Detection of unlabelled gluten in meat products and gluten-free flour by PCR and ELISA methods

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Abstract. Over the past decade, there has been an increase in the prevalence of gluten intolerance. Since wheat protein is widely used in the food industry, in particular in the meat industry, consumers prone to gluten intolerance should be aware of its presence in food products through the information on the label. Often, however, unintentional contamination of gluten-free products occurs. The aim of this study was to study the prevalence and level of gluten contamination of meat products and gluten-free flour used for the production of Russian-made meat products, which do not contain ingredients containing gluten. To determine gluten, PCR and ELISA methods were used. In four of the nine tested samples, gluten was found at a level exceeding 20 mg/kg.

1. Introduction
Celiac disease is an autoimmune disease caused by the ingestion of gluten proteins from wheat and related cereals (rye, barley and triticale) by genetically susceptible individuals [1]. The prevalence of celiac disease affecting both adults and children in the Western world is estimated at about 1-2% of the population [2]. In the Russian Federation, some data on the incidence of celiac disease diagnose an estimated prevalence in the range from 1:100 to 1:250 [3]. The overall prevalence of this disease in the world is estimated at 1:184. The disease leads to inflammation, atrophy of the intestinal villi and hyperplasia of the crypts of the small intestine. In addition to intestinal symptoms, celiac disease is associated with a variety of extraintestinal complications, including bone and skin diseases, anaemia, and endocrine and neurological disorders.

Given the widespread use of cereals containing gluten in the food industry (for example, pasta, breakfast cereals, most types of bread, thickeners and stabilizers used in soups, meat products, etc.), excluding wheat and other sources of gluten from the diet presents a challenge for people with celiac disease [5].

The production of gluten-free products is based on the food standards of the international commission, Codex Alimentarius, a joint body of the UN Industrial and Agricultural Organization and the World Health Organization. According to the ALINORM 08/31/26 Codex Alimentarius standard, gluten-free products can be considered gluten-free in which the gluten content does not exceed 20 ppm (20 mg/kg).

Currently, in the Russian Federation, in accordance with the technical regulations of the Customs Union TR CU 027/2012, gluten-free food products must be made from one or more components that do not contain wheat, rye, barley, oats or their crossbred variants and (or) must consist or be made in a
special way (to reduce the level of gluten) from one or more components that are obtained from wheat, rye, barley, oats or their crossbred variants, and in which the level of gluten in the ready-to-eat product is not more than 20 mg/kg.

Several studies have shown the likelihood and levels of contamination of gluten-free products. A 2011 Canadian study found that 82% of oat samples tested (n = 131) were also contaminated with high levels of gluten [6]. Another 2013 study by a group of Canadian scientists found that 32% of gluten-free flour (n = 640) sold at retail outlets in Canada was contaminated with gluten, which can pose health problems for people with celiac disease [7]. A study in Brazil found that 2.8% (n = 180) of traditional Brazilian food samples were inadvertently contaminated with gluten [8]. These studies highlight the risk of gluten-free cross-contamination of gluten-free products.

Unintentional gluten contamination of products that do not have labelling information about the presence of gluten or of gluten-free products is a serious problem. This type of information is important for consumers affected by celiac disease. The study described herein was conducted to determine the prevalence and level of gluten contamination in meat products and gluten-free flour used for the production of Russian-made meat products, which do not contain ingredients containing gluten.

2. Materials and Methods
To determine gluten, six different types of sausages of local specifications and lentil, corn and buckwheat flours were purchased in Moscow retail outlets. The labels of these products did not contain information about the presence of gluten.

2.1. ELISA method
ELISA test to detect wheat protein was performed using the Romer Labs Agra Quant® Gluten test kit, which is a screening test for examination of gluten-free products for the presence of gliadins and prolamins of wheat, barley and rye, and, as indicated by the manufacturer, it demonstrates presence of 4 mg/kg up to 120 mg/kg of gluten. The test is based on the principle of sandwich-like ELISA method, where gliadin was first extracted with 40% ethanol. The acquired extract was diluted with phosphate buffered saline and applied to test wells with conjugated antibodies against gliadin. After incubation and washing off redundant gluten, enzymatically marked antibody was bound to conjugated gliadin if present. A blue colour detected in the last stage was considered to be positive, while light pink colour was negative. Colour was compared to negative and positive controls. The test was performed in compliance with the manufacturer’s instructions.

2.1.1. Statistical data processing. For the calculations, the STATISTICA 10 program was used, the results were presented as “Mean ± SD”, and the statistical significance was calculated using the nonparametric Kruskal-Wallis test (for three or more independent groups). A probability of 0.05 was chosen as a significant level.

2.2. PCR method
2.2.1. Sample selection. Sausage samples were ground with Retsch GM200 blade homogenizer. Ten samples of 5 ± 1 g were selected from each sausage.

2.2.2. Isolation of DNA. DNA was isolated by the Sorb-GMO-B kit (Syntol CJSC, Russia) according to the instructions.

2.2.3. Conditions for real-time PCR. Primers and probes used in this work were taken from GOST 31719-2012 and MP 4.2.0019-11. The reaction mixture (30 µl) contained 2.5 µl of 10X PCR buffer, 2.5 µl of 2.5 mM MgCl₂, 2.0 µl of dNTP, nucleotides at a concentration of 25 mM, 2.5 U of SynTaqpolymerase, primers at a concentration of 300 nM, and 2 µl of DNA. The reagents were manufactured by Syntol CJSC, Russia. Amplification mode: pre-denaturation — 95°C, 420 seconds;
annealing-elongation — 60°C, 40 seconds; denaturation — 95°C, 15 seconds, 45 cycles. Real-time PCR was performed on a ANK-32 thermocycler (Syntol CJSC, Russia). PCR amplification was performed using a tenfold dilution of the original DNA.

2.2.4. Statistical data processing. Statistical analysis of the PCR results was performed using the software supplied with ANK-32 thermocycler.

3. Results and Discussion

It was found that the gluten content exceeded 20 mg/kg in the Braunschweig sausage (manufacturer 2) and the Krakowska sausage (manufacturer 3). The presence of a component potentially containing gluten was not indicated on the labels. The gluten in the sausages could be due to the presence of wheat flour impurities in some ingredients used in the formulation of these sausages, milk powder and dry egg powder. Gluten at levels significantly higher than the acceptable level was found in the studied samples of corn and lentil flour, which can be ingredients of meat products (Table 1).

Table 1. Gluten content in the tested sausages and flour

| Samples                          | Gluten content (mg/kg) detected by PCR | Gluten content (mg/kg) detected by ELISA |
|----------------------------------|----------------------------------------|---------------------------------------|
| Doktorskaya sausage, Manufacturer 1 | <20                                    | <20                                   |
| Russkaya sausage, Manufacturer 1  | <20                                    | <20                                   |
| Doktorskaya sausage, Manufacturer 2 | <20                                    | <20                                   |
| Braunschweig sausage, Manufacturer 2 | 30.12                                   | 23.98                                 |
| Krakowska sausage, Manufacturer 3 | 22.34                                   | 21.18                                 |
| Russkaya sausage, Manufacturer 3  | <20                                    | <20                                   |
| Corn flour, Manufacturer 4        | 80.35                                   | 91.21                                 |
| Lentil flour, Manufacturer 5      | 150.64                                  | 120                                   |
| Buckwheat flour, Manufacturer 6    | <20                                    | <20                                   |

All sausages selected for the analysis were produced in accordance with the national standards of the Russian Federation, and they assume gluten-containing ingredients are not present in the products. No gluten warning label was on any of the examined sausages. The same requirement applied to cereal and legume flours.

The most common causes of gluten appearing in meat products are: (1) unreliable suppliers, (2) non-compliance with good manufacturing practices, (3) inadequate analytical method used, (4) improper staff training and (5) inappropriate use of gluten warning labels.
According to the results of the study, the presence of gluten in sausages and flour was confirmed by PCR and ELISA methods. However, there were differences in the gluten levels, from which it can be concluded that further development of the analysis procedure is necessary, especially with respect to cereals and legumes.

4. Conclusions
To reduce production costs, cheaper vegetable proteins are often used in the meat industry. Certain plant sources, such as wheat, are also used as fillers and binders in meat products, but these plants can contain gluten, which causes celiac disease in consumers who are prone to celiac disease. Thus, information on the presence of gluten in such components of composite foods should be available on the label in accordance with the legislation. In addition, gluten can be inadvertently introduced into gluten-free products during transportation, production, storage through cross-contamination. As a result of the conducted research, gluten was detected in two samples of sausages, as well as corn and lentil flour.

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