Determination of grain product safety by high-performance liquid chromatography

T Yu Gumerov¹², L Z Gabdukaeva³, A R Nurgalieva³, I A Abrosimov¹

¹Kazan National Research Technical University named after A.N. Tupolev-KAI, 10, K. Marx St., Kazan, 420111, Russia
²Kazan National Research Technical University, 68, K. Marx St., Kazan, 420015, Russia
³Kazan Cooperative Institute (branch) of the Russian University of Cooperation, 58, N. Ershov St., Kazan, 420081, Russia

E-mail: tt-timofei@mail.ru

Abstract. The paper evaluates the safety of cereal stock developed as a supplement to the food ration of persons working in especially harmful labor conditions in accordance with the order of the Ministry of Health and Social Development of the Russian Federation No. 46n of February 16, 2009 (Annex 2). The performed sanitary and chemical studies revealed that the composition of considered products does not contain biological contaminants – mycotoxins. Such substances are natural contaminants of grains, cereals, legumes, vegetables and fruits and represent low molecular secondary metabolites produced by microscopic mold fungi.

1. Introduction

Mycotoxins are poisonous substances that may be formed during storage in many products under the influence of microscopic fungi. A common feature of all mycotoxins is toxicity. The most common classification of mycotoxins is by molecular structure, according to which there are aflatoxins, ochratoxins, zearalenone, deoxynivalenol and other substances. The following substances are considered in this study:

Deoxynivalenol (DON C₁₅H₂₀O₆) – organic substance, trichotecene mycotoxin, produced by several micromycete species – microscopic mold fungi of the Fusarium genus (Fusarium culmorum, Fusarium graminearum). DON has an immunosuppressive effect and is the world’s most common mycotoxin found in food and animal feed. It is highly toxic, a contaminant, causes severe poisoning, and also leads to mycotoxicosis – alimentary toxic aleukia (“septic angina”). DON negatively affects protein metabolism, leads to inflammation and necrosis of skin, mucous membranes. Other symptoms may damage the alveoli, lead to motor dysfunction, degeneration of neuronal cells and toxic effects on the bone marrow, which reduces the blood cell formation.

Zearalenone (ZEA C₁₈H₂₂O₅) – mycotoxin, has a direct estrogen effect, is a powerful estrogen metabolite produced by some species of Fusarium and Gibberella. Unlike DON, ZEA toxicity is further enhanced by conversion to α- and β-zearalenol.

Aflatoxin B1 (AFB1 C₁₇H₁₂O₆) is an organic compound from the group of polyketides, belongs to mycotoxins, extremely toxic, the strongest hepatocancerogene. One of the most common aflatoxins. It is a contaminant, a secondary metabolite that is produced by some species of microscopic mold fungi.
of the Aspergillus genus.

2. Materials and methods
The study was carried out to determine DON, ZEA and AFB1 in a cereal product using high-performance liquid chromatography. The cereal product “Cereal Bar” produced according to TU 10.61.33-009-23333135-2020 was selected as the subject of the study. The raw materials shown in Table 1 were used for the production of cereal bars.

Table 1. Raw materials for cereal products

| Name                  | Statutory document       | Raw materials consumption, kg |
|-----------------------|--------------------------|-------------------------------|
| Oat shorts            | TU 9295-006-35760517-12  | 100                           |
| Wheat-cedar fiber     | TU 9197-004-72551266-07  | 70                            |
| Whole grain corn flour| GOST 26791-18; GOST 14176-69 | 50                            |
| Spelt flour           | GOST 27558-87            | 40                            |
| Ground medic          | TU 9185-192-14721358-10  | 30                            |
| Coriander fruits      | GOST 17081-97            | 20                            |
| Fresh dill            | GOST 32856-2014          | 50                            |
| Artichokes            | GOST 31853-2012          | 70                            |
| Bulb onion            | GOST 34306-2017          | 60                            |
| Peanut butter         | GOST 7981-68             | 60                            |
| Carrot                | GOST 33540-2015          | 60                            |
| Broccoli              | GOST 33854-2016          | 80                            |
| Knob celery           | GOST 34320-2017          | 70                            |
| Blackberry            | GOST 34219-2017          | 50                            |
| Gooseberry            | GOST 33485-2015          | 40                            |
| Blueberry             | GOST 33915-2016          | 50                            |
| Total raw materials   |                          | 1000                          |
| Output, %             |                          | 905.7                         |

The procedure for grain mass preparation is as follows: a mixture of oat bran, wheat-cedar fiber, whole grain corn and spelt flour was prepared. Then ground medic, crushed dill and coriander fruits were added. All ingredients were thoroughly mixed for uniform distribution in the cereal mixture. After that, crushed sassafras was added and once again everything was thoroughly mixed. Next, vegetable puree and crushed carrots were added. Then, the vegetable puree was combined with the cereal mixture and thoroughly mixed until smooth, and then the berry mixture and peanut butter were added. The mass was mixed for 5-7 minutes. The average temperature was 32-35 °C. After mixing the obtained mass was put for sweating.

After the sweating stage, the mass was uniformly put in a mold, distributed with a thickness of 9-10 mm, baked in a cabinet oven for 20-25 minutes at a temperature of 180-190 °C until brown. The baked semi-product was completely cooled, taken from the mold and cut into long rectangles weighing 30 g.

To prepare the vegetable puree the washed vegetables: artichokes, apple knob celery, broccoli, bulb onions were cut into small cubes and crushed with a blender until pureed. Crushed carrots were added to the obtained vegetable mass.

To prepare the berry puree, a mixture of blueberries, blackberries and gooseberries was crushed with a blender to a puree-like state.

The nutritional value of the finished product is shown in Table 2.

Table 2. Nutritional value in 100g of product (calculated values)

| Product      | Protein, g | Fat, g | Carbohydrates, g | Caloric content, kcal | Energy, kJ |
|--------------|------------|--------|------------------|-----------------------|------------|
| Cereal product | 5.0        | 7.0    | 16.0             | 150.0                 | 630.0      |
The experiment was conducted at the test laboratory center of the Center for Hygiene and Epidemiology in Kazan in the Republic of Tatarstan (13a Sechenova Str.). The measurement procedure applies to bread and bakery products; confectionery; soybeans, spices, nuts, sunflower seeds; flour of various crops, cereals, flakes; and establishes a method to determine the mass concentration of mycotoxins using high-performance liquid chromatography via spectrophotometric determination (GOST 34140-2017).

The content of mycotoxins – DON, ZEA and AFB1 – is controlled in food raw materials and food products of plant origin. The priority contaminants are the following: for cereal products – deoxynivalenol; for nuts and oilseeds – aflatoxin B; for fruit and vegetable products – patulin.

According to SanPiN 2.3.2.1078-01, the maximum allowable concentration (MAC) for deoxynivalenol in wheat flour, pasta, rye, triticale, corn, millet, rice, buckwheat, sorghum, nuts, confectionery (cakes, cookies), doughnut bakery, sugar products, bread and bakery products (bread rolls) is 0.7 mg/kg (700 μg/kg). The MPC value for deoxynivalenol in barley flour, barley cereal, barley flakes according to SanPiN 2.3.2.1078-01 is 1.0 mg/kg (1000 μg/kg).

The MAC for ZEA in cereals is 1 mg/kg. The MAC for AFB1 is specified for food raw materials and food products of plant origin – 0.005 mg/kg (SanPiN 2.3.2.1078-01 Hygienic Requirements for Safety and Nutritional Value of Food Products: Sanitary and Epidemiological Rules and Norms).

3. Determination of mycotoxins by high-performance liquid chromatography with mass spectrometric detection

The isocratic high-performance liquid chromatography with spectrophotometric determination (HPLC-MS/MS) was used for the chromatographic detection of mycotoxins.

The method is based on extracting mycotoxins from an analyzed sample of a cereal product, identifying and quantifying them by the areas of ion product peaks using a calibration characteristic by HPLC-MS/MS through the monitoring of selected reactions.

HPLC-MS/MS analysis was performed as a series of measurements of the following samples: mobile phase A (B); calibration solutions and extracts of analyzed samples.

Thus, 890 (20) ml of deionized water, 100 (970) ml of methanol, 10 ml of acetic acid and 0.2 g of ammonium acetate were poured into a 1000 ml measuring flask to prepare the mobile phase A (B). Besides, 790 ml of acetonitrile, 200 ml of deionized water and 10 ml of acetic acid were added to a 1000 ml measuring flask to prepare the extracting solution.

For the preparation of calibration solutions, the initial \( C_0 \) solutions with a mass concentration of 1000 μg/ml or with a mass concentration of 100 μg/ml for each mycotoxin, the required mass of the \( i \) substance, mg, was calculated within the first decimal place taking into account the content of the base substance according to the formula

\[
m_i = \frac{C_0 \cdot V}{P_i} \cdot 100;
\]

where \( C_0 \) – concentration of the feed solution, μg/ml; \( V \) – volume of a measuring flask, ml; \( P_i \) – mass fraction of the base substance, %.

To prepare the sample extracts, 100 g of the average grain product sample was taken, ground in a laboratory mill and sieved through a sieve with holes of 0.5 mm in diameter. Next, 5 g of the prepared sample was placed in a 50 ml polypropylene tube, 25 ml of extraction solution was added and placed for 30 seconds in a vibration shaker, then on a rocking shaker for mixing during 60 minutes. Then, it was centrifuged at 3500 rpm for 20 minutes.

Thereafter, 500 ml of mobile phase A and 500 ml of the extract upper layer were added to a microfuge tube. It was then centrifuged at 15,000 rpm at 4 °C for 20 min. 700 ml of the extract upper layer was taken by pipette and filtered through a microfilter.

A 20 ml sample was placed into the chromatograph injector and measured (recorded) on a
chromatogram to determine the retention time of peaks of the two product ions of each mycotoxin.

To determine the quantitative content of the sample component, the peaks of chromatographic curves were decrypted using the Multi-Chrome for Windows XP software. The mass concentration of mycotoxins in the analyzed sample (in the initial sample) $X$, $\mu g/kg$ was calculated by the formula:

$$X = \frac{\hat{C}_{ch} \cdot V_3}{m_s \cdot K_{ext.1} \cdot K_{SPEext.2} \cdot V_2},$$

where $\hat{C}_{ch}$ – mean mycotoxin concentration obtained by chromatography in two parallel measurements [ng/ml];

$V_1$ – volume of the original extract equal to the volume of methylene chloride taken for primary extraction (60 ml) [ml];

$V_2$ – volume of filtered extract (filtrate) taken for further treatment (25 ml) [ml];

$V_3$ – eluate volume after SFE (about 1.5 ml, volume to be measured by measuring graduated glass end pipette per 2 ml) [ml];

$K_{ext.1}$ – primary liquid extraction coefficient with methylene chloride;

$K_{SPEext.2}$ – mycotoxin solid-phase extraction coefficient;

$m_s$ – mass of product sample taken for analysis [g].

The obtained data are summarized in Table 3.

| n/n | Indicator | Result, mg/kg |
|-----|-----------|--------------|
| 1   | DON       | less than 0.1|
| 2   | ZEA       | less than 0.02|
| 3   | AFB1      | less than 0.001|

The validity check and precision of measurements within the laboratory was carried out according to GOST ISO 5725-6-2003 (item 6.2.3) using Shewhart control charts.

4. Conclusion

Thus, the experiment made it possible to establish that the proposed cereal product does not contain highly toxic, poisonous substances and may be recommended as a supplement to the available food ration of people working in harmful labor conditions.

Besides, the proposed cereal product made according to TU 10.61.33-009-23333135-2020 contains adaptogene of plant origin in optimal ratio and amount, as well as preventive food components, proteins of high biological value, dietary fibers, vitamins that bind and effectively eliminate harmful chemical compounds out of the body. This product is capable of replenishing the energy value in nutrition.

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