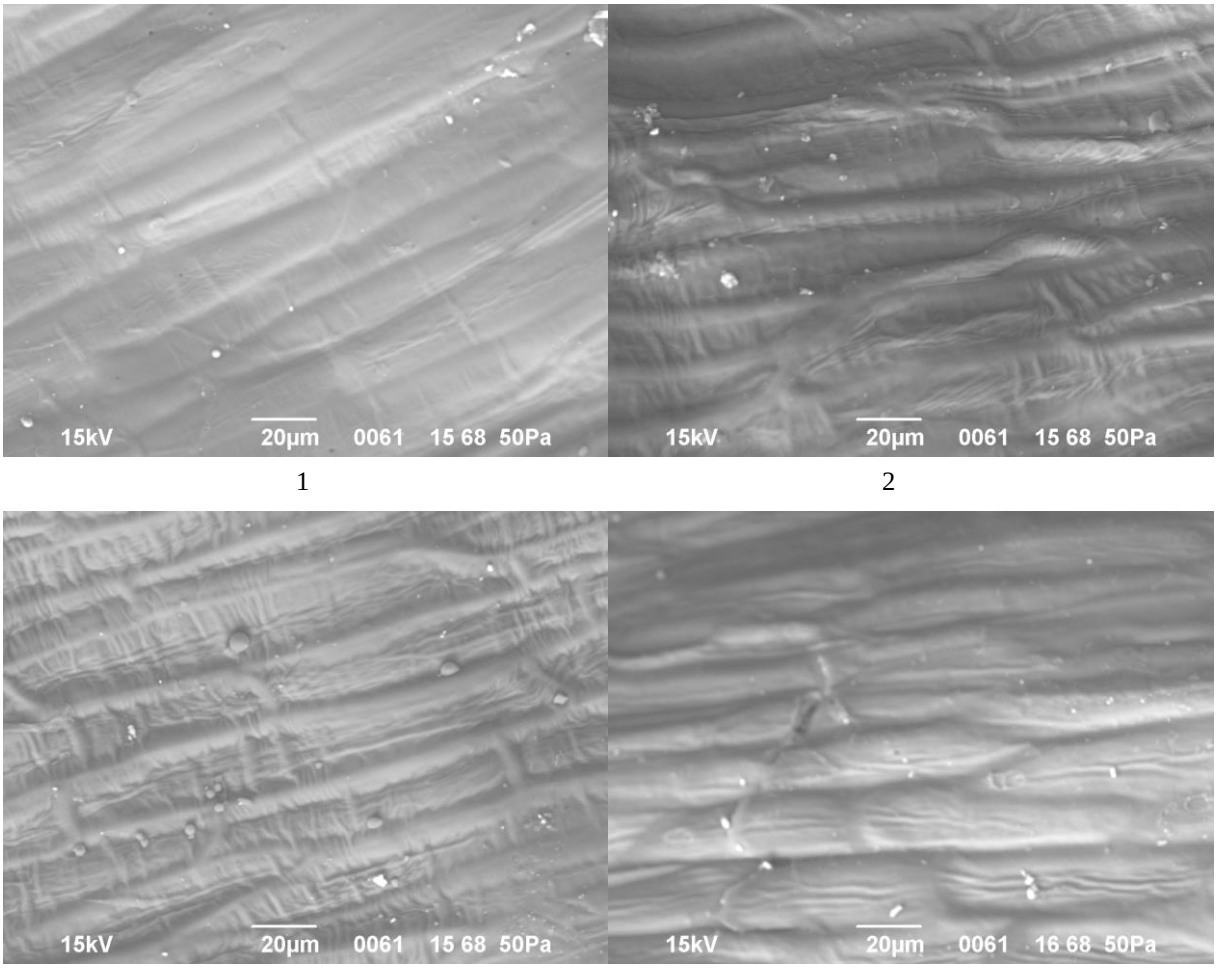
Table 1 Composition of non-starch polysaccharides of dry triticale grain soaked in a citrate buffer and treated with enzyme preparations, in %.

Experiment variation	Cellulose	Ratio of amorphous and crystalline cellulose	Hemicellulose	Soluble pentosans
		Antaeus		
Dry grain	2.20 ±0.04	2.33 ±0.04	7.93 ±0.04	5.46 ± 0.04
Control grain	2.08 ±0.05	2.24 ±0.04	7.24 ±0.05	5.88 ±0.06
Complex preparation	1.96 ±0.03	2.00 ±0.04	6.70 ±0.05	7.60 ±0.03
Xylanase	1.98 ±0.04	2.08 ±0.03	6.96 ±0.04	7.34 ±0.03
β- glucanase	2.04 ±0.03	2.18 ±0.03	7.18 ±0.03	6.66 ±0.05
		Talva 100		
Dry grain	2.14 ±0.04	2.60 ±0.04	7.94 ±0.02	5.84 ±0.03
Control grain	2.02 ±0.03	2.48 ±0.04	7.38 ±0.02	6.15 ±0.02
Complex preparation	1.85 ±0.02	2.21 ±0.04	6.95 ±0.04	7.96 ±0.03
Xylanase	1.90 ±0.05	2.26 ±0.03	7.12 ±0.06	7.68 ±0.03
β- glucanase	2.00 ±0.03	2.34 ±0.03	7.24 ±0.02	6.74 ±0.04

As a result of grain processing by enzyme complex concentration of water-soluble pentosans increased by 36.3 – 39.2%, depending on the sort of grain. Processing of grain by individual enzymes like hemicellulase comprised in complex preparation (xylanase and β-glucanase), showed that more significant role in polysaccharide complex composition of grain surface structures alteration belongs to xylanase. These results are consistent with data

Table 2 Carbohydrate composition of dry triticale grain, soaked in citrate buffer and treated with enzyme preparations, $g.L^{-1}$.

Sugar	Dry grain	Control grain	Complex preparation	Xylanase	β-glucanase
Arabinose	0.00	0.01	0.03	0.02	0.02
Galactose	0.00	0.00	0.00	0.00	0.00
Glucose	0.31	0.35	0.43	0.40	0.37
Xylose	0.00	0.00	0.04	0.02	0.01
Fructose	0.26	0.24	0.22	0.23	0.24
Raffinose	0.00	0.01	0.03	0.03	0.02
Unidentifiedsugar	0.07	0.08	0.12	0.10	0.09
Cellobiose	0.00	0.00	0.00	0.00	0.00
Maltose	1.68	1.98	2.08	2.05	2.02
Total	2.32	2.67	2.95	2.85	2.77



1 2

3 4

Figure 1 Microstructure of triticale grain surface (1 – grain soaked in buffer – control; 2 - treated by complex enzyme preparation; 3 – by xylanase preparation; 4 – by β-glucanase preparation), an increase of x 700. Photo: S. Motyleva, 2014.

from **(Havrlentova et al., 2011)**, where it was found that the level of soluble dietary fiber in wheat bran increases under the influence of enzyme preparations – hemicellulases, especially those containing endoxylanase.

Since varietal differences in non-starch polysaccharides of triticale grain shells content alteration after enzymes processing (cellulase complex *Penicillium canescens*) is not significant, the determination of triticale grain carbohydrate composition by chromatographic method was carried out for the average triticale grain sample, composed of two represented sorts. Hydrolysis of glycosidic linkages in polysaccharides molecules is occurred and partially collapsed matrix carcass nodes,

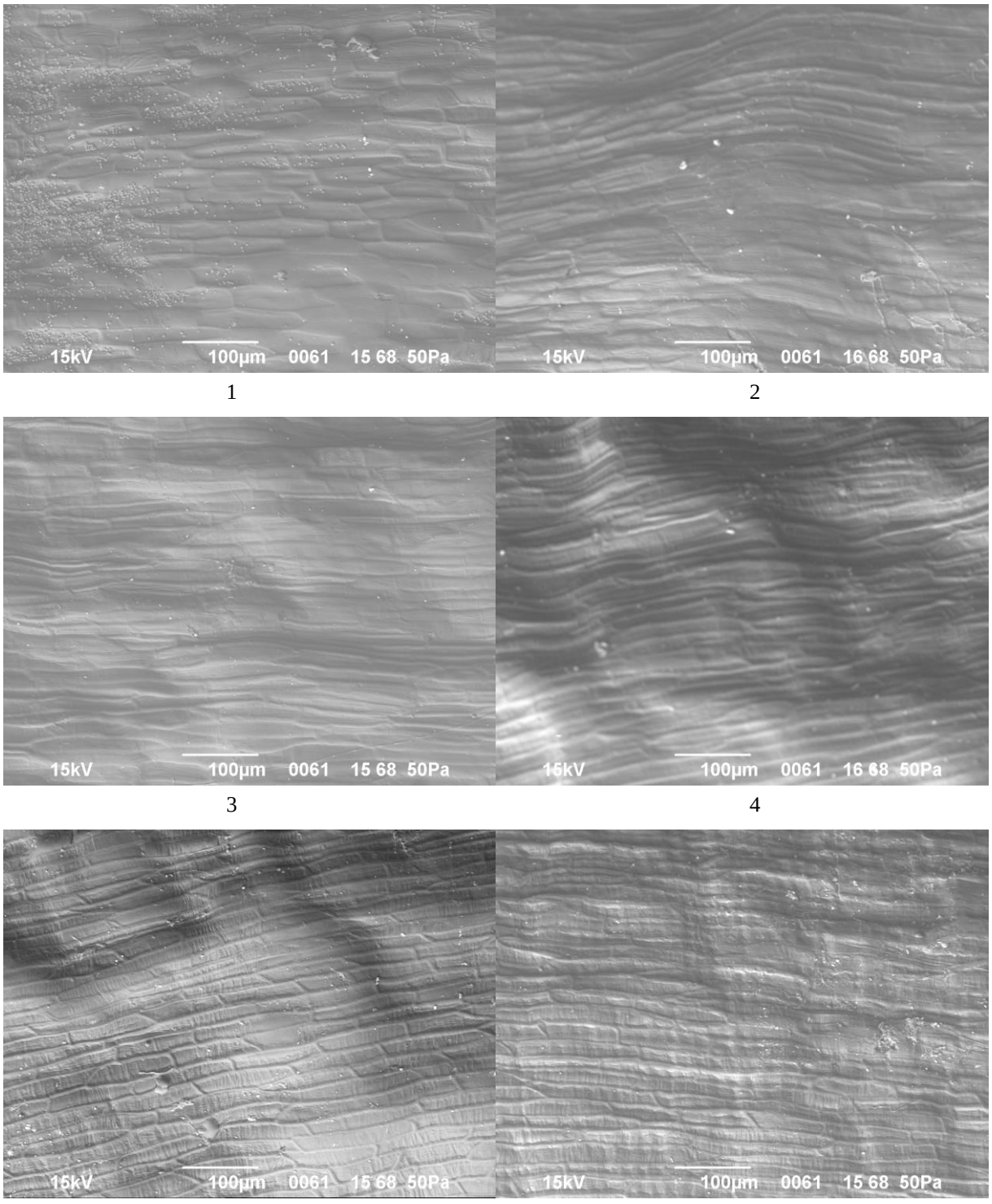


Figure 2 The surface microstructure of triticale grain, soaked in a solution of a complex preparation (cellulase, β-glucanase, xylanase) during different periods (1 – immediately after being placed in a solution; 2 – 2 hours; 3 – 4 hours; 4 – 6 hours; 5 – 8 hours; 6 – 10 hours) x 200 magnification. Photo: S. Motyleva 2014.

wherein substances with low molecular weight and high solubility was formed (see Table 2).

The content of arabinose (0.02 – 0.03 $g.L^{-1}$) and xylose (0.01 – 0.04 $g.L^{-1}$) in grain extracts indicate occurred biochemical processes in arabinoxylan chains. Such processes can be caused by the presence of hydrolyzing glycosidic linkages in the enzyme complex of hemicellulases preparations. Results of chemical composition alteration of the cell walls in wheat grain shells under the influence of xylanase reconciled with scanning electron microscopy **(Tervilä-Wilo et al., 1996; Parkkonen et al., 1997)**. Figure 1 shows microphotographs of dry triticale grain surface structure, soaked in citrate buffer and treated with enzyme preparations.

Xylanase have an influence on both type of hemicellulose fibers with longitudinal and radial orientation. Channels on the surface of the grain shells, having various directions are found. This fact shows that endoxylanases *Penicillium canescens* have much stronger destructive forces for non-starch polysaccharides in outer integument of triticale grain compared to β-glucanase.

Figure 2 shows photographs of the surface microstructure of triticale grain soaked in a solution of a complex preparation for adifferent time.

Microphotographs shows triticale grain surface alteration during hydrolysis by complex enzyme preparation in the dynamics. First of all microfibrils having a longitudinal orientation became bare because hemicellulose shielding layer exposed to degradation influence. Hollows having a radial orientation appear on the surface of the grain shells after 6-8 hours of hydrolysis. It means that deeper processes affecting both arabinoxylan molecules and cellulose matrix microfibrils.

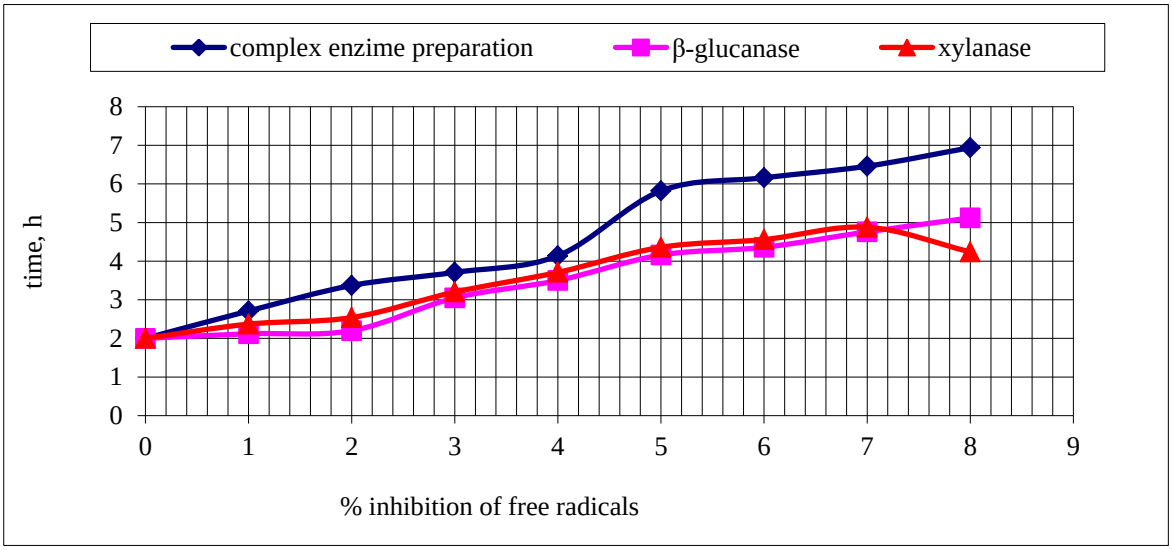


Figure 3 Triticale grain antioxidant activity alteration in the process of enzymatic hydrolysis by cellulase preparations.

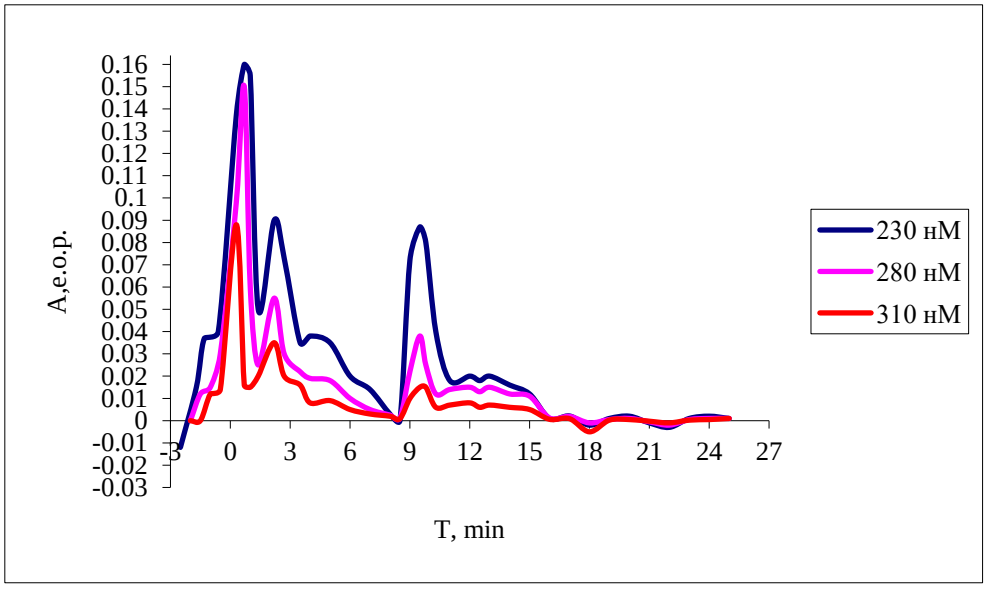


Figure 4 The chromatogram of triticale grain extract after enzymatic hydrolysis with a complex enzyme preparation (cellulase, β-glucanase, xylanase) made of *Penicillium canescens*.

The degradation of xylan from cell walls matrix under the influence of endo-xylanase and β-glucanase leads to destruction of the natural triticale grain shells structure and to increase of the water-soluble pentosans concentration.

Determination of antioxidant activity (Figure 3) for alcoholic extract of triticale grain treated for 8 hours by a complex enzyme preparation, β-glucanase and xylanase, show that the percentage of DPPG free radicals inhibition increasing with extension of hydrolysis duration.

Composition of phenolic compounds in triticale grain extract after enzymatic hydrolysis with a complex enzyme preparation was determined by HPLC method. Chromatogram is shown in Figure 4.

Chromatogram of alcoholic extract allowed to identify organic and hydroxycinnamic acids. Ferulic acid was identified (VR = 9,6; RS = 0,533). These findings are consistent with the results of **(de Vries et al., 2000)**, where stated that after the degradation of xylan chain by endo-xylanase, the antioxidant activity of cereal substrates increases by the release of ferulic acid.

CONCLUSION

During experiment it is found that triticale grain processing by enzymes of cellulase complex leads to an increase in the content of water-soluble pentosans by 36.3 – 39.2% and carbohydrates with a low molecular weight and high solubility. Xylan degradation of the cell walls matrix under the influence of endo-xylanase and β-glucanase leads to the destruction of the natural structure of triticale grain shells, that is consistent with data on the content increase of water-soluble pentosans. Application of cellulase complex enzymes (producer is *Penicillium canescens*) for the treatment of triticale grain increases the content of water-soluble pentosans, low molecular carbohydrates, the antioxidant activity of raw material that has positive implications for the future grain usage in bread baking. Grain surface microstructure alteration leads to modifications of non-starch polysaccharides, that may decrease their strength and improved sensory characteristics of the product.

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