



Received: 31.5.2022  
Revised: 15.6.2022  
Accepted: 28.6.2022  
Published: 4.7.2022

*Potravinárstvo Slovak Journal of Food Sciences*  
vol. 16, 2022, p. 320-340  
<https://doi.org/10.5219/1767>  
ISSN: 1337-0960 online  
[www.potravinarstvo.com](http://www.potravinarstvo.com)  
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## The influence of grain mixtures on the quality and nutritional value of bread

*Zhuldyz Nurgozhina, Dinara Shansharova, Gulzhanat Umirzakova,  
Pernekul Maliktayeva, Madina Yakiyayeva*

### ABSTRACT

The desire to survive in a competitive environment mobilizes managers to make unconventional decisions to increase their product range, quality, and safety. This study aims to create a technology of bread with increased nutritional value using bioactivated cereal mixtures and develop new bread recipes. The experiment used bioactivated wheat and maize grains, flax, rye flour, 1st-graduate wheat flour, spontaneous fermentation starter, salt, and water. Vegetable components such as dried crushed hawthorn berries, jaggery, and barberry were also used. Standard, generally accepted chemical and organoleptic methods of examining raw materials, semi-finished and finished products were used. It was found that the best physical and chemical indices were possessed by testing the bread prepared with the addition of a 20% grain mixture. All experimental analyses improved several parameters compared to the control sample. The nutritional value of obtained products was increased from 0.5 to 3 times. According to the obtained results, it is possible to conclude the relevance of this topic is getting a new range of bread products with increased nutritional value.

**Keywords:** nutritional value, bread, grain, bioactivation, microbiological safety, grain mix

### INTRODUCTION

Currently, a wide range of bread products are baked according to different recipes and correspond to the taste preferences of different population segments. The deterioration of the environment, the rapid pace of life, the reduction in the quantity and deterioration in the quality of food products lead to the maturing problem of developing food with increased nutritional value, and bread as one of the most widely distributed products is very important in the human diet. That is why the technology of obtaining grain bread as a healthy, "live" product is becoming increasingly popular.

An important feature of grain mixtures is their increased hydration capacity. The dough with such grain mixtures has a significant water-absorbing ability, allowing the binding free water in the dough. In turn, this leads to a decrease in the stove, a significant increase in the weight of the products, and a substantial reduction in moisture loss in the storage of finished products – to a slowdown in staling [1], [2], [3]. Bread with the addition of bioactivated grains and dry vegetable ingredients has many advantages compared to bread made from wheat flour, prepared according to a traditional recipe. So, it differs in that it contains almost entirely preserved proteins, fats, micro- and macronutrients, vitamins, and dietary fiber [4], [5].

Moreover, dry vegetable ingredients improve the color, taste, and aroma of the finished product and, importantly, further enrich the bread contained in the composition of many valuable nutrients [6], [7], [8]. In choosing grains for bio-activation and their further use, the composition of grain mixtures was because they have a high nutritional and biological value. Grain bioactivation is a controlled process of grain moisture saturation that occurs in the presence of water, heat, and air and is the beginning of germination. High-molecular substances are transformed into easily accessible forms. Due to this, bioactivated grain is a source of biologically active substances [9], [10]. We have developed a technology and a range of bakery products made from bioactivated grain. However, despite the advantages of bread made from bioactivated grain, which is characterized by an

increased content of dietary fiber, minerals and vitamins compared to traditional types of bread, it has reduced protein content and a lack of lysine [11], [12], [13]. Bioactivated grains with sprouts (no longer than 5 mm in length) contain sufficient antioxidants, which in low concentrations slow down or prevent oxidative processes [14], [15]. In addition, in germination in the grain, the enzyme systems are activated. There is a breakdown of complex nutrients into simpler, easily digestible by the human body [16], [17]. A mixture of each component enriches it with certain useful substances for humans, and the product acquires a preventive orientation. The naturalness in the ratio of the natural raw materials components of food nutrients contributes to the increase in the nutritional value of products and their better absorption [18], [19], [20].

Grain mixtures differ from other food groups by their low water content, energy saturation, transportability, long shelf life (up to one year), and the presence of functionally active ingredients in their composition. According to the grain, the mixture's rationally and purposefully formulated recipe, and you can get a product with an exceptional value proven in this article.

### Scientific Hypothesis

The increased nutritional value of bread will depend on the beneficial properties of grain mixtures.

## MATERIAL AND METHODOLOGY

### Samples

The objects of the study were: bioactivated grains of wheat and corn, flax, rye flour, wheat flour of the 1st grade, spontaneous fermentation starter, salt, and water. Some herbal ingredients were used, such as dry crushed hawthorn berries, horseradish, and barberry. All analyzes were carried out in an accredited laboratory of the Almaty Technological University.

### Chemicals

All reagents were of analytical grade and were purchased from Laborfarm (Kazakhstan) and Sigma Aldrich (USA).

### Animals and Biological Material

Animal and biological materials were not used in this study.

### Instruments

We used automatic fat extractor SER 148/3 (Velp Scientifica, Italy), Kjeldahl VELP UDK 129 (Velp Scientifica, Italy), atomic absorption spectrometer KVANT-Z-ETA-T (OJSC Kortek, Russia), convection oven UNOX XB693 (UNOX, Italy).

### Laboratory Methods

In work, the following indicators of the raw materials used and the resulting assortments of bakery products were investigated: organoleptic indicators by GOST 5667-85 [21], mass fraction of moisture by GOST 21094-75 [22], mass fraction of fat by GOST 5668-68 [23], a mass fraction the proportion of protein by GOST 10846-91 [24], mass fraction of sugar by GOST 5672-68 [25], mass fraction of carbohydrates by GOST 25832-89 [26], the mass fraction of ash by GOST 5901-2014 [27], mass fraction of porosity by GOST 5669-96 [28], acidity content by GOST 5670-96 [29], iron content by GOST 26928-86 [30], vitamin A content by GOST R 54635-2011 [31], and others. Some standards generally accepted chemical and organoleptic methods were used to study raw materials, semi-finished products, and prepared products.

### Description of the Experiment

The methodological basis of the study was a systematic analysis of the technology used in the production of bakery products enriched with useful herbal ingredients. The following main tasks were performed sequentially: 1) selection and justification of the method for introducing herbal ingredients into the recipe for bakery products and 2) improvement of the technology used in bakery products by incorporating proper plant ingredients. The theoretical basis of the research consisted of general scientific and unique research methods, methods of system analysis, and experimental planning.

**Number of samples analyzed:** We analyzed 3 bread samples.

**Number of repeated analyses:** All tests were performed in triplicate.

**Number of experiment replication:** 2 times.

**Design of the experiment:** Bioactivation was carried out under the following modes such as washing the grain in clean drinking water, washing the grain 5% for disinfection, washing the grains the second time, soaking the grain in water for 24 hours at a temperature of 22-25 °C, bioactivation the grains at a temperature of 20-30 °C, dispersing the bioactivated grains to a humidity of 13-14% and crushing the bioactivated grains to pass a sieve

with round holes with a diameter of 1.5 mm, with a residue on the sieve of 4.0%. The main controlled indicator of wet sprouted grain was the presence of a germ root no longer than 5 mm in length in 90% of seeds. After the bioactivation process of grains and drafting of compounding of grain mixtures, compounding of bread were made with an increasing food value and the physicochemical indices of baked foods are certain. The research applied the generally accepted and special methods of estimating raw material properties and the quality of baked foods.

### Statistical Analysis

Data processing and calculations were performed using the Statistica 12.0 (StatSoft, Tulsa, OK, USA) sequential regression analysis program. The program first gives the values of all the regression coefficients and their confidence intervals (errors), then checks the significance of the regression coefficients. After removing all the insignificant coefficients, the remaining significant coefficients and their confidence errors are printed. Also, the data were analysed using MS Excel for Windows version 10 Pro, 2010. The data collected during the study were subjected to independent testing, and questionnaires were conducted to assess the organoleptic characteristics of control and test samples. The analysis process used absolute and relative statistical indicators and tabular and graphical methods for presenting the results. The student's t-test was used to evaluate bread yield, crumb porosity, and crumb moisture. To check the quality of the obtained regression equations, the multiple correlation coefficient R, the coefficient of determination R<sup>2</sup>, the Fisher Criterion F and the Durbin-Watson criterion d were calculated. On their basis, response surfaces and lines of equal levels (isolines) of indicators of the content of grain mixtures on technological indicators of the quality and safety of bread were obtained and built, depending on various combinations of the studied parameters: the dependence of the content of grain mixtures on the content of sourdough.

### RESULTS AND DISCUSSION

Physical and chemical indices and the quality indicators of two samples of rye flour and wheat flour of the 1st grade were studied further and shown in Table 1 and Table 2.

**Table 1** Physical and chemical indices of the medium rye flour.

Indicators	Sample 1	Sample 2
Humidity, %	13.5 ±0.3	13.7 ±0.5
Ash content, %	1.43 ±0.11	1.40 ±0.10
Acidity, deg.	2.0 ±0.07	2.0 ±0.04
Falling number, sec	158 ±0.2	155 ±0.3
Whiteness, the device unit	6 ±0.09	6 ±0.08
Pest infestation	Does not found	
Mineral impurity	The crunch is not felt	

Note: ± standard deviation.

**Table 2** The 1st grade wheat baking flour's quality indicators.

Indicators	Sample 1	Sample 2
Humidity, %	13.7 ±0.1	14.0 ±0.4
Ash content, %	0.54 ±0.18	0.60 ±0.12
Acidity, deg.	2.0 ±0.04	2.0 ±0.03
Falling number, sec	250 ±0.3	246 ±0.8
Whiteness, the device unit	52.4 ±0.07	53.2 ±0.07
Amount of gluten, %	63 ±0.3	62 ±0.05
Gas-forming capacity, cm <sup>3</sup>	1264 ±0.9	1300 ±0.2
Pest infestation	Not present	
Mineral impurity	The crunch not detected	

Note: ± standard deviation.

Lean on the research's result, it can be concluded that the flour samples used in this research correspond to the requirements of state standards. Pest infestation and crunch in flour were not detected in all samples.

Rye sourdoughs were prepared from medium rye flour to baking bread. The starter culture was prepared firstly for these purposes. The resulting thick starter culture was fed and brought to readiness by fermentation.

Some laboratory research has been carried out on the quality of rye sourdough. The results of the research are shown in the following Table 3. Following physical and chemical indices as humidity, acidity, and lifting force of the "ball" were studied.

**Table 3** Physical and chemical indices of the rye sourdough's quality.

Names of the indices	Received results
Length of soaking, h	20
Temperature, °C	26-30
Mass fraction of moisture, %	48
Acidity, deg.	13.5
Lifting force of the "ball", min	18

As a result of sourdough's quality analysis, lifting force indicators can be noted as good. The acidity and humidity correspond to the used grade of rye flour and the type of sourdough.

Wheat and maize grains were used for bioactivation. Grains for bioactivation should be of good quality, mature, and without impurities, and when submerged in water, 90-95% of the grains should sink to the bottom. One hundred large grains are selected and lowered into the water to do this. The surfaced grains are removed and replaced with other, good-quality grains. After that, the grains are laid out, covered with a wet cloth and put in a warm place at a temperature of 20-25 °C. After 72 hours, check the germinating of the grain, i.e. the presence of roots and seedlings. After that, the number of germinating grains is calculated as a percentage of the number of 100 grains initially taken for bioactivation.

The grain chosen for bioactivation was cleared of impurities. The water used in bioactivation should be potable and clean of impurities. Washing is carried out at room temperature.

Bioactivation was carried out as follows: washing grain in clean drinking water, washing grain with 5% for disinfection, washing grain a second time, and soaking grain in water for 24 hours at 22-25 °C. After washing, the grains are soaked in water at 20-25 °C. Then soak in water for 2-3 hours. After 4-6 hours, 90-95% of the grains should emerge. The bio-activated grains are then crushed until they pass through a 1.5 mm round hole sieve with a 4.0% residue on the sieve. A germinal root with a length of no more than 5 mm in 90% of the seeds was regarded as the main indicator to be monitored for moist germinated grains.

After the bioactivation process of grains and drafting of compounding of grain mixtures, compounding of bread were made with an increasing food value and the physical and chemical indices of baked foods are certain. The research applied the generally accepted and special methods of estimating raw material properties and quality of baked foods.

Three types of grain mixes were developed to further increase the nutritional value of bread: grain mixes "Kopzhasar", "Khanshaiym", and "Arman". The recipe and the percentage of ingredients to each other are shown in Tables 4-6.

The bread recipe was developed by using grain mixtures. Samples of dry bioactivated grains were stored at a temperature of 17±3 °C to determine the laboratory's acceptable shelf life and relative humidity of 75%. Under such conditions, all samples were kept for 12 months. No significant losses were observed, and no changes in organoleptic parameters were observed. After this period, deterioration in the quality of the grains was observed: the appearance of an unpleasant, musty smell, plaque on the grains, and deterioration in taste. Thus, bioactivated grains are conveniently stored and transported over long distances for no more than 12 months, with all the conditions being met.

**Table 4** Recipes of control sample and grain mixture "Kopzhasar" and their nutritional values.

Name	Control	Consumption of raw materials, %					
		Kopzhasar					
Variants' number	0	1	2	3	4	5	6
Rye medium flour mixture and wheat baking grade 1	200	181	167	155	135	115	95
Bioactivated wheat	-	10	15	20	25	30	35
Bioactivated corn	-	3	5	10	15	20	25
Flax seeds	-	1	3	5	10	15	20
Dzhigida	-	3	5	10	15	20	25
Barberry	-	-	-	-	-	-	-
Sea-buckthorn	-	-	-	-	-	-	-
Hawthorn	-	-	-	-	-	-	-
Salt	1.5	1.5	1.5	1.5	1.5	1.5	1.5

**Table 5** Recipes of grain mixture "Arman" and its indicators of nutritional values.

Name	Consumption of raw materials, %					
	Arman					
Variants' number	7	8	9	10	11	12
Rye medium flour mixture and wheat baking grade 1	175	155	135	115	95	75
Bioactivated wheat	10	15	20	25	30	35
Bioactivated corn	5	10	15	20	25	30
Flax seeds	-	-	-	-	-	-
Dzhigida	-	-	-	-	-	-
Barberry	5	10	15	20	25	30
Sea-buckthorn	-	-	-	-	-	-
Hawthorn	5	10	15	20	25	30
Salt	1.5	1.5	1.5	1.5	1.5	1.5

**Table 6** Recipes of grain mixture "Khanshaiym" and its indicators of nutritional values.

Name	Consumption of raw materials, %					
	Khanshaiym					
Variants' number	13	14	15	16	17	18
Rye medium flour mixture and wheat baking grade 1	175	155	135	115	95	75
Bioactivated wheat	10	15	20	25	30	35
Bioactivated corn	-	-	-	-	-	-
Flax seeds	5	10	15	20	25	30
Dzhigida	-	-	-	-	-	-
Barberry	-	-	-	-	-	-
Sea-buckthorn	5	10	15	20	25	30
Hawthorn	5	10	15	20	25	30
Salt	1.5	1.5	1.5	1.5	1.5	1.5

Then, guided by this recipe, we selected the most optimal dosage options for grain mixtures compared with the quality indicators of the control sample of bread. For a full and fair assessment of the quality, organoleptic and physical and chemical indicators of the quality of bread were studied.

After moisture was determined by the physical and chemical indices of the selected dosages of grain mixtures, acidity, protein, fatty acids, fiber, minerals, and vitamins were examined (Table 7).

**Table 7** Chemical indices of the grain mixtures per 100 g of product.

Name of the indicators	Name of grain mixtures		
	Kopzhasar	Arman	Khanshaiym
Mass fraction of moisture, %	14.5 ±0.08	14.0 ±0.1	14.5 ±0.11
Protein, g	42.0 ±0.06	17.6 ±0.12	33.2 ±0.04
Dietary fiber, g	54.3 ±0.3	68.7 ±0.5	47.8 ±0.9
Carbohydrates, g	150.1 ±0.2	134.0 ±0.4	94.6 ±0.1
Fat, g	97.6 ±0.11	99.4 ±0.08	52.5 ±0.15
Vitamin B1, g	2.0 ±0.04	0.8 ±0.07	1.7 ±0.02
Vitamin B2, g	0.3 ±0.02	0.41 ±0.02	1.0 ±0.05
Vitamin B6, g	1.5 ±0.08	1.0 ±0.04	1.2 ±0.08
Iron, mg	15.0 ±0.11	38.7 ±0.12	12.0 ±0.08
Magnesium, mg	256.4 ±0.4	307.2 ±0.3	183.4 ±0.7
Potassium, mg	1566.0 ±0.15	2075.1 ±0.18	1814.0 ±0.11

Note: ± – standard deviation.

Thus, the optimal formulations of grain mixtures were selected, and grain mixtures' physical and chemical parameters were determined. Grain mixtures can be distinguished visually because it is easy to distinguish the colors and structure of the components in the composition.

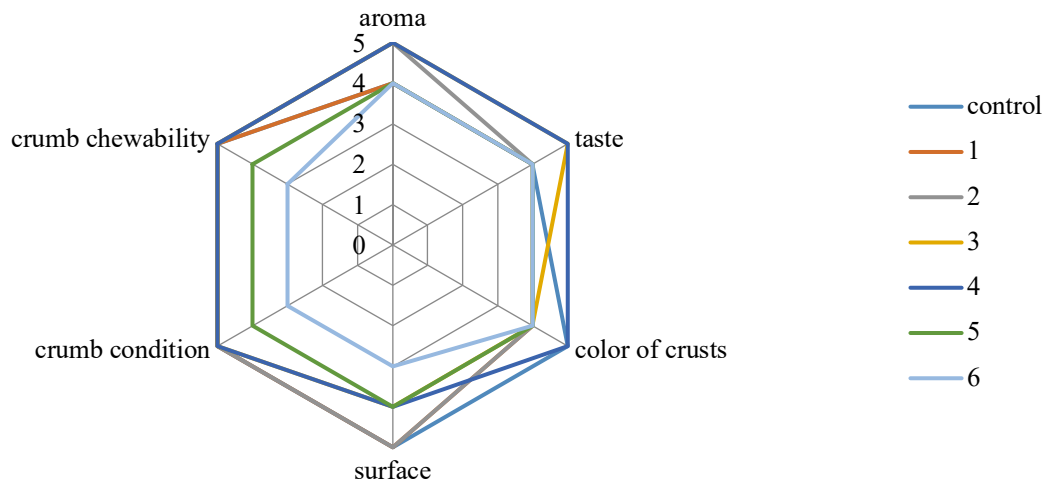
During the study of the quality indicators of prepared bread using promising phyto-enriching agents, namely, grain mixtures, the dough was prepared in a non-paired way. The bread dough was kneaded by hand, following the calculated values of the components according to the recipe (Table 7).

In recipes, all components are mixed until a homogeneous dough consistency is obtained. The dough was fermented in a thermostat for 150-180 minutes at 30-35 °C. After 60 and 120 minutes, the test was wrapped. The final acidity of the test was not more than 3 degrees. The fermented dough was divided into dough pieces weighing 350 g, rounded, and placed in moulds and sheets. The duration of proofing of the dough at a temperature of 37-38 °C and relative humidity of 75-80% was 40-50 minutes. The products were baked in a humidified baking chamber for about 30-35 minutes at 230-240 °C.

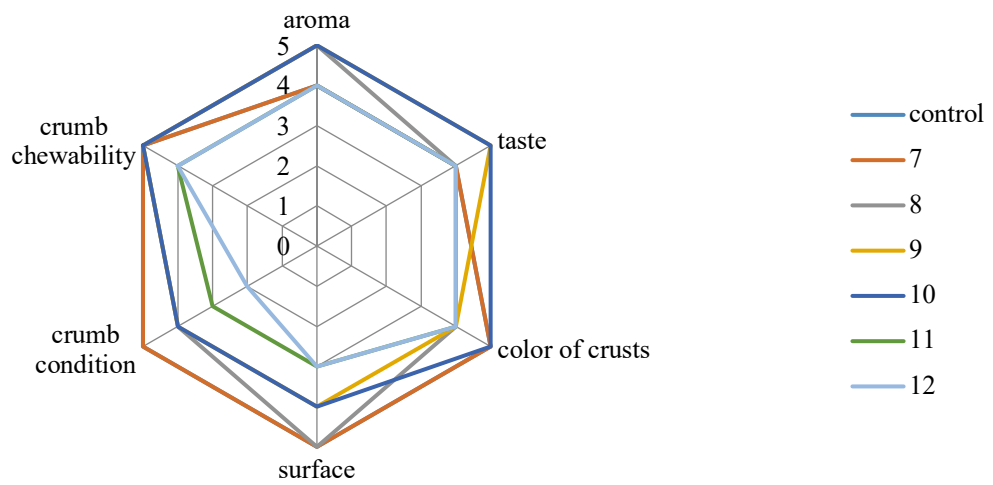
The test properties were evaluated by organoleptic and physical and chemical indices that were determined following standard methods for determining the quality of raw materials and products.

Organoleptic parameters were determined on a five-point scale. For this case, the finished bread was cooled for 1 hour at room temperature, and then the organoleptic parameters were evaluated. Figure 1 shows the organoleptic indicators in the five-point rating system. Samples of bread without bioactivated grains and sourdough were taken as a control version of loaves of increased nutritional value.

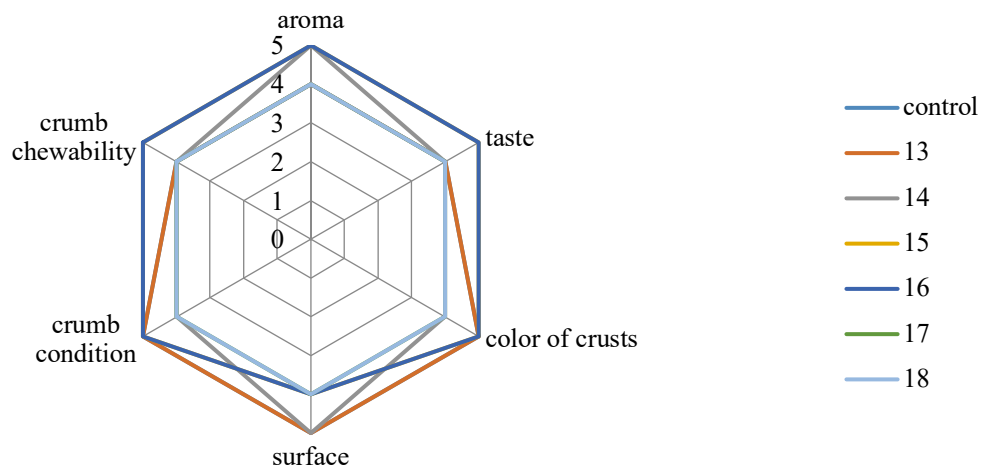
In some variants, the colour of the bread was rated higher than the previous value, while the proportion of rye-wheat bread decreased. The colour was less bright and greyish in the lower fractions of the flour content. In general, there is a noticeable similarity in the indicators. Colour with a decrease in the amount of flour became more intense and attractive, to a point after which the colour changed to dark, closer to black, inclusions of grain mixtures became more noticeable, and the crumb became denser less attractive to the consumer.



a)



b)



c)

**Figure 1** The effect of grain mixture on the organoleptic indicators of bread: a – with a grain mixture "Kopzhasar"; b – with a grain mixture "Arman"; c – with a grain mixture "Hanshaiym".

After the organoleptic estimation of all samples, the results were calculated in the arithmetic mean and clearly expressed in Figure 1. Table 8 shows the characteristics of organoleptic indicators.

The bread samples had the correct shape, the colour of the crust was smooth, dark brown, the crumb was elastic, dark-coloured with grain inclusions, and the taste and aroma were pleasant, characteristic of the appearance and content of the grain mixture. Samples of grain bread can be characterized as products with a good volume, regular shape, and a slightly convex crust. Grain inclusions in all the studied samples make the developed bread attractive from a consumer point of view.

Generally, all studied bread samples had positive organoleptic indicators. According to organoleptic studies, samples of grain bread with 15 and 20% inclusion of grain mixtures have the best results.



**Figure 2** Obtained bread samples: 1 – bread with a grain mixture "Kopzhasar", 2 – bread with a grain mixture "Arman", 3 – bread with a grain mixture " Khanshaiym».

Microbiological parameters were determined, and the results are shown in Table 8.

**Table 8** Effect of fermentation on the microflora of bread with grain mixture.

Indicators	Control sample	A grain mixture Kopzhasar	A grain mixture Arman	A grain mixture Hanshaiym
Mesophilic anaerobic and facultative microorganisms, CFU/g	$3.2 \times 10^4$	$3.3 \times 10^4$	$3.0 \times 10^3$	$3.0 \times 10^3$
Mold fungi, CFU/g	28.0	29.6	29.5	30.0
<i>E. coli</i> bacteria, CFU/g	Does not found	Does not found	Does not found	Does not found
Pathogenic microorganisms, salmonella, g	Does not found	Does not found	Does not found	Does not found
<i>S.aureus</i> staphylococci, g/cm	Does not found	Does not found	Does not found	Does not found

Adding starter cultures, rather than using yeast in the preparation of the dough, has a positive effect on the structure of the dough and, consequently, on the prepared bread. Lactic acid bacteria found in sourdough have many advantages, as they can reduce the growth and development of bacteria and mold fungi in bread. This significantly reduced microorganisms' content, which was proved by our microbiological study (Table 8).

According to the requirements, negative microbiological activity can be contained in the grain no more than 50 CFU/g. The microbiological studies also showed no pathogenic microorganisms of the salmonella group, staphylococci, or bacteria of the coli group in bread by adding grain mixtures. The physicochemical parameters of bread were studied (Table 9).



**Table 9** Results of physical and chemical parameters in grain loaves per 100 g of bread, %.

Indicators	Control sample	A grain mixture Kopzhasar	A grain mixture Arman	A grain mixture Hanshaiym
protein, g	6.30 ±0.08	10.11±0.02	11.70 ±0.04	11.00 ±0.081
carbohydrates, g	30.0 ±0.04	45.1 ±0.07	43.2 ±0.09	45.0 ±0.05
fat, g	0.20 ±0.01	0.60 ±0.04	0.81 ±0.07	0.74 ±0.03
dietary fiber, g	1.30 ±0.04	2.71 ±0.08	3.10 ±0.02	2.65 ±0.07
Minerals, mg:				
calcium	25.0 ±0.04	34.0 ±0.02	43.6 ±0.08	38.5 ±0.11
magnesium	73.3 ±0.09	83.3 ±0.02	86.7 ±0.04	85.0 ±0.01
phosphorus	210.0 ±0.07	255.0 ±0.08	283.0 ±0.11	231.0 ±0.07
iron	3.25 ±0.02	3.38 ±0.01	3.68 ±0.02	3.38 ±0.09
Vitamins, mg:				
thiamine	0.310 ±0.01	0.49 ±0.08	0.51 ±0.04	0.51 ±0.05
riboflavin	0.11 ±0.02	0.14 ±0.02	0.20 ±0.04	0.14 ±0.07
Antioxidant activity	1.70 ±0.08	3.57 ±0.02	4.00 ±0.08	4.63 ±0.06
Content of polyunsaturated fatty acids	Does not found	33.60 ±0.08	34.8 ±0.01	38.50 ±0.02

Note: ± – standard deviation.

The analysis of the obtained physical and chemical parameters data shows that the obtained bread samples are rich in minerals and vitamins. The amount of chemicals in bread using grain mixture increased by up to 70% compared to the control sample. In comparison with the control sample, the studied samples showed an increase in the content of protein, phosphorus, dietary fiber, and antioxidant activity in grain bread using various starter cultures.

Judging by the taken results, the antioxidant activity increases in bread with bioactivated grains, depending on the recipe of the grain mixture, by 2-2.5 times. The study's results on the content of polyunsaturated fatty acids look to more potential due to the high content. They were not found in the control sample in the grain mixture, while in bread with sprouted grains, their content increases by 33-34 times. This is due to the content of large amounts of flax seeds in grain mixtures.

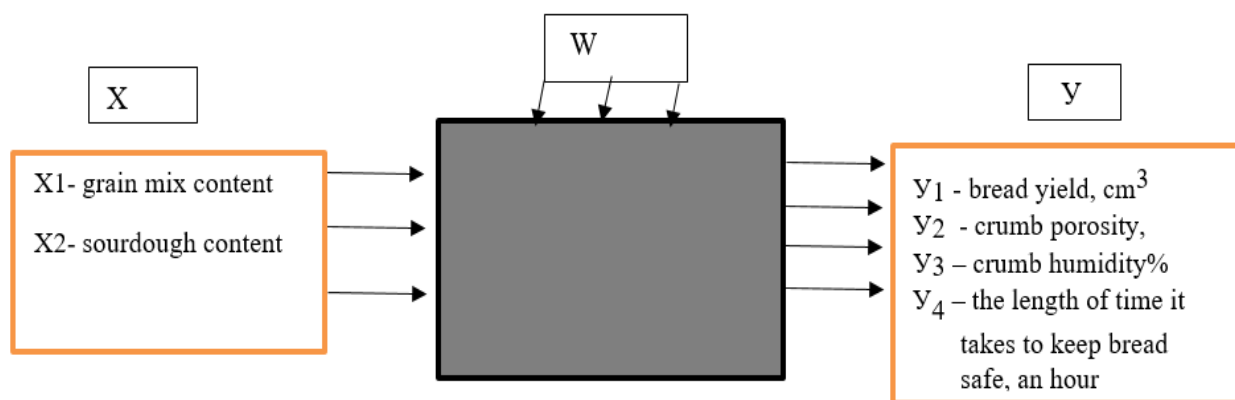
To sum up, the consumption of 100 g of grain bread with the addition of grain mixtures "Kopzhasar" and "Arman" satisfies the daily need of a healthy person in polyunsaturated fatty acids by 30%, and the other nutrients are satisfied from 1 up to 30%.

The results show that changing the recipe and using bio-activated grains and starters can slow down the process of moisture loss and thus the drying of bread during the storage time studied. These processes occur most intensively in the first 24 hours of storage.

Mathematical models adequate to the set experiment with some accuracy and simplicity make it possible to see a mathematical description of the simulation of the influence of grain mixtures' content on the bread's technological parameters. Start with it is necessary to establish the setting and the choice of the experimental model for determining the influence of the content of grain mixtures and leaven on the technological parameters of the bread.

To denote the process in question, whose mechanism of functioning is complex and unknown, we use the "black box" concept in the framework (Figure 3). The idea of a "black box" is when you need to determine what is required at the entrance to the system and what should be at the exit from it, no matter what is inside the system.

Our process, which is influenced by random influences  $W$ , has some "input" to input information about the regulated parameters of grain mixture content and an "output" to control the results characterized by optimization criteria. The state of the outputs  $Y$  is presumably functionally dependent on the state of the inputs  $X: Y = f(X)$ . However, the type of dependence of the results on the inputs is unknown.



**Figure 3** Model for determining the effect of grain mixture and sourdough content on bread quality and safety.

To solve the set tasks, the + planning of multifactorial experiments, statistical processing of the results and search optimization were planned: the optimization parameters and the most important factors that can affect the indicators of bread quality were selected, the research plan was defined, and a mathematical model based on the results was developed. This model was used to study the influence of controllable factors on the output parameters of the process in the stationary area of the factor space.

**Table 10** Researched parameters of grain mixtures content's influence on bread's technological parameters and their levels of variation in laboratory conditions.

Adjustable parameters: coded (natural)	Coded levels			Variation interval
	-1	0	+1	
X1 - the content of grain mixtures, %	0.25	0.643	1.0	0.375
X2- the content of rye sourdough, g	318	150.0	339	10.5

The laboratory research on the effect of grain mixture content on bread's quality and safety parameters was carried out according to the scheme of two-factor planning of experiments.

If the type of dependence of the response on the studied parameters is unknown, the regression equation is shown as a polynomial of the second degree. The central point, together with other points of the plan, allows to estimate coefficients of the full quadratic regression model from  $k = 2$  coded variables  $x_1, x_2$  in the area of factor space by this formula:

$$Y = b_0 + \sum b_i x_i + \sum b_{ij} x_i x_j, \tag{1}$$

The quadratic regression equation (1) has linear (main) effects  $x_1, \dots, x_k$ . The second-order terms  $x_i x_j$  at  $i \neq j$  account for interaction effects, i.e. effects of joint action  $x_i$  and  $x_j$  on the value  $Y$ , and terms  $x_i x_j$  at  $i = j$  (i.e.  $x_i^2$  - squared arguments) – non-linearity of response function  $Y$  at change of  $i$ -th argument. In this case, the effect of the  $i$ -th factor on the studied indicator of the impact of grain mixture content on technological indicators of bread is estimated by regression equation coefficients (1).

To begin with, it is necessary to calculate the statistical characteristics of the main indicators of the impact of the content of grain mixtures on technological indicators of quality and safety of bread and then analyze the obtained set of experimental data (Table 11).

According to the data of the experiment in Table 11, for each indicator, the following parameters are estimated: the mean ( $M$ ) and the error ( $m$ ), the median ( $med$ ) and mode ( $mod$ ), the standard deviation  $s$  and the variance  $s^2$ , the smallest ( $min$  – minimum) and the largest ( $max$  – maximum) values, the spread  $R$ , the skewness  $A$  and the excess  $E$  and the variation coefficient  $V$ .

**Table 11** Statistical characteristics of indicators of the influence of grain mixtures on the quality and safety of bread.

Statistical characteristics	Unit designation	Parameter		Y1	Y2	Y3	Y4
		X1	X2				
Scope of observations	<i>N</i>	4	4	4	4	4	4
Arithmetic mean reading	<i>M</i>	0.643	327.100	2330.000	68.250	46.375	45
Standard error	<i>m</i>	0.247	6.024	60.553	3.326	1.068	10.247
Standard error, % of arithmetic mean	<i>m, %</i>	3.641	1.845	2.607	4.873	2.303	25.237
Median	<i>med</i>	0.643	321.131	2380.000	70.200	46.250	42
Mode	<i>mod</i>	1.000	337.000	#N/D	#N/D	#N/D	#N/D
Standard deviation	<i>s</i>	0.433	12.455	121.106	6.463	2.136	20.494
Sampling variance	<i>s2</i>	0.188	147.000	1434.667	44.250	4.563	420
Excess	<i>E</i>	-6.000	-6.000	3.642	-1.700	-0.543	0.343
Skewness	<i>A</i>	0.000	0.000	-1.87 877724461	-0.482	0.292	0.753
Spread	<i>R</i>	0.750	21.000	260.000	15.763	5.000	48
Minimum	<i>min</i>	0.250	318.000	2150.000	60.000	44.000	24
Maximum	<i>max</i>	1.000	339.000	2410.000	75.000	49.000	72
Coefficient of variation, %	<i>V</i>	69.28	3.45	5.20	9.75	4.61	45.765

The statistical characteristics of Table 12 provide a quantitative view of the empirical data (the position of the mean, its dispersion – scatter, skewness) and, as a first approximation, test the assumptions underlying the regression analysis. The resulting measures' standard errors are small and less than 4% of the corresponding mean values. Approximate equality of the mean and median is observed.

There is no mode for Y1, Y2, Y3 and Y4, while the kurtosis and skewness values are negative; the minimum and maximum values are approximately equidistant from the mean, and the coefficients of variation are less than 9 % for the resultant indicators. This indicates the closeness of empirical and normal or generalized-normal distributions.

After that, we began to study the influence of a set of production factors on each of the indicators. That is, a system of regression equations is determined that reflects this value:

$$F_i(\bar{y}) = f(x_{m+1}, x_{m+2}, \dots, x_n) \quad (i = 1, 2, \dots, m), \quad (2)$$

Consequently, there are *m* indicators that act as optimality criteria, that is, *y*1, *y*2, ..., *y**m* and *n* factors affecting these indicators - *x*<sub>*m*+1</sub>, *x*<sub>*m*+2</sub>, ..., *x*<sub>*n*</sub> (in our case, *m* = 6, *n* = 7). The form of communication between effective indicators and factors is assumed to be non-linear.

stepwise regression methods chose the optimal set of components of the model (2). The most common and effective methods are Forward, Backward and Stepwise.

The approximation of the optimality criteria *Y*<sub>*i*</sub> by a polynomial provides a good indication of the shape of the response surface.

As a result of the implementation of computational procedures implemented in the computer program Excel, Statistica 12, calculated four b-coefficients of nonlinear regression for the coded variables *x*<sub>1</sub>, *x*<sub>2</sub>, their standard errors, Student's t-tests to test the significance regression components, *p* probability levels, upper and lower 95 % confidence limits.

**Table 12** Results of regression analysis of regulated parameters of grain mixture content on technological indicators of bread: coded variables.

Factor	Regression coefficient	Standard error	Student's t-test	p-value of significance	95% confidence limits	
					bottom	top
<b>Y1 – bread yield, cm<sup>3</sup></b>						
–	2330	60	38.35465	0.01639	1567.628	3092.372
x1	-50	60	-0.83333	0.557716	-812.372	712.3723
x2	70	60	1.166667	0.451125	-692.372	832.3723
x1x2	60	60	1.178667	0.735047	-0.099	0.0713
<b>Y2 – crumb porosity, %</b>						
–	68.25	2.25	30.33333	0.02098	39.66104	96.83896
x1	-0.75	2.25	-0.33333	0.795167	-29.365	27.83896
x2	5.25	2.25	2.333333	0.354650	-23.339	33.42655
x1x2	2.25	2.25	1.96234	0.066305	-0.0020	0.0556
<b>Y3 – moisture content of crumb, %</b>						
–	46.375	0.875	53	0,01201	35.25707	57.49293
x1	0.125	0.875	0.513221	0.909666	-10.9929	11.24293
x2	1.625	0.875	1.857143	0.314453	-9.49293	12.74293
x1x2	0.875	0.875	1.96234	0.3213321	-0.0020	0.0556

Thus, the following regression equation can be written in coded and natural values of factor X (Table 13).

**Table 13** Equation of regression in coded and natural values of factor X.

The equation in coded values	The equation in natural values
<b>Y1 - bread yield, cm<sup>3</sup> (3)</b>	
Y1 = 2330 - 50 X1 + 70 X2 + 60 X1X2	Y1=3351.9-5139.05*X1- 2.85714*X2+15.2381*X1*X2
<b>Y2 - crumb porosity, % (8)</b>	
Y2 = 68. 25 – 0.75 X1 + 5.25 X2 + 2.25 X1*X2	Y2=22.5714- 189.714*X1+0.14286*X2+0.57143*X1*X2
<b>Y3- crumb moisture content, % (4)</b>	
Y3 = 46.375 + 0.125 X1 + 1.625 X2 + 0.875 X1*X2	Y3=40.95238- 72.6667*X1+0.015873*X2+0.222222*X1*X2
<b>Y4 - the length of time it takes to keep bread safe, an hour (5)</b>	
Y4 = 45.3 X1 - 15 X2 - 9 X1*X2	Y4=40+758.8571*X1+0*X2-2.28571*X1*X2

Checking the correctness of the calculations. If we substitute the natural values of factors X1 and X2 in equation (3-5), the value of Yi at each level will be the same as the corresponding coded values of factors X1 and X2 in equation (3-5) (Table 13).

The analysis of the obtained values of the Student's t-test and appropriate levels of significance *p* confirms a significant impact on the resulting indicators of the content of grain mixtures on technological indicators of bread: *x*<sub>1</sub> – grain mixture content, %; *x*<sub>2</sub> – water content, g. Thus, the linear components *x*<sub>1</sub> and *x*<sub>2</sub> with *p* < 0.06 proved significant. *x*<sub>1</sub> – grain mixture content, %; *x*<sub>2</sub> – negative impact on the resulting criteria Y<sub>1</sub> and Y<sub>2</sub>.

Thus, based on the data received in experiments, by the method of least squares, the regression equations (3-5), depending on two investigated parameters *x*<sub>1</sub>, and *x*<sub>2</sub> presented in the standardized scale, were calculated.

**Table 14** Values of input factors in natural and coded form.

Number of an experiment	Values of X1 at X2 equal to				Yi	Relative change, %
	in kind values		in coded values			
<b>Y1 – bread yield, cm<sup>3</sup></b>						
1	1	339	1	1	2413	0
2	0.25	318	-1	-1	2370	0
3	1	318	1	-1	2532	0
4	0.25	339	-1	1	2734	0
<b>Y2 – crumb porosity, %</b>						
1	1	339	1	1	74	0
2	0.25	318	-1	-1	67	0
3	1	318	1	-1	62	0
4	0.25	339	-1	1	73	0
<b>Y3 – humidity of crumb, %</b>						
1	1	339	1	1	75	0
2	0.25	318	-1	-1	63	0
3	1	318	1	-1	62	0
4	0.25	339	-1	1	77	0
<b>Y4 – the length of time it takes to keep bread safe, an hour</b>						
1	1	339	1	1	23	0
2	0.25	318	-1	-1	41	0
3	1	318	1	-1	78	0
4	0.25	339	-1	1	33	0

To check the quality of the obtained regression equations (3-5) we calculated the multiple correlation coefficient  $R$ , the coefficient of determination  $R^2$ , the Fisher Test  $F$  and the Durbin-Watson test  $d$  (Table 14). The values of statistical criteria given in Table 14 indicate that the obtained regression equations with 95% confidence probability reliably and adequately describe the impact of the studied parameters of grain mixtures content on technological indicators of bread.

Sufficiently high values of the multiple correlation coefficient ( $R = 0.8202 - 0.9206$ ) indicate a very close relationship between the resulting indicators  $y_1$ ,  $y_2$ , and  $y_3$ . The included in the study regulated parameters of grain mixtures content on technological indicators of bread. The coefficient of determination ( $R^2 = 0.643 - 0.8475$ ) describes 64.3% and 84.6% variation of the corresponding response in the experimental data.

Fisher criterion  $F$  values calculated significance levels  $p < 0.3905$  indicate the obtained equations' sufficiently good approximating ability.

The serial correlation coefficients are weak and insignificant for the regression residuals of the equations. As evidenced by the values of the Durbin-Watson criterion  $d$ , we can assume that there is no serial correlation.

Thus, reliable and adequate regression equations of controlled parameters, fully characterizing the content of grain mixtures on safe storage time and technological parameters of bread, were obtained. Further, we present the values of estimated parameters (regression coefficients) –  $b_0$ ,  $b_1$ ,  $b_2$ ,  $b_{12}$  obtained in Statistica 12.

The resulting loss is 0.000000000. The regression coefficient is 1. Explained variance (adequacy variance) is 100%, i.e. the hypothesis of equation adequacy to experimental data is accepted.

Then the analysis of response surfaces for indicators of grain mixture content on technological indicators of bread was carried out. First, a complete factor experiment  $2^2$  was conducted to study the dependence. For this purpose, planning matrices of the complete factor experiment were made (Tables 16-19).

**Table 15** Statistical indicators of quality and characteristics of the adequacy of regression models.

Statistical quality indicators and adequacy criteria	Response		
	Y1	Y2	Y3
Multiple correlation $R$	0.8202	0.945310	0.854240
Determination coefficient $R^2$	0.676540	0.847458	0.776256
Normalized $R$ -square	0.018182	0.542373	0.345642
Standard deviation	120	4.5	1.75
Number of degrees of freedom $df: k_1; k_2$	2	2	2
Fisher criterion $F$	1.027778	2.777778	1.71234
Significance $F$	0.572078	0.390567	0.473016
Durbin-Watson criterion $d$	2.253	2.378	2.274
Coefficient of serial correlation $r$	-0.133	-0.231	-0.124

Note:  $k_1$  and  $k_2$  are the number of degrees of freedom for numerator and denominator, respectively.

**Table 16** Planning matrix of the full factor experiment for two factors for Y1.

Experience number	Factors on an in-kind scale		Factors in a dimensionless coordinate system			Output parameter
	$z_1$	$z_2$	X0	X1	X2	
1	1	343	0	1	1	2452
2	0.25	342	0	-1	-1	237425
3	1	342	0	1	-1	2113
4	0.25	379	0	-1	1	23736

**Table 17** Planning matrix of the full factor experiment for two factors for Y2.

Experience number	Factors on an in-kind scale		Factors in a dimensionless coordinate system			Output parameter
	$z_1$	$z_2$	X0	X1	X2	
1	1	3321	0	1	1	76
2	0.25	3524	0	-1	-1	65
3	1	3432	0	1	-1	61
4	0.25	343	0	-1	1	70

**Table 18** Planning matrix of the full factor experiment for two factors for Y3.

Experience number	Factors on an in-kind scale		Factors in a dimensionless coordinate system			Output parameter
	$z_1$	$z_2$	X0	X1	X2	
1	1	342	0	1	1	51
2	0.25	347	0	-1	-1	47.5
3	1	315	0	1	-1	42
4	0.25	346	0	-1	1	48

**Table 19** Planning matrix of the full factor experiment for two factors for Y4.

Experience number	Factors on an in-kind scale		Factors in a dimensionless coordinate system			Output parameter
	$z_1$	$z_2$	X0	X1	X2	
1	1	342	0	1	1	25
2	0.25	340	0	-1	-1	42
3	1	342	0	1	-1	73
4	0.25	347	0	-1	1	37

Substitute different values of  $y$  into the obtained expression and make the corresponding Table 20.

**Table 20** Equal level lines for Y1.

	2410	2310	2210	2110	2010
-1	3	-7	-17	-27	-37
-0.75	1.7	-2.3	-6.3	-10.3	-14.3
-0.5	1.355	-1.125	-3.625	-6.125	-8.625
-0.25	1.227243	-0.59054	-2.40922	-4.22743	-6.04543
0	1.142853	-0.28543	-1.71445	-3.14243	-4.57143
0.25	1.0882786	-0.08843	-1.26443	-2.44143	-3.61742
0.5	1.05	0.05	-0.95	-1.95	-2.95
0.75	1.021739	0.152145	-0.71535	-1.58673	-2.45667
1	1	0.230743	-0.53843	-1.30769	-2.07642

The color marks show the value of the corresponding indicator by means of intensity. According to them, it is possible to determine the range of values of the variables, where the indicator of the quality of the bread is of the most significant importance.

To get a clearer idea of how the quality indicators of bread are related to the dosage of the grain mixture, volumetric graphs are built. The same levels of the displayed values of the quality indicators of bread are highlighted in the volumetric drawings using a "wire" mesh, different shading, and shading. The levels have the same values where these surface chart elements are the same.

The constructed surface diagram allows you to find the best combination of mixture components, which is difficult to identify in any other way from the available values.

Response functions are best represented graphically. Figures 4-6 show response surfaces and lines of equal levels (isolines) of grain mixture content indicators on technological indicators of bread quality and safety depending on different combinations of the studied parameters: X1 – grain mixture content, %, X2 – sourdough content, g.

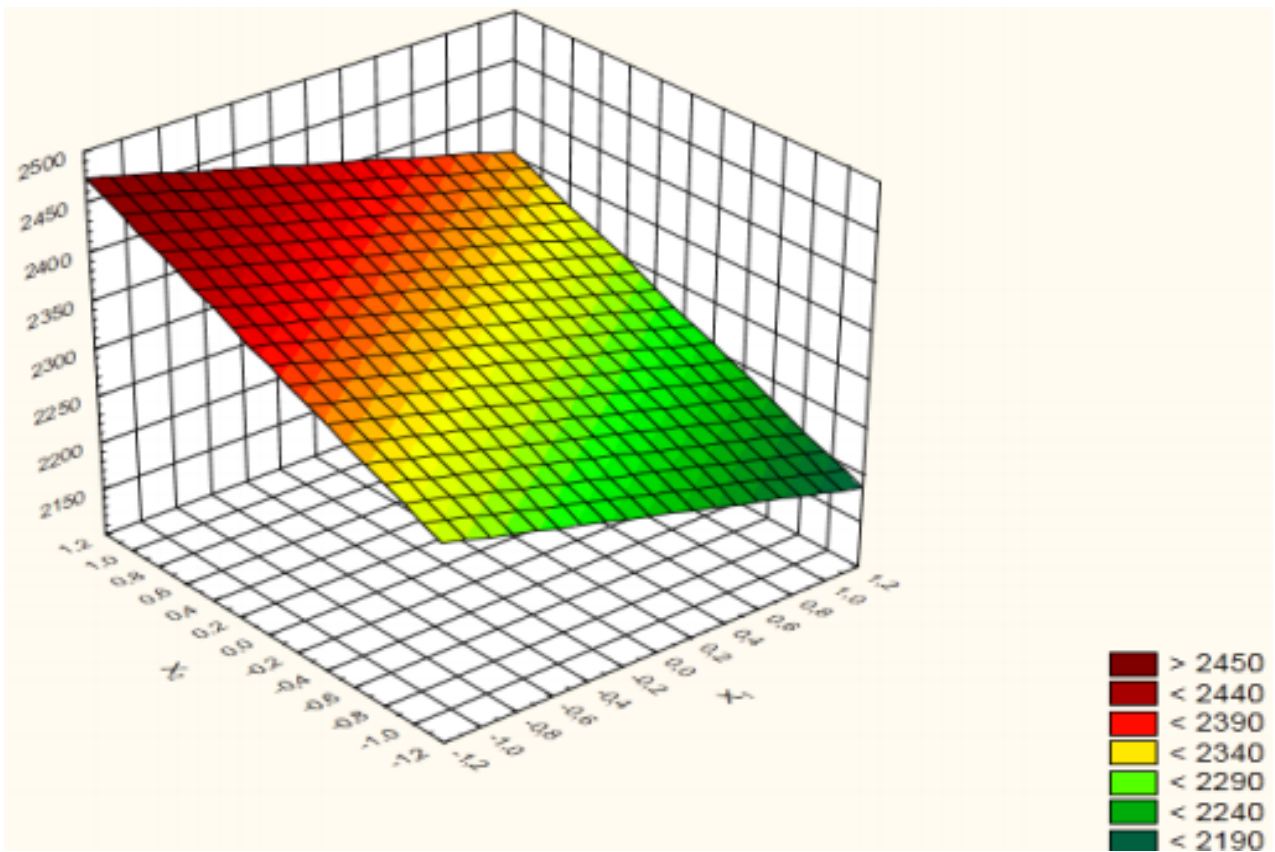


Figure 4 Response surface of grain mixture and starter content on technological indicators of bread for  $y_1$ .

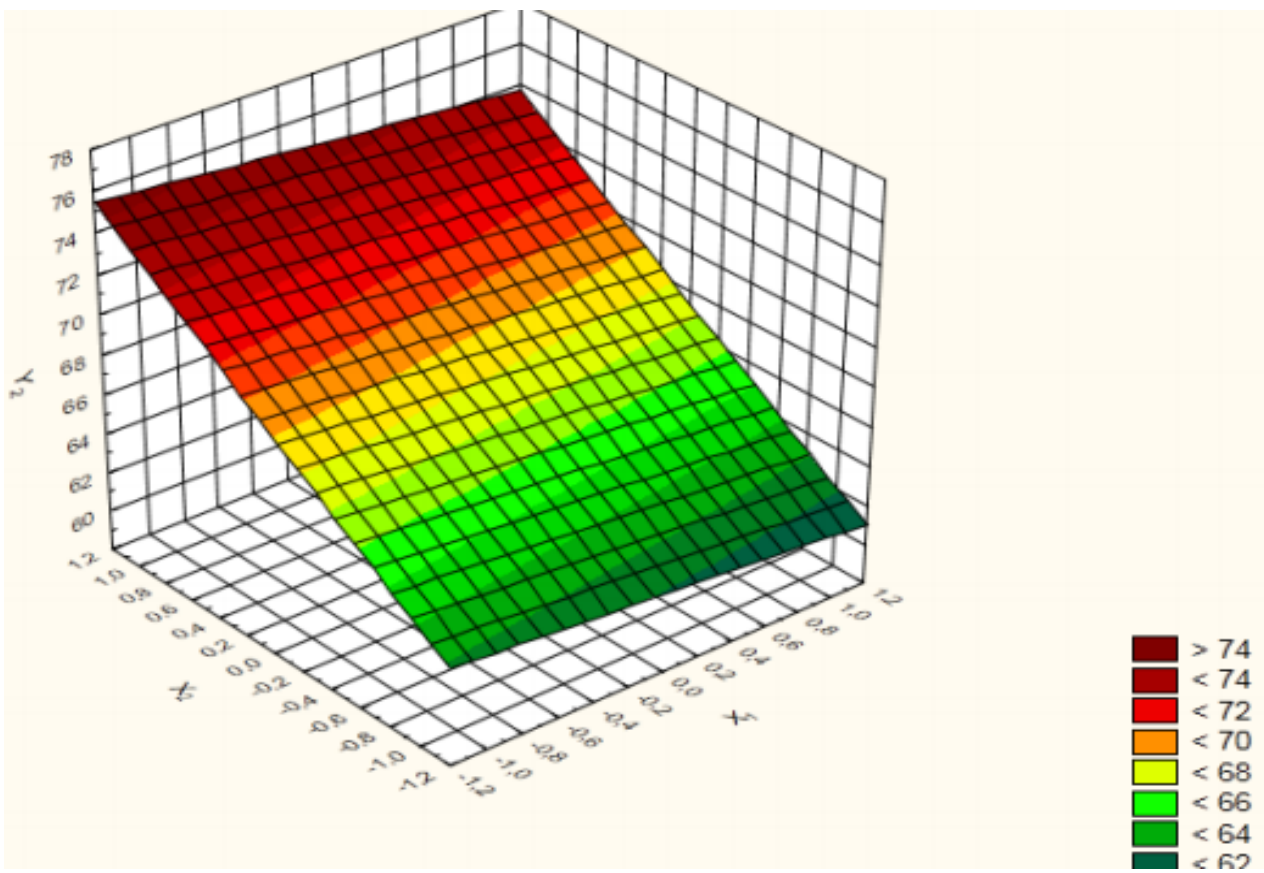
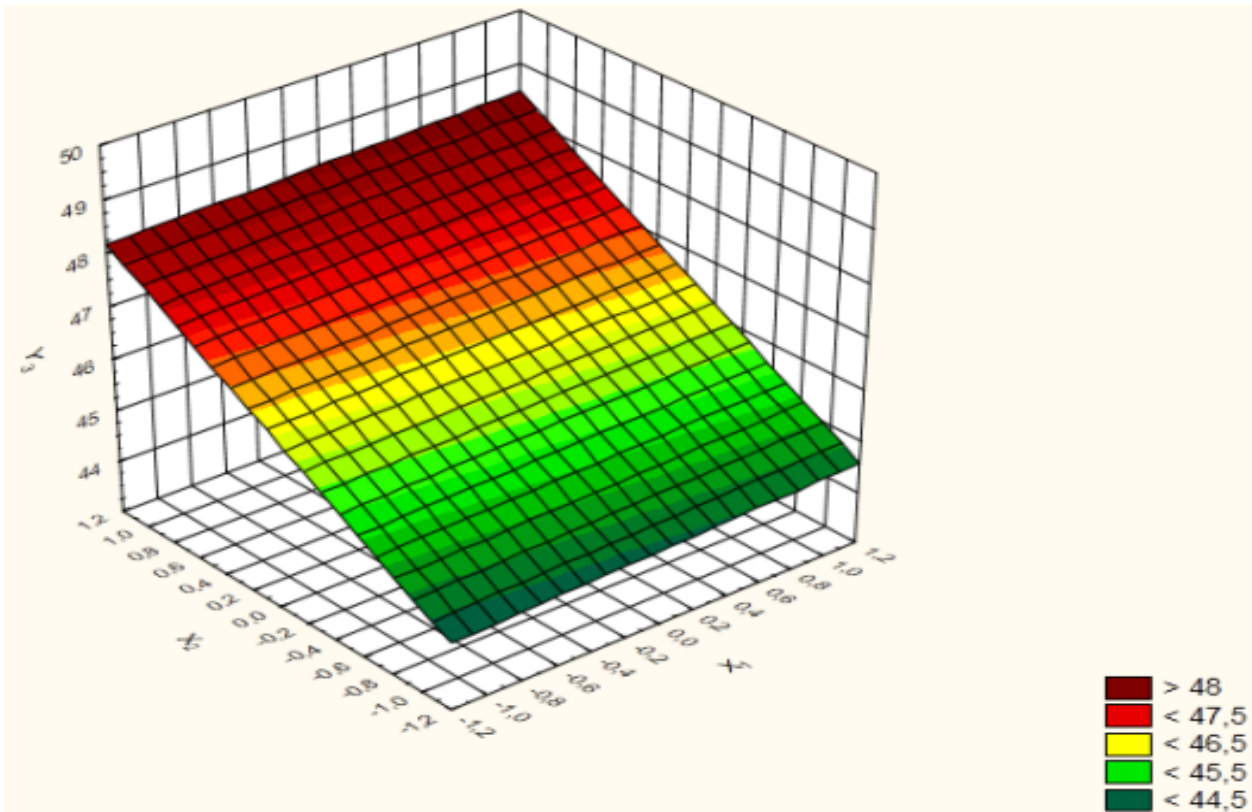


Figure 5 Response surface of grain mixture and starter content on technological indicators of bread for  $y_2$ .





**Figure 6** Response surface of grain mixture and starter content on technological indicators of bread for  $y_3$ .

After that, the changes occurring in bread samples during storage were investigated. Changes in the bread of increased nutritional value with grain mixtures compared with the control sample were investigated. The researched bread samples were stored at  $20 \pm 5$  °C and  $70 \pm 5\%$  relative humidity. Assessment of bread quality was carried out 6, 24, and 48 hours after baking. The dynamics of changes are presented in Figure 7. Also, organoleptic analysis of the degree of freshness using an eight-point evaluation scale was carried out.

According to the data obtained, shown in Figure 7, the most significant difference was noticed at the end of the study period, when the control sample received 0 points (the control sample began to go moldy and lost its consumer appeal), and the bread using grain mixtures in the same study period of 72 hours showed 4 points, and no signs of mold and spoilage were noticed, only the consumer turgor was lost, the appearance of staling processes, etc. After baking, all the samples received the highest score, and a few hours after the end of the shelf life, all the samples showed such a big difference in scores.

A long-time of samples freshness explains due to the feature of the formulations of different samples of bread, especially in the presence of starter cultures and the presence of bioactivation grains due to the high moisture content, lactic acid bacteria, the activity of enzymes, beans, etc.

## DISCUSSION

The quality indicators of bread crumbs using grain mixtures significantly differed from the control sample in terms of specific volume and porosity structure. According to organoleptic parameters, the experimental samples of grain bread, compared to the control sample, were more attractive in appearance, porosity, crumb color, taste, and aroma. It was found that the introduction of a grain mixture in the amount of 5-25% of the total mass of flour has a positive effect on the quality of bread. At the same time, the physical and chemical indicators of the quality of bread decrease with an increase in the organoleptic characteristics of the finished products. It was found that the best physical and chemical parameters were obtained from bread samples prepared with the addition of 20% of the grain mixture. In general, all types of experimental analyses performed showed an improvement in a number of indicators compared to the control sample. The nutritional value of the resulting products was increased from 0.5 to 3 times. The content of polyunsaturated fatty acids, vitamins, and minerals increased. Mathematical

modelling analyses proved the positive effect of the addition of grain mixtures on the structure and technological parameters of the bread.

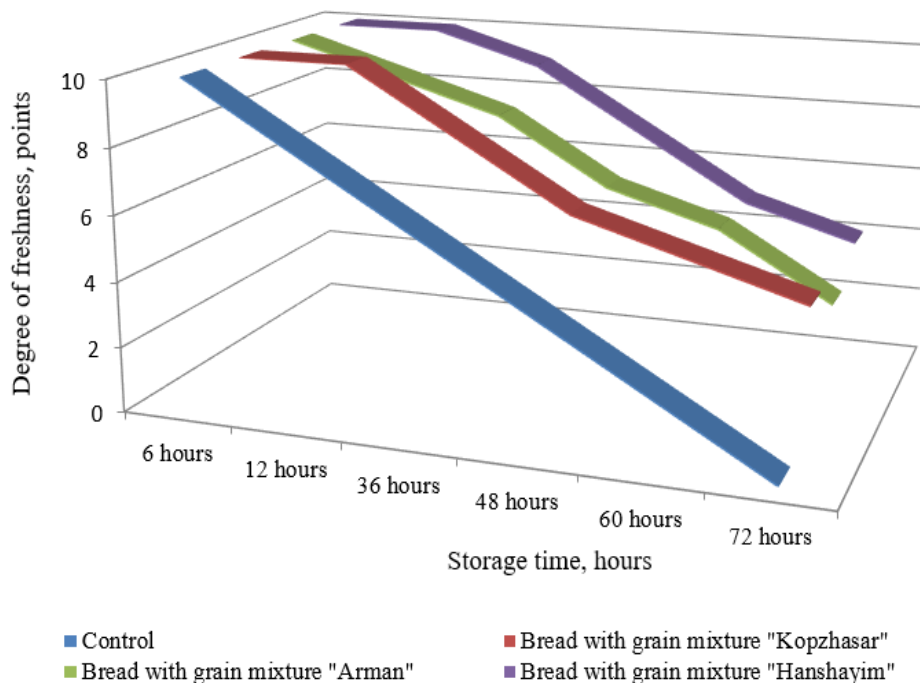


Figure 7 Changes in the degree of freshness of the tested bread samples.

## CONCLUSION

The additive of grain mixtures and rye sourdough impact bread quality has been researched. The surface response of the content of grain mixtures and sourdough on the bread's technological parameters was obtained as bread's yield, crumb porosity, crumb moisture, and the duration of safe storage of the bread. All researched indicators showed an excellent result demonstrating the prospects and effectiveness of adding grain mixtures to the quality of the bread. Consequently, it has been experimentally proved that the introduction of grain mixtures into the bread recipe significantly increases the nutritional value of bread. Generally, the technology of bread using grain mixes and starter cultures is relevant for the modern world. It offers great opportunities to expand the range and ensure the microbiological safety of grain bakery products, especially with the addition of bioactivated grains.

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**Funds:**

This work was supported by a scholarship from the Ministry of Education and Science of the Republic of Kazakhstan.

**Acknowledgments:**

The authors express their gratitude to the Mendel University (Czech Republic) and the administration of the Almaty Technological University. Special thanks are expressed to the Department of "Technology of bread products and processing industries" of the Almaty Technological University.

**Conflict of Interest:**

The authors declare no conflict of interest.

**Ethical Statement:**

This article does not contain any studies that would require an ethical statement.

**Contact Address:**

Zhuldyz Nurgozhina, Almaty Technological University, Faculty of Food Technology, Department of Technology of Bread Products and Processing Industries, Tole bi 100, 050012, Almaty, Republic of Kazakhstan  
Tel.: +77074718620,

E-mail: [juldyz-900@mail.ru](mailto:juldyz-900@mail.ru)

ORCID: <https://orcid.org/0000-0002-6576-4445>

Dinara Shansharova, Almaty Technological University, Faculty of Food Technology, Department of Technology of Bread Products and Processing Industries, Tole bi 100, 050012, Almaty, Republic of Kazakhstan,  
Tel.: +77077540290,

E-mail: [dinara-shansharova@mail.ru](mailto:dinara-shansharova@mail.ru)

ORCID: <https://orcid.org/0000-0002-7346-2658>

Gulzhanat Umirzakova, West Kazakhstan Agrarian and Technical University named after Zhangir Khan, Higher School of Technology of food and processing industries, Zhangir Khan street 51, 090009, Uralsk, Republic of Kazakhstan,  
Tel.: +77479263035,

E-mail: [zhan\\_u\\_al@mail.ru](mailto:zhan_u_al@mail.ru)

ORCID: <https://orcid.org/0000-0001-6988-9520>

Pernekul Maliktayeva, International Taraz Innovation Institute, Faculty of natural science, Department of Standardization and veterinary sanitation, Zheltoksan, 69B, 080000, Taraz, Republic of Kazakhstan,  
Tel.: +77021072844,

E-mail: [pernekul.78@mail.ru](mailto:pernekul.78@mail.ru)

ORCID: <https://orcid.org/0000-0002-1251-811X>

\*Madina Yakiyayeva, Almaty Technological University, Research Institute of Food Technologies, Tole bi 100, 050012, Almaty, Republic of Kazakhstan,  
Tel.: +77011626749,

E-mail: [yamadina88@mail.ru](mailto:yamadina88@mail.ru)

ORCID: <https://orcid.org/0000-0002-8564-2912>

Corresponding author: \*

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