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Bread made from *Triticum dicoccum* grain with buckwheat starter culture as a source of valuable nutrients

Elena Kuznetsova, Maksim Rebezov, Zhyldyz Irmatova, Viktoria Simonova, Elena Kuznetsova, Sukhrab Makhmudov, Sanavar Azimova, Damira Tattibayeva

ABSTRACT

This study aimed to evaluate the nutritional value of bread prepared from *Triticum dicoccum* grain on thick buckwheat sourdough. Products made from *Triticum dicoccum* grain are attracting significant interest among consumers and researchers due to their higher nutritional value, unique taste and aroma, and beneficial properties. Our data showed that the studied variety of Triticum dicoccum grain had a higher protein content than commercial wheat. The quantity and quality of gluten in *Triticum dicoccum* grain are inferior to those of commercial wheat (*Triticum aestivum*). The ratio of gliadin to glutenin in emmer was higher than in common wheat. This indicates that to obtain high-quality bread from *Triticum dicoccum* grain, technological methods are necessary. *Triticum dicoccum* grain has hard, tightly adhering husks. Therefore, to soften the husks and improve bread quality, the grain was pre-treated with an enzyme preparation containing cellulase, β-glucanase, and xylanase. Fermentation of the grain led to changes in the microstructure of the surface and cross-sections. After fermentation under optimal enzyme conditions, the *Triticum dicoccum* grain was dispersed. Thick buckwheat sourdough was prepared using flour from whole-ground buckwheat grain and kefir grain culture Lc3/P1/Ac1. The kefir grain culture contained the following microorganisms: *Lactococcus lactis, Lactococcus cremoris, Leuconostoc dextranicum, Lactobacillus bulgaricus, Lactobacillus acidophilus, Lactobacillus fermenti, Acetobacter aceti, Saccharomyces cerevisiae, Saccharomyces lactis*. Additionally, no baker's yeast was added. Experimental data show that the sourdough exhibits high fermentative activity. To improve the bread's physicochemical properties, 4% dry wheat gluten was added. It was established that the optimal amount of thick buckwheat sourdough is 50% of the mass of dispersed *Triticum dicoccum* grain. The resulting bread possessed good sensory, physicochemical, and nutritional properties. The bread was found to contain higher levels of the essential amino acids lysine, phenylalanine, leucine, and isoleucine, methionine, and valine, as well as vitamins B6, B2, B3, B1, PP, and E, and the trace elements Fe, Mn, Zn, Cu, Ni, and Co. The antioxidant activity was 3.2 times ($p<0.01$) higher than that of bread made from commercial Triticum aestivum grains. Bread made from Triticum dicoccum grains, when baked with a thick buckwheat starter culture, can expand the range of functional bread types with potential beneficial properties.

Keywords: *Triticum dicoccum* grain, buckwheat sourdough, bread, chemical composition

INTRODUCTION

In recent years, it has been demonstrated that consuming whole-grain products reduces the risk of several chronic diseases, including type 2 diabetes, cardiovascular disease, coronary heart disease, stroke, and certain types of cancer **[1]**, **[2]**, **[3]**, and **[4]**. It has been reported that a lack of whole grains in the diet is a major factor contributing to reduced life expectancy **[5]**, **[6]**, and **[7]**. These effects are explained by the higher content of dietary fiber present in whole-grain products compared to refined ones, as well as by the synergistic effect of numerous plant-derived bioactive molecules abundantly found in unrefined cereal-based foods **[8]**, **[9]**, **[10]**, and **[11]**.

The phytochemical compounds in whole-grain products include several classes of compounds, among them alkaloids, coumarins, phenolic acids, polyphenols, and terpenoids **[12]**, **[13]**, and **[14]**, which may exert beneficial health effects through several mechanisms, including modulation of cell differentiation and gene expression **[15]**, **[16]**, **[17]**, and **[18]**.

Due to the growing consumer interest in natural, unrefined products made from ancient wheat varieties – owing to their higher nutritional value, unique taste, aroma, and associated health benefits **[19]**, **[20]**, **[21]**, and **[22]** – researchers are increasingly turning to the study of the potential use of ancient wheat species such as *Triticum monococcum*, *Triticum dicoccum*, and *Triticum spelta* in food technologies.

The main value of these species lies in their ability to produce high yields on poor soils, their low fertilizer requirements, adaptability to various conditions, and resistance to stress and phytopathogens. Nutritionists consider them valuable for developing specialized products with improved organoleptic and nutritional properties, despite challenges associated with functionality, primarily due to the low quality of gluten **[23]**, and **[24]**. Some studies have identified high levels of bioactive or functional compounds in ancient wheat varieties, indicating their enhanced health benefits and positive physiological effects **[25]**, and **[26]**.

The nutritional value of *Triticum dicoccum* grain is mainly determined by its high protein content (18–23%) and the overall proportion of essential amino acids in the protein **[27]**, and **[28]**. Grain starch is predominantly composed of resistant fractions, leading to slower carbohydrate absorption **[29]**, and **[30]**. Indigestible resistant starch is one factor that enhances the functionality of food products **[31]**. According to recent studies, ancient wheat is a potential source of various health-promoting compounds, including alkylresorcinols, phenolic acids, and carotenoids **[32]**.

The grains of *Triticum dicoccum* contain a hard hull that closely adheres to the aleurone layer. *Triticum dicoccum* is used worldwide to produce traditional foods such as roasted flakes, breakfast cereals, pancakes, porridges, baby food, and local types of pasta. It has been noted that Triticum dicoccum wheat can be used for bread-making, though the bread is of lower quality compared to that made from traditional wheat varieties **[33]**. It has been established that although whole-grain bread generally has a smaller volume, replacing 30–50% of whole-grain flour with regular wheat flour significantly increases the volume, improves crumb structure and elasticity, and results in higher organoleptic evaluation **[34]**. In another study, bread baked from 100% emmer flour showed lower overall and specific volume, as well as an open, coarse, and granular texture. Formulations containing 15% emmer flour were found to be the most acceptable in terms of organoleptic properties **[35]**.

The addition of larger amounts of sprouted emmer flour significantly improved both the functional properties and the nutritional value of bread. The use of small amounts of this flour improved the final product **[36]**.

In recent years, there has been a growing trend toward using ancient wheat varieties for sourdough bread-making due to their nutritional value and distinctive flavors **[37].** In the scientific work of Getman I. A. and Mikhonik L. R., the possibility of using buckwheat flour in the nutrient mixture of bakery sourdoughs was examined. The addition of 10–15% sourdough relative to the total flour amount shortened the technological process, improved the bread's taste and aroma, and increased its nutritional value **[38]**. In the study by **[39]**, the effect of adding buckwheat sourdough to wheat bread at 10% and 20% concentrations was investigated. The use of buckwheat sourdough significantly influenced the rheological properties of wheat dough, increasing its resistance to deformation and maximum viscosity. The inclusion of buckwheat sourdough improved the overall quality of the final product. The addition of 10% buckwheat sourdough increased the specific volume of bread by approximately 20% and significantly reduced crumb hardness. The use of buckwheat sourdough also helped extend the shelf life of rye bread **[40]**.

Buckwheat grain contains a well-balanced chemical composition, including protein with an amino acid profile characterized by high concentrations of lysine and arginine compared to cereals **[41]**, and **[42]**. Buckwheat grain exhibits a high level of antioxidant activity due to its flavonoid content **[43]**. Rutin, quercetin, and flavone C-glycosides have been detected in buckwheat grain **[44]**. It has been found that in wheat bread containing buckwheat flour, the overall antioxidant activity increases with higher proportions of buckwheat flour, and the rutin content in such bread ranged from 7.76 to 26.90 mg/kg **[45]**, **[46]**, and **[47]**. Despite the large number of published research results, the literature provides no information on the use of whole-grain Triticum dicoccum in bread technology. There is also no information about the use of symbiotic starter culture based on kefir mushrooms and unpeeled buckwheat grains in baking. This is the first study to combine enzymatic hydrolysis of Triticum dicoccum with a thick buckwheat sourdough prepared from kefir grain culture.

Scientific Hypothesis

The scientific hypothesis is that bread made from *Triticum dicoccum* grain on thick buckwheat sourdough possesses improved properties and nutritional value compared to bread baked from *Triticum aestivum* grain.

Objectives

To develop technological solutions for the use of enzymatic processing of *Triticum dicoccum* grain before dispersion, thick buckwheat sourdough, and dry wheat gluten in bread preparation to obtain a product of high quality and nutritional value.

MATERIAL AND METHODS

Samples

Samples description: We analyzed the technological quality parameters of emmer wheat (*Triticum dicoccum*) of the Runo variety and compared them with winter wheat (*Triticum aestivum*) of the Moskovskaya 39 variety. Buckwheat (*Fagopyrum esculentum*) of the Dikul variety was used to prepare a thick buckwheat sourdough for bread-making.

Sample collection: The samples were collected and stored at room temperature.

Samples preparation: The samples were collected and freed from visible impurities, then rinsed with plenty of tap water. For the examination, 1 kg of the average sample was taken from each sample.

Number of samples analysed: 16 grain samples were analyzed to determine the essential nutrient content of *Triticum dicoccum* grain and *Triticum aestivum* grain. 12 samples of the finished bread were analyzed.

Chemicals

All chemical reagents were supplied by Merck (Germany).

Animals, Plants and Biological Materials

The study aims to analyze the technological quality indicators of spelt (*Triticum dicoccum*) variety Runo in comparison with winter wheat (*Triticum aestivum*) variety Moskovskaya 39.

Instruments

Homogenizer 1094 disperser (Tecator, Sweden), mill SM 200 (Retsch, Haan, Germany), proofing cabinets PRSh-1 and PRSh-11 (Techno-Sib, Russia), amino acid analyzer BIOCHROM (Biochrom Ltd., UK), “Milichrom-5” instrument (ZAO “Nauchpribor,” Russia), UniChrom software (ZAO “Nauchpribor,” Russia), Hitachi 170-70 (Japan), electron microscope JEOL JSM 6390 (JEOL, Japan), IDK-1M device (Biophyspribor, Ukraine).

Laboratory Methods

The buckwheat grains were first milled into flour using an SM 200 mill (Retsch, Haan, Germany) to obtain particles smaller than 0.35 mm. Proofing and baking were conducted in a laboratory oven with proofing cabinets PRSh-1 and PRSh-11 (Techno-Sib, Russia). Four hours after baking, the bread was analyzed for organoleptic and physicochemical parameters. The content of crude protein, starch, fiber, and fat was determined according to the methods described by Yermakov **[48]**. The content of free and protein-bound amino acids was determined after hydrolysis of suspensions in sealed ampoules with 6 N HCl for 24 hours, using ion-exchange chromatography with electrochemical detection on a BIOCHROM amino acid analyzer (Biochrom Ltd., UK). Antioxidant activity was measured spectrophotometrically in an alcoholic extract according to Silva et al. **[49]**, based on the percentage inhibition of the DPPH radical (2,2-diphenyl-1-picrylhydrazyl). Optical density of the solutions during interaction was measured according to specification M40 (Carl Zeiss Industriel Messtechnik GmbH, Germany) at 515 nm. The Trolox equivalent was the standard for DPPH calibration. Vitamin content was determined by reversed-phase HPLC using a “Milichrom-5” instrument (ZAO “Nauchpribor,” Russia) with a separon-SGX-C18 analytical column (internal diameter 2 mm, length 70 mm) and UniChrom software (ZAO “Nauchpribor,” Russia). A water extract of buckwheat grain (pH 3) was used; the eluent composition was acetonitrile: aqueous solution of sodium heptanesulfonate and monopotassium phosphate (pH 3.0, 20/80), mobile phase flow 1 $cm^3 \cdot min^{-1}$, isocratic elution, detection at 200–400 nm, analysis time 12–25 min, sample volume 2–6 µL. HPLC determined the total phenolic compounds on the same “Milichrom-5” instrument. An alcoholic extract of buckwheat grains was used; the eluent composition was acetonitrile: aqueous solution of trifluoroacetic acid (pH 2.5, 15/85), isocratic elution, analysis time 12–25 min, sample volume 2–6 µL. The content of trace elements was determined after mineralization in a 1:1 mixture of 10% hydrochloric and nitric acids using atomic absorption spectroscopy on a Hitachi 170-70 instrument (Japan). Microstructural studies were performed using a scanning electron microscope JEOL JSM 6390 (JEOL, Japan). Pre-prepared samples were placed on a copper stub, coated with a layer of platinum using a JEOL JEE 44E vacuum evaporator, and scanned with the scanning electron microscope at an accelerating voltage of 15 kV. Gluten quality was determined using an IDK-1M device (Biophyspribor, Ukraine).

Description of the Experiment

Study flow: For the fermentation of emmer wheat grains, a dry, complex enzymatic preparation containing cellulase, β-glucanase, and xylanase (produced by Trichoderma reesei) was emplyed (Agroferment, Russia). The enzymes had the following activities: cellulase – 3522 U/g, β-glucanase – 3084 U/g, xylanase – 728 U/g. The powdered enzyme preparation was mixed with citrate buffer (pH 4.5) using a magnetic stirrer for 0.5 h at a concentration of 0.6 $g \cdot L^{-1}$, after which the emmer wheat grains were added to the solution. A solution of the enzyme preparation in water was used as the control sample. Whole emmer wheat grains were incubated in the enzyme solution at a 1:2 ratio for 10 hours at 50 °C in a thermostat. The hydrolysis conditions (t = 50 °C, pH 4.5) were optimal for enzyme activity. After incubation, enzyme inactivation was not performed. During the 10-hour fermentation, the moisture content of the emmer wheat grains reached over 42%, which is optimal for grain dispersion in cereal bread technology. At the end of the enzymatic hydrolysis period, the grains were rinsed with running water at 18–20 °C for 5–10 minutes.

Bread from fermented emmer wheat grains was prepared using thick buckwheat sourdough. The ground grain mass was mixed with thick grain sourdough, 4% dry wheat gluten, and 1.5% table salt. The thick buckwheat sourdough was prepared using the kefir grain culture Lc3/P1/Ac1. The kefir grain culture contains the following microorganisms: *Lactococcus lactis, Lactococcus cremoris, Leuconostoc dextranicum, Lactobacillus bulgaricus, Lactobacillus acidophilus, Lactobacillus fermenti, Acetobacter aceti, Saccharomyces cerevisiae,* and *Saccharomyces lactis*.

The preparation of thick buckwheat sourdough was carried out according to the scheme shown in Figure 1.

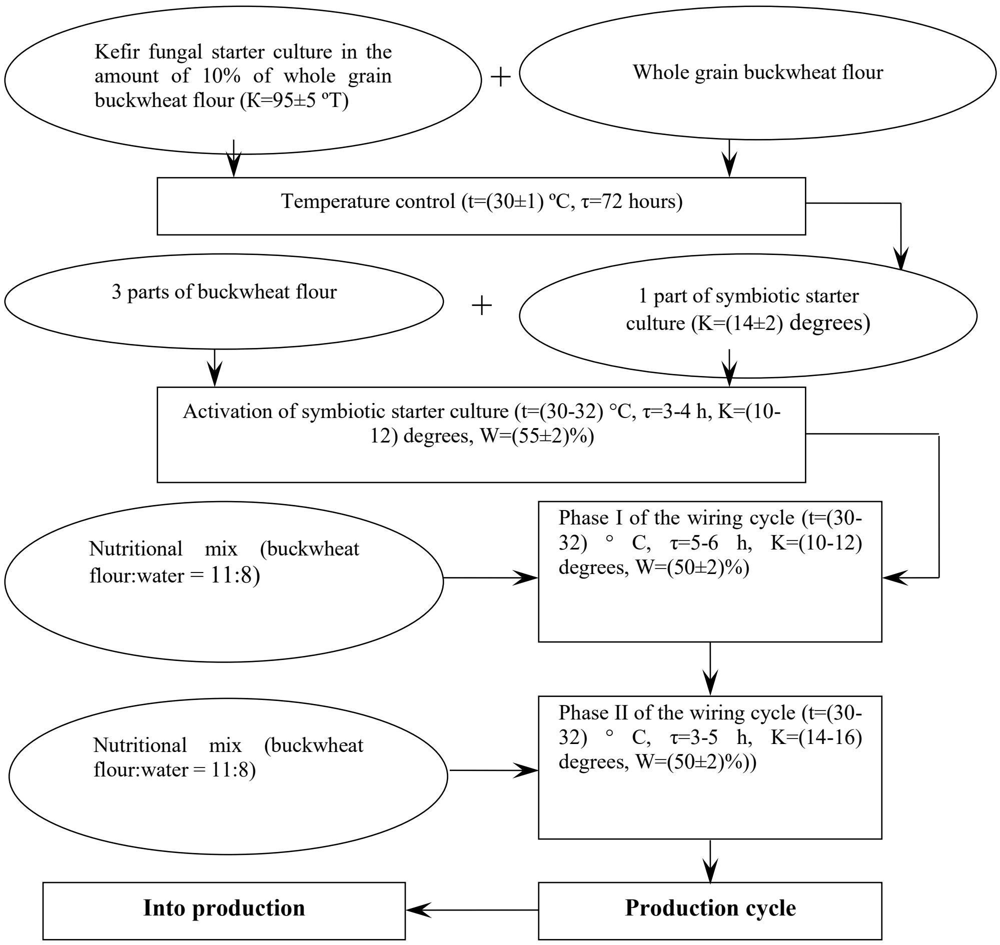


Figure 1 The scheme of cooking thick buckwheat starter culture.

To determine the optimal amount of sourdough in the bread-making process from fermented emmer wheat grains, sourdough was added at 20, 30, 40, 50, 60, and 70% of the mass of dispersed grains.

Dough mixing was carried out for 15 minutes. The dough fermentation lasted 2.0–2.5 hours at a dough temperature of 32–33 °C. The final dough acidity at the end of fermentation reached 9–10°. The prepared dough was divided into 350 g portions, placed in baking molds, and allowed to proof. The proofing time was 35 minutes at 35 °C with a relative humidity of 75–80%. Baking of the bread was carried out for 30–35 minutes at 200–220 °C.

Quality Assurance

Number of repeated analyses: Eight samples of each raw material and three samples of the finished product were taken for analysis.

Number of experiment replication: The samples were analyzed three times.

Reference materials: The equipment manufacturer provided instructions for checking the equipment's performance.

Calibration: Each instrument was calibrated before each experiment, and calibration checks were performed regularly to ensure measurement accuracy. Each instrument was calibrated before each experiment, and calibration checks were performed periodically to ensure the accuracy of measurements.

Laboratory accreditation: The experiments were performed in a laboratory accredited according to the international standard ISO 17025.

Data Access

The data supporting the findings of this study are not publicly available.

Statistical Analysis

Statistical processing of the obtained data was performed on a personal computer using Microsoft Excel 2019 (Microsoft, USA) and Statistica 13.0 (TIBCO Software Inc., USA). The arithmetic mean and the standard error of the arithmetic mean were determined. The Student's t-test was used to assess the statistical significance of the differences in the averages. A preliminary check for normality was carried out before applying the t-test. The differences were considered statistically significant at the $p<0.05$ level.

RESULTS AND DISCUSSION

In some countries, traditional foods are prepared from *Triticum dicoccum*. It is considered rich in bioactive compounds, and the body digests its starch slowly. However, the content and composition of bioactive compounds may vary depending on geographic location, seasonal fluctuations, wheat varieties, and analytical methods.

Table 1 presents the main biochemical and technological parameters of *Triticum dicoccum* and *Triticum aestivum* grains.

Table 1 Essential nutrient content of *Triticum dicoccum* grain and *Triticum aestivum* grain.

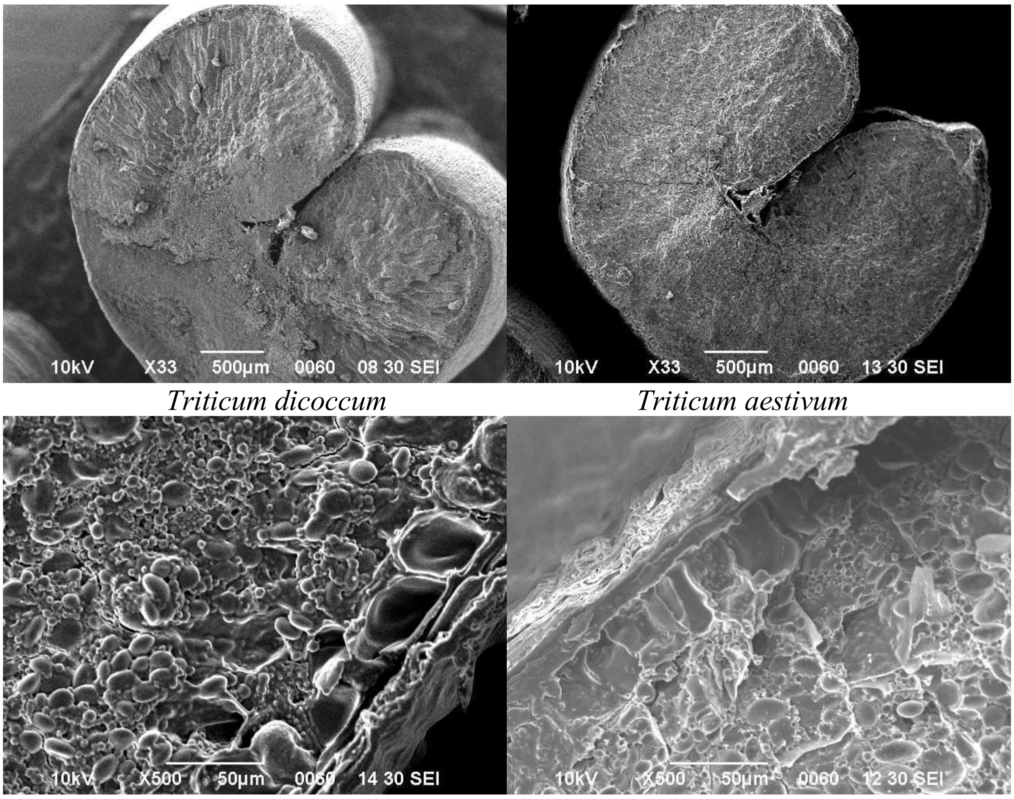
Component	***Triticum dicoccum*** **(n=8)**	***Triticum aestivum*** **(n=8)**
Raw protein, (%) *	14.0±0.18***	12.8±0.16
Starch, (%)*	59.4±0.46***	63.2±0.38
Fat, (%)*	1.80±0.02**	1.90±0.02
Mass of raw gluten, g.100g^{-1} (t/p)	20.4±0.21	28.0±0.25
Instrument reading IDK-1M, unit. (t/p)*	78.0±0.52***	65.0±0.48
GLI / GLU	1.86	1.50

Note: ** $p<0.01$; ***$p<0.001$ (t - student's criterion, p - level of significance)

Our data showed that the protein content in the grain of the studied *Triticum dicoccum* variety was 9.4% higher than that of commercial wheat. The revealed differences were statistically significant ($p<0.001$). Additionally, the gliadin-to-glutenin ratio in emmer wheat was higher than in common wheat. The high protein content in *Triticum dicoccum* grain is consistent with other studies **[50]**. It was found that the gliadin-to-glutenin ratio in common wheat is lower than in emmer wheat. Achieving optimal dough characteristics requires a proper balance between gliadin, which influences dough viscosity, and glutenin, which affects dough strength and elasticity **[51]**.

The gluten content and quality in *Triticum dicoccum* are lower than those in *Triticum aestivum*, but these parameters are crucial for obtaining good bread volume **[23]**.

Figure 2 shows microphotographs of the cross-sectional surface of the *Triticum dicoccum* grain. The hulls are tightly adherent to the endosperm and embryo, whereas in common wheat, voids are observed between the hulls and the internal grain structures. These data are consistent with reports that *Triticum dicoccum* grains have hard hulls closely attached to the aleurone layer **[33]**, **[52]**. The grain surface exhibits a characteristic primary relief formed by parallel cellulose fiber structures coated with non-starch polysaccharide derivatives. The microstructure of the endosperm in *Triticum dicoccum* and *Triticum aestivum* grains is identical. Starch granules of various sizes and shapes are embedded in the protein matrix.



Triticum dicoccum *Triticum aestivum*

Figure 2 Microphotographs of cross-sectional structures of the grain of *Triticum dicoccum* and *Triticum aestivum*. Increase x33 and x500 zoom.

To produce bread from emmer wheat grain with high sensory quality, we propose modifying the hull structure of *Triticum dicoccum* using a complex enzymatic preparation based on cellulase and hemicellulase.

Figure 3 shows microphotographs of the surface of the hulls of native *Triticum dicoccum* grains and grains after enzymatic hydrolysis.

Kuznetsova et al. reported **[53]**, **[54]** changes in the surface microstructure of wheat, rye, and triticale grains under the action of complex enzymatic preparations based on cellulases. In native *Triticum dicoccum* grains (/1/), the surface exhibits the characteristic relief of seed hulls, characterized by parallel cellulose microfibrils overlaid with surface layers of hemicelluloses. As a result of enzymatic hydrolysis (/2/), the hemicellulose coverings are degraded, the cellulose microfibrils become accessible to enzyme action, and undergo structural modification.

The cross-section of native grains (/3/) showed that the seed hulls of *Triticum dicoccum* are tightly adherent to the endosperm. After enzymatic hydrolysis, a hollow space of 75–100 µm forms between the hulls and the internal grain structure (/4/). Due to these changes, grains are more easily dispersed, producing the grain mass used in cereal bread production.

An important technological approach to improving bread quality from *Triticum dicoccum* is the use of sourdough. Fermented baked products, thanks to sourdough, have greater specific volume, a softer and more elastic texture, and a longer shelf life compared to unfermented products. The use of sourdough improves flavor, texture, and stability of baked goods. Compared with pure yeast, sourdough with lactic acid bacteria as the dominant microbial flora attracts research interest for improving the overall quality of whole-grain products **[55]**, and **[56]**.

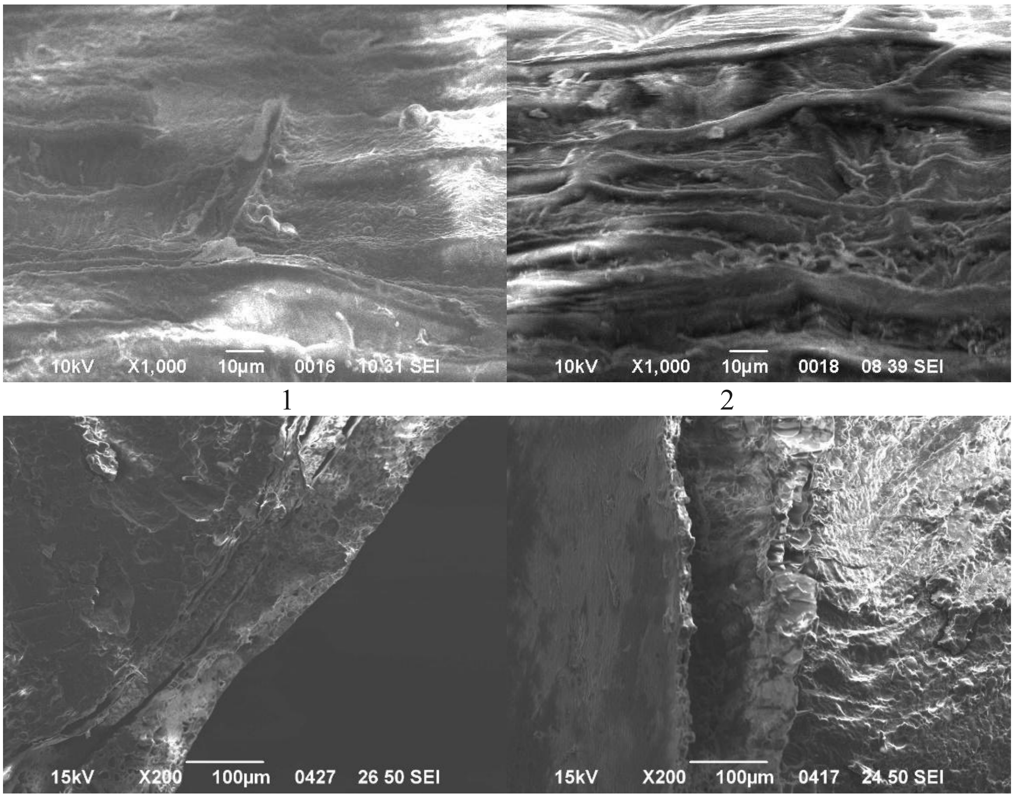


Figure 3 Microphotographs of superficial structures and cross-sections of the grain of *Triticum dicoccum* after enzymatic hydrolysis (1, 3 – control grain; 2, 4 - grain after enzymatic hydrolysis). Increase x1000 and x200 zoom.

In this study, a thick buckwheat sourdough was developed, prepared using flour from whole buckwheat (*Fagopyrum esculentum*) grains. Table 2 presents selected compositional parameters of the whole buckwheat flour.

Table 2 Some indicators of the composition of wholegrain buckwheat flour.

Component	Content
Raw protein, %	13.20
Starch, %	63.50
Vitamins	
B_6, mg/100g	4.40
B_3, mg/100g	5.90
B_1, mg/100g	1.60
P, mg/g	0.50
E, mcg/100 g	87.62
Trace elements, mg/kg	
Fe	45.22
Mn	2.95
Zn	10.36
Cu	2.27

The characteristics of the thick buckwheat sourdough are presented in Table 3. Experimental data indicate that the sourdough exhibits high fermentative activity, even though the fact that the yeast content in the sourdough is significantly lower than that of bacteria. This can likely be explained by the heterofermentative lactic acid bacteria in the kefir grain culture Lc3/P1/Ac1, which produce gas at rates comparable to yeast.

Table 3 Characteristics of the Thick Buckwheat Sourdough.

Indicators	Value
Mass fraction of moisture, %	49.93
Titrated acidity, degrees	15.0
Fermentation activity, min	7
Lifting force, min	45
Quantity of microorganisms in the semi-finished product:	
In total	123×10^6
Yeast	8×10^6
Bacteria	115×10^6

Sourdough based on kefir grains is a good alternative to baker's yeast. Bread made with kefir grains is similar in quality to traditional sourdough bread. The crumb retains moisture better, has a firmer structure, higher acidity, improved flavor and aroma according to consumer evaluation, and maintains freshness for a more extended period **[57]**.

The data obtained during the determination of the gas-forming ability of the microorganisms in the starter culture are shown in Figure 4.

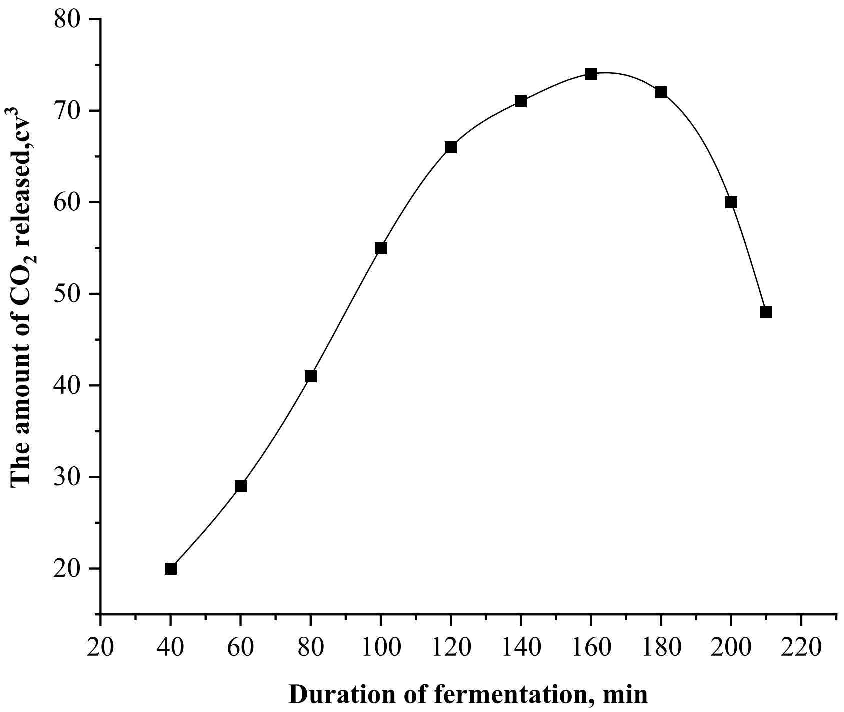


Figure 4 Dynamics of changes in the amount of carbon dioxide released during fermentation of starter cultures.

The amount of sourdough significantly affects the technological parameters of dough processing and the quality of bread. Therefore, the optimal amount of sourdough was determined experimentally. Data on the physicochemical quality parameters of the products are presented in Table 4.

Table 4 Effect of Different Doses of Thick Buckwheat Sourdough on the Quality Parameters of Whole-Grain from *Triticum dicoccum* Baked Products.

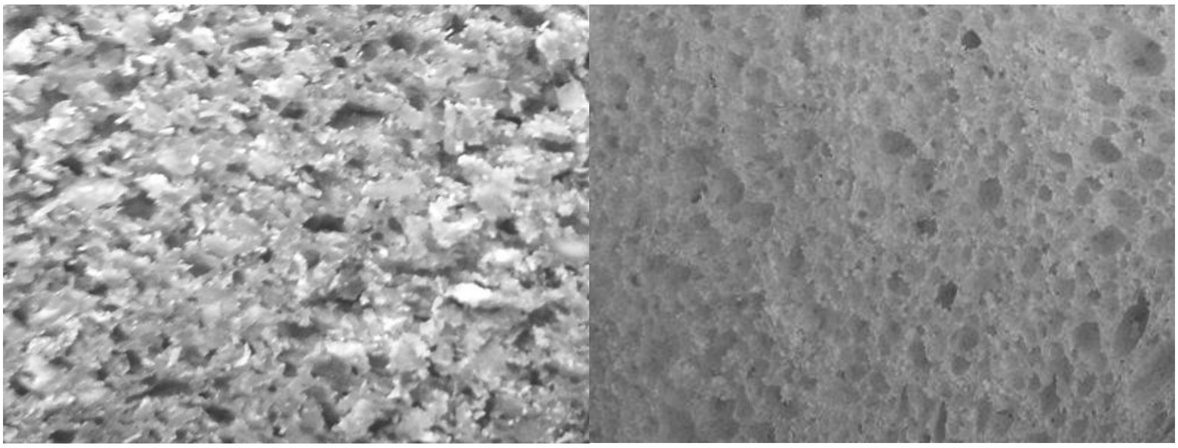
The name of the investigated indicator of the bakery product	**The amount of thick buckwheat starter culture added to the dough, % by weight of the dispersed emmer wheat grain**						
	0	**20**	**30**	**40**	**50**	**60**	**70**
Mass fraction of moisture, %	47.0 ±0.18	47.1 ±0.15	47.85 ±0.16*	46.00 ±0.25*	44.39 ±0.23***	44.08 ±0.18***	43.71 ±0.28***
Acidity, degree	6.2 ±0.07	6.4 ±0.14	7.0 ±0.19*	7.8 ±0.14***	8.2 ±0.19***	9.6 ±0.19***	10.8 ±0.15***
Porosity, %	47.5 ±0.35	47.8 ±0.21	47.95 ±0.25	49.53 ±0.33*	51.49 ±0.31**	51.98 ±0.20***	52.76 ±0.23***
Specific volume, cm^3/g	1.46	1.47	1.47	1.49	1.53	1.55	1.55

Note: n=3, * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

Increasing the amount of sourdough improved the physicochemical parameters of the final product: moisture decreased, while porosity and specific volume increased. At the same time, the organoleptic properties of the bread deteriorated due to excessive acid accumulation in the crumb. When 60-70% of the starter is added, the dough has a sour taste. Therefore, the most appropriate amount of thick buckwheat sourdough is 50% of the mass of dispersed *Triticum dicoccum* grain.

The resulting bread exhibited high sensory qualities, including taste, aroma, pore structure, color, and crumb elasticity, surpassing those of whole wheat bread prepared using a straight-dough method. These findings are consistent with reports that emmer sourdough bread is perceived as less acidic in taste, aroma, and odor, making it more appealing than bread from common wheat **[37]**.

Figure 5 shows photographs of cross-sections of the finished products. The images indicate that in bread made from *Triticum dicoccum* grains using buckwheat sourdough, the porosity is thin-walled and more pronounced. In the control variant, fragments of whole spelt grains are observed. The use of buckwheat kefir starter culture in the technology of bread made from Triticum dicoccum grain treated with an enzyme preparation leads to an increase in the sugar content in the dough, intensification of the fermentation process and release of carbon dioxide. These processes contribute to the loosening of the dough and make the crumb airy and porous.



1 2

Figure 5 Wholemeal bakery products: (1) control bread; (2) *Triticum dicoccum* grain bread with buckwheat starter culture.

Figure 6 shows microphotographs of the bread's cross-section. Unlike the control variant, the micrographs show no inclusions of whole grains

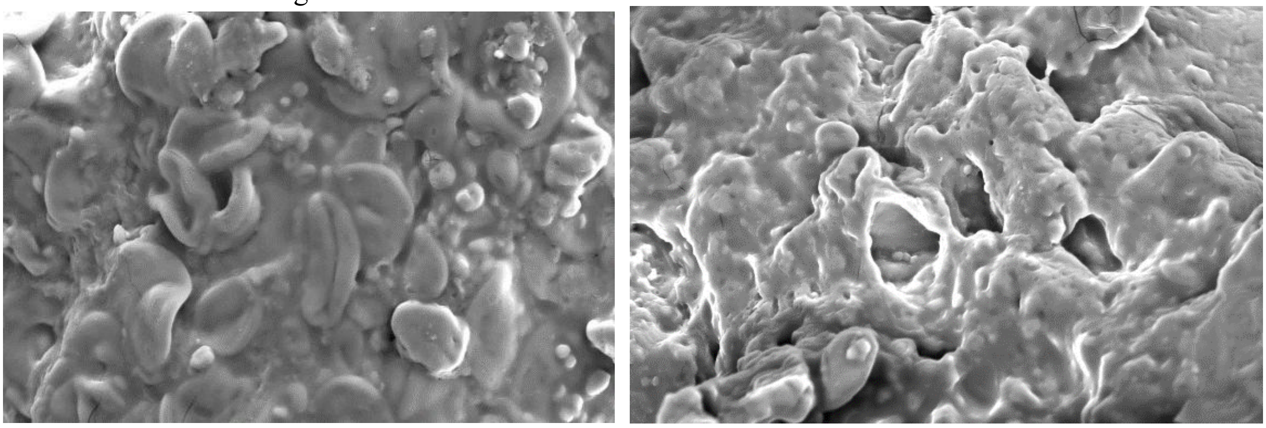


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Figure 6 Microphotographs of cross-sections structures of the bread: (1) control bread; (2) *Triticum dicoccum* bread with buckwheat starter culture. Increasex 950 zoom.

This can likely be explained by the use of a complex enzymatic preparation based on cellulases during the soaking stage of *Triticum dicoccum* grains, which promotes more thorough grain dispersion by partially modifying the structural components of the grain hulls, combined with the fermentation of the dispersed grain using sourdough in the bread-making process.

Bread made from whole emmer wheat grains using thick buckwheat sourdough is enriched with bioactive compounds due to the use of *Triticum dicoccum* grains as raw material and whole buckwheat (*Fagopyrum esculentum*) grains for sourdough preparation. Bogatyreva et al. reported that bread prepared with buckwheat sourdough enhances the nutritional and biological value of the baked product and improves bread quality parameters **[40]**.

Table 5 presents the amino acid content in the protein of emmer sourdough bread compared with whole wheat bread prepared using a straight-dough method.

The bread made from Triticum dicoccum grains using thick buckwheat sourdough has high nutritional value. It was found to have a higher content of essential amino acids compared to bread made from commercial wheat *Triticum aestivum*. In particular, in terms of the content of the limiting amino acid lysine, the protein of bread from Triticum dicoccum grain on buckwheat starter culture exceeded the protein of bread from wheat grain Triticum aestivum by 22.9% ($p<0.05$).

The content of the limiting amino acid lysine in the protein of *Triticum dicoccum* sourdough bread was 19% higher than in *Triticum aestivum* bread. *Triticum dicoccum* grains are richer in lysine than modern commercial wheat, which is consistent with previous reports **[58]**. The phenylalanine content in the protein of bread from Triticum dicoccum grain on buckwheat starter culture was higher than in the protein of bread from commercial wheat by 6.8%, leucine and isoleucine by 6.9%, methionine by 27.8% ($p<0.05$), and valine by 8.6%.

Experimental data indicate that the protein of *Triticum dicoccum* sourdough bread contains 25.8% more of the amino acid proline and 19.5% more glycine compared with the protein of commercial wheat bread.

Proline and glycine are key amino acids involved in collagen synthesis in the human body. These amino acids play important roles in musculoskeletal function, skin health, wound healing, and blood sugar balance. Proline and glutamine are abundant in the gliadin storage proteins **[59]**. Probably, a statistically significant reduced glutamine content in the bread protein of Triticum dicoccum grain bread on buckwheat starter culture ($p<0.01$) determines the ratio of gliadin to glutenin.

In the developed *Triticum dicoccum* sourdough bread, bioactive compounds, including vitamins, trace elements, and antioxidant activity, were also determined. The experimental results are presented in Table 6.

Table 5 Amino acid composition of bread protein.

Amino acid	Content, %	
	spelt bread with buckwheat starter culture	wheat bread
Arginine	0.56±0.02*	0.40±0.03
Lysine	0.43±0.02*	0.35±0.01
Tyrosine	0.25±0.02	0.28±0.03
Phenylalanine	0.78±0.04	0.73±0.04
Histidine	0.28±0.02	0.23±0.03
Leucine + isoleucine	1.08±0.05	1.01±0.05
Methionine	0.22±0.01*	0.18±0.01
Valin	0.62±0.03	0.58±0.05
Proline	1.22±0.05	0.97±0.04
Serine	0.53±0.04	0.49±0.05
Alanine	0.64±0.04	0.60±0.03
Glycine	0.49±0.03	0.41±0.02
Cysteine	0.17±0.01	0.18±0.01
Glutamic acid	3.42±0.08**	4.18±0.06
Aspartic acid	0.92±0.04**	0.68±0.03
Tryptophan	0.13±0.02	0.12±0.01

Note: n=3, * p<0.05; ** p<0.01.

Table 6 Vitamins, minerals and AOA of *Triticum dicoccum* bread.

Biologically active substances and antioxidant activity	Content, %	
	***Triticum dicoccum* bread** (n=3)	**wheat bread** (n=3)
Vitamins, mg/100 g		
B_6	1.2±0.09***	0.16±0.04
B_2	0.36±0.04*	0.14±0.03
B_3	1.6±0.14**	0.7±0.12
B_1	1.5±0.19**	0.23±0.04
PP	5.18±0.13**	3.61±0.19
E	1.12±0.04*	0.91±0.05
Trace elements, mg/kg		
Fe	46.5±1.06**	31.7±1.84
Mn	23.2±2.14**	13.4±0.97
Zn	32.4±1.81***	7.5±0.32
Cu	3.2±0.21**	1.4±0.25
Ni	0.41±0.04	0.28±0.03
Co	0.54±0.05**	0.11±0.01
AOA, % inhibition of the DPH radical	26.8±1.26**	8.5±0.39

Note: * p<0.05; ** p<0.01; ***p<0.001.

It was found that the amount of vitamins in bread made from dispersed *Triticum dicoccum* grain on buckwheat sourdough is 7.5 times higher for vitamin B_6, 2.6 times higher for B_2, 2.3 times higher for B_3, 6.5 times higher for B_1, 1.4 times higher for PP, and 1.2 times higher for E compared with bread made from commercial wheat. The amount of trace elements is 1.5-4.9 times higher in bread made from dispersed Triticum dicoccum grain on buckwheat sourdough. This is likely due to the significantly higher vitamin and trace element content in emmer wheat compared with *Triticum aestivum*, particularly Fe, Zn, and Cu **[60]**. Buckwheat also contains substantial amounts of Fe, Cu, Co, and Zn in its grains **[61]**. Ancient wheat species, such as *Triticum dicoccum*, are among the best dietary sources of Zn and Fe for human consumption **[62]**. High concentrations of iron and manganese were found in buckwheat grains, and emmer wheat grains also contained considerable manganese **[60]**.

Antioxidant activity in bread made from *Triticum dicoccum* grain using buckwheat sourdough was 3.2 times higher than the corresponding indicator of bread made from *Triticum aestivum* wheat grain (p<0.01). This is consistent with reports of elevated antioxidant concentrations in *Triticum dicoccum* and *Fagopyrum esculentum* grains **[63]**, and **[64]**.

These findings indicate that high-quality bread can be produced from *Triticum dicoccum* grains using thick buckwheat sourdough. Such bread serves as a valuable source of nutrients for consumers.

CONCLUSION

Experimental results demonstrated that the nutritional properties of *Triticum dicoccum* grains are comparable to those of modern commercial wheat (*Triticum aestivum*). Our data showed that the studied *Triticum dicoccum* grains had higher protein content (14.0±0.21 %), than commercial wheat (12.8±0.16 %). At the same time, the gluten content and its quality in *Triticum dicoccum* were lower compared with *Triticum aestivum*. The gliadin-to-glutenin ratio in emmer wheat was also higher (1.86) than in common wheat (1.50). These parameters are essential for achieving good bread volume.

Microscopic analysis of the surface and cross-sections of *Triticum dicoccum* and *Triticum aestivum* grains revealed that *Triticum dicoccum* grains have rigid and more tightly adhering husks. To soften the husks and improve the sensory properties of bread, the grains were pretreated with a complex enzymatic preparation containing cellulase, β-glucanase, and xylanase. After enzymatic hydrolysis under optimal enzyme conditions, a hollow space measuring 75–100 μm formed between the husks and the internal grain structure. The treated grains dispersed more easily.

To enhance the quality and nutritional value of bread from dispersed *Triticum dicoccum* grains, a thick buckwheat sourdough was used. The sourdough was prepared using the kefir culture Lc3/P1/Ac1, which contains the following microorganisms: *Lactococcus lactis, Lactococcus cremoris, Leuconostoc dextranicum, Lactobacillus bulgaricus, Lactobacillus acidophilus, Lactobacillus fermenti, Acetobacter aceti, Saccharomyces cerevisiae*, and *Saccharomyces lactis*.

No additional baker's yeast was added. However, experimental data indicate that the sourdough exhibits high fermentative activity. To improve the bread's physicochemical properties 4% dry wheat gluten was added. It was established that the optimal amount of thick buckwheat sourdough is 50% of the mass of dispersed *Triticum dicoccum* grains. The resulting bread demonstrated excellent sensory properties, including taste, aroma, crumb porosity, color, and elasticity, surpassing bread made from whole wheat grains using a straight-dough method.

The bread contained higher levels of essential amino acids, including lysine (0.43±0.02%), phenylalanine (0.78±0.04 %), leucine+isoleucine (1.08±0.05 %), methionine (0.22±0.01 %), and valine (0.62±0.03 %), compared to bread made from commercial *Triticum aestivum* wheat. Bread made from *Triticum dicoccum* using thick buckwheat sourdough also exhibited increased levels of vitamins B6, B2, B3, B1, PP, and E, as well as microelements Fe, Mn, Zn, Cu, Ni, and Co. The antioxidant activity was 3.2 times ($p<0.01$) higher than that of bread made from commercial *Triticum aestivum* grains.

This is the first study to combine enzymatic hydrolysis of Triticum dicoccum with a thick buckwheat sourdough prepared from kefir grain culture. Thus, the use of *Triticum dicoccum* grains in bread-making with thick buckwheat sourdough can expand the range of functional breads that contain bioactive components and possess potential health-promoting properties. Triticum dicoccum grain has a good potential for industrial bread production and is of interest to the functional food market.

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Contact Address:

Elena Kuznetsova
Affiliation: Orel State University named after I. S. Turgenev, Institute of Natural Sciences and Biotechnology, Department of Industrial Chemistry and Biotechnology, 302026, Orel, 95 Komsomolskaya str., Russia,
Tel.: +79102661634,
E-mail: elkuznetcova@yandex.ru
ORCID: https://orcid.org/0000-0001-7165-3517
Author contribution: conceptualisation, methodology, investigation, resources, data curation, writing –original draft, writing –review & editing, visualisation, project administration, funding acquisition

Maksim Rebezov
Affiliation: Gorbatov Research Center for Food Systems, Department of Scientific Research, 26 Talalikhin Str., Moscow, 109316, Russia;
Affiliation: Ural State Agrarian University, Department of Biotechnology and Food, 42 Karl Liebknecht str., Yekaterinburg, 620075, Russia
Tel.: +79999002365
E-mail: rebezov@ya.ru
ORCID: https://orcid.org/0000-0003-0857-5143
Author contribution: conceptualisation, methodology, investigation, resources, data curation, writing –original draft, writing –review & editing, visualisation, project administration.

Zhyldyz Irmatova
Affiliation: Osh Technological University named after M.M. Adysheva, Institute of Technology and Environmental Management, Department of Technology of agricultural products processing, Kyrgyzstan, Osh, 81 Isanov str.
Tel.: +996553457368
E-mail: julduz75@mail.ru
ORCID: https://orcid.org/0000-0002-8749-4802
Authorcontribution: writing –review&editing

Victoria Simonova
Affiliation: Orel State University named I. S. Turgeneva, Medical Institute, Department of Public Health, Healthcare and Hygiene, 302026, Orel, 95 Komsomolskaya str., Russia,
Tel.: +79192601974,
E-mail: segeja36@mail.ru
ORCID: https://orcid.org/0000-0003-4639-5311
Author contribution: writing –review&editing, validation, formal analysis, investigation

Elena Kuznetsova
Affiliation: Orel State University named after I. S. Turgenev, Institute of Natural Sciences and Biotechnology, Department of Industrial Chemistry and Biotechnology, 302026, Orel, 95 Komsomolskaya str., Russia,
Tel.: +79192022345,
E-mail: 1408199714@rambler.ru
ORCID: https://orcid.org/0000-0001-9518-6968
Author contribution: investigation, data curation, writing –review & editing, project administration.

Sukhrab Makhmudov
Affiliation: Almaty Technological University, Research Institute of Food Safety, Tole bi 100, 050012, Almaty, Kazakhstan,
Tel.: +77471553957
E-mail: laalan143@gmail.com
ORCID: https://orcid.org/0009-0005-6124-7941
Author contribution: writing –review&editing, validation, formal analysis, investigation

Sanavar Azimova
Affiliation: Almaty Technological University, Research Institute of Food Safety, Tole bi 100, 050012, Almaty, Kazakhstan,
Tel.: +77018011414
E-mail: sanakz2525@gmail.com
ORCID: https://orcid.org/0000-0002-8992-8889
Author contribution: writing –review&editing, validation, formal analysis, investigation

Damira Tattibayeva
Affiliation: International Engeeniring Technological University, The Department of Technique and technology of food production, Al-Farabi Ave., 93G/5, 050060, Almaty, Kazakhstan,
Tel.: +77479646183
E-mail: d.tattibaeva@metu.kz
ORCID: https://orcid.org/0009-0001-6810-176X
Author contribution: writing –review&editing, validation, formal analysis, investigation

Corresponding author: **Elena Kuznetsova**