

**CHANGES IN THE MICROSTRUCTURE OF THE CEREALS SHELLS CELL WALLS UNDER
THE INFLUENCE OF BIOCATALYSTS ON THE BASIS OF CELLULASE
(PRODUCER *Penicillium Canescens*)**

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ABSTRACT

*The grain has structural features. Some food technologies to improve sensor performance product used grain handling biocatalysts based on cellulases to modify the non-starch polysaccharides. Wheat, rye, triticale, oats and barley were treated with complex enzyme preparation comprising cellulose, β -glucanase, xylanase (producing *Penicillium canescens*). Under the action of biocatalysts have been changed in the microstructure of the surface of cereals. It has been established that the layered structure of the outer and inner pericarp grains regardless of a botanic accessory predominantly longitudinal orientation to a lesser extent radially layers occur. Found that the depth and direction of the process of degradation of non-starch polysaccharides covers grain determined by the composition of the enzyme complex preparation and duration of exposure.*

Key words: corn, the cell wall, the non-starch polysaccharides, complex enzyme preparation, microstructure

The surface of each plant organ is a part of the overall body structure and has its own characteristics. Surface structure of a plant seeds, that include such formations as micro extensions, cells, folds representing directly topography [Kochetova et al. 1982]. Surface of cereals shells cell walls is a structural unit, which primarily responds to the impact of water and biocatalysts solutions based on cellulases. Cell walls of cereals are presented by non-starch polysaccharides consisting mainly of arabinoxylan, cellulase and β -glucan. [Prosky et al. 1985, Saulnier et al. 1995, Phillippe et al. 2006, Gebruers et al. 2008]. These polysaccharides are determined by the strength of the grain shells, forming crosslinks in the matrix structure of the cell wall [Mabille et al. 2000, Cui, S.W. et al. 2009]. The outer pericarp, which surrounds the outer layers of the grain, is a thin and weak tissue is attached to the intermediate layer. An intermediate layer comprising various tissues (nucellar epidermis, inner pericarp), has a complex and heterogeneous structure. These two structures are grain fiber-rich fraction [Antoine et al. 2004].

Some food technologies to improve the sensory characteristics of the product are increasing instead of chemical treatment plant usage of a soft biotech processing [Jiang et al., 2005, Kuznetsova et al. 2008, Ulvskov et al. 2011]. To modify the non-starch polysaccharides used enzyme preparations, which contain cellulase endoxylanase, β -glucanase and other enzymes [Dijkerman et al. 1997]. The use of hemicellulases hydrolysis of water-insoluble non-starch polysaccharides of the cell walls leads to an increase in the degree of swelling of bread improving sensory performance and slowed the process of retrogradation of starch [Gruppen et al. 1998, Andlauer et al. 2002, Charalampopoulos et al. 2002, Jiang et al., 2005].

Objective of this study is to examine the nature of changes in the microstructure of the surface of the cell walls of shells grain cereals in conditions of application of biocatalysts based cellulases (producer *Penicillium canescens*) in the preparation of grain for the production of grain bakery.

MATERIAL AND METHODS

For the study took the grain of wheat varieties (sort) Moscow 139, rye varieties (sort) Talovskaya 33, triticale varieties (sort) Nemchinovsky 56 varieties of oats and barley varieties Racehorse Vakula grown in the Central Region of the Russian Federation. Used dry complex enzyme preparation containing cellulase, β -glucanase, xylanase (P-215, producing *Penicillium canescens*, IBPM RAS). Enzymes had the following activity: cellulase - 58711 nkat / g, xylanase - 12135 nkat / g, β -glucanase - 51317 nkat / g and were given laboratory physical and chemical transformation of polymers chemical faculty of Moscow State University [Sinityna et al. 2003].

Enzyme preparation in powder c was mixed using a magnetic stirrer with a citrate buffer (pH 4.5) for 0.5 hours at a concentration of 0.6 g / L before the analysis. This concentration corresponds to the optimum enzyme in the production of grain concentrate for use in technology grain bakery products [Kuznetsova et al. 2014]. The whole cereal grains in a solution maintained at a ratio of enzyme preparation grains: 1:1.5 solution for 8 hours at 50 ° C in an incubator. Temperature 50 ° C and pH 4.5 is optimal for the action of the enzymes studied.

Microstructural studies of the longitudinal and transverse sections grains were performed using a scanning electron microscope ZEISS EVO LS. Survey was carried out at an accelerated voltage of 15 kV.

Determination of cellulose content, the ratio of amorphous and crystalline cellulose and the total amount of hemicellulose were carried out according to procedures described by Yermakov [Ermakov 1972].

RESULTS

Figure 1 shows photomicrographs of the anatomical surface of the peripheral parts of grains of cereals studied in the cross sections after enzymatic hydrolysis.

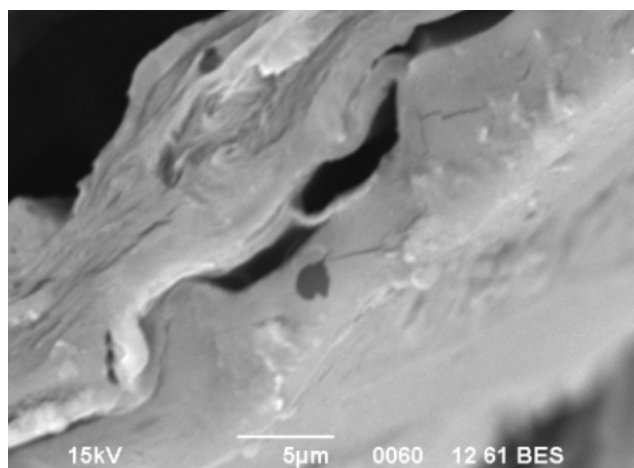
Images show that fruit and seed coat of wheat and triticale have a distinct layered structure between the fruit and the seed coat are voids up to 2.5 microns. The layered structure is predominantly longitudinal orientation, but there are radially arranged layers. The images obtained are consistent with the studies of the microstructure of the external hull, middleware (including inner pericarp and nucellar tissue) of wheat, obtained by confocal laser scanning microscopy. 50 % of the pericarp layers have a longitudinal orientation, which determines the elasticity, strength and rigidity of the structure. The intermediate layer to a greater extent presents layers having both longitudinal and radial orientation [Antoine et al. 2003].

We also found rye layered structure in the peripheral parts of grains, but the microstructure of the shells are packed more compactly, the layers closely adhere to each other, the distance between the layers - up to 3.0 microns. Shell grains oats have a loose microstructure, the individual layers are superimposed on each other. There are gaps between the layers of 0.1-0.25 microns. Shell barley grain in contrast to all other cereals are the densest packing.

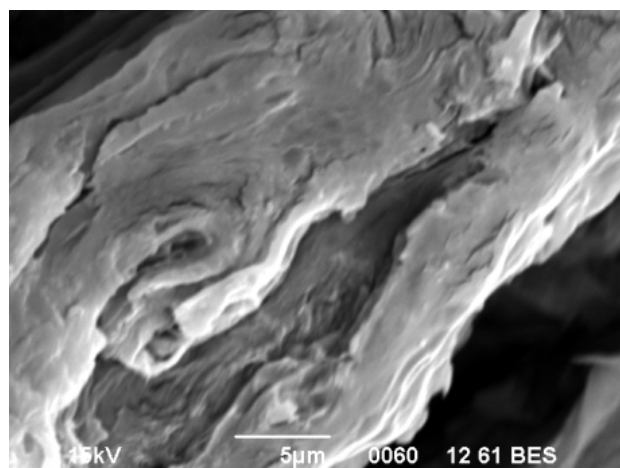
Table 1 shows the results of determining the content of non-starch polysaccharides in the cereal grain.

grain	cellulose, %	The ratio of crystalline and amorphous cellulose	The total amount of hemicelluloses, %
wheat	2,7	2,62	7,7
rye	3,0	2,75	9,1
triticale	3,0	2,33	9,9
oat	13,8	2,11	10,3
barley	7,2	3,37	7,9

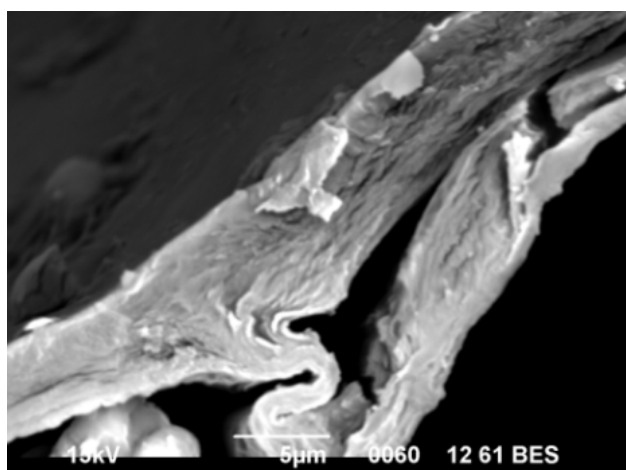
Despite the fact that the highest fiber content oat characteristic, the ratio of crystalline and amorphous cellulose therein is 2.11, whereas in wheat - 2.62, rye - 2.75, triticale - 2.33, barley - 3.37. Regardless of the botanical species of grasses in cross sections of the outer layers of the grain shells presented longitudinally oriented fibers.



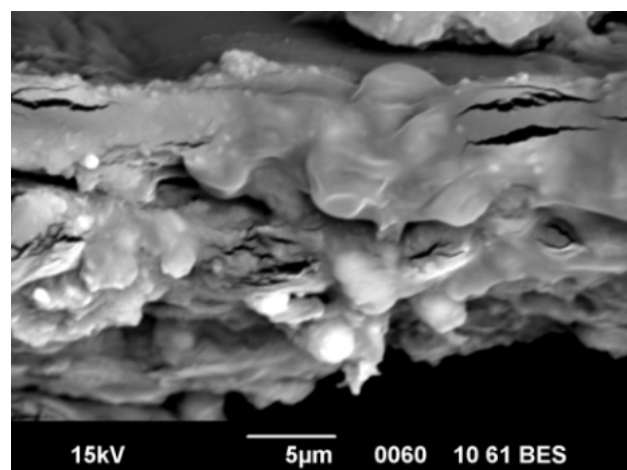
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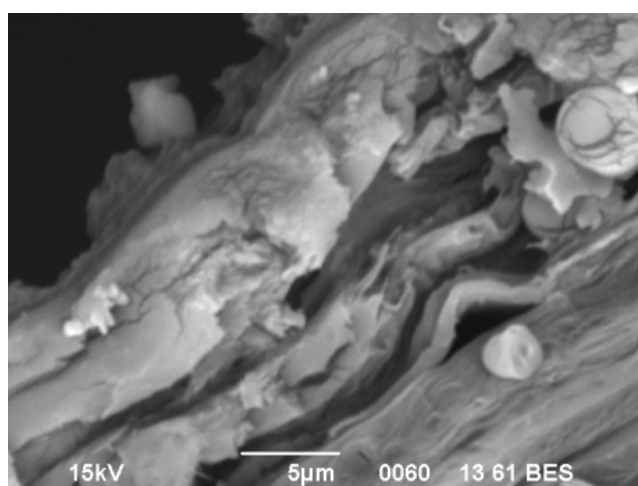
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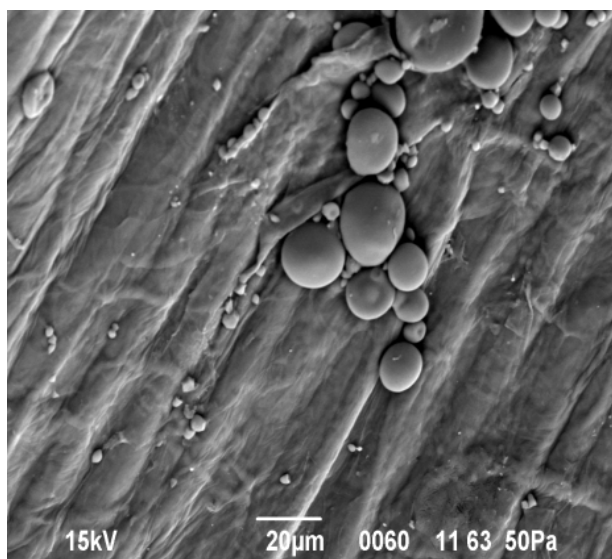
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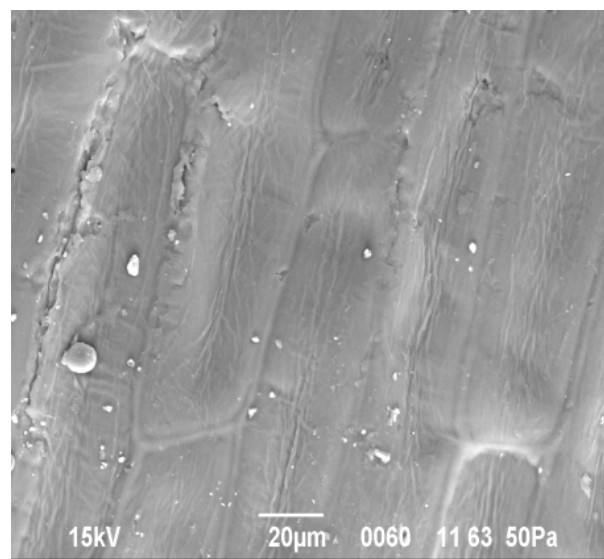
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Figure 1 Photomicrographs of the surface membranes of grain cereals in cross section, an increase 4000h (1 - wheat, 2 - rye, 3 - triticale, 4 - oats, 5 - barley). Photo: Motyleva 2014.

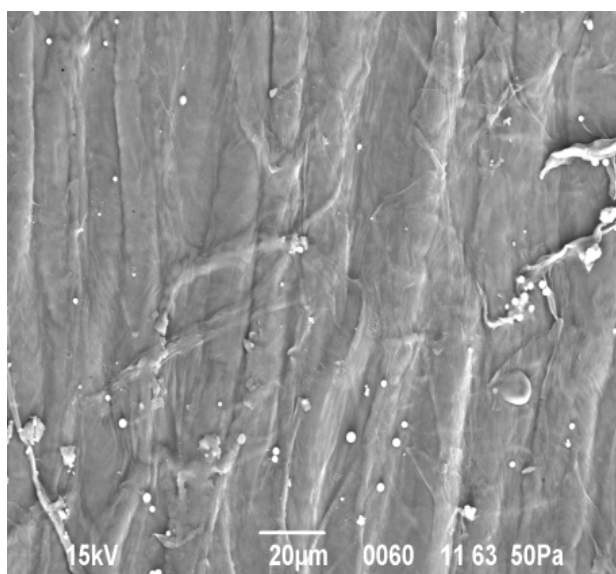
To assess changes in the character of the surface structure of shells of wheat, rye, triticale, oats and barley by the action of a complex enzyme preparation prepared longitudinal sections grains which were studied using scanning electron microscopy with an increase X700. Micrographs studied herbal preparations are shown in Figure 2.



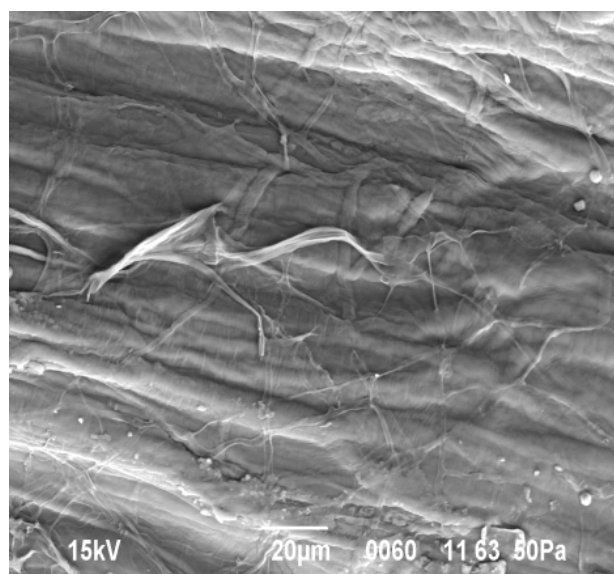
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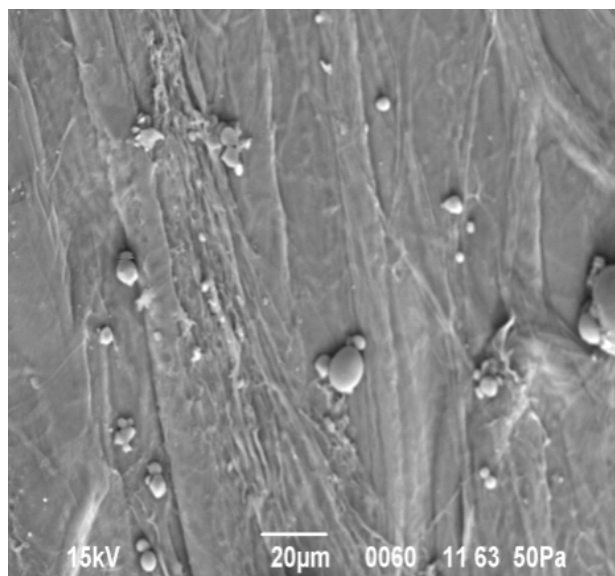
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Figure 2 - Photomicrographs of the surface of cereal grains after treatment with an enzyme preparation based on phytase X700 (1 - Wheat 2 - rye, 3 - triticale, 4 - oats, 5 - barley). Photo: Motyleva 2014.

Images of the surface grains show that under the action of the enzyme preparation in a complex surface topography of wheat / 1 / the most significant changes have occurred. Destruction observed strands of cellulose microfibrils and interfibrillar crosslinks built from molecules of hemicellulose. Prevails on the surface texture parallel microfibrils. The structure of the enzymatic complex of the drug enters the enzyme cellulase, which are subjected to the action of destruction of the outer layers of cellulose microfibrils having amorphous structure. The grain rye has not undergone such visible changes, but noticeable depressed areas with good discernible boundary represented horizontally spaced parallel strands and crosslinked hemicellulose. Surface modified triticale grain occupies an intermediate position between the grain of wheat and rye. To enjoy a special oat grain microstructure surface: microfibrils thinner, their average width of 3-4 microns, well distinguishable microfibrils having radial orientation. These fibers have a repeated bending, each link has a fiber length of 40 mm and a bend extending approximately at right angles to the long average 10 microns. If other grain cereals studied preponderance mikrotызhey longitudinal texture, the surface topography oat presents alternating longitudinal and radial thin microfibrils. With this arrangement, microfibrils can lose packing, and therefore, despite the high cellulose content (14 %), the ratio of crystalline and amorphous cellulose is the smallest (2.11), indicating a high content of amorphous fraction.

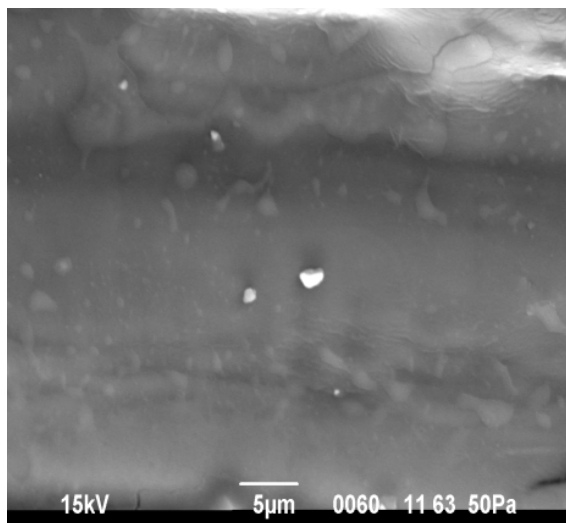
The structure of a complex enzyme preparation consists of biocatalysts: cellulase , xylanase , β - glucanase. Each of these enzymes is of a specific action on the substrate complex multi - cell walls of grain shells. To study the influence of individual enzymes that are part of the complex preparation and combinations thereof, investigated the effects of experimental drugs on the microstructure of the surface of the grain of wheat (Figure 3).

In the control variant in which the grain was kept in citrate buffer / 1 / , the destruction of the surface layer of the matrix polysaccharides weakly expressed and surface topography grain is parallel strands cellulose fibril strands overlapped smaller hemicellulose nature. The preparation, the effect of which is presented in the case / 2 / , includes cellulase enzyme, whose influence was subjected to destruction of the outer layers of cellulose fibrils with amorphous structure. In the microstructure of the surface texture prevails parallel fibrils and interfibrillar baring paracrystalline areas.

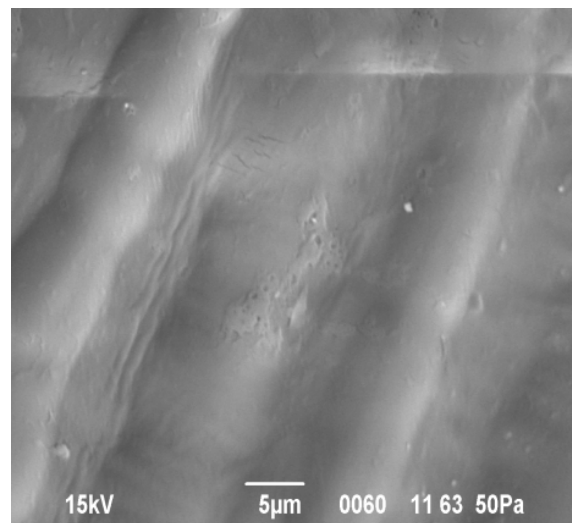
Under the action of the enzyme β -glucanase / 3 / and combinations of two cellobiohydrolase enzymes and β -glucanase / 4 / has a surface topography similar grain shape. Denudation observed parallel strands of cellulose microfibrils of varying thickness and tortuosity. The differences are expressed in a large deep ulceration of the surface cuticular strands under the action of the preparation containing cellobiohydrolase enzyme complex and β -glucanase. Xylanase enzyme action leads to

preferential destruction of hemicellulose, the depth direction of fabric / 5 /. Under the influence of complex enzymes cellobiohydrolase and xylanase / 6 / masonry surface relief grains are formed deep enough, they are represented by parallel strands almost devoid of cuticular crosslinks.

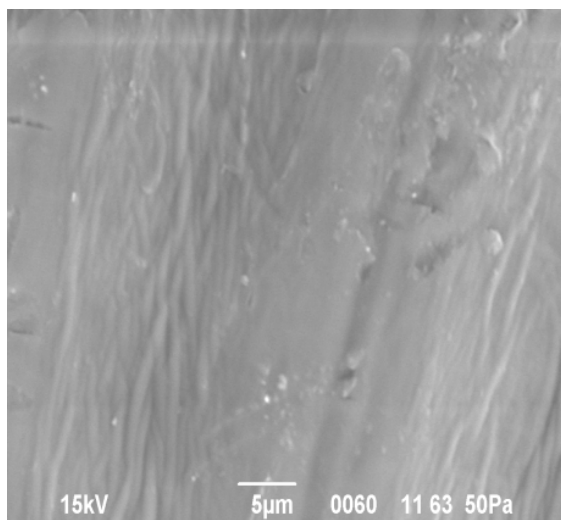
Microscopic studies of wheat bran to the endoxylanase and after treatment showed that aleurone and inner bran layers (seed coat and nucellus) were completely reorganized and outer (fruit shell , epidermis and the subcutaneous layer) only stable to endoxylanase [Benamrouche et al. 2002] . According to the analysis in the work of Peterson et al. 2013 xylanase enzyme plays a leading role hydrolysis of arabinoxylans of wheat and rye bran, facilitating the degradation of cellulose and β - glucan. Marked synergism in action endoxylanase and β -glucanase during degradation of cell wall polysaccharides. Release of hydrolysis products of low molecular weight with a combination of these enzymes increases 2-2.5 times relative to the individual effect [Ronald et al. 2000] .



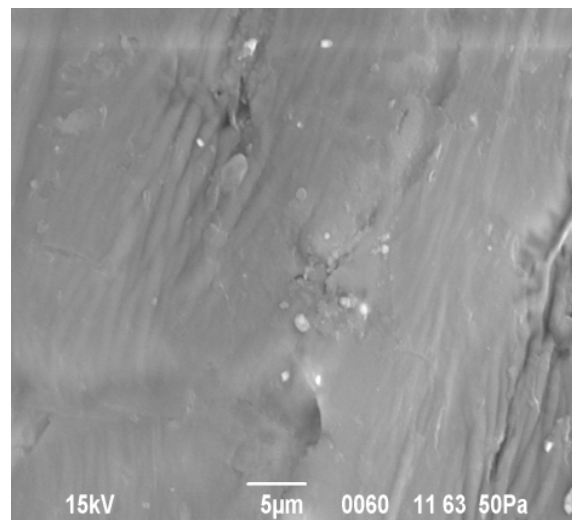
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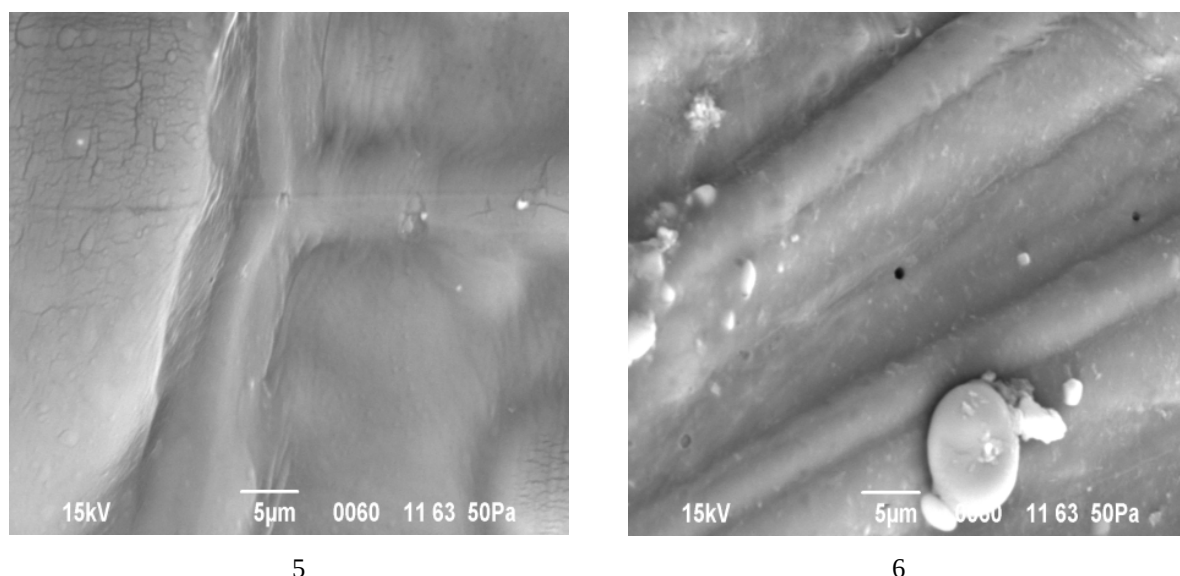


Figure 3 - Photomicrographs of the surface of wheat treated with enzymes of the cellulase complex h2700 (1 - control without enzyme, 2 - cellobiohydrolase, 3 - β -glucanase, 4 - cellobiohydrolase + β -glucanase, 5 - xylanase, 6 - cellobiohydrolase + xylanase. Photo: Motyleva 2014

CONCLUSION

The experimental data show a deep enough destructive action with respect to the cell wall membranes grain endoxylanases *Penicillium canescens* compared with β -glucanase and cellulase. Using enzyme preparations containing endoxylanase allows through the hydrolysis of arabinoxylan open structure of the cell wall matrix to modify the water-insoluble arabinoxylan. This can lead to higher water absorption of the coating material for the cell wall due to cleavage of arabinoxylan, thereby exposing the surface structure of the membrane and increasing the degree of swelling.

Cellulase complex enzymes have different substrate selectivity and differ in their preference for the hydrolysis of hemicellulose. Therefore, understanding the specificity and selectivity of enzymes is critical to achieve the desired hydrolysis.

Changes in the grain surface microstructure leads to modifications of non-starch polysaccharides, which may contribute to a decrease in their strength and improved sensory performance of the product using the modified raw material in breadmaking.

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