Development, properties and sanitary-hygienic assessment of selenium-containing cereal food additives

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Abstract. Background: Selenium is an essential element in the diet and in living systems. Thus, the development of new food sources of selenium is relevant. Methods: The object of the study was the sprouted grain of wheat varieties “Alaya Zarya” bred in Voronezh state agricultural university. Sodium selenite solution or dimetidipirazoliliselenide (DMDPS) were used at the stages of soaking and germination. For analytical studies of selenium-containing cereal food additive generally accepted standard and modified methods were used. To assess the total toxicity and biosafety of selenium-containing additives the express methods based on biotesting samples. The total content of antioxidants in wheat samples was determined by amperometric method on liquid chromatograph “Tsvetyauza-01-AA” - flow-injection system.

Results: The cereal additives correspond to the requirements of TR TS 021/2011 for sanitary and hygienic indicators. In the case of using DMDPS germination, the content of vitamin C in grain increases from 0.3 to 4.75 g/100 g, vitamin E from 2.05 to 3.1 g/100 g. The content of selenium is 0.81 µg/g of cereal additive after germination of wheat grain with DMDPS and drying with an infrared energy source. Discussion: The duration of germination is reduced from 48 to 24 hours in conditions of optimal moisture. Authors important contribution is established the fact that wheat germination in aqueous solutions of selenium preparations increases microbiological stability of additives during storage. Conclusions: The method of obtaining selenium-containing cereal supplements is justified and developed. It is advisable to use DMDPS with a mass fraction of 0.005% as a biostimulator and source of selenium.

1. Introduction
Great opportunities in the organization of production of combined food with enriched composition obtain cereals bioactivated in special conditions [1-4]. The seeds represent a kind of structurally complex biosystem, for the development of which the necessary conditions are, in addition to its readiness and ability to function, the presence of a trigger mechanism for the transfer of the system from inactive to active state [5-7]. Considering the general sequence of processes in the process of seed germination, which were previously in a state of forced rest, it is necessary to position water as a trigger of biochemical transformations of biopolymers of morphological parts of the grain accompanying the germination processes [8-10]. The introduction of various components into the steep water that activates the processes accompanying the germination of the seeds is a physiologically sound approach to the stimulation of grain germination [11-13].

In connection with the role of selenium as an essential element in nutrition and the variety of its biological functions in the living systems [14-20], the actual direction of the research is the development of new food sources of selenium, including those with multifunctional properties [21-26].
Grain and forage crops through the biochemical conversion of trans-form selenium mainly in selenomethionine, which is incorporated into proteins instead of methionine as the t-PHKMet not able to distinguish between these two compounds [20, 23, 27, 28]. In this regard, the research of biochemical processes accompanying the germination of wheat, as well as a comprehensive assessment of the quality and safety of the products obtained by the end of the process, from the point of view of their use as new food sources of selenium is of scientific interest and practical importance.

The purpose of the study is to substantiate and develop the method for obtaining selenium – containing cereal additive based on bioactivated wheat grain, comprehensive assessment of its composition and properties, including sanitary and hygienic indicators.

2. Materials and methods

| Table 1. Methods of investigation of sanitary-hygienic and toxicological indicators |
|----------------------------------|---------------------------------|---------------------------------|---------------------------------|
| The defined indicators, units of measurement | Documents regulating the research methods | Principle of the method |
| **Toxic elements** |
| Cadmium, mg / kg | GOST 30178-96 Raw material and food-stuffs. Atomic absorption method for determination of toxic elements | Atomic absorption. The method is based on the mineralization of the product by the method of dry or wet salinization and determination of the concentration of the element in the mineral solution by the method of flame atomic absorption. |
| Lead, mg / kg | GOST 26927-86 Raw material and food-stuffs. Methods for determination of mercury | Colorimetric method. The method is based on the destruction of the analyzed sample of nitric and sulfuric acids, the deposition of mercury by copper iodide and the subsequent calorimetric determination in the form of copper tetrayodomercuroate by comparison with the standard scale |
| Mercury, mg / kg | GOST 26930-86 Raw material and food-stuffs. Method for determination of arsenic | Colorimetric method. The method is based on measuring the intensity of coloring of the complex arsenic compound with silver diethyldithiocarbamate in chloroform. |
| Arsenic, mg / kg | GOST 26930-86 Raw material and food-stuffs. Method for determination of arsenic | |
| **Mycotoxins** |
| T-2 toxin | MU 3184-80 Guidelines for the detection, identification and determination of T-2 toxin in food and food raw materials | To the base of the method for the determination of T-2 toxin gas-liquid chromatography triptoreline derivative of the toxin with the use of electron capture detectors (ECD), which can detect 1-2 ng of toxin at the peak is considered. |
| Aflatoxin b-1 | GOST R 53162-2008 Foodstuffs. Determination of aflatoxin B and the total content of aflatoxin B, B, G and G in cereals, nuts and derived products. High-performance liquid chromatographic method | Selected aflatoxin on the column for affinity chromatography determined using high-performance liquid chromatography with the reversed phase with the definition of fluorescence and post column derivatization |
| **Pesticides** |
| Hexachlorocyclohexan-Methods of determining micro-(HCH), mg / kg quality of pesticides in food, feed DDT, mg / kg and the environment [31] | The technique is based on thin-layer chromatographic determination of gamma-hexachlorocyclohexane, its alpha-, beta - and delta-isomers and metabolites in the presence in biological material |
The object of the study was the sprouted grain of wheat varieties "Alaya Zarya" bred in Voronezh state agricultural university. Modification of technological modes of germination in comparison with traditional ones in malting invoked the use of sodium selenite solution or dimetidipirazolilselenide (DMDPS) with a mass fraction of 0.005% at the stages of soaking and germination, as well as in the reduction of germination from 48 to 24 hours under optimal humidification [29].

For analytical studies of FDD generally accepted standard and modified methods were used. The quality indicators of the study objects were determined using freshly sprouted wheat grain and after its dehydration using the dryer with infrared heating of the SUPRA DFS-301 brand. Mass fraction of moisture was determined according to GOST 13586.5-93 Grain. The method of moisture content determination; protein - according to GOST 10846-91 Grain and products of its processing. The method for determination of protein; fat - according to GOST 29033-91 Grain and derived products. Determination of fat content; carbohydrates - according to GOST 10845-98 Cereals and cereal milled products. The method for determination of starch; vitamin C - according to GOST 30627.2 Methods for determination of mass part of vitamin C (Acidum ascorbinium), vitamin E – according to GOST R 52147-2003 Protein-vitamin-mineral and amide-vitamin-mineral additives. The methods for the determination of retinol-acetate (vitamin A), argocalciferol (holecalciferol) (vitamin D), tokoferylacetate (vitamin E). Selenium content was determined by fluorimetric method in accordance with the recommendations [30].

The residual amounts of toxic substances were determined in the selenium-containing cereal additive according to the methods, specified in Table 1. In order to assess the total toxicity and biosafety of selenium-containing additives the express methods based on biotesting samples using simple single-celled organisms: Paramecium caudatum were used [32].

The total content of antioxidants in wheat samples was determined by amperometric method on liquid chromatograph "Tsvetyauza-01-AA" -flow-injection system with amperometric detection. The method is direct, expressive and is specific to the determination of antioxidants. Mean square deviation of the successive measurements is less than 5 %, the sample was introduced using six-step valve. The measurement results were recorded and processed in real time [33].

### 3. Results and discussion

| Step                                           | Conditions                  |
|------------------------------------------------|-----------------------------|
| Washing                                        | $t=18-22^\circ C$, $\tau=5$ min |
| Disinfection                                   | 2.5% KMnO$_4$, $\tau=5-10$ min |
| Steeping                                       | $\tau=4$ h, 0.005 % solution of selenium water (Na$_2$SeO$_3$ or C$_8$H$_{10}$N$_4$Se(DMDPS)) |
| Germination on pallets in thermostat TSU-02-200 | $\tau=20-24$ h, $t=18-22^\circ C$, in conditions of optimal moisture |
| Drying with infrared heating SUPRA DFS-301     | $\tau=20-24$ h, $t=35^\circ C$ |
| Grinding                                       | $d_{hole}=3-4$ mm            |
| Storage, 10 months                             | $\varphi=11\%$, $t=12-16$   |
| Storage, 24 h                                  | $t=3-6^\circ C$             |

![Figure 1. Technological scheme of SCA production.](image-url)
Functional orientation of food products, including probiotic products based on milk, can be additionally provided with plant ingredients rich in vitamins, enzymes, mineral trace elements, antioxidants and other biologically active substances. Wheat grain contains proteins, carbohydrates, represented by fiber and starch, B vitamins, trace elements. After germination, the content of these components in the grain increases markedly.

The established regularities of micro-phenological phases of germination of wheat grain with DMDPS, accumulation of low molecular weight components of antioxidant system of bioactivated grain products, activation of enzyme complexes, comparative evaluation of free amino acids that exhibit affinity to selenium, form the basis of the developed technological scheme for obtaining a selenium-containing cereal additive (SCA), which is shown in Figure 1. Indicators of cereal additives after germination for 24 h in the conditions of optimal moisture and drying with infrared heating are presented in Table 2.

Table 2. Chemical composition of cereal additives during germination with different selenium sources

| Sample SCA          | Moisture, % | Protein, % | Fat, % | Ash, % | Selenium, µg/g | Carbohydrates, % |
|---------------------|-------------|------------|--------|--------|----------------|------------------|
| Wheat               | 12.50       | 15.08      | 2.42   | 1.60   | 0.01           | 68.30            |
| Wheat + DMDPS       | 14.50       | 11.65      | 2.12   | 1.68   | 0.81           | 70.05            |
| Wheat + Na₂SeO₃     | 14.30       | 10.48      | 2.18   | 1.67   | 0.11           | 71.37            |
| Wheat + H₂O         | 14.50       | 10.98      | 2.13   | 1.65   | 0.023          | 70.74            |

As a result, the bioassay on toxicity (GOST R 52337-2005), the survival rate of infusorians for the sample "Wheat+DMDPS" was 98%, for "Wheat+H₂O" - 97%, for "Wheat+Na₂SeO₃" - 82%, which implies that the addition of "Wheat+DMDPS" has a beneficial effect on the life processes of the test objects (Figure 2).

The content of vitamins in the samples was determined by liquid chromatograph Milichrom-5. The content of some vitamins in the samples of the sprouted wheat is shown in Figure 3, which shows the positive effect of bioactivation, especially in the presence of selenium sources, on the processes of biosynthesis of vitamins in the investigated biological objects. The content of vitamin C increases 15 times (from 0.3 to 4.75 g/100g), vitamin E 1.5 times (from 2.05 to 3.1 g/100g).
Figure 3. Effect of selenium sources on the vitamin composition of wheat during germination: a-vitamin C; b-vitamin E.

The study of sanitary-hygienic and toxicological indicators of new food sources of selenium was carried out in accordance with the requirements of the Federal law "On sanitary-epidemiological welfare of the population" dated March 30, 1999 № 52-FZ; HRSFV 2.3.2.10.78 01 "Hygienic requirements of safety and food value”; technical regulations of the Customs Union "On food safety" TR CU 021/2011. The results of the determination of toxic residues in the SCA samples are presented in Table 3, which indicates that they do not exceed the acceptable levels.

Table 3. Content of residual quantities of toxic substances in the SCA samples.

| Indicators, units of measurement | Results of research of samples of SCA | Admissible level |
|---------------------------------|--------------------------------------|------------------|
|                                 | Wheat + DMDMS | Wheat + Na₂SeO₃ | Wheat |
| Hexachlorocyclohexane (α,β,γ - isomers), mg / kg | - | Less than 0.006-0.5 | - | 0.5 |
| DDT, mg / kg | - | Less than 0.008-0.02 | - | 0.02 |
| Cadmium, mg/kg | - | 0.004 | - | 0.1 |
| Aflatoxin B₁ | - | Less than 0.004 | - | 0.005 |
| Mercury, mg / kg | 0.001 | 0.005 | - | 0.03 |
| Arsenic, mg / kg | 0.01 | 0.07 | 0.01 | 0.2 |
| Lead, mg / kg | 0.001 | 0.01 | 0.001 | 0.5 |
| T - 2 toxin | - | Less than 0.08 | - | 0.1 |
| Mercury containing pesticides | - | - | - | Not allowed |

According to the hygienic safety standards the SCA comply with the technical regulations of the Customs Union TR CU 021 / 2011 "On Food Safety”, as well as HRSF 2.3.2.1078-01 (index 1.9.4.1). In terms of colony-forming units of mesophilic aerobic and facultative anaerobic microorganisms and molds, SCA samples do not exceed the threshold values for 12 months of storage (Table 4).

The total content of antioxidants (CSA) in terms of quercetin in the samples of sprouted cereals is different and varies in the range from 30 to 71g/100g (Figure 4). Most of the antioxidants are contained in the sample, germinated with organic selenium. It was found that dehydration with IR heating leads to a decrease in antioxidant activity being not higher than 8% due to gentle drying modes (20-24 h, 35 C).
Table 4. Safety indicators of SCA.

| Sample                | Quantity of mesophilic aerobic and optional and anaerobic microorganisms (QMA&OAMO), CFU/g | The performance of SCA samples for the duration of storage, months |
|-----------------------|------------------------------------------------------------------------------------------------|------------------------------------------------------------------|
|                       |                                                                                               | 0                   | 2     | 6     | 10    | 12    |
| Wheat+ H₂O            | 5·10³                                                                                           | 5·10²               | 8·10² | 3·10³ | 4·10³ | 5·10³ |
| Wheat+ DMDPS          | 5·10³                                                                                           | 2·10²               | 5·10² | 8·10² | 3·10³ | 4·10³ |
| Wheat+ Na₂SeO₃        | 5·10³                                                                                           | 3·10²               | 6·10² | 9·10² | 3·10³ | 4·10³ |
| Wheat + H₂O           |                                                                                                 | 50                 | 10    | 15    | 25    | 45    | 50    |
| Wheat + DMDPS         |                                                                                                 | 50                 | 10    | 15    | 25    | 35    | 40    |
| Wheat + Na₂SeO₃       |                                                                                                 | 50                 | 10    | 15    | 25    | 35    | 40    |

Considering the high bio damaging potential of free radicals and the products of their metabolism, the antioxidant system of the cell plays an important role in ensuring the physiological functions of the human body [25, 34]. In order to maintain the antioxidant system in working condition requires selenium. Therefore, the enrichment of selenium products of mass consumption is the most urgent issue in modern nutrition [13, 19, 26, 28].

Biochemical processes during wheat grain germination are accompanied by synthesis and accumulation of water and fat-soluble vitamins [13, 23]. Thus, organic selenium has a favourable biochemical background for effective inclusion in the antioxidant system, which indicates in favour of the chosen method of complex enrichment of food additives obtained from wheat seedlings using an organic source of selenium with antioxidants.

In accordance with the recommendations [35], physiologically-based norms of daily consumption of antioxidants are: for a healthy person in the range of 350 mg per day; for sick people and people with intense physical activity (athletes)-more than 1200 mg per day. Thus, the use of 100 g of wheat grain at rest stage provides 8.6 % of the average daily physiological norm of antioxidant consumption for a healthy person and 20% of the same norm, if the grain was previously activated by germination in DMDPS solution.

4. Conclusion

According to sanitary and hygienic indicators, cereal additives obtained by bioactivation in the presence of selenium sources meet the requirements of TR CU 021/2011. It was found that the

Figure 4. Total content of antioxidants in dry seeds (Wheat), and wheat germ, obtained without the use of (Wheat+H₂O) and organic (Wheat+DMDMS) and inorganic (Wheat +Na₂SeO₃) of selenium.

AOA antioxidant activity of fresh wheatgrass.
germination of wheat in aqueous solutions of selenium preparations increases the microbiological stability of the additives during storage. This is consistent with the previously formulated hypothesis on the stimulating effect of DMDPS on the biosynthesis of mucoid substances with bactericidal action [29]. At the same time, the content of vitamin C in the grain increases 15 times (from 0.3 to 4.75 g/100 g), vitamin E-1.5 times (from 2.05 to 3.1 g/100 g) in the case of using DMDPS germination. The content of selenium is 0.81 µg/g of cereal supplement after germination of wheat with DMDPS and drying with an infrared energy source.

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