A study of the quantitative content of gluten in dietary products using the enzyme immunoassay system RIDASCREEN® Gliadin competitive

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Abstract. In the work, the quantitative content of gluten in our developed dietary cereal culinary and flour confectionery products was measured. The fractional composition of several types of flour and their mixtures used in our developed products by calculation was determined, which revealed that the gliadin content in the flour types used was less than 20 mg/kg. The amount of gluten in our developed products was experimentally estimated using an enzyme immunoassay — the system RIDASCREEN® Gliadin competitive. As a result of our research, it was proven that the use of gluten — free raw materials (rice, corn, pumpkin and flax flour) in the development of dietary products allowed reducing gluten levels in muffins, casseroles and cereals, and cookies on average 124, 143 and 130 times, respectively. According to the data obtained, our developed products can be labeled as “gluten-free”, since the gluten level therein does not exceed the standard indicator of 20 mg/kg and therefore are recommended for people suffering from celiac disease.

1. Introduction

Nowadays, food products of specialized, dietary and therapeutic purposes are in high demand among the population, both because of an individual’s choice (religion, fashion, professional activity), and as a result of his/her state of health. For example, such types of food intolerance and allergies are widely known, such as lactose, casein and gluten ones. Gluten intolerance, or celiac disease, is a chronic hereditary disease characterized by persistent intolerance to the proteins of cereal crops with the development of atrophy of the mucous membrane of the small intestine and the associated malabsorption syndrome. This disease is lifelong, and self-healing never occurs. Today, it is characterized not only by intestinal damage, but is a reaction of the whole organism to gluten: as a result, almost all organs or systems are affected [1]. The main components of gluten are prolaminos (wheat gliadin, rye secalin, barley hordein, and oat avenin), which make up 5 – 50% of the total protein, soluble in 60 – 80% ethanol solution, and glutelins soluble in 0.1 – 0.2% alkaline solutions. By present, it has been proven that glutelins have toxic amino acid sequences identical to pelamine peptides, therefore, only one common name for toxic cereal proteins is commonly used – gluten [2].
| Content of protein fractions, % | Wheat | Barley | Rye | Oats |
|--------------------------------|-------|--------|-----|------|
| Albumins                       | 1.2   | 12.5   | 25.3| 12.5 |
| Globulins                      | 2.8   | 12.7   | 19.2| 17.3 |
| Prolamins                      | 43.5  | 34.4   | 25.4| 23.1 |
| Glutelins                      | 36.0  | 29.6   | 16.5| 29.3 |

From the data presented in Table 1 it can be seen that the maximum amount of alcohol-soluble (prolamins) and alkali-soluble (glutelins) protein fractions are found in cereals such as wheat and barley, rye has a lower one, and the alkali-soluble fraction predominates in oats. These cultures are widely used in the production of flour confectionery and bakery products, at the same time, they are not permissible for use in the production of gluten-free products.

Along with gluten intolerance, such patients often have lactose deficiency or intolerance to components of other foods, such as cow's milk, bananas, chicken egg protein, etc.

2. Problem statement
According to the information set forth in the National Technology Initiative (NTI) of December 4, 2014, supported by the President of the Russian Federation V.V. Putin, two innovative modern directions in areas related to human health and nutrition were proposed. The first one, HealthNet, aims to create a market for personalized medical services and medicines that would provide growth and longevity, as well as obtaining new effective means of prevention and treatment of various diseases.

The second direction is FoodNet. Its trend is the development of the production and sale of nutrients and finished types of food products (personalized and common, based on both traditional raw materials and their substitutes), as well as related solutions (for example, providing logistics and individual nutrition selection services). The national idea to address these initiatives is the creation by 2035 of “smart” services and products that would become competitive in the world market due to the best technological solutions for human food security. According to the forecasts of the Government of the Russian Federation, domestic companies should occupy significant shares of the world market and make up from 5 to 15% depending on the segment [3].

In recent years, experts have relied on general dietary rules to draw up a diet for celiac disease patients, namely, the complete exclusion of gluten-containing raw materials. In these cases, the carbohydrate component is compensated by cereals (rice, buckwheat, corn, and millet), vegetables, potatoes, fruits and berries. Proteins and fats are replenished with meat, eggs, dairy products, vegetable oil and butter, therefore, the diet for celiac disease is often called the “diet from the market”. Also in the gluten-free diet it is very important to raise the content of hematopoietic components (iron, copper, calcium, B vitamins and complete proteins of animal origin), since with such nutrition a deficiency of these substances is usually observed.

In most developed countries, the development and production of gluten-free products is at a high level and is universally developed. At the same time, in Russia this market is at the initial stage of development, therefore, domestic experts are urgently faced with the question of creating a wide range of products from gluten-free raw materials, which would provide high-quality, competitive products.

Objective of the work: to analyze the gluten content in our developed dietary culinary and confectionery products using the enzyme immunoassay system RIDASCREEN® Gliadin competitive.

3. Objects and methods
A number of culinary products developed by us were selected as objects of our study, namely: rice cereal casserole with almond milk and corn flour, rice cereal casserole with almond milk and linseed flour, buckwheat groats with goat milk and rice flour and flour confectionery: cookies from a mixture
of flax and corn flour, cake with the addition of flax and rice flour, cake with the addition of corn and pumpkin flour, and cake with the addition of corn and rice flour.

Quantitative analysis of gluten content in our developed products was carried out using the enzyme immunoassay system RIDASCREEN® Gliadincompetitive [4].

Statistical processing of the results of our study was carried out using Microsoft Excel 2010.

4. Discussion of the results
Based on our previously published works [5, 6, 7] on the development of gluten-free food products, presented in Table 2, recommendations were made for people suffering from various types of food intolerance.

Table 2. Recommendations for the use of our developed dietary culinary and confectionery products for certain types of food intolerance

| Product                                                                 | Celiac disease (gluten intolerance) | Intolerance to the components of cow’s milk | Comorbidity (gluten-free casein-free diet) |
|------------------------------------------------------------------------|-------------------------------------|---------------------------------------------|--------------------------------------------|
| Rice cereal casserole with almond milk and cornmeal                   | +                                   | +                                           | +                                          |
| Rice cereal casserole with almond milk and flax flour                 | +                                   | +                                           | +                                          |
| Buckwheat groats with goat milk and rice flour                        | +                                   |                                             |                                            |
| Flax and Corn Flour Cookies                                            | +                                   | +                                           |                                            |
| Gluten-Free Cake with Flaxseed and Rice Flour                         | +                                   | -                                           | -                                          |
| Gluten-Free Cake with Corn and Pumpkin Flour                          | +                                   | -                                           | -                                          |
| Gluten-Free Cake with Corn and Rice Flour                             | +                                   |                                             |                                            |

Currently, in accordance with TR TS 027/2012 “On the Safety of Certain Types of Specialized Food Products, including Dietary and Preventive Nutrition”, gluten-free food products must be made from one or more components that do not contain wheat, rye, barley, oats or their crossbred options and/or must consist or be made in a special way (to reduce a gluten level) of one or more components obtained from wheat, rye, barley, oats or their crossbred options, so as the level of gluten in ready-to-eat products wouldn’t be higher than 20 mg/kg [8].

In the course of our research, we initially evaluated the fractional composition of the types of flour and their mixtures used in the developed products by calculation. Table 3 presents the results.

Table 3. Mass fraction of protein fractions of various types of flour and their mixtures

| Type of flour                          | Mass fraction of protein fractions, % |
|---------------------------------------|---------------------------------------|
|                                      | Albumins | Globulins | Glutelins | Insoluble proteins | Prolamins (gliadin) | Zein |
| Wheat                                 | 1.20     | 2.80      | 36.00     | 8.70               | 43.50              | –    |
| Pumpkin                               | 25.20    | 42.80     | 21.80     | 10.20              | –                  | –    |
| Corn                                  | 8.10     | 5.90      | 80.00     | –                  | –                  | 5.90 |
| Rice                                  | 5.84     | 9.17      | 70.90     | –                  | 14.17              | –    |
| Linen                                 | 0.92     | 1.48      | 2.20      | –                  | 0.92               | –    |
| Pumpkin and corn composite mix        | 16.65    | 24.35     | 50.90     | 5.10               | –                  | 2.95 |
| Flax and rice composite mix           | 4.36     | 6.85      | 50.29     | –                  | 10.18              | –    |
| Corn and rice composite mix           | 6.97     | 7.53      | 75.45     | –                  | 7.10               | 2.95 |
Based on the data of Table 2 it is seen that the gliadin content in the types of flour used is less than 20 mg/kg. Gliadin is known to play the main role in the cause of the disease, since it reacts most intensely with anti-gliadin antibodies IgA and IgG, appearing in the blood of patients with celiac disease [9, 10, 11].

Then, to confirm our calculated data, we experimentally analyzed the quantitative content of gluten in the developed products using the enzyme immunoassay system RIDASCREEN® Gliadin competitive [4].

Figures 1–3 show the results of gluten levels obtained at a wavelength of 450 nm.

Thus, the use of gluten-free raw materials (rice, corn, pumpkin and flax flour) in the development of dietary products has allowed reducing the gluten level in muffins, in casseroles and cereals, and in cookies on average 124, 143 and 130 times, respectively.

Figure 1. Gluten content in our developed diet cakes

Figure 2. Gluten content in our developed dietary cereal culinary products

Figure 3. Gluten content in our developed diet cookie
5. Conclusion
For the first time using the enzyme immunoassay system RIDASCREEN® Gliadin competitive, the quantitative content of gluten was analyzed in our developed dietary culinary and confectionery products. According to the data obtained, our developed products can be labeled as “gluten-free,” since the gluten level therein does not exceed the standard threshold of 20 mg/kg and therefore are recommended for people suffering from celiac disease.

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