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REDISTRIBUTION OF MINERAL ELEMENTS IN WHEAT GRAIN WHEN APPLYING THE COMPLEX ENZYME PREPARATIONS BASED ON PHYTASE

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ABSTRACT

Biogenic minerals play an important role in the whole human nutrition, but they are included in the grain of the phytates that reduces their bioavailability. Whole wheat bread is generally considered a healthy food, but the presence of mineral elements in it is insignificant, because of weak phytate degradation. From all sources of exogenous phytase the most productive are microscopic fungi. To accelerate the process of transition hard mineral elements are mobilized to implement integrated cellulolytic enzyme preparation based on the actions of phytase (producer is *Penicillium canescens*). Phytase activity was assessed indirectly by the rate of release of phosphate from the substrate. It has been established that the release rate of the phosphoric acid substrate is dependent on the composition of the drug and the enzyme complex is determined by the presence of xylanase. The presented experimental data shows that a cellulase treatment of the grain in conjunction with the β-glucanase or xylanase leading to an increase in phytase activity could be 1.4 – 2.3 times as compared with the individual enzymes. As a result of concerted action of enzymes complex preparation varies topography grain, increase the pore sizes in seed and fruit shells that facilitate the penetration of the enzyme phytase in the aleurone layer to the site of phytin hydrolysis and leads to an increase in phytase activity. In terms of rational parameters of enzymatic hydrolysis, the distribution of mineral elements in the anatomical parts of the grain after processing complex enzyme preparation with the help of X-ray detector EMF miniCup system in a scanning electron microscope JEOL JSM 6390 were investigated. When processing enzyme preparation wheat trend in the distribution of mineral elements, characteristic of grain - the proportion of these elements in the aleurone layer decreases, and in the endosperm increases. Because dietary fiber and phytate found together in the peripheral layers of fiber-rich grains, it is difficult to separate the effects of degradation processes nonstarch polysaccharides and fiberphytate redistribution of polyvalent metal ions. However, studies have shown that phytase - an effective mechanism for regulating mineral nutrient diet. Application of phytase in grain bakery technology will increase the biological value of the product.

Keywords: phytin; phytase; complex enzyme preparation; microstructure; mineral element; grain of wheat

INTRODUCTION

Phytic acid is found in many plant systems. In beans and cereal grain, it is approximately 5.1% by weight. This compound is of vital importance for the successful development of the seed and growth of plants. However, phytic acid is a strong chelator and in the interaction with the polyvalent cations and the formation of complexes with proteins - phytate - reduces the bioavailability of many vital mineral compounds **(Cheryan, 1980; Bergman et al., 1997)**. Studies in animal and human as subjects showed that a diet high in phytic acid leads to a deficiency of zinc, calcium, magnesium, phosphorus. This can cause immunodeficiency and lead to cognitive and growth disorders **(Erdman, 1979)**. At the same time, as anti-nutrients in the human diet, phytates can carry a positive role in the nutrition as antioxidants and anti-cancer drugs **(Graf et al., 1987; Lott et al., 2000; Urbano et al., 2000; Hyun-Joo et al., 2004)**.

Reduction of phytate content in the diet is one way to improve nutrient absorption of mineral elements. This may be achieved through use of cooking methods that lead to activation of the endogenous phytase, through the action of microorganisms, such as yeast in baking, with the proviso that the pH and other environmental conditions are favorable, or by application of exogenous phytase technology **(Lonnerdal, 2002; Lestienne et al., 2005; Eklund-Jonsson et. al., 2006)**. Wholewheat bread, usually considered more healthy food than of high-grade flour because different high content of dietary fiber, vitamins (especially B and E) and biogenic minerals. However, whole-grain bread also contains large amounts of phytate. A reduction in the level of phytate in whole meal flour, rye, oats and wheat after fermentation, a figure only slightly dependent on the temperature of the process **(García-Estepa et al., 1999; Buddrick et al., 2014)**. When making bread from whole grain wheat in establishing pH 5.0 during fermentation phytate level was reduced by 64% **(Türk et al., 1996)**. Phytate degradation to the free ends of phytic acid in the production of almost rye bread with a long fermentation time, but if the bread is made from whole grains, minor degradation of phytate **(McKenzie-Parnell and Davies, 1986; Nielsen et al., 2007)**.

From all sources of exogenous phytase, which have been studied (plants, animals, microorganisms), the most

productive are microscopic fungi **(Wodzinski and Ullah, 1996)**. All commercial phytase preparations containing enzymes of microbial origin, produced by fermentation **(Haefner et al., 2005)**. The main application of phytase are found in feeding monogastric animals **(Madrid et al., 2013)**, but it is also used for the treatment of raw materials destined for human nutrition. This enzyme has already found use in breadmaking, the production of vegetable protein isolates, the wet milling of corn, bran fractionation **(Greiner and Konietzny, 2006)**.

The use of phytase in the art from the bread wheat results in a significant increase in its specific volume and improves the texture and shape. Phytase in baking as improver has two benefits: it improves the nutritional status by reducing the phytate content and promotes activation of endogenous a- amylase, which improves the quality of the product **(Haros et al., 2001)**.

The purpose of the presented work was to study the redistribution of trace elements within the grain by the enzymes of the cellulase complex and phytase (producer *Penicillium canescens*).

MATERIAL AND METHODOLOGY

For the study, we took winter wheat varieties obtained in Moscow 139 Moscow Research Institute of Agriculture "Nemchinovka". Dry using a complex enzyme preparation comprising cellulase, β- glucanase, xylanase, phytase, as well as formulations containing the individual enzymes of the complex or combination thereof (P-215, producing *Penicillium canescens*, IBPM RAS). Enzymes had the following activity: cellulase 58711nkat/g, xylanase 12135 nkat/g, β- glucanase 51317 nkat/g, phytase 205268 nkat/g and were given laboratory physical and chemical transformation of polymers chemical faculty of Moscow State University. MV Lomonosov **(Sinitsyna et al., 2003)**.

Enzyme preparations in powdered form were mixed using a magnetic stirrer with citrate buffer (pH 4.5) for 0.5 hours at a concentration of 0.6 $g.L^{-1}$ in the solution before placing grain. This concentration corresponds to the optimum enzyme in the production of bread from whole grain **(Kuznetsova et al., 2007; 2013).** Whole grain incubated enzyme preparation in solution at the ratio of grains: 1 : 1.5 solution for 8 hours at 50 ±2 °C in an incubator. Modes hydrolysis (t = 50 °C, pH 4.5) are optimum for the operation of the enzymes studied. Duration of cereal substrate hydrolysis determined by the time during which the grain moisture is 40% or more, which is necessary to obtain the grain mass, the ability to undergo dispersion and allow the use of grain raw material for the production of grain bakery. After incubation, the inactivation of enzymes not performed.

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

Phytase activity was assessed indirectly by the rate of release of phosphate from the substrate spectrophotometrically. To a 1 cm^3 of fluid was poured keyhole 1 cm^3 of 10% trichloroacetic acid solution and 2 cm^3 reagent "C" (3.66 g iron sulfate (II) was dissolved in a solution of ammonium molybdate (2.5 g of ammonium molybdate was dissolved in pre- 8 cm^3 of sulfuric acid and adjusted to 250 cm^3 with distilled water). Absorbance of the test solution after 30 minutes of soaking at room temperature for CK-3 for a wavelength of 750 nm in a cuvette with a distance of 1 cm between the faces against distilled water. Calibration curve found the mass concentration of phosphorus using standard aqueous solutions of known concentration of KH_2PO_4. Phytase activity was calculated using the formula:

$$FA = ([PO_4] * 106 * Rrs * Rs) / (M * 103 * t_p), \quad (1)$$

where Rrs - dilution of the enzyme preparation in the reaction mixture;

Rs - pre-dilution of the enzyme preparation (before adding to the reaction mixture);

M - molecular weight phosphate;

t_p - the reaction time.

Determination of trace performed after dry digestion in a muffle furnace at 450 °C and dissolving the ash in the mixture of 10% hydrochloric acid and nitric acid by atomic absorption spectrophotometry, the air-acetylene flame device firm HITACHI 180-80 with deuterium background corrector. For calibration using standard solutions of elements of the company (Merck).

Analysis of the distribution of mineral elements in the anatomical parts of grains and the relative content of mineral elements in the washings were performed using X-ray detector EMF miniCup system in a scanning electron microscope JEOL JSM 6390.

RESULTS AND DISCUSSION

Cereal products provide delivery 20 – 30% minerals (Cu, Zn, Mg, Mn, etc.) in the human diet **(Gyori et al., 1996)**. Table 1 shows the results of the determination of certain mineral element nutrients in wheat.

A number of studies on the processes of distribution of manganese and iron in plant tissues. These elements exhibit a strong affinity for moving organic chelates and complexes. However, when the supply of manganese in

Table 1 Mineral content in wheat grain.

Mineral element	Content [$mg.kg^{-1}$ DM]
Zinc	22.43 ±1.23
Copper	2.13 ±0.13
Manganese	37.50 ±2.10
Iron	64.30 ±4.50
Cobalt	0.04 ±0.01

small plants, its mobility is very limited in tissues. Transfer of iron in plant tissues is difficult. Manganese is a specific component of two enzymes - arginase and phosphotransferase, moreover it increases the activity of certain oxidases. Iron - an essential metal involved in the transformation of the energy required for synthetic processes in the cells. Zinc is part of multiple enzymes - dehydrogenase, peptidases, proteinases and fosfohydrolase. Basic functions related to zinc metabolism of carbohydrates, protein and phosphate. Copper is part of

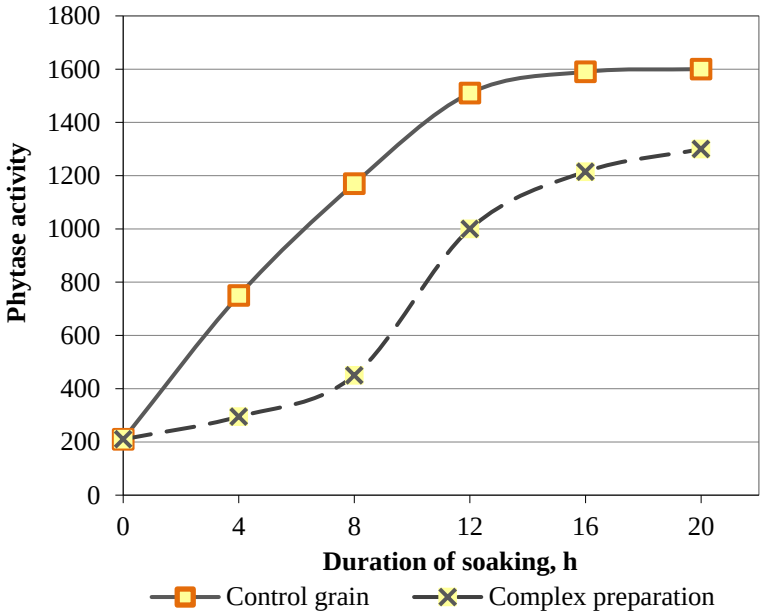

Figure 1 The change in phytase activity of the substrate in the processing of wheat complex enzyme preparation.

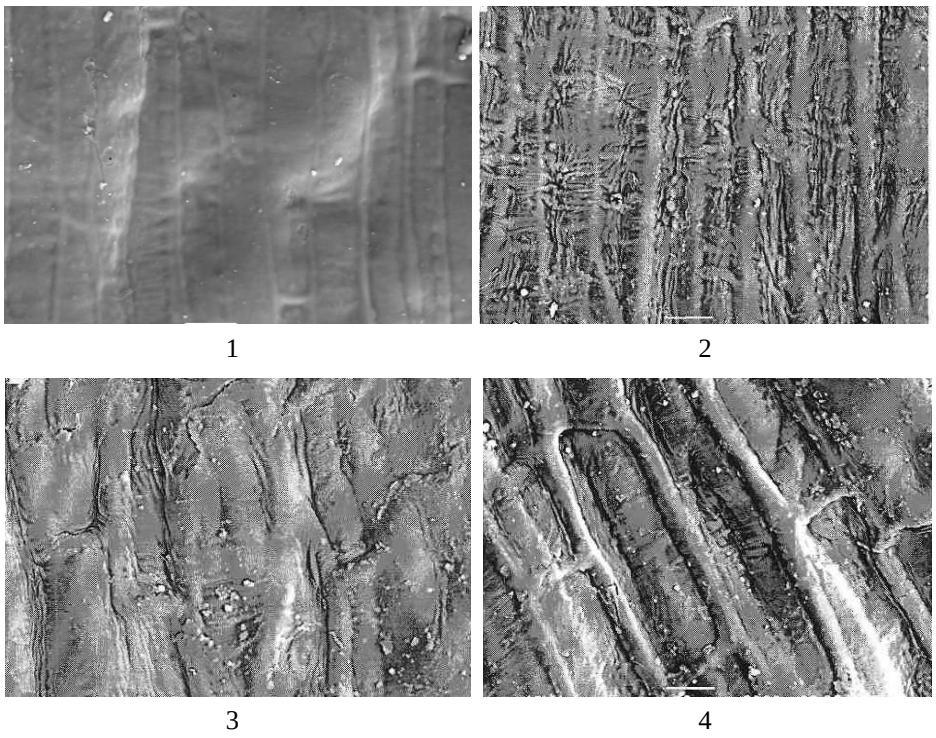

Figure 2 Photomicrographs of the surface of wheat treated with enzymes of the cellulase complex (1 – control without enzyme, 2 – β-glucanase-phytase, 3 – xylanase+phytase, 4 – complex enzyme preparation. An increase of x700. Photo: S. Motyleva, 2013).

enzymes that regulate the processes of respiration, redistribution of carbohydrates, protein metabolism **(Kabata-Pendias and Pendias, 1989)**. Therefore, these trace nutrients plays a key role in the processes of waking up and swelling of the grain as a result of the activation of metabolic processes need to move the movable element in the form increases.

It is known **(Betchel et al., 1981; Jacobsen et al., 1981)**, that it is located in special phytates aleurone grains of the aleurone layer and the embryo and associated biogenic minerals in remote systems. To accelerate the process of transition hard mineral elements are mobilized to implement integrated cellulolytic enzyme preparation based on the actions of phytase.

Treatment of wheat grains complex enzyme preparation during 20 hours was observed changes in the activity of phytase substrate. The experimental data is presented in Figure 1.

Studies have shown that in the first 8 hours of soaking the grain in buffer solution pH 4.5, the activity of phytase substrate is slowly increased as the grains swell and increased by 1.7 times compared to the control. In the period from 8 to 12 hours of hydrolysis observed maximum phytase activity values increase. In the next 8 hours of exposure to the substrate preparation phytase activity in the grain did not undergo significant changes. Phytase activity, the values presented is total value of the substrate activity of endogenous and exogenous phytase.

Table 2 shows the values of the phytase activity of the substrate after 8 hour treatment wheat individual enzymes that are part of a complex enzyme preparation, and their combination in the obligatory presence of exogenous phytase.

From the experimental data presented shows that

Table 2 Effect of enzyme complexes to change the phytase activity of the substrate.

The composition of the enzyme complex	Phytase activity [unit activity]
Cellulase + phytase	685 ±12
β- glucanase + phytase	880 ±21
Xylanase + phytase	940 ±22
Cellulase + β-glucanase + phytase	1050 ±33
Xylanase + cellulase + phytase	1180 ±27

Table 3 Distribution of mineral elements in the anatomical parts of the grain after processing complex enzyme preparation in mass%.

Chemical element	Morphological parts of the grain													
	Germ		The surface of the fruit shell		Fruit shell		Seed coat		Aleurone layer		Endosperm		Barb	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2
C+N+O	93.39	98.37	96.77	97.48	99.18	98.95	98.98	99.42	90.08	98.11	99.00	95.86	93.02	94.95
Na	0.03	–	0.01	–	0.03	0.06	0.01	–	–	–	0.02	0.05	0.03	–
Mg	0.10	0.19	0.12	0.22	0.06	0.11	0.08	0.04	2.03	–	0.05	0.08	0.20	0.13
Al	0.06	0.04	0.06	–	0.03	0.05	–	0.01	–	–	0.01	0.01	0.33	-
P	0.31	0.54	0.15	0.13	0.06	0.06	0.04	0.09	3.95	0.25	0.02	0.78	0.30	0.91
S	0.16	0.24	0.18	0.21	0.07	0.10	0.13	0.12	0.13	0.14	0.03	0.93	0.06	0.51
K	0.55	0.21	0.10	0.13	0.08	0.13	0.21	0.02	2.71	0.31	0.09	0.12	0.03	0.32
Ca	0.15	0.06	0.33	0.34	0.10	0.20	0.18	0.10	0.05	0.19	0.01	0.47	0.49	0.61
Cr	0.01	–	0.12	0.14	0,01	0.03	0.01	0.06	–	–	0.05	0.07	0.84	–
Mn	0.92	0.02	–	0.13	–	–	0.01	0.02	0.02	0.23	–	0.24	–	0.32
Fe	0.89	0.03	0.04	0.03	0.02	0.06	0.01	0.02	0.11	0.11	–	0.38	–	0.01
Co	0.02	–	0.08	–	–	0.03	0.01	–	0.03	0.04	0.03	0.13	1.19	0.26
Ni	0.02	0.05	0.03	0.24	0.01	0.01	0.06	–	0.05	–	0.07	0.09	0.14	–
Cu	1.71	–	0.12	0.12	0.10	0.06	0.15	0.03	0.07	0.16	0.10	0.19	2.36	0.45
Zn	1.41	0.06	0.05	0.20	0.03	0.01	0.03	–	0.01	0.24	–	0.46	–	0.28
Se	0.17	0.19	0.20	0.46	0.05	0.05	0.05	–	0.11	0.17	0.06	0.09	0.88	0.46

1 - The distribution of chemical elements in the morphological parts of wheat (control variant), mass%;
2 - Distribution of chemical elements in morphological parts of wheat treated complex enzyme preparation, mass%

a cellulase treatment of the grain in conjunction with the β-glucanase or xylanase leading to an increase in phytase activity could be 1.4 – 2.3 times as compared with the individual enzymes.

This indicates a synergistic effect on the action of enzyme complex which is caused by the action of enzymes on successive substrates entering into the matrix of the cell walls, which is a complex composition of the substrate.

Figure 2 shows photomicrographs of the surface of the wheat treated with the enzyme solution of the test drug under the optimum conditions of hydrolysis.

Pictures were made with a scanning electron microscope at 700x magnification.

Under the action of biocatalysts based cellulases has changed the surface topography of grain. In control variant the relief of the grain surface is parallel strands of cellulose fibrils, hemicellulose polysaccharides layer overlain nature (1). Under the action of the enzyme β-glucanase grain surface topography changes. Denudation observed parallel strands of cellulose microfibrils of varying thickness and tortuosity. Probably destroy exposed top layer of hemicellulose (2).

Xylanase enzyme action causes destruction layer hemicelluloses tissue depth direction. Modification of the surface structures occurs both in longitudinal and radial direction (3). Under the action of the complex enzymes cellulase, β-glucanase and xylanase (4) masonry surface relief grains are formed deep enough, they are represented by parallel strands almost devoid of cuticular crosslinks. As a result of concerted action of enzymes complex preparation varies topography grain, increase the pore sizes in seed and fruit shells that facilitates the penetration of the enzyme phytase in the aleurone layer to the site of phytin hydrolysis and leads to an increase in phytase activity. The article **Haraldsson et al. (2004)** also points to the possibility of combining the degradation of phytate degradation β-glucan under the joint action of phytase and β-glucanase during malting, which is of interest for the production of cereal products with high nutritional value. However, according to our studies listed, phytate degradation to a greater extent due to the presence in the complex enzyme preparation comprising xylanase and phytin hydrolysis, intensity is a maximum when the cereal substrate operates complex enzyme preparation comprising cellulase, β-glucanase, xylanase.

To analyze the distribution of trace elements P, K, Mg, Ca, Fe, Mn in the outer layers of the wheat grain has been used and the X-ray structure analysis. It has been found that the studied elements are concentrated in the aleurone layer. In particular, P, Mg, K were concentrated in the aleurone layer of subcellular particles and outer layers of the wheat grain; Ca was found in abundance in the tissues of the pericarp **(Tanaka et al., 1974)**.

In terms of rational parameters of enzymatic hydrolysis, investigated the distribution of mineral elements in the anatomical parts of the grain after processing complex enzyme preparation with the help of X-ray detector EMF miniCup system in a scanning electron microscope JEOL JSM 6390.

Gained data relative content of mineral nutrient elements are presented in Table 3.

The studies showed that after soaking in solution of the enzyme preparation on the basis of phytase migrates within the mineral grains.

Reduces the number of elements studied in the aleurone layer and significantly increased in the endosperm.

Under the influence of enzyme preparations polysaccharides constituting the matrix of the cell walls are modified, the system is broken native intermolecular bonds between the main structural components of the polysaccharide complex, the process of maceration and partial structures shells fragmentation polymers themselves. This ensures destruction of intercellular substance, leading to the separation of cells, solubilization hydrolysis products. Electrostatic forces arising due to the functional group having affinity for the metal ions at the micelle surface terminate. The process is accompanied by desorption of ions, molecules associated with non-starch polysaccharides. Experimental studies of morphological parts of wheat showed that under the action of biocatalysts based cellulases distribution of chemical elements in the caryopsis changed. There is a tendency in the distribution of mineral elements, characteristic of grain - the proportion of these elements in the aleurone layer (5) is reduced, and in the endosperm (6) - increases. The relative content of nutrients that are part of metalloenzymes and biologically active compounds increased in the endosperm, where during swelling grain intensified oxidative decomposition processes of high- replacement compounds. The chemical elements which have a high mobility, potassium and sodium are moved from the central portion to the peripheral weevil. Because dietary fiber and phytate found together in the peripheral layers of fiber-rich grains, it is difficult to separate the effects of degradation processes nonstarch polysaccharides and fiber phytate redistribution of polyvalent metal ions **(Torre M. et al., 1991)**.

Distribution of chemical elements in the morphological parts of the grain shows that activation occurred own enzyme systems. This is evidenced by the increase in the relative content of sulfur, which is part of the proteins, enzymes and free amino acids, as well as phosphorus, participating in all the processes of metabolism. Increase in the relative amount of phosphorus, sulfur, potassium, magnesium, selenium in the bud indicates the activation of the synthesis of organic compounds necessary for the construction of the developing plant tissues. Reduction in the relative content of trace elements in the aleurone layer speaks about embryonic germ awakening, intensifying the process of synthesis and migration of enzymes in the endosperm. The preferential increase in the endosperm fraction trace indicates that after 8 hours of soaking wheat germination basic feature consists in the biochemical processes in direction towards hydrolysis. The appearance of selenium, known for their antioxidant properties, morphological parts of grains indicates the incorporation of plant protection from the negative effects of oxidation products - free radicals, peroxides and hydroperoxides.

These data confirm that in the hydrolysis of phytin complexes decompose exogenous phytase, phytin formed with mineral elements: calcium, magnesium, iron, copper and zinc. These chemicals migrate into the endosperm where the basic seed and nutrients included in the modification process and replacement of biological

polymers. These experimental data are in agreement with the findings of previous studies that during imbibition of wheat mineral elements (magnesium, calcium and potassium) redistributed from the aleurone layer and mobilized for the development of seedlings **(Eastwood and Laidman, 1971)**.

CONCLUSION

As a result of a complex enzyme preparation based on phytase (producer *Penicillium canescens*) for the treatment of wheat has been a change of the surface microstructure of grain. Microstructural changes and phytase activity indicator substrate, characterizing the rate of release of phosphate caused enzyme complex composition of the drug and determined the presence of xylanase enzyme. Availability phytin phytase is associated with the degree of degradation of the hemicellulose. It has been established that the release rate of the phosphoric acid substrate is dependent on the composition of the drug and the enzyme complex is determined by the presence of xylanase. When processing enzyme preparation wheat trend in the distribution of mineral elements, characteristic of grain - the proportion of these elements in the aleurone layer decreases, and in the endosperm - increases. These data confirm the results of microstructural studies of chemical analysis.

Thus, studies have shown that phytase - an effective mechanism for regulating mineral nutrient diet. Application of phytase in grain bakery technology will increase the biological value of the product.

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