Prospects for the use of phytase to keep the freshness of bread

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Abstract. The results of studies on the effect of enzyme preparation with phytase activity on the quality and preservation of bread freshness from composite flour are described. In grain products there is rather a higher content of the antinutrient – phytic acid, which reduces the bioavailability of phosphorus, calcium, magnesium and other minerals, and thus it reduces the quality of bread. In order to solve this problem, it is proposed to add an enzyme preparation with phytase activity to the formula of bread from composite flour. The degree of staleness of bread samples was estimated by changing the loading force on the indenter in the process of deforming the crumb and removing the load. The total deformation of bread crumb was determined on the “ST-2 Structurometer.” Assessment of the loading force on the indenter during the crumb deformation was carried out 24 and 72 hours after baking the bread. The control sample was the bread without enzyme preparation. It was found that the crumb of the bread sample from composite flour with the introduction of 0.08% of the enzyme preparation with phytase activity is characterized by the lowest staling rate 72 hours after baking. It is concluded that it is appropriate to include the enzyme preparation with phytase activity in composite bakery mixtures in the amount of 0.08%. The use of the enzyme preparation with phytase activity will not only improve the quality of bread from composite mixtures with whole grain flour and bioavailability of mineral substances but is also provide prolonged preservation of bread freshness.

1. Introduction
Following the concept of state policy in the field of healthy nutrition of the population of the Russian Federation, the task is to improve the structure of nutrition by increasing the share of quality and affordable products of mass consumption with increased nutritional value. The relationship between nutrition quality and quality of life is scientifically sound, and the existence of such correlation is confirmed by both Western and Eastern researchers [1, 2, 3, 4, 5, 6].

From the second half of the last century to the present scientists offer various solutions on designing composite formulas of products with increased nutritional and biological value [7, 8, 9]. A promising way to improve the quality of one of the most mass-consumed products – the bread – is to use scientifically based flour composite mixtures. Various ready-made mixtures are increasingly used in the production of bakery products for healthy nutrition, including the components which give the desired volume, texture, taste, and colour to the product. This, in its turn, ensures the preservation of the properties of the products during storage, as well as resistance to foreign microflora. This trend is very effective, as it provides using various raw materials, including non-traditional and by-product raw
materials; applying useful properties of individual components; achieving a better balance of nutrients in the finished product and, eventually, obtaining high-quality products with predetermined composition and properties [10, 11, 12, 13, 14]. Such mixtures are called composite, compound, ready, and flour ones. The possibility of varying the composition of such mixtures makes it possible to use them to obtain functional products with predetermined properties and nutritional value. The introduction of such products into the daily diet of the population allows improving the quality of life through the improvement of nutrition quality, to effect the nutritive status of the nation and to reduce the risk of alimentally dependent diseases [15, 16, 17, 18].

In the light of the global trend towards a healthy lifestyle, including the use of healthy foods, many countries have seen the increase in demand for high-cereal bakery products in recent years (grain, cereal, grit). However, the high share of grain products in the formula leads to receiving bread with a rough crumb, insufficient porosity, low volume, that limits demand for such grades of bread. However, the main problem is instead a higher content of the antinutrient - phytic acid - in grain products. This antinutrient reduces the bioavailability of phosphorus, calcium, magnesium and other minerals and, accordingly, diminishes the quality of bread. There are several ways to solve this problem. For example, Japanese scientists have studied the impact of the use of brown rice flour on bread quality [19]. In order to increase bread food value, wheat flour in the formula was replaced with flour from sprouted brown rice in the amount from 10% to 30%. It was found that when 30% of flour was replaced with rice in the final product, phytic acid was destroyed by 30-54% and it was concluded that partial replacement of wheat flour with flour from sprouted brown rice could positively affect the quality of bread.

However, a more effective way to solve this problem is to use an enzyme with phytase activity to improve the quality of bakery grain products [20]. For example, Spanish scientists have studied the effectiveness of using bacterial phytase to decompose phytates in whole ground grain bread [21]. Purified Bifidobacterium longum spp Infants and Bifidobacterium pseudocatenulatum were used. Control bread samples were made from conventional wheat flour and whole ground wheat grain flour. All-ground amaranth flour in the amount of 25% or 50% was used in the test samples. The dough was prepared in a sponge way; phytase was introduced before the main dough kneading. The own phytase activity of the flour used in the test was also determined, which proved to be quite high but insufficient to decompose all the phytates present in the flour. The addition of phytase contributed to a more pronounced decomposition of phytates. In essence, bacterial phytase reduced phytate content so much that they no longer affected the bioavailability of iron and zinc to the human body. The tested phytase is promising for use in the production of whole grain bakery products. Another team has studied the effect of phytase addition on the quality of bran-enriched wheat bread and the bioavailability of the iron contained therein [22].

Wheat, lentils, brown rice and their processing products are known to contain phytic acid. The content of phytic acid in wheat is 1.03 g. per 100 g. of product, in lentils 1.15 g. per 100 g. of product, in brown rice - 0.88 g. per 100 g. of product. The active phytase content in wheat flour is 3.08 standard units and in brown rice flour, only 0.19 standard units [23, 24].

2. The purpose of the study
The research aims to study the effect of enzyme preparation with phytase activity on quality, increased bioavailability of mineral substances and freshness preservation of bread from composite flour.

3. The object of the study
Bakery wheat flour of the first grade (GOST R 52189-2003); whole-ground lentil flour (CTO 38744625-001-2012); whole ground brown rice flour (CTO 38744625-001-2012); food salt (GOST R 51574-2018); dry wheat gluten (GOST 31934-2012); pressed bakery yeast (GOST R 54731-2011); white sugar (GOST 33222-2015); enzyme preparation with phytase activity ”Agrofit” (manufacturer ”Agroferment”, Russia); drinking water (GOST R 51232-98, SanPin 2.1.4.1074-01).

The enzyme preparation (EP) contains the phytase enzyme with the activity of at least 5000 units/g., cellulase, β-glucanase, protease and xylanase. The produce is the fungus of the
strain *Penicillium canescens PhPl-33VKM F-3867D*, which has not undergone genetic engineering intervention. The biological activity of the drug is based on the stability of the phytase enzyme to break phytates down, thereby significantly increasing the digestibility of phosphorus, as well as calcium, magnesium, trace elements, crude protein and amino acids and increasing the energy value of food products.

4. Materials and methods

The formula and technological dough preparation parameters for bread from flour composite mixture are presented in Table 1. Dough kneading was carried out in laboratory dough machine BEAR VARIMIXER TEDDY for 2-5 min. Pressed bakery yeast was added as yeast suspension in ratio 1:3. The enzyme preparation with phytase activity was added in the amount of 0.02%, 0.04%, 0.06% and 0.08% of flour weight.

| Name of raw materials, semi-finished products and process indicators | Raw material consumption (g) and parameters of the dough preparation process |
|---------------------------------------------------------------------|--------------------------------------------------------------------------------|
| First-grade wheat flour                                             | 78.6                                                                            |
| Whole-ground brown rice flour                                       | 11.3                                                                           |
| Whole-ground lentil flour                                           | 4.7                                                                            |
| Dry wheat gluten                                                    | 2.0                                                                            |
| Food salt                                                           | 1.5                                                                            |
| White sugar                                                         | 1.9                                                                            |
| Pressed bakery yeast                                                | 2.0                                                                            |
| Refined deodorized sunflower oil                                    | 5.0                                                                            |
| Drinking water                                                      | 60.0                                                                           |
| Dough humidity, %                                                   | 41.0-45.0                                                                     |
| Final dough acidity, deg                                            | 2.5-3.5                                                                        |
| The duration of dough fermentation, min                             | 60                                                                             |

Dough temperature after kneading was 26-28°C. Dough fermentation was carried out in a thermostat at temperature 33-35 °C and relative air humidity 75-80% for 60 minutes. Then the dough was divided into pieces weighing 0.5 kg for mould bread. Cutting and moulding were carried out manually. The separated dough pieces were placed in moulds and sheets for baking and sent to final proofing at 36-38 °C and 75-80% relative air humidity. The baking readiness of the dough pieces was determined organoleptically. The products were baked in a laboratory electric furnace at the medium temperature of the baking chamber 170-190 °C. The duration of bread baking was 30 min.

The bread analysis was carried out in 14-16 hours after baking according to the following indicators. In essence, the moisture of the bread crumb was determined by drying it in an oven at 130 °C for 40 minutes and expressed as a percentage according to Standard 21094 "Bread and bakery products. Method for the determination of moisture." The acidity of the finished products was determined by titration of the filtrate obtained from crumbs of bread products with sodium hydroxide solution with the addition of phenolphthalein indicator and expressed in degrees of acidity (Standard 5670 "Bread, rolls and buns. Methods for determination of acidity"). Bread crumb porosity was estimated by the ratio of crumb pore volume to total crumb volume and expressed in percentage (Standard 5669 "Bakery products. Method for determination of porosity"). Specific volume was determined by the ratio of the volume of loose fine-grain displaced by bread to its mass (Standard 27669 "Wheat bread flour. Method for experimental laboratory bread making"), expressed in cm³/g. The appearance, colour of the crusts, elasticity and baking of the crumb, the state of porosity, taste, smell (Standard 5667 "Bread and bakery products. Rules of acceptance, methods of sampling, methods for determination of organoleptic characteristics and mass") were evaluated in the process of organoleptic bread assessment.
The degree of staleness of the bread samples was estimated by the rheological properties of the crumb, in particular - by changing the loading force on the indenter in the process of deforming the crumb and removing the load. The total deformation of the crumb was determined on the ST-2 Structurometer of the Laboratory of Quality, Russia. The principle of operation of the device is based on the measurement of mechanical load on the nozzle-indenter at its introduction at the specified speed into the prepared product sample. The required indenter is attached to the strain block moved in a vertical direction through a ball-screw pair according to the specified program. Assessment of the loading force on the indenter during crumb deformation was carried out in 24 and 72 hours after bread baking.

Bioavailability of bread was carried out according to the method during 16 replicates: a culture medium was prepared to contain 0.87 g. of pancreatic casein hydrolyzate, 0.87 g. of glucose, 0.2 g. of yeast extract and 0.2 g. of sodium chloride per 100 ml of distilled water; it was poured into the tubes and sterilized. After that, the Tetrahymena pyriformis ciliates were introduced into the tubes, which were grown in this medium for 48-96 hours at the temperature of 25 ± 2 °C. The samples were mixed in a multi-well plate and the number of Tetrahymena pyriformis ciliates in the wells was counted after 24 and 48 hours. At the same time, test samples of the products were prepared by grinding them to a particle size of fewer than 200 \( \mu \)m, weighed in distilled water. Hydrochloric acid was added until the pH of the solution was pH 3; pepsin was added, and the suspension was extracted for 0.5-2 hours. Then sodium hydroxide and pancreatin were added up to pH eight and extraction was carried out for 0.5-2 hours, hydrochloric acid was added to pH 6. The living biomaterial was fed by diluting a working culture of ciliates in the extract, the samples were placed in the multi-well plate, and the number of Tetrahymena pyriformis ciliates in the wells was counted after 24 and 48 hours.

5. Discussion of the results
Physicochemical indicators of the bread quality of experimental samples with the addition of the enzyme preparation remained at the level of the control sample. They amounted to specific bread volume 2.00 \( \text{cm}^3/\text{g} \), porosity 80.0%, crumb moisture 42.0%, crumb acidity 1.8 deg.

The indenter loading force indices were determined at four points of the crumb of each bread sample; as a result, diagrams of changes in the loading force on the indenter during crumb deformation were obtained. The average value of the loading force was obtained as a result of data processing of the loading force diagrams on the indenter. The bread without enzyme preparation with phytase activity was the control sample

The indicators of average values of loading and displacement forces on the indenter obtained in the study of the bread crumb are shown in Figures 1 and 2.

The assessment of the change in loading and displacement forces on the indenter during crumb deformation and load removal revealed that the crumb of composite flour bread with the addition of 0.08% enzyme preparation with phytase activity was characterized by the lowest staling rate 72 hours after baking compared with the same control sample. According to organoleptic indicators, the crumb of all bread samples with the addition of the enzyme preparation with phytase activity was elastic and non-crumbling, in contrast to the control sample. The enzyme preparation with phytase activity in the studied dosages did not affect the bread appearance, taste, and smell.
In order to determine the bioavailability of bread prepared from a flour composite mixture, the effect of the enzyme preparation with phytase activity in the amount of 0.02, 0.04, 0.06 and 0.08% by weight of flour on the growth of *Tetrahymena pyriformis* ciliates was studied. As control samples, bread samples without enzyme preparation were used. The indicators of average values of the number of *Tetrahymena pyriformis* ciliates determined by counting them during the storage of bread after 24 and 48 hours are presented in Figure 3.
The data obtained showed that the use of the enzyme preparation as a prescription bread component contributed to an increase in the number of *Tetrahymena pyriformis* infusoria by 26-165% depending on its dosage, compared to the control sample. After 48 hours of storage of bread from the flour composite mixture with the addition of the enzyme preparation in the dosage of 0.08%, the number of *Tetrahymena pyriformis* ciliates was 84.6% more than in the control sample. Moreover, in the experimental samples, the ciliates of *Tetrahymena pyriformis* were characterized as large, very active and fast ones, compared to the control sample.

![Figure 3. The average value of the number of *Tetrahymena pyriformis* during bread storage prepared from flour composite mixture with the addition of the enzyme preparation](image)

**6. Conclusion**

Based on the studies, it can be concluded that it is advisable to introduce an enzyme preparation with phytase activity in the composition of composite baking mixtures in the amount of 0.08%. The use of the enzyme preparation with phytase activity did not impair the physicochemical and organoleptic quality indicators of bread from flour composite mixtures. The introduction of the enzyme preparation with phytase activity in the composition of the flour composite mixture increased the preservation of bread freshness and the number of *Tetrahymena pyriformis* ciliates during bread storage, increasing the bioavailability of mineral substances.

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