УДК 633.12:577.1
Код ВАК 3.1.2
https://doi.org/10.32417/1997-4868-2025-25-09-1395-1405

Biochemical indicators of the composition and properties of *Fagopyrum tataricum* (L.) *Gaertn*. grain and changes in some of them during enzymatic hydrolysis

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Abstract. **Relevance.** Tartary buckwheat (*Fagopyrum tataricum* (L.) *Gaertn*) is characterized by high nutritional value and is a source of biologically active compounds (polyphenols, vitamins, microelements), as well as having strong antioxidant activity. **The purpose** is to study the biochemical parameters, antioxidant activity, microstructure and mineral composition of Tatar buckwheat grain and the effect of enzymatic hydrolysis during preparation for use in food technologies on changes in some of its properties. **Methods.** Experimental data are presented that confirm the high nutritional value and antioxidant activity of Tartary buckwheat grain compared to common buckwheat grain. The results of a study are provided on the effect of a complex cellulolytic enzyme preparation – used for enzymatic hydrolysis of the tough structures of the fruit coats – on changes in the microstructure of the grain surface, antioxidant activity, and the fractional composition of proteins. **Results.** Tartary buckwheat grain was found to have a high content of vitamins, microelements, flavonoids, a well-balanced amino acid composition of proteins, and significant antioxidant activity. After enzymatic hydrolysis of Tartary buckwheat grain using the complex enzyme preparation “Celloviridin G20x” at a temperature of 50 °C and pH 5.0 for 28 hours, changes were observed in the microstructure of the grain surface, along with biopolymer modification. Antioxidant activity of the grain increased by 6.2 % in DPPH radical inhibition, and the fractional composition of Tartary buckwheat grain proteins was altered. **Scientific novelty.** The use of enzymatic hydrolysis with the help of complex enzymatic preparations with cellulolytic action allowed for controlled modification of grain husk polysaccharides and opened up prospects for using Tatar buckwheat grain in the production of functional food products.

Keywords: Tartary buckwheat, grain, biochemical properties, antioxidant activity, microstructure, enzymatic hydrolysis

Acknowledgements. The authors express their gratitude to the Russian Science Foundation for financial support of the research conducted within the framework of grant No. 24-26-00259.

For citation: Kuznetsova E. A., Rebezov M. B., Kuznetsova E. Al. Biochemical indicators of the composition and properties of *Fagopyrum tataricum* (L.) *Gaertn*. grain and changes in some of them during enzymatic hydrolysis. *Agrarian Bulletin of the Urals*. 2025; 25 (09): 1395–1405. https://doi.org/10.32417/1997-4868-2025-25-09-1395-1405.

Date of paper submission: 10.07.2025, ***date of review:*** 14.07.2025, ***date of acceptance:*** 19.08.2025.

Биохимические показатели состава и свойств зерна *Fagopyrum tataricum* (L.) *Gaertn.* и изменение некоторых из них при ферментативном гидролизе

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Аннотация. **Актуальность.** Гречиха татарская (***Fagopyrum tataricum*** **(L.)** ***Gaertn***) характеризуется высокой пищевой ценностью, является источником биологически активных соединений (полифенолов, витаминов, микроэлементов) и обладает высокой антиоксидантной активностью. **Цель исследования** – изучение биохимических показателей, антиоксидантной активности, микроструктуры и минерального состава зерна гречихи татарской и влияние ферментативного гидролиза в процессе подготовки к использованию в пищевых технологиях на изменение некоторых его свойств. **Методы.** Представлены экспериментальные данные, подтверждающие высокую пищевую ценность и антиоксидантную активность зерна гречихи татарской по сравнению с зерном гречихи посевной. Приведены результаты исследования влияния комплексного ферментного препарата целлюлолитического действия, применяемого для ферментативного гидролиза жестких структур плодовых оболочек зерна, на изменение микроструктуры поверхности зерна, антиоксидантную активность и изменения фракционного состава белков. **Результаты.** Установлены высокое содержание витаминов, микроэлементов, флавоноидов, сбалансированный аминокислотный состав белков, антиоксидантная активность в зерне гречихи татарской. После проведения ферментативного гидролиза зерна гречихи татарской с использованием комплексного ферментного препарата «ЦелловиридинГ20х» при температуре 50 °С, рН 5,0 в течение 28 часов происходят изменение микроструктуры поверхности зерна, модификация биополимеров, увеличиваются антиоксидантная активность зерна на 6,2 % ингибирования радикала ДФПГ, изменяется фракционный состав белков зерна гречихи татарской. **Научная новизна.** Использование ферментативного гидролиза с помощью комплексных ферментных препаратов целлюлолитического действия позволило провести регулируемую модификацию полисахаридов оболочек зерна и открыло перспективу для использования зерна гречихи татарской в производстве функциональных продуктов питания.

Ключевые слова: гречиха татарская, зерно, биохимические свойства, антиоксидантная активность, микроструктура, ферментативный гидролиз

Благодарности. Авторы выражают благодарность Российскому научному фонду за финансовую поддержку исследований, проведенных в рамках выполнения гранта № 24-26-00259.

Для цитирования: Кузнецова Е. А., Ребезов М. Б., Кузнецова Е. Ал. Биохимические показатели состава и свойств зерна *Fagopyrum tataricum* (L.) *Gaertn.* и изменение некоторых из них при ферментативном гидролизе // Аграрный вестник Урала. 2025. Т. 25, № 09. С. 1395–1405. https://doi.org/10.32417/1997-4868-2025-25-09-1395-1405.

Дата поступления статьи: 10.07.2025, ***дата рецензирования:*** 14.07.2025, ***дата принятия:*** 19.08.2025.

Introduction

The most widely cultivated buckwheat species are common buckwheat (Fagopyrum esculentum Moench) and Tartary buckwheat (*Fagopyrum tataricum* (L.) *Gaertn*). The first species accounts for 90 % of global buckwheat production [1]. Buckwheat grain is used primarily in the form of flour or groats [2–4].

Buckwheat groats are believed to possess high nutritional value and medicinal properties [5; 6]. It is known that buckwheat is gluten-free, making it attractive for developing product lines for individuals with celiac disease. Buckwheat grain contains a well-balanced amino acid composition, with a high concentration of lysine and arginine compared to cereals. It is also rich in dietary fiber, minerals, and vitamins [7–9].

Buckwheat grain contains high levels of antioxidants such as polyphenols and flavonoids [2].

Buckwheat grain is widely used as a raw material for the creation of functional food products containing various phenolic compounds, such as rutin, quercetin, and C-glycosylflavones, which have beneficial therapeutic effects in supporting human health [6].

Tartary buckwheat is traditionally cultivated in mountainous regions of China, Bhutan, northern India, and Nepal. It is also grown in Belgium, Germany, Slovenia, Italy, Bosnia and Herzegovina, Korea, and Japan. In Russia, the use of this buckwheat species has been limited by the lack of cultivars suitable for groat production, due to the tight adherence of the fruit coat to the endosperm in Tartary buckwheat grain. In addition, Tartary buckwheat grain has a bitter taste, caused by the activity of the enzyme rutinosidase, which hydrolyzes rutin into quercetin upon contact with water [10]. The bitterness can be reduced by soaking the grain overnight and draining the water [11].

Tartary buckwheat grain is a rich source of biologically active components such as polyphenolic compounds – particularly rutin and quercetin – soluble and insoluble fractions of dietary fiber, valuable vitamins, microelements, and beneficial proteins, including all eight essential amino acids, the content of which is higher than in traditional cereal grains [12; 13].

Buckwheat grain is characterized by a high starch content, protein with a favorable amino acid profile, and low levels of α-gliadin. The proteins in the grain are of high biological value and are mainly represented by albumin and globulin. In Tartary buckwheat, the content of prolamins ranges from 1.1 % to 1.2 %, and glutelins from 10 % to 12 % [14]. The amino acid composition of Tartary buckwheat remains relatively stable during ripening, in contrast to the changes observed in cereal crops.

Tartary buckwheat also contains a higher amount of lysine (the limiting amino acid) and a lower amount of glutamic acid (a major storage protein amino acid) [15; 16].

Tartary buckwheat grain contains 0.8–2.9 % rutin [17], which is 100 times more than in common buckwheat grain [18].

Tartary buckwheat grain contains three classes of flavonoids: flavonols, anthocyanins, and C-glycosylflavones, which have been reported to possess beneficial properties as dietary components. These compounds exhibit antioxidant, hypocholesterolemic, and antidiabetic effects. It has been shown that the consumption of Tartary buckwheat products alleviated symptoms in patients with type I and type II diabetes. In addition, the consumption of these products led to a reduction in fasting blood glucose levels, glycosylated hemoglobin (GHb), and glycosylated serum proteins, as well as an increase in fasting serum insulin levels [19].

Flavonoids are of particular interest in food technology due to their antioxidant properties and their potential to prevent diabetes, oxidative stress, and neurodegenerative diseases [17].

The hypoglycemic effects of *Fagopyrum tataricum* are widely used in traditional medicine for the treatment of diabetes. Its compounds influence blood glucose levels in diabetic laboratory rats and may prevent insulin resistance [20]. Tartary buckwheat grain significantly reduces blood lipid and cholesterol levels, preventing hyperlipidemia induced by high-fat diets [21]. Extracts from Tartary buckwheat grain inhibit the oxidation of low-density lipoproteins, preventing atherosclerosis by scavenging free radicals, inhibiting endogenous vitamin E, chelating metal ions, and affecting the activity of related enzymes [22]. They may also prevent atherosclerosis by suppressing the secretion of various proinflammatory cytokines or gene expression [23].

Iminosugars from Tartary buckwheat are attracting increasing interest due to their high biological activity as glycosidase inhibitors. When used as a dietary supplement or functional food component, D-fagomine may reduce the risk of developing insulin resistance and excessive weight gain. It has also been found that fagomine possesses strong antihyperglycemic effects [24].

The vitamins present in Tartary buckwheat grain (mg per 100 g) are: B1 – 0.53; B2 – 0.24; B9 (folic acid) – 0.032; PP (niacin) – 4.3; E – 6.65. The mineral elements (mg per 100 g) include: calcium – 70; phosphorus – 298; magnesium – 200; potassium – 380; silicon – 81; iron – 8; zinc – 2.1; manganese – 1.6; copper – 0.64; sulfur – 48 [25].

Thus, Tartary buckwheat grain possesses great potential due to its content of biologically active substances (phenolic compounds and proteins) that promote human health. This fact has increased the demand for buckwheat groats in recent years and attracted the attention of nutrition scientists to more in-depth studies of Tartary buckwheat grain [6; 26; 27].

However, in order to soften the tough fruit coat, increase the bioavailability of the grain's biologically active substances, and eliminate bitterness during the processing of Tartary buckwheat grain for functional food production, it is necessary to develop technological methods that would accelerate the introduction of this valuable crop species into food technologies. One such technological approach is the use of biocatalytic technologies.

The aim of the study was to investigate the biochemical indicators, antioxidant activity, microstructure, and mineral composition of Tartary buckwheat grain, as well as the effect of enzymatic hydrolysis during preparation for use in food technologies on the alteration of some of its properties.

Methods

The objects of the study were Tartary buckwheat grain (*Fagopyrum tataricum* (L.) *Gaertn*) of the Kurab variety and common buckwheat grain (Fagopyrum esculentum Moench) of the Dikul variety, kindly provided by the Buckwheat Breeding Laboratory of the Federal Scientific Center for Legumes and Groat Crops (FGBNU). To modify the polysaccharides of the fruit coats of Tartary buckwheat grain, a complex enzyme preparation "Celloviridin G20x" was used, produced by Trichoderma reesei. The optimal conditions for its action are pH 4.5–5.5 and a temperature of 50 °C. The composition includes cellulobioglucanase (activity – 3522 U/g), β-glucanase (activity – 3084 U/g), and xylanase (activity – 728 U/g).

Tartary buckwheat grain was freed from various impurities and washed thoroughly with tap water. The powdered enzyme preparation was added to a citrate buffer solution (pH 5.0) to a concentration of 0.6 g/L and mixed using a magnetic stirrer for 0.4 hours. The prepared grain was then soaked in the enzyme solution in citrate buffer at a grain-to-buffer ratio of 1:2.5. Soaking was carried out in a thermostat at 50 °C. The soaking duration was determined by studying the water absorption dynamics over 36 hours. It was established that the optimal soaking time for Tartary buckwheat grain was 28 hours. After soaking the grain with the complex enzyme preparation, the grain was rinsed with tap water. The enzymatic hydrolysis of the polysaccharides in the fruit coats of Tartary buckwheat grain followed by water rinsing helps to eliminate bitterness and soften the tough cell walls.

The studies were conducted using dry native grains of the two buckwheat species and grains after enzymatic hydrolysis. The antioxidant activity of the grain was determined by a spectrophotometric method using an alcoholic extract. This method is based on measuring the percentage inhibition of the DPPH radical (2,2-diphenyl-1-picrylhydrazyl). Optical density was measured on a "Spekord M40" spectrophotometer at a wavelength of 515 nm. The total flavonoid content was determined using a standard spectrophotometric method. This method is based on studying the absorption spectra of the products formed by the reaction of flavonoids with a 3 % aluminum chloride solution. The optical density of the solutions was measured on a "SF200" spectrophotometer. Microstructural studies were carried out using a ZEISS EVO LS scanning electron microscope with SmartSEM 5.06 software. Mineral elements were determined by atomic absorption spectrophotometry using a HITACHI 180-80 instrument. The amino acid composition and fat-soluble vitamins were analyzed using an Agilent 1260 Infinity II liquid chromatograph.

Results

Table 1 presents the results of experimental studies on the content of certain biochemical indicators in the composition of Tartary buckwheat grain and common buckwheat grain.

The obtained data on protein and starch content are consistent with those of other researchers. The protein content in Tartary buckwheat grain is slightly lower than that reported for common buckwheat grain [28]. The starch in Tartary buckwheat belongs to the resistant starch group; it is not digested or absorbed in the upper gastrointestinal tract but passes into the large intestine, where it is fermented by microorganisms [16].

Table 1

Some biochemical indicators of buckwheat grain composition

Indicator	***The content of buckwheat in the grain***	
	Fagopyrum tataricum	***Fagopyrum esculentum***
Mass fraction of moisture, %	*8.40*	*9.10*
Protein, %	*9.80*	*13.20*
Starch, %	*57.45*	*63.50*
Amount of flavonoids, %	*4.18*	*0.05*
Vitamins		
C, mg / 100 g	*1.70*	*0.70*
B_6, mg / 100 g	*4.70*	*4.40*
B_3, mg / 100 g	*6.80*	*5.90*
B_1, mg / 100 g	*9.60*	*1.60*
P, mg/g	*1.70*	*0.50*
D_2, μg / 100 g	*21.40*	*6.79*
E, μg / 100 g	*254.32*	*87.62*
Trace elements, mg/kg		
Fe	*77.00*	*45.22*
Mn	*15.60*	*2.95*
Zn	*18.90*	*10.36*
Cu	*4.32*	*2.27*
AOA, % inhibition of the DPH radical	*48.00*	*28.8*

Table 2

Amino acid composition of Tartary buckwheat and common buckwheat grain

Amino acid, mg / 100 g	***Composition***	
	Fagopyrum tataricum	***Fagopyrum esculentum***
Aspartic acid	*1 182*	*1 042*
Glutamic acid	*1 961*	*2 016*
Serin	*502*	*445*
Histidine	*509*	*246*
Glycine	*554*	*448*
Threonine	*507*	*441*
Arginine	*961*	*686*
Alanine	*527*	*558*
Tyrosine	*300*	*224*
Cystine	*74*	*196*
Valin	*475*	*378*
Methionine	*160*	*150*
Phenylalanine	*519*	*425*
Isoleucine	*452*	*342*
Leucine	*678*	*484*
Lysine	*564*	*430*
Proline	*177*	*360*
The sum of amino acids	*10 102*	*8 871*

It was found that Tartary buckwheat grain is characterized by a high content of vitamin E and flavonoids (252.32 µg / 100 g and 4.18 %, respectively), which are among the strongest natural antioxidants. The detected content of microelements in Tartary buckwheat grain indicates that this buckwheat species can be considered a source of iron and zinc in human nutrition. The iron and zinc content in Tartary buckwheat grain is 77.0 and 18.9 mg/kg of absolutely dry weight, respectively.

Data on the composition and content of amino acids in buckwheat grain, depending on origin, show species and varietal differences in amino acid content [15].

Table 2 presents the amino acid composition of the grain of the two buckwheat species.

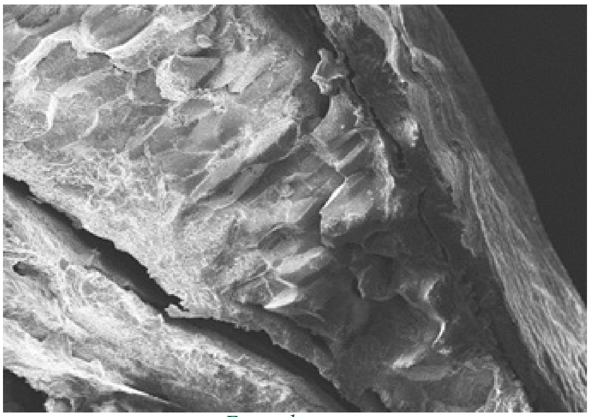
The conducted studies show that the total amino acid content in Tartary buckwheat grain is higher compared to common buckwheat. It was found that the content of essential amino acids – lysine, methionine, phenylalanine, leucine, isoleucine, valine, and threonine – is higher in Tartary buckwheat grain compared to common buckwheat grain. The content of the limiting amino acid lysine in Tartary buckwheat grain is 23.7 % higher than in common buckwheat grain. Common buckwheat grain is characterized by a higher content of cystine, proline, and glutamic acid, indicating a predominance of storage protein amino acids in common buckwheat grain.

The obtained data are consistent with those of other researchers, who showed that both buckwheat species have a balanced amino acid composition with a high proportion of essential amino acids such as leucine and lysine. Tartary buckwheat grain was also found to contain higher levels of these essential amino acids [29].

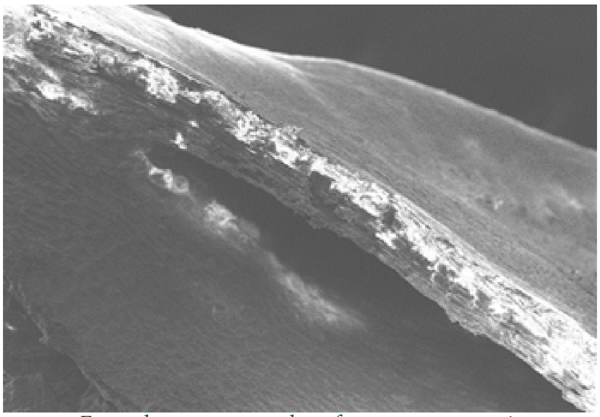
The obtained experimental data indicate that the protein of the Tatar buckwheat grain contains 2 times more proline from essential amino acids and 15 % more glycine compared to the grain of common buckwheat. Proline and glycine are the most important amino acids involved in the synthesis of collagen in the human body. These amino acids play an important role in the functioning of the musculoskeletal system, in ensuring healthy skin, in the process of wound healing, and are also able to balance blood sugar levels [30].

The conducted studies of certain biochemical indicators of Tartary buckwheat grain of the Kurab variety showed that this buckwheat species surpasses the nutritional value of common buckwheat grain of the Dikul variety. To modify the polysaccharides of the fruit coats of Tartary buckwheat grain of the Kurab variety and increase the bioavailability of biologically active substances, enzymatic hydrolysis of the grain was carried out using the complex enzyme preparation “Celloviridin G20x”.

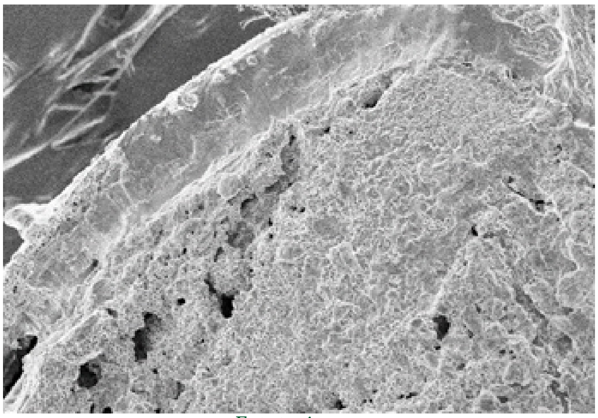
Fig. 1 shows microphotographs of cross-sections of buckwheat grains taken with a ZEISS EVO LS scanning electron microscope, illustrating the effect of the complex enzyme preparation on the outer layers of the grains of the two buckwheat species. The images show that Fagopyrum esculentum grain is characterized by cellulose microfibrils that are less densely packed. After enzymatic hydrolysis, relatively wide voids ranging from 43 to 60 µm were observed between the coats. In contrast, *Fagopyrum tataricum* grain has more tightly adhering and mechanically strong coats, which are difficult to break down.



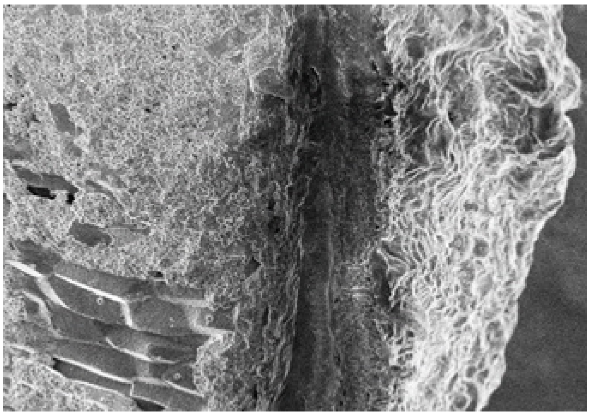
F. esculentum



F. esculentum + complex of enzyme preparation



F. tataricum



F. tataricum + complex of enzyme preparation

Fig. 1. Microphotographs of the edges of cross-sections of native and complex enzyme preparation-treated buckwheat grain of two species, magnification: 250×

As a result of the enzymatic hydrolysis, the coats became more porous, and small voids appeared between the layers (up to 15–25 µm). Microphotographs of the grain surface of the two buckwheat species (Fig. 2) show that the microrelief of the surface changed after treatment with the complex cellulolytic enzyme preparation. Initial hydrolysis of hemicelluloses facilitates increased accessibility of cellulose for hydrolysis and structural modification due to the action of the enzyme preparation.

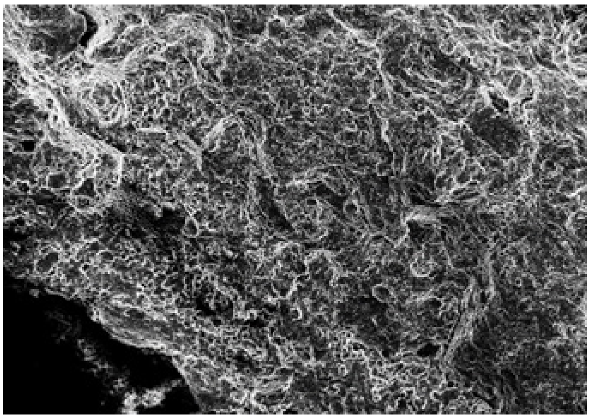
The application of biocatalytic technologies is an approach that allows modification of non-starch polysaccharides forming plant cell walls, thereby enhancing the potential use of seeds with tough coats in food technologies to improve their sensory properties and increase the bioavailability of biologically active compounds.

Fig. 3 shows microphotographs of the endosperm of Tartary buckwheat grain after enzyme treatment during extraction, taken using a scanning electron microscope.

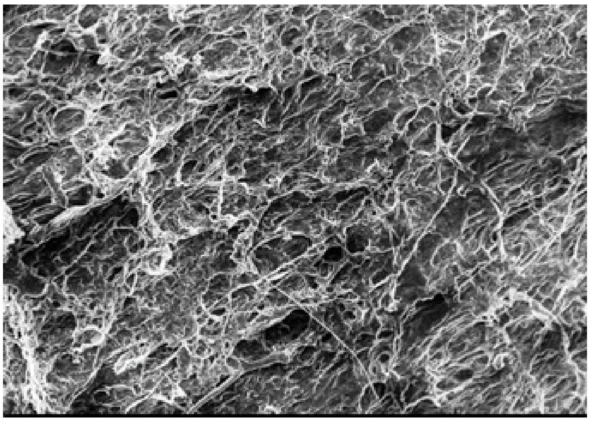
Enzymatic hydrolysis of the polysaccharides in the buckwheat grain coats was carried out in the aqueous phase at a temperature of 50 °C for 28 hours. During this period, a series of processes took place, including moisture filling the pores in the fruit coats, water absorption by hemicelluloses, followed by starch and proteins. As a result of these processes, the conformation of biopolymer molecules changes, the microstructure of protein globules is disrupted, they increase in size and acquire an amorphous, diffuse structure. It is known that heating to 50 °C reduces proteolytic activity and protein solubility in the grain. Water- and salt-soluble protein fractions respond first to the temperature increase, undergoing partial denaturation. Denatured albumins and globulins gain the ability to dissolve in a 0.2 % alkali solution, leading to an increase in the amount of glutenin fraction proteins.

As a result of using cellulolytic enzyme preparations, the protein fraction composition of the grain undergoes somewhat more significant changes. After enzymatic hydrolysis with the complex enzyme preparation "Celloviridin G20x" for 28 hours, the content of the albumin protein fraction in Tartary buckwheat grain increases by 4 %, the globulin fraction by 11 %, and the glutenin fraction by 12 %, while the amount of prolamins decreases by 9.5 %. Additionally, enzymatic hydrolysis leads to a 6.2 % increase in antioxidant activity measured by DPPH radical inhibition, and the total flavonoid content rises by 0.4 %.

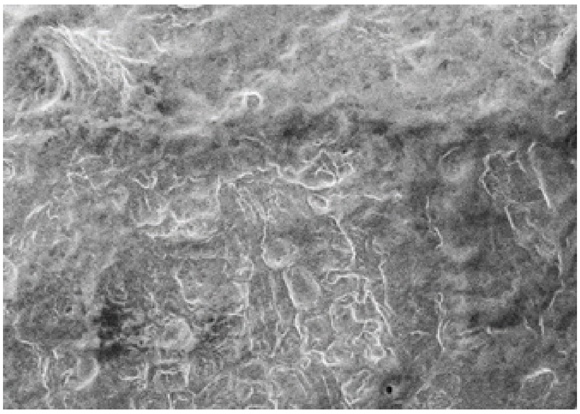
Tartary buckwheat grain prepared using biocatalytic technologies can be used in food technologies to enrich products with functional ingredients.



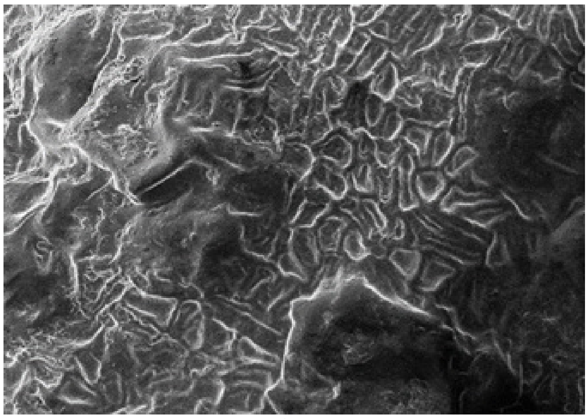
F. esculentum



F. esculentum + complex enzyme preparation

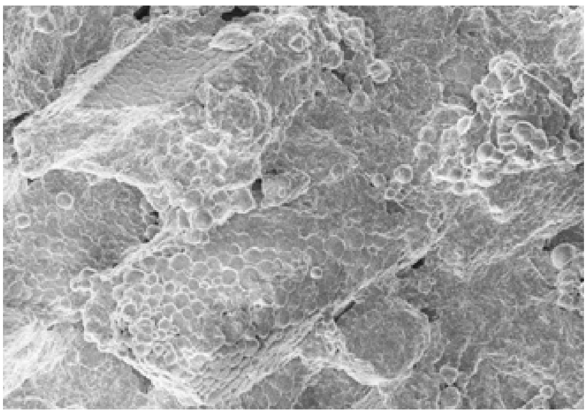


F. tataricum



F. tataricum + complex enzyme preparation

Fig. 2. Microphotographs of the surface of native and complex enzyme preparation -treated buckwheat grain of two species, magnification: 500×



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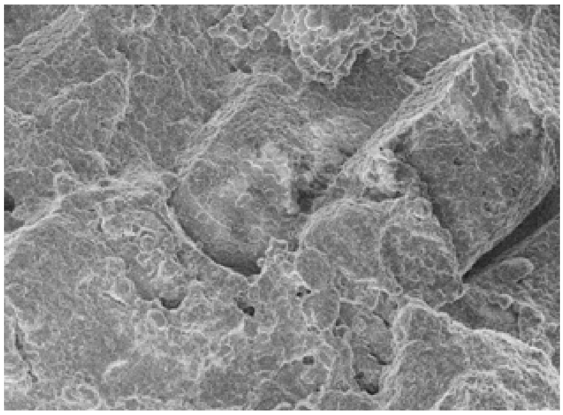

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Fig. 3. Microphotographs of the surface of the endosperm of Tartary buckwheat grain (1 – native grain, 2 – treated with enzyme preparation), magnification: 1000×

Discussion and Conclusion

The determination of certain biochemical composition indicators of Tartary buckwheat grain of the Kurab variety showed that, despite a slightly lower protein content compared to common buckwheat grain of the Dikul variety, the amino acid composition of the proteins is more balanced. The amino acid profile of Tartary buckwheat grain shows an increase in essential and functional amino acids and a decrease in the amino acids of storage proteins compared to common buckwheat grain. The vitamin E content in common buckwheat grain is three times lower, and the total flavonoid content is 100 times lower than in Tartary buckwheat grain.

The antioxidant activity of Tartary buckwheat grain is also high, with 48.00 % inhibition of the DPPH radical, which is 1.7 times higher than that of common buckwheat grain. The content of vitamin B1 in Tartary buckwheat grain is six times higher, vitamin C is 2.5 times higher, and vitamin D2 is three times higher than in common buckwheat grain. Additionally, Tartary buckwheat grain can be considered a source of iron, zinc, and manganese.

The obtained results demonstrate the promising potential of using Tartary buckwheat grain in biotechnology and food technologies as a source of biologically active substances and antioxidants. The application of enzymatic hydrolysis in the preparation of Tartary buckwheat grain for use as a functional ingredient in food technologies shows that the cellulolytic enzyme preparation alters the grain's microstructure.

Modification of the polysaccharides in the fruit coats of Tartary buckwheat grain leads to a reduction in their hardness, detachment from the endosperm, and changes in the microstructure of the protein globules in the endosperm. After enzymatic hydrolysis, the protein fraction composition of Tartary buckwheat grain shifts towards an increased amount of the biologically active globulin fraction and elevated levels of albumins and glutenins.

Furthermore, antioxidant activity increases by 6.2 % in terms of DPPH radical inhibition, and the bitterness characteristic of Tartary buckwheat grain disappears.

Thus, Tartary buckwheat grain has high potential for use in food technologies, and the application of enzymatic hydrolysis using a complex cellulolytic enzyme preparation makes it possible to obtain a functional ingredient based on Tartary buckwheat grain with excellent sensory characteristics and beneficial properties for improving consumer health.

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