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EXPERIMENTAL SUBSTANTIATION OF THE APPLICATION OF PLANT EXTRACTS AND ENZYMES TO OBTAIN SAFE RAW MATERIALS FOR WHOLE GRAIN BREAD TECHNOLOGY

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Currently, ensuring the high quality and safety of food is the most important condition for a rational diet, maintaining health, mental and physical performance and supporting the body's defense systems. The modern environmental situation is one of the conditioning factors of the nutritional status of the population. It is known that more than 70 % of the total amount of pollutants entering the human body falls on food.

The purpose of this work is to improve the safety and quality of raw materials and grain products for whole-grain bread technology using enzyme preparations and extracts of plant origin during grain defrosting.

Reasonable effective doses of enzyme preparations have been determined. Cellolux was introduced at a dose of 0.03–0.08 % by weight of grain solids. To reduce the content of toxic elements in the grain, 0.05 % of the mass of grain solids was chosen, the optimal duration of defrosting is 6 and 12 hours, but depending on the efficiency of the time – 6 hours. The grain was soaked at a temperature of 23 °C (room temperature).

Extracts of leaves and stems of rosehip, sea buckthorn and barberry were used as plant material. The use of these plant raw materials is explained by the fact that their leaves and bran extracts contain polyphenols with antioxidant, antimicrobial and carcinogenic properties. For disinfection and purification of grain from heavy metals, a content of 0.05 % of the grain weight was selected.

The effect of plant extracts on the microbiological contamination of grain during soaking and in preparation for the production of grain bread and preventing microbial spoilage of bakery products during storage has been studied. And extracts of rosehip leaves and sea buckthorn stalk have a pronounced antimicrobial effect against fungi of the genus Pénicillium. That will make it possible to exclude diseases of bread and improve its rheology

Keywords: enzyme preparations, whole grain bread, sea buckthorn, rosehip and barberry extracts

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1. Introduction

The modern environmental situation is one of the conditioning factors of the nutritional status of the population and food safety [1]. Every year the problem of environmental degradation, removal of heavy and radioactive metals from the human body is becoming more and more urgent all over the world [2].

Grains are the main source of energy and carbohydrates for human nutrition. The predominant "consumption" of refined products leads to a decrease in the content of ballast substances and valuable trace elements in the modern diet of the population of industrialized countries [3, 4]. Whole grains usually contain more contaminants than refined products. However, whole grains also contain more nutrients that can reduce exposure to these contaminants [5].

Currently, insufficient attention is paid to the development of ways to reduce the content of toxic elements in grain raw materials. In this regard, soaking with enzyme preparations is relevant to reduce the content of heavy metals in grain raw materials. The enzyme preparation hydrolyzes non-starchy polysaccharides of wheat bran and affects the aleurone layer of wheat bran, increasing their digestibility. When moistened with an enzyme preparation, the outer shell of wheat grain softens and heavy metals are released [6].

It has an optimal effect in moistening grain with vegetable raw materials to increase the microbiological safety of finished grain products and enrichment with antioxidants, and also helps to prolong the shelf life of finished products [7].

Due to this, the application of plant extracts (sea buckthorn, rosehip and barberry), which have an antimicrobial effect, is prevalent in grain soaking.

Sea buckthorn (Hippophae rhamnoides) is well known for its antioxidant content (ascorbic acid, polyphenols, carotenoids), high acidity, bright yellow color, pleasant taste and smell. The antimicrobial properties of sea buckthorn affect the sensory properties of the finished product, improve the antioxidant potential, influence the microbiological stability and shelf life of the products [8].

The use of rosehip fruit (Rosa canina) has been registered in many countries for a long time. These berries contain some of the main active ingredients, such as flavonoids, tannins, anthocyanins, phenolic compounds, fatty oil, organic acids and inorganic compounds. In particular, it was reported that rosehip fruit powder and extract have a therapeutic effect on arthritis [9].

The types of barberry (Berberis) are of great current interest in the medical and food industry, including a wide range of the most common biologically active compounds. They contain carbohydrates – free sugars, pectins and protopectins, phenolic compounds – catechins, anthocyanins and tannins, as well as organic acids that cause the sour taste of fruits and vegetables. It exhibits antimicrobial activity, since the element berberine is found in barberry bushes. In precisely tested doses, the element has a beneficial effect [10].

This vegetable raw material is one of the main sources of biologically active substances with even minimal bactericidal effect. This factor is associated with the presence of components such as phenols, vitamins, flavonoids, carotenoids, phytoncides, tannins in the plant material. In addition, as a regulator of the rheological properties of dough, when soaking grain with sea buckthorn, rosehip and barberry extracts, it increases the gas-retaining ability of wheat flour, strengthens gluten, increases water absorption capacity, and affects the reduction of dough dilution [11].

The information given above indicates that the quality of food products, including grain, is commonly understood as a set of characteristics determining its consumer properties and ensuring the safety of the product for humans. Ensuring safety, in turn, is associated with the absence of toxic, carcinogenic, mutagenic or any adverse effects on the human body when using the product in generally accepted quantities. In this regard, it is urgent to apply the cellulolytic enzyme and extracts from plant raw materials in grain soaking for reducing the content of heavy metals in grain raw materials.

2. Literature review and problem statement

The paper [12] presents the results of a study of grain soaking in order to remove heavy metals. It is shown that hydrothermal treatment effectively removes heavy metals, but there are unresolved issues related to the organoleptic characteristics of the grain. The reason for this may be objective difficulties associated with an increase in grain temperature during hydrothermal treatment, as well as changes in chemical composition. Changes in the chemical composition adversely affect the shelf life and quality of raw materials. Raising the temperature is not cost-effective. Soaking of grain can be a way to overcome difficulties. This approach was used in [13], however, the soaking process does not lead to the expected result, because soaking occurs in water. All this suggests that it is advisable to conduct a study on the soaking process using plant extracts and enzyme preparations.

The soaking stage is the longest and most influential process in the formation of the quality of grain bread. The studies proposed by scientists from different countries [14, 15] are aimed at solving the problem of reducing the duration of this stage by taking into account soaking at higher temperatures, preliminary wet-heat and mechanical processing. The use of cleaning methods for soaking grain by subsequent treatment with gaseous preservatives, chemical disinfectants, organic acids or their solutions, extracts of medicinal plants, etc. is also noted. The use of such approaches helps to increase the specific volume of bakery products, improve the rheological properties of the dough and slow down the processes of their solidification. The removal of heavy metals remains an unresolved issue.

The research work [16] shows that it is advisable to use enzyme preparations of hemicellulase and cellulase to improve the quality of grain bread. Given the significant amount of non-starchy polysaccharides in grain raw materials, these enzyme preparations support the normal hydrolysis of grain shells, increase the viscosity of the dough, improve its ability to retain water and gas, which has a positive effect on the formation of high-quality products. The use of an enzyme preparation at the product preparation stage is not as effective as the use of an enzyme preparation on raw materials. In the research work [17, 18], it was shown that the use of the enzyme preparation xylanase during grain purification affects its structural and mechanical properties in the technology of whole grain bread. This helps to increase the specific volume, improves the rheological properties of the dough and slows down the processes of its solidification. The authors do not consider the quality of raw materials and the removal of heavy metals.

In [19], hemicellase and cellulase were used at the stage of grain soaking to remove heavy metal ions from the shell in grain bread technology. An additional effect of their use is reduced duration of grain soaking and improved rheological properties of dough and bread. However, the implementation of this technical solution is associated with the need to create favorable conditions for the action of enzymes and involves the use of high temperatures for grain soaking, which requires special equipment, leading to an increase in the cost of production. In addition, the use of enzymes at the stage of kneading grain dough may be justified, since its fermentation temperature (30±2 °C) is more favorable for the effects of enzymes than the absorption temperature, which is usually 20±2 °C. Proteolysis. One of the effective ways to regulate the properties of gluten is the use of oxidoreductases in the preparation of dough [20], which is accompanied by an improvement in the rheological properties of the dough, an increase in its gas-holding capacity and an improvement in the quality of bread. In addition, many modern studies [21] discuss the feasibility of the combined use of xylanase and glucose oxidase enzyme preparations due to the synergism of their action.

Thus, thanks to the use of enzyme preparations during grain defrosting, the grain relief changes, the pore size in the peel of seeds and fruits increases, which promotes the penetration of the phytase enzyme into the aleurone layer instead of hydrolysis of phytin and leads to increased phytoactivity. When processing wheat with an enzyme preparation, there is a tendency for the spread of mineral elements, in the aleurone layer the proportion of these elements characteristic of grain decreases, and in the endosperm layer increases. Since dietary fiber and phytate occur together in the peripheral layers of fiber-rich grains, it is difficult to isolate the effect of the decomposition of insoluble polysaccharides and redistribution of fibrous ions of polyvalent metals. However, studies have shown that the enzyme preparation is an effective mechanism for regulating the metabolism of minerals and nutrients [22].

The analysis of scientific sources [22] revealed the prospects for the use of enzyme preparations at the stage of grain soaking, which requires a systematic study of their influence on the formation of grain properties.

There is a constant need to protect stored products from spoilage, loss of food quality and especially during storage, mainly due to fungi and insects, which usually act in concert. Most of the grain products stored in warehouses for restocking are an important part of the world's food stocks.

Among the studies, there is a lot of evidence that extracts of plant raw materials are secondary metabolites, biosynthesized by plants to prevent pathogen attack, ultraviolet stress or attract pollinating insects. Phenolic compounds, including flavonoids, phenolic acid and tannin, are the main group of phytochemicals that exhibit strong antioxidant and antibacterial activity [23–25].

3. The aim and objectives of the study

The aim of the study is the optimization of the parameters of the grain soaking process using enzyme and plant extracts in the removal of heavy metals to obtain whole-grain bread with increased safety.

To achieve this aim, the following objectives have been formulated:

– to determine the effect of cellulolytic enzyme and plant extracts on the indicators of heavy metals Cd and Pb in grain;

– to determine the effect of extracts of plant raw materials on the microbiological parameters of grain.

4. Materials and methods of research

4. 1. Research materials

The object of research was grain Egemen 20, enzyme preparation Cellolux and extracts from plant raw materials (barberry, wild rose, sea buckthorn).

Grain Egemen 20 – a variety of semi-intensive type, mid-season, vegetation period 260–270 days. Spires and ripens 2–3 days earlier than the zoned variety Almaly. The weight of 1000 grains is above average to high (45–50 g). The straw of medium length (105–125 cm), resistant to lodging (9 points). According to the competitive variety testing of KazRIAPg, on average for three years (2009–2011), the yield was 70.2 centners per hectare, i.e., 4.6 centners per hectare higher than that of the Almaly variety. The variety is distinguished by relatively field resistance (tolerance) to yellow and brown rust and septoria. In terms of flour-grinding and baking qualities, the variety belongs to valuable wheat. The content of crude gluten is 27–38 %, the protein content in grain is 13–18.0 %, the volume of bread is 850–890 ml, the total bakery score is 3.6 points [26].

Enzyme preparation Cellolux is a preparation containing an enzyme complex of cellulase, β-glucanase and xylanase. When wheat grains are soaked with the Cellolux enzyme preparation, heavy metals are released from the outer shell of wheat grains. Also, under the action of xylanase contained in the enzyme preparation, in the protective layer of cellulose, xylan is hydrolyzed and quickly transported to the cellulose enzyme, splitting cellulose, which acts on β-glucanase, into carbohydrates. As a result, there is an accumulation of monosaccharides and disaccharides, which become an additive to yeast as an additional nutrition, which enhances the process of alcoholic fermentation and leads to gas formation. To improve the microbiological safety of bakery products from grain and enrichment with antioxidants, enzyme preparations that have a cellulose effect, together with plant raw materials, have an optimal effect on moistening the grain, and also help soften the grain shells [27].

Extracts from plant materials (sea buckthorn, rosehip and barberry) were obtained by extraction. Leaves and stems of sea buckthorn, rosehip and barberry were collected in the mountainous regions of the Almaty region, the Republic of Kazakhstan in the fall of 2019. Leaves and stems were thoroughly washed in distilled water and dried at room temperature under sterile conditions. Dried leaves and stems were crushed and extracted with 50 % ethanol for 20 hours at room temperature (25±2 °C). The supernatant was dried in a vacuum using a rotary evaporator at 50 °C. The dried extract was stored at 4 °C.

4. 2. Methods of investigation of grain soaking using an enzyme and plant extracts during purification from heavy metals

Statistical data processing was carried out using the MS Excel spreadsheet processing software package. The test of the hypothesis about the normality of the distribution of quantitative traits was carried out using the Shapiro-Wilk test. Descriptive statistics for quantitative traits with a normal distribution are represented by means and standard deviations or 95 % confidence intervals ($X \pm S$, $X \pm \Delta x$). Characterization of variation series for quantitative traits with non-parametric distribution is represented by the median (Me) and percentiles [Q1; Q3]. Comparison of indicators in two samples with a normal distribution of the trait was carried out using Student's t-criterion. For a comparison of the independent data series that do not follow a normal distribution, the Mann-Whitney U-test was used. Interrelations of indicators were calculated by Pearson's correlation coefficient. Further, to determine the differences in the effectiveness of the methods, the obtained variables were compared as independent data series. Statistical significance was taken at $p \leq 0.05$.

The reproducibility of the analysis was evaluated by the relative standard deviation S_r – the ratio of the standard deviation to the mean value:

$$S_r = \frac{S}{\overline{X}}.$$

The content of heavy metals was determined by atomic absorption spectroscopy (ASS) on a KVANT-Z.ETA-T spectrometer with electric atomization (SPF Kortek, RF). The samples were prepared by the dry mineralization method, which is based on the complete decomposition of organic matter by burning the sample in an electric furnace under controlled temperature conditions. A bowl with a weight of 20 g was placed on an electric stove and charring was carried out carefully, avoiding strong smoke. After the cessation of smoke emission, the bowl was placed in the PDP Analytics electric furnace, previously adjusted to a temperature of about 250 °C. After the end of charring, the mineralization of the samples was carried out in an electric furnace, gradually (by 50 °C every 30 min) raising the temperature to 450 °C. Mineralization was continued at this temperature until gray ash was obtained. The samples were ashed for 10–15 hours, cooled to room temperature, and the contents were moistened drop by drop with a minimum amount of nitric acid solution (1:1). The acid was evaporated to dryness in a water bath, followed by holding in an oven at temperatures up to 140 °C, either under an infrared lamp or on an electric stove with low heat. After cooling, the bowl with the sample was placed again in the cooled electric furnace. The temperature was gradually brought to 300 °C and held for 0.5 h. This cycle was repeated several times. Mineralization is considered complete when the ash becomes white or slightly colored, without charred particles. After the mineralization of the samples, lead and cadmium were determined by atomic absorption spectroscopy [28].

The falling number of grain samples was determined by a method based on rapid pasteurization of an aqueous suspension of flour in a water bath with a PCHP-3 device (Ukraine) and subsequent measurement of the degree of dilution of the paste under the action of alpha-amylase in the sample [29].

When studying the effect of plant extracts on the microbiological parameters of grain, classical methods of microbiological analysis were used: methods for sampling and preparing samples for microbiological analyses [30, 31], methods for cultivating microorganisms [32, 32].

Mass fractions of protein in grain were determined by the Kjeldahl method using a DK6 automated combustion furnace (VELPSCIENTIFICA, Italy) and a UDK129 steam distillation apparatus (VELPSCIENTIFICA, Italy) [33].

5. Results of the study of optimization of the parameters of grain soaking duration

5. 1. Results of studying the effect of the enzyme Cellolux and plant extracts on the indicators of heavy metals in grain

In order to study how the content of heavy metals in the grain decreases, the grain was contaminated in a solution of cadmium and lead. The content of cadmium and lead in uncontaminated grain was 0.0860 mg/kg, in contaminated grain, the content of heavy metals was 3.3655 mg/kg.

For the modification of the structure of the fruit and seed coats of wheat grain in order to reduce the content of toxic elements, the cellulolytic enzyme preparation Cellolux and extracts from plant raw materials were used.

The selection of rational doses of the enzyme preparation was carried out experimentally by determining the amount of toxic elements of lead and cadmium in the grain after soaking at a process duration of 4, 6, 12, 18 and 24 hours (Table 1).

In this context, it was found that the optimal dosage of the enzyme preparation to reduce the content of toxic elements in the grain is 0.05 % of the mass of dry matter of the grain, the optimal duration of soaking is 6 and 12 hours, but depending on the efficiency of the time, 6 hours are chosen. Grain soaking was carried out at a temperature of 23 °C (at room temperature).

From the obtained results, it can be seen that the most significant decrease in metals is observed during the 24-hour soaking of wheat grain, depending on the type of enzyme preparation used. During the period of 4 hours of grain soaking, it can be seen that the content of heavy metals in the grain did not change compared to the control.

But long-term soaking of grain in the period of 18–24 hours is ineffective due to the influence of the duration of soaking of enzyme preparations on changes in grain moisture.

Plant raw materials contain biologically active substances (flavonoids, tannins, glycosides, alkaloids, organic acids, and others) belonging to various classes of compounds. In this regard, the effectiveness of the plant extract in relation to microflora is determined by the peculiarities of the chemical structure and the concentration of biologically active substances. It is known that the antimicrobial activity of plants is determined by the high content of phenolic compounds of substances containing aromatic rings with a hydroxyl group and their functional derivatives, in particular, tannins, flavonoids, glycosides, phenolcarboxylic acids, phenol alcohols, anthocyanins, bitter substances, simple phenols [20]. The use of plant extracts with organic acid contents also contributes to the chelation of heavy metals and their transport [34]. As plant raw materials, extracts of leaves and stems of rosehip, sea buckthorn and barberry were used. These medicinal plants, in particular, their berries are used in bakery to improve the safety and quality of bakery products.

Table 1

The influence on the degree of distribution of heavy metals in grain raw materials when using the enzyme preparation Cellolux during grain soaking (P=0.95, n=10)

No.	Kind of processing	Cadmium, mg/kg (X±Δx)					
		start	4 h	6 h	12 h	18 h	24 h
1	Control	0.0860±0.0015	0.0860±0.0015	0.0860±0.0015	0.0860±0.0015	0.0860±0.0015	0.0860±0.0015
2	Control contaminated grain (soaking in water)	3.3665±0.01	0.0859±0.0013	0.0857±0.0015	0.0857±0.0011	0.0856±0.0013	0.0855±0.0016
3	Cellolux enzyme (0.03 %)	3.3665±0.01	0.0859±0.0011	0.0855±0.0013	0.0854±0.0015	0.0854±0.0015	0.0848±0.0015
4	Cellolux enzyme (0.05 %)	3.3665±0.01	0.0853±0.0013	0.0564±0.0015	0.0564±0.0011	0.0530±0.0015	0.0511±0.0013
5	Cellolux enzyme (0.08 %)	3.3665±0.01	0.0854±0.0015	0.0570±0.0015	0.0567±0.0012	0.0564±0.0016	0.0562±0.0015
Lead, mg/kg (X±Δx)							
1	Control contaminated grain (soaking in water)	2.0975±0.01	0.05330.001±	0.05330.001±	0.05330.001±	0.05320.001±	0.0531±0.001
2	Cellolux enzyme (0.03 %)	2.0975±0.01	0.0532±0.001	0.0532±0.001	0.0531±0.001	0.0530±0.001	0.0529±0.001
3	Cellolux enzyme (0.05 %)	2.0975±0.01	0.0528±0.001	0.0344±0.001	0.0344±0.001	0.0338±0.001	0.0332±0.001
4	Cellolux enzyme (0.08 %)	2.0975±0.01	0.0532±0.001	0.0352±0.001	0.0352±0.001	0.0350±0.001	0.0349±0.001

The application for disinfection and cleaning of grain from heavy metals in the amount of 0.05 % by weight of the grain is substantiated for a number of plant extracts [35]. The same concentration of solutions of plant extracts (barberry, rosehip and sea buckthorn) was taken for experiments to determine the optimal soaking time for grains (Table 2).

Table 2 shows that rosehip leaves and sea buckthorn stem had the highest bactericidal effect. When soaking grain with extracts of rosehip leaves and sea buckthorn stem for 6–12 hours, the content of heavy metals in grain, i.e. cadmium and lead is reduced by 4 times. During the period of 4 hours of grain soaking, it can be seen that the content of heavy metals in the grain did not change compared to the control.

Soaking grain with extracts of plant materials for 18–24 hours is ineffective in accordance with the data given above.

In the production of grain wheat bread, there are problems of increasing its environmental safety and quality. Thus, the combined application of an enzyme preparation with cellulolytic action and rosehip leaf extracts with antimicrobial action in a ratio of 1:1, 1:2, 1:3 for the enzymatic process leads to a change in the pores of the seed coats of wheat grain, which contributes to a deeper penetration of chelators (organic acids, flavonoids, possibly products of glutathione metabolism). The resulting complexes with heavy metals freely pass into the liquid phase and are taken out of the grain.

Therefore, a concentration of 0.05 % extract of rosehip leaves and Cellolux enzyme preparation was chosen in a ratio of 1:1 (Table 3).

The effect of the complex application of the Cellolux enzyme preparation and rosehip leaf extract on the grain quality index can also be seen in Table 3.

Table 2

The influence of extracts of plant raw materials on the degree of grain cleaning from heavy metals (P=0.95, n=10)

No.	Kind of processing	Cadmium, mg/kg (X±Δx)					
		start	4 h	6 h	12 h	18 h	24 h
1	Control	0.0860	0.0860	0.0860	0.0860	0.0860	0.0860
2	Control contaminated grain (soaking in water)	3.3665±0.01	0.0859±0.0013	0.0857±0.0015	0.0857±0.0011	0.0856±0.0013	0.0855±0.0016
3	Soaking with 0.05 % sea buckathorn leaf extract	3.3665±0.01	0.0760±0.0013	0.0588±0.001	0.0587±0.0011	0.0576±0.001	0.0573±0.001
4	Soaking with 0.05 % sea buckathorn stem extract	3.3665±0.01	0.0588±0.001	0.0514±0.001	0.0513±0.001	0.0512±0.001	0.0511±0.001
5	soaking with 0.05 % rosehip leaf extract	3.3665±0.01	0.0577±0.001	0.0539±0.001	0.0536±0.0012	0.0535±0.001	0.0533±0.001
6	soaking with 0.05 % rosehip stem extract	3.3665±0.01	0.0732±0.0013	0.0566±0.001	0.0566±0.001	0.0564±0.001	0.0563±0.001
7	soaking with 0.05 % barberry leaf extract	3.3665±0.01	0.0707±0.0012	0.0660±0.0011	0.0659±0.0011	0.0629±0.001	0.060±0.001
8	soaking with 0.05 % barberry stem extract	3.3665±0.01	0.0754±0.0013	0.0619±0.0011	0.0616±0.001	0.060±0.001	0.0587±0.001
Lead, mg/kg (X±Δx)							
1	Control contaminated grain (soaking in water)	2.0975±0.01	0.0534±0.001	0.0533±0.001	0.0533±0.001	0.0532±0.001	0.00531±0.0005
2	soaking with 0.05 % sea buckathorn leaf extract	2.0975±0.01	0.0447±0.0006	0.0340±0.0005	0.0339±0.0005	0.0338±0.0005	0.0337±0.0005
3	soaking with 0.05 % sea buckathorn stem extract	2.0975±0.01	0.0361±0.0005	0.0345±0.0005	0.0345±0.0005	0.0343±0.0005	0.0342±0.0005
4	soaking with 0.05 % rosehip leaves	2.0975±0.01	0.0336±0.0005	0.0322±0.0005	0.0322±0.0005	0.0321±0.0005	0.0319±0.0005
5	soaking with 0.05 % rosehip stem	2.0975±0.01	0.0456±0.0006	0.0391±0.0005	0.0389±0.0005	0.0373±0.0005	0.0367±0.0005
6	soaking with 0.05 % barberry leaves	2.0975±0.01	0.0436±0.0005	0.0340±0.0005	0.0340±0.0005	0.0381±0.0005	0.0373±0.0005
7	soaking with 0.05 % barberry stem	2.0975±0.01	0.0454±0.0006	0.0365±0.0005	0.0365±0.0005	0.0354±0.0005	0.0353±0.0005

Table 3

The effect of enzyme preparations and rosehip leaf extracts on the degree of grain cleaning from heavy metals (P=0.95, n=10)

No.	Kind of processing	Cadmium, mg/kg ($X\pm\Delta x$)					
		Start	4 h	6 h	12 h	18 h	24 h
1	Control	0.0860	0.0860	0.0860	0.0860	0.0860	0.0860
2	Control contaminated grain (soaking in water)	3.3665±0.01	0.0859±0.0013	0.0857±0.0015	0.0857±0.0011	0.0856±0.0013	0.0855±0.0016
3	Enzyme and extract of rosehip leaves in the ratios: 1:1	3.3665	0.0585±0.001	0.0315±0.0005	0.0315±0.0005	0.0314±0.0005	0.0290±0.0005
	1:2	3.3665	0.0586±0.001	0.0321±0.0005	0.0320±0.0005	0.0320±0.0005	0.0315±0.0005
	2:1	3.3665	0.0589±0.001	0.0334±0.0005	0.0332±0.0005	0.0331±0.0005	0.0329±0.0005
Lead, mg/kg ($X\pm\Delta x$)							
1	Control contaminated grain (soaking in water)	2.0975±0.01	0.0535±0.001	0.0533±0.001	0.0533±0.001	0.0532±0.001	0.0531±0.001
	Enzyme and extract of rosehip leaves: 1:1	2.0975±0.01	0.0387±0.0006	0.0181±0.0002	0.0168±0.0002	0.0162±0.0002	0.0160±0.0002
	1:2	2.0975±0.01	0.0390±0.0006	0.0361±0.0005	0.0188±0.0002	0.0187±0.0002	0.0186±0.0002
	2:1	2.0975±0.01	0.0429±0.001	0.0205±0.0003	0.0204±0.0003	0.0203±0.0003	0.0202±0.0003
Indicators of grain quality (the application of enzyme preparations and extracts of rosehip leaves in a ratio of 1:1)							
2	Protein including:	13±0.2	11.70±0.2	9.90±0.2	9.80±0.2	9.60±0.2	9.58±0.2
	Albumins, %	1.04±0.01	1.16±0.01	1.20±0.01	1.30±0.01	1.15±0.01	1.15±0.01
	Globulins, %	3.68±0.05	3.24±0.05	2.52±0.05	2.45±0.04	2.38±0.04	2.36±0.04
	Gliadins, %	4.59±0.05	4.02±0.05	3.21±0.05	3.30±0.04	3.14±0.05	3.13±0.05
	Glutenins,%	3.07±0.05	2.84±0.04	2.56±0.04	2.54±0.04	2.50±0.04	2.50±0.04
3	Falling number	288±4.32	274±4.35	263±4.73	262±4.71	254±4.7	252±4.7

When grain is soaked, the proportion of the albumin fraction increases, which consists of biologically active proteins, including enzymes that are actively synthesized when the seed germ awakens. The percentage of other protein fractions of wheat grain decreases during soaking, and in solutions of an enzyme preparation based on cellulases and extracts of plant materials [18].

The obtained experimental data indicate that significant changes in the protein complex occur during the wheat grain soaking and in the enzyme preparation based on cellulases and extracts of plant materials. The changes are associated with the process of seed germination, as a result of which the enzyme of the proteolytic complex is activated, endosperm proteins undergo hydrolysis, and qualitative and quantitative changes in protein components occur.

Long-term presence of grain in water leads to the activation of biochemical processes, in particular, to an increase in the activity of amylolysis enzymes. This can drastically impair the baking properties of wheat grain and result in sticky and crumbling bread. We have studied the effect of grain soaking time in the presence of enzyme preparations on enzyme activity, which was measured by the "falling number" indicator (Table 3).

As can be seen from the research results, when grain is soaked, the falling number decreases (from 288 to 247–256 s), which indicates an increase in the activity of amylolysis enzymes. The amount of water-soluble substances increases as a result of the breakdown of starch, and the viscosity of the starch paste decreases. When using enzyme preparations and extracts of plant raw materials at the stage of grain soaking, the decrease in the falling number occurs to the greatest extent compared to the control option, on average, by 5.0–14.2 %. Obviously, this is due to the fact that the enzyme preparations and extracts of plant materials used, by hydrolyzing cellulose and hemicellulose, increase the availability of starch to the action of its own active amylases. When using rosehip leaves, a maximum decrease in the falling number is observed, since it has not only pentosanes, but also amylase activity. However, despite the fact that the process of soaking is accompanied by an increase in the autolytic activity of the grain, it belongs to the category of "average" and does not exceed the established standards.

5. 2. Determination of the effect of plant extracts on the microbiological parameters of grain

Grain products are the most important source of indigestible carbohydrates and dietary fiber for the human body.

However, in the production of bread from whole grains, there is always a problem of microbiological purity and quality improvement of bread. Therefore, extracts of leaves and stems of plant raw materials were used to improve the microbiological safety of bread.

The choice of extracts of leaves and stems of plant raw materials as antiseptics is explained by the fact that they contain polyphenols with antioxidant, antimicrobial, and carcinogenic properties [36], as well as volatile substances that are bactericidal substances.

The use of plant raw materials with antimicrobial properties when soaking grain in the process of preparing for the production of grain bread will reduce the microbial contamination of grain and prevent microbial spoilage of bread products during storage.

Extracts of leaves and stems of rosehip, sea buckthorn and barberry were used as plant raw materials. Plant raw materials used in baking, in order to improve the safety and quality of bakery products are shown in Fig. 1.

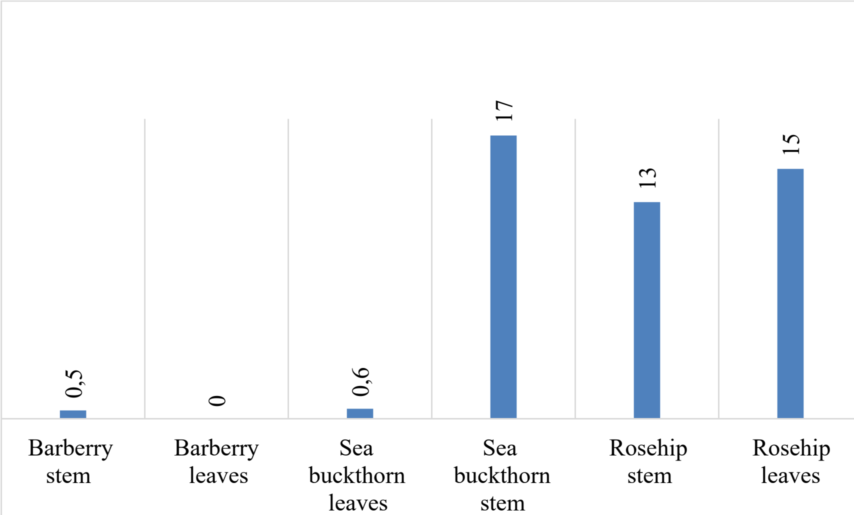

Fig. 1. Antimicrobial effect of plant extracts on fungi of the genus *Pénicillium*

As a result of the research, it was found that extracts of rosehip leaves and sea buckthorn stem have a stronger antimicrobial effect against fungi of the genus *Pénicillium*.

Thus, the diameters of the zones of inhibition, the growth of fungal microflora under the action of sea buckthorn stem extract was 17 mm, stem and rosehip extracts – 13–15 mm. The lowest results were shown by extracts from sea buckthorn leaves and barberry stem. Barberry leaf extract did not show antimicrobial activity against fungi of the genus *Pénicillium*.

In order to increase the microbiological purity of grain, comparative studies of the effectiveness of the influence of substances of natural origin with antiseptic action (extracts from the stem and leaves of rosehip, sea buckthorn and barberry) on the change in the number of grain microflora during soaking were carried out (Table 4).

The optimal amount of plant raw material extract during soaking is 0.05 % by weight of the grain. The duration of soaking is 6 hours, the temperature is 23–24 °C, at which the extract has the best antiseptic properties.

Table 4

The effect of plant material extracts on the number of microorganisms in the grain after soaking

Plant raw material extracts	Groups of microorganisms, CFU/g	
	NMAFAnM	Mold
Norms on requirements TP TC 2011/021	$5*10^3$	50
Control grain (water treatment)	$3*10^3$	23
rosehip leaves	$0.27*10^3$	1
rosehip stem	$1.1*10^3$	6
sea buckthorn leaves	$1.2*10^3$	7
sea buckthorn stem	$0.7*10^3$	1
barberry stem	$2.1*10^3$	11
barberry leaves	$2.1*10^3$	10

From Table 4, it can be seen that the use of rosehip leaf extract during grain soaking can reduce the value of NMAFAnM by 90.0 %, mold – by 96.6 % compared to the control. Extracts from the rosehip stem and sea buckthorn leaves provide a decrease in NMAFAnM by 63.0 %, mold – by 80 %, and the extract of the sea buckthorn stem – by 76 %, mold – by 96.6 %. In extracts of leaves and stems of barberry, in comparison with the control, a lower indicator was noted.

Under the action of extracts from the rosehip stem and sea buckthorn leaves, a decrease in NMAFAnM by 63.0 %, mold – by 80 % can be observed, and the extract of sea buckthorn stem – by 76 %, mold – by 96.6 %.

As a result of the research, it was found that extracts of rosehip leaves and sea buckthorn stem had the greatest effect on the reduction of microorganisms in grain after soaking.

6. Discussion of research results to obtain safe grain products

In [37], extracts of plant raw materials, such as mint leaves (Mentha longifolia), bakain leaves, sprouts and seeds of harmala (pegnum harmala) and lemongrass roots (citrates of cymbopogon), were used to control pests by wetting the grain. And in [38], by treating grain with aqueous extracts of medicinal plants (Asclepias sinaica, Farsetia aegyptia, Hypericum sinaicum, Phagnalon sinaicum and Salvia Aegyptiaca, Aspergillus flavus, A. mold niger, Curvularia lunata, Fusarium moniliforme and Penicillium chrysogenum) were used against shingles. But many studies have not studied the effect of vegetable raw materials on heavy metals in grain during grain watering. Therefore, the conducted scientific research allowed substantiating the application of an enzyme preparation and extracts from plant raw materials in the process of grain soaking to reduce the content of heavy metals in the grain.

At the first stage of the study, grain soaking with the Cellolux enzyme preparation in doses of 0.03–0.08 % of the dry matter weight of the grain was carried out. In studies with the use of an enzyme preparation at doses of 0.03 %, when soaking grain, the effect on the content of heavy metals in grain did not change compared to the control. When used in doses of 0.08 % of the enzyme preparation, the structure of grain membranes is deeply disturbed and, as a result, cellulose microfibrils weakly bind to the grain cell wall matrix, while the reduction of heavy metals in the grain decreases. This is probably due to the fact that heavy metals are mainly present in the form of complex compounds with non-starchy polysaccharides on the cell walls of grain shells, they contribute to the deep hydrolysis of cellulose and hemicellulose during grain soaking [12].

When grain is soaked with an enzyme preparation at a concentration of 0.05 % (Table 1), it affects the local structure of the matrix of the outer cell walls of the grain, which consists mainly of fruit and seed coats of caryopsis. Softens the outer shells of the grain and promotes the reduction of heavy metals in the grain. For 6 hours of grain soaking, the content of cadmium and lead decreased by 4 times.

Prolonged soaking of the grain led to a change in the moisture content of the grain. To obtain a grain mass capable of being dispersed, the moisture content of the grain after soaking should be at least 43–45 %. In the first hours of soaking (0–4 hours), there is a sharp increase in the moisture content of dry grain, which is associated with intensive absorption of moisture through the embryo and groove. This period is characterized by an abrupt increase in grain moisture, and when enzyme preparations are used, the rate of moisture absorption by the grain increases. At the second stage (6–12 hours), there is a gradual distribution of moisture over the anatomical parts of the grain in an equilibrium ratio in accordance with their structural features and thermodynamic characteristics of moisture transfer. With a soaking time of 12 hours, the final moisture content of the grain using enzyme preparations is higher. At the same time, the grain achieves the technological value of moisture (43–45 %), which is necessary to obtain a finely dispersed grain mass. At the third stage of soaking (18–24 hours), the rate of moisture absorption by the grain decreases. This is due to the fact that the swollen grain shells close the microcapillaries of the endosperm, which makes it difficult for moisture to move in the grain. The enzyme preparations introduced during grain soaking act on the components of the peripheral layers of the grain (cellulose and hemicellulose), contributing to the unimpeded penetration of moisture into the endosperm. In this case, the cell walls of the grain shells are partially destroyed, and moisture quickly moves [18].

In the study, soaking grain with extracts from plant raw materials (Table 2) showed high rates of rosehip leaves and sea buckthorn stem in reducing the content of heavy metals in grain. This is due to the fact that plant raw materials contain biologically active substances (flavonoids, tannins, glycosides, alkaloids, organic acids, and others) belonging to various classes of compounds. In this regard, the effectiveness of the plant extract in relation to microflora is determined by the peculiarities of the chemical structure and the concentration of biologically active substances [20]. Thus, the complex application of an enzyme preparation with an extract of rosehip leaves and sea buckthorn stems was established, which showed high rates among plant raw materials.

With the combined use of rosehip leaf extract, which has antimicrobial properties, and the enzyme preparation Cellolux, which is part of the preparation, the enzyme complex in a ratio of 1:1 (Table 3), the content of cadmium and lead in the grain is reduced by 4 times in 6 hours of soaking and is sorbed and concentrated on areas of non-starch polysaccharides with structural defects. This puts pressure on the walls of pores and microcracks in cellulose micelles, and the distances between macromolecules increase. Water, citrate ions, biologically active compounds of plant extracts penetrate into the formed space. This leads to the rupture of hydrogen bonds in the matrix of cell walls of caryopsis shells, solvation and separation of microfibrils. Enzyme molecules are adsorbed on the amorphized sections of cellulose and defects in the crystal structure are fixed.

Heavy metals adsorbed on cell walls or bound into complexes are desorbed as a result of changes occurring in the cell wall matrix. Organic acids, flavonoids, and other chelating agents are probably involved in new complexation processes, form hydrophilic channels, and some of these complexes with heavy metals can be easily washed out by washing the grain with water.

It was found that extracts from rosehip leaves and sea buckthorn stems, due to biologically active compounds (phytoncides, polyphenols, organic acids) with bactericidal properties, also showed an antiseptic effect, which made it possible to increase the microbiological purity of wheat.

A further increase in the content of the components of the enzyme preparation and extracts from plant raw materials during grain irrigation leads to a deep violation of the structure of grain membranes and, as a consequence, a change in the composition of the grain shell. This affects the organoleptic characteristics of the product. Therefore, when soaking grain, we recommend observing optimal dosages.

Prospects for the following studies: finding critical points in the technology of obtaining whole-grain bread with the addition of vegetable raw materials with an enzyme preparation, conducting critical control and developing measures to bring products to the consumer market.

7. Conclusions

1. To improve grain safety, it is advisable to apply biochemical treatment by soaking it with an enzyme preparation – Cellolux, as well as extracts of plant materials.

The optimal dosage of the enzyme preparation and the extract of plant materials to reduce the content of toxic elements in the grain is 0.05 % by weight of dry matter, the optimal duration of soaking is 6–12 hours. Grain soaking was carried out at a temperature of 23–25 °C.

2. The expediency of increasing the microbiological purity of grain is shown. Substances with an antiseptic effect (rosehip leaves and sea buckthorn stem) used for grain soaking can improve the microbiological purity of wheat.

Conflict of interest

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this paper.

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Data availability

Data will be made available on reasonable request, open access.

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