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Study of Changes in Antioxidant Activity, Microstructure, and Mineral Composition of Nadir Wheat Grain During Preparation for Whole Grain Bread Production

ABSTRACT

Relevance. Whole grain products are becoming increasingly popular in industrialized countries due to their high content of dietary fiber, vitamins, and minerals. Soaking wheat grain is a crucial stage in the production of whole grain bread. To improve the quality and health benefits of whole grain bread, enzyme preparations based on cellulase are used during the grain soaking stage. This biotechnological technique influences the localization and content of key biologically active substances in the grain. New knowledge about the redistribution of chemical compounds within wheat grain during enzymatic hydrolysis will help develop guidelines for optimizing the soaking process.

Methods. The study presents experimental data on the effect of a complex enzyme preparation based on cellulase and sodium selenite, introduced during wheat grain soaking, on changes in the microstructure of the main morphological parts of the grain, antioxidant activity, the content of chemical compounds determining antioxidant activity, and the distribution of mineral elements within the grain kernel.

Results. Soaking Nadir wheat grain in a buffered solution of an enzyme preparation based on cellulase and sodium selenite for 10 hours under optimal conditions for enzyme action results in a modification of the microstructure of the grain's morphological parts. Antioxidant activity increases by 9.3% (DPPH radical inhibition), total flavonoid content (by 0.05%), anthocyanin content (by 0.132%), and glutathione content (by 12 mg%) compared to the grain soaked in water. Biologically active mineral elements and selenium accumulate in the germ after grain fermentation.

Key words: wheat grain, complex enzyme preparation, sodium selenite, antioxidant activity, microstructure, mineral elements

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Исследование изменения показателей антиоксидантной активности, микроструктуры и минерального состава зерна пшеницы сорта Надира при подготовке к производству зернового хлеба

РЕЗЮМЕ

Актуальность. Цельнозерновые продукты приобретают большую популярность у жителей индустриально развитых стран благодаря высокому содержанию пищевых волокон, витаминов и минеральных элементов. Замачивание зерна пшеницы является важнейшей стадией при производстве зернового хлеба. С целью улучшения качества и приобретения полезных свойств зерновым хлебом применяют ферментные препараты на основе целлюлаз на стадии замачивания зерна. Этот биотехнологический прием влияет на изменение локализации и содержания важнейших биологически активных веществ в зерне. Новые знания о перераспределении химических соединений в пределах зерна пшеницы в процессе ферментативного гидролиза позволят разработать основы регулирования процесса замачивания.

Методы. Представлены экспериментальные данные о влиянии комплексного ферментного препарата на основе целлюлаз и селенита натрия, вносимых при замачивании зерна пшеницы, на изменение микроструктуры основных морфологических частей зерна, антиоксидантную активность, содержание химических соединений, определяющих антиоксидантную активность, и распределение минеральных элементов в пределах зерновки.

Результаты. При замачивании зерна пшеницы сорта Надира в буферном растворе ферментного препарата на основе целлюлаз и селенита натрия в течение 10 часов в оптимальных для действия ферментов условиях происходит модификация микроструктуры морфологических частей зерна, увеличиваются антиоксидантная активность зерна на 9,3% ингибирования радикала ДФПГ, содержание суммы флавоноидов (на 0,05%), антоцианов (на 0,132%) и глутатиона (на 12 мг%) по сравнению с вариантом, в котором зерно замачивали в воде. Биологически активные минеральные элементы и селен накапливаются после ферментации зерна в зародыше.

Ключевые слова: зерно пшеницы, комплексный ферментный препарат, селенит натрия, антиоксидантная активность, микроструктура, минеральные элементы

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Introduction/Введение

Diets rich in whole grain products help reduce the risk of several chronic diseases, including Type 2 diabetes, cardiovascular diseases, coronary heart disease, stroke, and certain types of cancer [1–3]. This can be attributed to the high fiber content found in whole grain products compared to refined ones, as well as the synergistic effect of many bioactive molecules present in unrefined cereal products [4–6]. Wheat is one of the most cultivated agricultural crops in the world. Milling wheat grain produces flour that is lower in mineral elements, vitamins, and fiber compared to the whole grain [7–9].

High-grade wheat flour has excellent baking qualities but is deficient in biologically active substances [10–12]. When milling, bran, which consists of the outer layers of the grain, makes up 15% of the wheat grain [13]. Using whole wheat grain in bread production is challenging due to the negative impact of the rough grain shells on the taste and technological properties of the product [14, 15].

The use of whole unpeeled grain in cereal-based products can address many issues, including improving product quality, shelf life, and maintaining consumer health [16].

New products with improved rheology, thanks to the presence of carbohydrate components from the outer layers of the grain, can be introduced to the market as functional foods aimed at preventing various diseases, such as diabetes, cancer, and bacterial infections [17, 18].

The outer layers of cereal grains are plant cell walls, which form a matrix consisting of non-starch polysaccharides: cellulose and hemicelluloses, as well as lignin, minerals, and other components. These are tightly bound together, creating a hydrophobic network with high strength and stiffness [19, 20].

For this reason, the pre-treatment of raw materials is necessary for food technologies, and it represents a critical step in breaking down the natural structure of the plant's cell wall [21–23].

When producing baked goods from whole wheat grain, enzyme preparations based on cellulase are used. The use of enzyme preparations in bread technology improves the organoleptic and physicochemical properties of the product and extends its freshness [24–26].

Enzymatic treatment of wheat grain is carried out during the preparation stage for whole grain bread production [25, 27].

For this, the grain is soaked in a solution of the enzyme preparation and held until it reaches the moisture content (above 40%) necessary for the dispersion process. During this time, the grain swells and germination processes begin [28].

It is known that pre-treatment of the grain leads to the accumulation of biologically active compounds. However, suboptimal conditions during the first stage of germination may lead to the accumulation of harmful products due to the activation of secondary metabolism [29]. Rehydration, during which seeds that were in an inactive state awaken and metabolism begins, is associated with a high level of oxidative stress [30].

The absorption of reactive oxygen species is crucial for germinating seeds and involves non-enzymatic components, mainly associated with the overproduction of antioxidants (such as phenolic compounds) [31]. It is also known that germination moderately increases the total content of polyphenols [32].

Thanks to advances in grain breeding aimed at improving quality, new varieties of colored wheat have been developed. These varieties can address nutritional

challenges by increasing the content of antioxidants, such as anthocyanins, carotenoids, flavonoids, polyphenols, and others. Colored wheat opens a new path for enhancing the nutritional value of grain-based products [33, 34]. Anthocyanins, located in the aleurone layer of the grain, give wheat a blue-violet color [35]. The high nutrient content of colored wheat makes it a promising ingredient for the baking industry [36].

The antioxidant defense system of the grain is enhanced by the addition of selenium in the form of sodium selenite or sodium selenate. This occurs due to an increase in the content of complex selenoproteins and soluble conjugated forms of phenolic acids in germinating seeds, as well as an increase in the activity of glutathione peroxidase and superoxide dismutase [37, 38].

Additionally, Se biofortification during germination can represent an effective strategy for increasing Se concentration in seeds [39].

Thus, in the production of baked goods from whole wheat grain, to obtain a product with enhanced antioxidant properties, one can use grain from colored wheat varieties, which inherently contain anthocyanins and flavonoids, along with carbohydrate-hydrolyzing enzymes and selenium-containing preparations during the grain soaking process in the production of whole grain bread [27, 40, 41].

The aim of the study was to investigate changes in antioxidant activity, microstructure, and mineral composition of wheat grain during preparation for whole grain bread production.

Materials and methods/

Материалы и методы исследования

The object of the study was the grain of purple wheat of the Nadir variety, which has a high content of anthocyanins that determine antioxidant properties. During the soaking stage, a complex enzymatic preparation with cellulolytic activity produced by *Penicillium canescens* was used, with an optimal action pH of 4.5–5.5 and a temperature of 40–45 °C. The preparation contains the following enzymes: cellobiase (activity — 469 U/g), β -glucanase (activity — 803 U/g), and xylanase (activity — 5719 U/g). To enhance the antioxidant activity of bread made from whole unpeeled wheat grain and for biofortification purposes, sodium selenite was added during the grain soaking stage.

The initial moisture content of the wheat grain was 12.0%. Before use in the production of whole grain bread, the grain was freed from various impurities and washed with a large amount of tap water. The powdered enzyme preparation was mixed using a magnetic stirrer with an acetate buffer (pH 5.0) at a concentration of 0.5 g/L and a selenium-containing preparation in powder form at a concentration of 3 μ g / 100 g of grain for 0.4 hours. Then, the grain was soaked in a solution of the cellulase-based enzyme preparation in acetate buffer at pH 5.0, with a grain-to-water ratio of 1:2. Soaking was carried out in a thermostat at a temperature of 40 °C. The rational dosage of the enzyme preparation was determined experimentally through trial baking of whole grain bread. The criteria for this were the organoleptic and physicochemical indicators of the quality of the baked goods.

It was established that the rational dosage of the enzyme preparation is 0.06% of the mass of dry grain solids. The duration of the grain soaking was determined after studying the dynamics of water absorption, which was conducted over 18 hours. It was found that the optimal soaking duration for wheat grain is 10 hours. During this time, the moisture content of the wheat grain reached 42%.

For the study, three variants of wheat grain were taken: native dry grain, control (grain soaked in tap water), and grain soaked in an acetate buffer solution of the complex enzyme preparation and sodium selenite (EP + buffer + Na₂SeO₃). The antioxidant activity of the grain was determined spectrophotometrically in an alcoholic extract. The method is based on determining the percentage inhibition of the DPPH radical (2,2-diphenyl-1-picrylhydrazyl). Optical density was measured using a "Spekord M40" spectrophotometer at a wavelength of 515 nm. The total flavonoid and anthocyanin content was determined using commonly accepted spectrophotometric methods. The method for determining the total flavonoids is based on studying the absorption spectra of the products of the reaction of total flavonoids with a 3% aluminum chloride solution. The total anthocyanins were determined in a 1% hydrochloric acid extract. The optical density of the solutions was measured using an "SF200" spectrophotometer. The glutathione content in the grain was determined using the iodometric method. Microstructural studies were conducted using a JEOL JSM 6390 scanning electron microscope. Energy dispersive spectroscopy (EDS) analysis of the chemical composition of the main components of the sample (Na, P, S, K, Mn, Fe, Mg, Ca, Al, Cr, Co, Ni, Zn, Cu, Se) was performed using energy-dispersive spectroscopy (EDS) in the JEOL JSM 6390 scanning electron microscope system. Cross-sectional samples of the grain were coated with platinum in a tabletop vacuum coater JEC-3000FC and placed on a sample stage coated with carbon dioxide. The microscope resolution is 4 nm at an accelerating voltage of 20 kV. Twenty points from each sample were examined. The local analysis area is 3 mm, and the scanning area is at least 12 μm. Statistical processing was carried out using Statgraphics Centurion XVII software.

**Results and discussion /
Результаты и обсуждение**

It was established that the use of an enzyme preparation for soaking wheat grain in a buffered solution in conjunction with sodium selenite leads to an increase in antioxidant activity of the grain after soaking by 9.3% (DPPH radical inhibition), as well as an increase in the total flavonoid content by 0.05%, anthocyanins by 0.132%, and glutathione by 12 mg% compared to the variant in which the grain was soaked in water. The increase in antioxidant activity, total flavonoid and anthocyanin levels, and glutathione content in the wheat grain during soaking is associated with the awakening of the germ and the initiation of the synthesis processes of biologically active compounds. The acceleration of metabolic processes in wheat grain when treated with a complex enzyme preparation and sodium selenite occurs due to the modification of the biopolymers in the cell wall matrix. This facilitates an increase in the rate of water absorption into the inner layers of the grain and accelerates the hydrolysis of reserve substances.

Figure 1 shows photographs of the germ, the surface of the grain, and the endosperm.

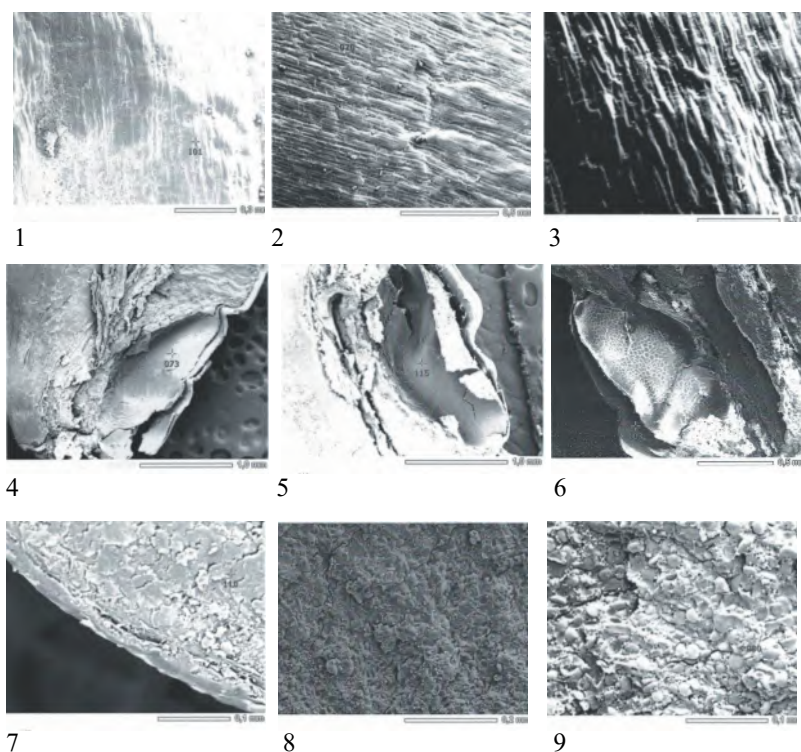
In photographs 1–3, the microstructure of the surface of native grain (1) and the altered microstructure of the grain surface under the action of water (2) and the complex of enzyme and selenium-containing preparations (3) are shown. During soaking, water penetrates into the grain. This is facilitated by the presence of biopolymers in the grain with varying hydrophilicity. They absorb water molecules, resulting in an increase in the volume of the grain by 40–45% over time. Water absorption in the first hours of soaking occurs through non-starch polysaccharides of the fruit envelopes, which have numerous pores and cavities where initial moisture accumulation takes place. Further water absorption is associated with the hydration of the seed coat tissues, aleurone layer, and germ, in which water forms complexes with carbohydrates and proteins, altering the conformation of their molecules. In the final stage, moisture spreads through the tissues in equilibrium proportions [18].

Under the action of the solution of the complex enzyme preparation in acetate buffer, the outer layers of hemicelluloses that lie on the surface of the microfibrils are primarily hydrolyzed. The surface of the grain becomes rougher, with depressions observed in the relief; the diameter and looseness of the fibers change, with polysaccharides swelling in some areas to form broad folds, while in others, microfibrils are exposed and become more accessible for hydrolysis. Due to the modification of the polysaccharides in the surface layers of the grain, the rate of moisture absorption into the endosperm and germ is accelerated, leading to an increase in metabolic processes.

Table 1. Antioxidant activity in wheat grain and the content of substances with antioxidant activity

Experimental Variant	Antioxidant Activity (AOA), % DPPH Radical Inhibition	Total Flavonoid Content, %	Total Anthocyanin Content, %	Glutathione Content, mg%
Native grain	5.8	0.040	0.027	18
Control (water)	9.2	0.060	0.056	40
Enzyme + Buffer + Na ₂ SeO ₃	18.5	0.110	0.188	52

Fig. 1. Microphotographs of the anatomical parts of wheat grain: 1–3 — germ, 4–6 — grain surface, 7–9 — endosperm



Photographs 4–6 show the germ of the wheat grain. Photograph 4 depicts the germ of native wheat grain, which is closely attached to the endosperm. Under the action of water during the soaking of wheat grain for 10 hours, the seed coat detaches in the area of the germ and the tissues crack (5). Treatment of the wheat grain with the enzyme and selenium-containing solution leads to the swelling of the germ, and a hollow space is found between the germ and the endosperm (6).

The microstructure of the endosperm also undergoes changes, as shown in photographs 7–9. In native wheat grain (7), the endosperm presents a dense structure. In the grain treated with the enzyme preparation in conjunction with sodium selenite (9), swelling of the starch granules and protein globules is observed to a greater extent compared to the grain soaked in water (8).

Figure 2 shows microphotographs of cross-sections of wheat grain, where changes in the microstructure of the peripheral parts of the grain can be observed.

Native wheat grain has a shell thickness of 6.26–8.24 μm. After soaking in water for 10 hours, the shell thickness increased to 13.68–19.50 μm, indicating the swelling of non-starch polysaccharides in the peripheral areas of the grain.

After soaking the grain in a buffered solution of the complex enzyme preparation and selenium-containing preparations, the shell thickness of the grain increased to 21.50–28.50 μm. As a result of the action of the enzymes in the preparation, changes occurred in the microstructure of the non-starch polysaccharides, which facilitated easier water penetration and increased accessibility to the crystalline areas within the structure of the cell wall matrix. This confirms the increase in the rate of water absorption into the inner layers of the grain and the acceleration of hydrolysis processes of reserve substances when soaking the grain in the solution of the enzyme preparation in conjunction with sodium selenite.

Using the EDS detector in the JEOL JSM 6390 scanning electron microscope system, the distribution of chemical elements in the anatomical parts of the grain was studied after treatment with the cellulase-based complex enzyme preparation and sodium selenite (Table 2).

It has been established that the primary chemical elements of organic compounds (C + N + O) prevail in the studied anatomical parts of the wheat grain. As a result of soaking and enzymatic hydrolysis of the grain, a redistribution of chemical elements occurs among the anatomical parts. A decrease in the content of mobile elements K, Na, Co, Ni, Zn, and Cu in the surface structures was observed after soaking the grain in a buffered solution of the enzyme preparation and sodium selenite. However, a slight increase in the relative quantity of chemical elements S, Al, P, Mg, and Cr on the surface of the grain was noted. As a result of the enzymatic hydrolysis of the non-starch polysaccharides in

the wheat grain husks, essential chemical elements for the synthesis of protein enzymes and other biologically active compounds (S, P, Fe, Mn, Co, Ni, Cu, Zn, Ca) entered the germ with the flow of water. Their quantity in the germ of the grain treated with the enzyme preparation and sodium selenite is higher compared to the grain soaked in water. This indicates a greater rate of synthetic processes compared to the grain soaked in water for the same duration. Conversely, in the endosperm, biogenic mineral elements predominate in the grain soaked in water. It is likely that, as a result of the slower transport of these elements into the germ, they have not yet accumulated in sufficient quantities to accelerate the synthesis of biologically active compounds.

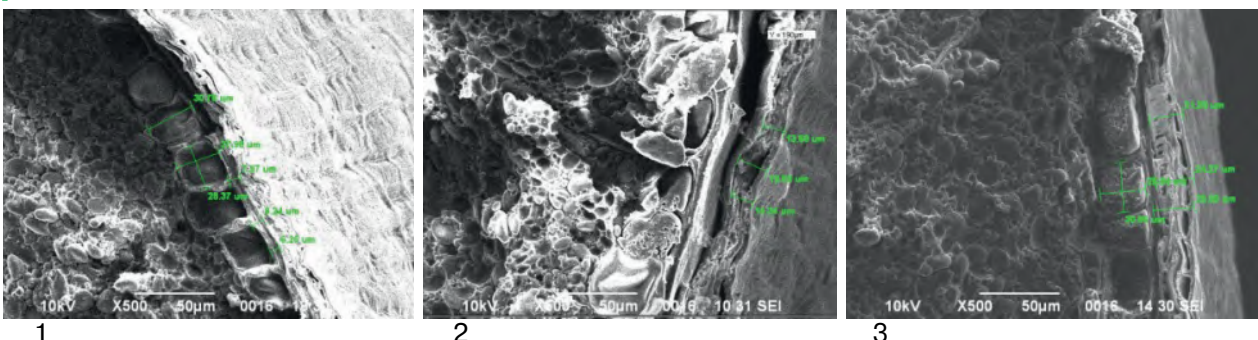
Selenium, as is well known, possesses antioxidant properties, which is one of the reasons for the increased antioxidant activity of wheat grain soaked in a solution of the enzyme preparation and sodium selenite. However, the enrichment of grain products with selenium is of great significance, as selenium has pronounced anticancer effects, not only preventing but also halting the development of malignant tumors. It strengthens cardiac muscles and is necessary for the synthesis of iodinated hormones of the thyroid gland. Selenium is particularly famous as a longevity

Table 2. Distribution of Chemical Elements in the Anatomical Parts of the Grain

Chemical element	Relative content, mass%								
	Surface			The embryo			Endosperm		
	1	2	3	4	5	6	7	8	9
C	33.70	32.23	31.89	33.20	35.72	31.72	33.68	29.50	32.83
N	36.51	32.75	30.11	38.16	17.79	30.27	38.01	25.41	31.93
O	27.97	33.86	36.49	27.34	20.29	36.66	27.09	32.81	34.21
Na	0.09	0.03	–	–	0.10	0.18	0.07	0.01	–
Mg	0.06	0.07	0.21	0.17	–	–	–	0.11	0.02
Al	0.01	0.01	0.09	–	1.07	2.20	–	–	–
P	0.13	0.15	0.19	0.08	0.32	1.78	0.25	1.85	0.01
S	0.12	0.19	0.25	0.22	0.39	2.04	0.33	2.05	0.05
K	0.48	0.34	0.16	0.32	6.01	0.41	0.01	3.03	0.08
Ca	0.15	0.09	0.17	0.40	1.23	5.17	–	–	–
Cr	–	0.01	0.10	0.01	–	–	0.04	0.78	0.07
Mn	–	–	–	–	0.20	0.75	0.11	0.97	–
Fe	–	0.06	0.06	0.01	0.09	0.14	0.06	0.57	–
Co	0.09	0.05	0.03	–	0.19	0.18	–	–	0.06
Ni	0.08	0.04	–	0.02	1.20	3.10	0.12	–	–
Cu	0.02	0.01	–	–	1.20	1.19	0.06	0.43	0.20
Zn	0.08	–	–	–	–	0.23	0.03	–	–
Se	–	–	0.22	–	–	1.24	–	–	–

Note: * 1 — Native grain, 2 — Control (water), 3 — Enzyme + Buffer + Na₂SeO₃.

Fig. 2. Microphotographs of cross-sections of wheat grain: 1 — native grain, 2 — grain soaked in water, 3 — grain soaked in a solution of the complex enzyme preparation and selenium-containing preparations



trace element because it not only protects cell membranes from damage by aggressive forms of oxygen but also actively helps vitamin E, a well-known antioxidant, to fully realize its antioxidant potential [42].

Grain treated with sodium selenite during soaking differs in selenium content. It has been established that selenium concentrates on the surface of the grain, but is found in greater quantities in the germ, where it plays a role in crucial processes of biosynthesis of selenium-containing proteins and other biologically active substances.

Conclusions/Выводы

Soaking Nadir wheat grain in a buffered solution of a cellulase-based complex enzyme preparation and sodium selenite for 10 hours at pH 5.0 and a temperature

of 40 °C in preparation for whole grain bread production leads to changes in the microstructure of the surface, peripheral parts of the grain, germ, and endosperm.

Additionally, antioxidant activity of the grain increases by 9.3% (DPPH radical inhibition), the total flavonoid content increases by 0.05%, anthocyanins by 0.132%, and glutathione by 12 mg% compared to the variant where the grain was soaked in water. Furthermore, there is a redistribution of mineral elements among the anatomical parts of the grain: their content decreases in the surface layers of the grain and increases in the germ, where chemical elements participate in the synthesis of enzymes and other biologically active compounds. Selenium, introduced during the soaking of wheat grain, concentrates on the surface of the grain and in the germ.

All authors bear responsibility for the work and presented data. All authors made an equal contribution to the work. The authors were equally involved in writing the manuscript and bear the equal responsibility for plagiarism. The authors declare no conflict of interest.

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