Study of enzymatic hydrolysis parameters for reducing anti-nutritional properties of flour

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Abstract. The paper addresses the problem of production of foodstuffs for people suffering from diagnosed celiacia. There are no ways of pharmacological treatment of this group of people. The only possibility to maintain their optimal living standards is a life-long gluten-free diet. Grain-based proteins (wheat gliadins, rye secalines, barley hordeins, and oat avenines) and products of their processing are the disease triggers. Most often one generally accepted term “wheat gluten” is used instead of all these species. Celiacia is one of the urgent social problems in many countries which can be explained by its wide morbidity rate, difficulties in its diagnostics and a lack of experience of its prophylaxes and treatment. Strict requirements to the chemical composition of gluten-free flour-based foodstuffs dictate their high retail prices. Market analysis has shown that gluten-free flour-based products on average are 240% more expensive than usual flour-based products. So crackers, butter biscuits, pizzas and pasta made from gluten-free flour are the most expensive products. However the market of gluten-free flour-based products is one of the most fast growing markets. In this connection there is a need to provide people suffering from diagnosed celiacia with inexpensive domestic gluten-free products of high quality including products suited for public catering facilities such as pre-school, school and higher education institutions, military army facilities, canteens and sanatorium-and-spa resorts. Developing specialized foodstuffs based on nontoxic raw materials having a positive effect on human organisms and able to decrease risks of disease recurrence is a challenge to the scientific community.

1. Introduction
Celiacia has long been considered a rare disease from which little kids suffer. In 2014, National Recommendations on diagnostics and treatment of celiacia of adults were adopted in Russia. In 2015, Federal Clinical Recommendations on the delivery of health-care services to children suffering from celiacia were approved. Application of advanced diagnostic methods in recent decades resulted in a considerable growth of celiacia patients due to the identification of latent or dormant forms of the disease. Lately the perception of celiacia has drastically changed. At present it is a disease with a high incidence rate (1-3% of the European population) and a chance of occurrence at any age [1]. Gastroenterologists develop algorithms of laboratory examination of patients with suspected celiacia and risk groups. These algorithms make it possible to timely diagnose the disease. Dietitians and food technologists try to monitor the results of diet therapy of patients sensitive to gluten enteropathy. The population groups mentioned above join in societies of celiacia patients in such cites as St.Petersburg, Moscow, Novosibirsk and other cities. The authorities of the Novosibirsk Oblast render financial assistance to such families with children. This money is used to purchase expensive foodstuffs.

It is known that for celiacia to actively show itself three factors are necessary, namely, heredity, the availability of gluten in food and a trigger mechanism, e.g. emotional stress, pregnancy, surgeries, virus
infections or early ablatation and passing to infant formulas, etc. A damage of the small bowel caused by gluten is a key factor of the disease. When gluten enters the digestive tract of a genetically predisposed person, antigliadin antibodies began to develop in the digestive tract. These antibodies join with a part of a gluten molecule, which causes damages and destruction of villi of the intestinal mucosa. As a result of an enzymatic deficiency the surface of the small bowel becomes smooth and practically unable to absorb nutritious substances necessary for normal functioning of the organism [2].

The elaboration and application of biotechnological approaches in food production is a dynamically developing area. Scientists are making attempts to find and study safe alternatives to the existing celiacia treatment, i.e. a gluten-free diet. One of them is selection of some high-yielding variety of wheat not containing toxic gluten fractions. Another direction is the development of processes of complete proteins degradation by enzymes which lead to hydrolysis of immunogenic peptides to biologically available amino acids and short peptides [3]. An effectively selected and stabilized enzyme will stimulate gluten digestion or can be used as a food additive [4].

To implement the research objective an analytical review of Russian and foreign literature was made. The RF patent No. 2057458 describes the method of obtaining a carbohydrate protein module from grain variety flour for children nutrition. In 1996, scientists from the Research Institute for Baby Food carried out amilosubtiline enzymatic hydrolysis G10x. The use of enzymes as technological additives in the biotechnological industry to detoxify gluten is investigated at the Chemical Faculty of the University of Salerno (Italy) and the European laboratory of food-borne disease study in the University of Naples Federico II (Italy) [5]. María Cristina Sotomayor Grijalva studies enzymatic hydrolysis of wheat gluten at different protein concentrations [6]. Doctor Atze Jan Van Der Goot describes research on determining an intensified process of wheat gluten hydrolysis. He argues that enzymatic hydrolysis is a process in which “soft” conditions are used i.e. pH from 6 to 8 and the temperature from 40 to 60° C. Their studies prove that understanding the enzymatic hydrolysis reactions can help to realize various functional properties of hydrolyzates of wheat gluten, for example solubility, emulsion and foam formation.

In 2017 and 2018, research results obtained by R.G. Kondratenko and E., N. Urbanchik and other scientists from the Mogilev State University for Food (Belarus), were published. Their papers address the problem of the chemical composition of flour made from enzymatic wheat grains. Their colleagues E.V. Nelyubina, E.V. Zakharova and others are involved in developing gluten-free products made from new bioavailable vegetable raw materials. A range of pea hydrolyzate crisps was developed at the department of technology and organization of food production in the Novosibirsk State Technical University. The complex enzymatic preparation Protosubtiline G3x was used.

It is known that wheat proteins are hydrolyzed with the use of acids, bases or enzymes. Compared to this, enzymatic hydrolysis of wheat flour is a softer process with a more selective hydrolysis. The development of specialized enzymatic gluten-free foodstuffs made from cheap raw materials available in the Novosibirsk Oblast for celiacia patients is quite relevant.

The objective of the research is to study the parameters of the raw material enzymatic hydrolysis to reduce anti-nutritional properties of wheat flour based on gluten degradation.

2. Materials and methods
Local flour of various varieties from NPO Avangard and the enzyme preparation Feedbest VGPro from OOO PO Sibbiofarm were used to prepare the samples. All the ingredients are produced in the Novosibirsk Oblast, Russia.

Research objects are:
- patent wheat flour (top grade), GOST 26574-2017, the gluten content is no less than 28%;
- light wheat flour (first grade), GOST 26574-2017, the gluten content is no less than 30%;
- second grade wheat flour, GOST 26574-2017, the gluten content is no less than 25%.

Feedbest-VGPro is an enzyme complex for feed production. It is used in rations with a high content of oil-bearing seed by-products (oilseed residues, oil cake) and grain legumes (soya peas, chicl-peas, lupine, etc.).

Feedbest-VGPro contains such enzymes as xylanase, β-glucanase and pectinase (table 1). They provide hydrolysis of non-starch polysaccharides and proteolytic enzyme complexes which destroy high molecular weight proteins to available peptides and amino acids. Enzymes contribute to the
neutralization of anti-nutritional substances in grain fodders (non-starch polysaccharides), which ensures an increase in fodder nutrient digestibility. This preparation is the most available on the market and economically more profitable as compared to foreign and Russian counterparts.

Table 1. Feedbest-vgpro composition.

| Characteristic                          | Enzyme activity |
|----------------------------------------|-----------------|
| Xylanase activity (XA), no less than U/g | 10000           |
| β-glucanase activity (β-glA), no less than U/g | 3500           |
| Pectinase activity, no less than U/g   | 5000            |
| Proteolytic activity, no less than U/g | 120             |

Advantages:
- Feedbest-VGPro increases the level of the ration metabolizable energy of by 4–7%.
- Animal and poultry products can be used for food after using Feedbest-VGPro without limitation.
- Feedbest VGPro is compatible with all ingredients of mixed fodder, all drugs and feed additives.
- The preparation is not toxic for people and does not pollute the environment.

The introduction of the enzyme complex into raw materials makes it possible to:
- Accelerate hydrolysis of high molecular weight proteins and polysaccharides;
- Increase nutritional substance utilization in the digestive tract;
- Offset the deficit of digestive enzymes.

General sequence of research stages is shown in Figure 1.

Figure 1. Diagram of research stages.
The first stage of sample preparation was carried out in the Institute of Solid State Chemistry and Mechanochemistry, SB RAS.

- enrichment by Feedbest-VGPro at the rate of 20.0 units per 1kg of flour;
- further mechanochemical treatment of the mix was carried out by the procedure [7] in the TM-3 centrifugal-roll mill.

The following samples were obtained:
- Sample No. 1 – enzymes with wheat flour, top grade;
- Sample No. 2 – enzymes with wheat flour, first grade;
- Sample No. 3 – enzymes with wheat flour, second grade.

At the next stage the samples were sifted through a sieve with meshes of 1mm in diameter. After that they were diluted with warm water in the proportion of 1:1.692 until receiving a homogeneous consistency.

Enzymatic hydrolysis was carried out at 50-55°C in the proofing oven during 5 hours with pH=6 and subsequent enzyme inactivation. The hydrolizate was tested for the residue of crude gluten in the samples by washing them with water at 16-18°C. Washing was not interrupted; washing water was changed several times and was filtered though a fine silk sieve. Residues of gluten on the sieve were weighed and photographed.

3. Results and discussion

Table 2 presents the content of crude gluten in the samples after 5 hours of enzymatic hydrolysis.

| Content of crude gluten | Samples |
|------------------------|---------|
| No.1                   | No. 2   | No. 3   |
| 0.116±0.015            | 0.061±0.001 | 0.049±0.005 |

The results in the samples after 5 hours of enzymatic hydrolysis and further washing of gluten are shown in Figures 2 and 3.

**Figure 2.** Traces of residual gluten.

**Figure 3.** Results in the samples.
The results given in table 3, Figures 2 and 3 allow us to assume that hydrolysis occurred at earlier stages and it was decided to control its occurrence every hour (table 3).

### Table 3. Grude gluten content in the samples by hours after enzymatic hydrolysis, %.

| Samples | Time of enzymatic hydrolysis, hours | 1     | 2     | 3     | 4     | 5     |
|---------|-------------------------------------|-------|-------|-------|-------|-------|
| No. 1   |                                     | 0.605±| 0.281±| 0.121±| 0.118±| 0.116±|
|         |                                     | 0.015 | 0.003 | 0.002 | 0.004 | 0.008 |
| No. 2   |                                     | 0.356±| 0.155±| 0.113±| 0.073±| 0.061±|
|         |                                     | 0.011 | 0.005 | 0.001 | 0.001 | 0.009 |
| No. 3   |                                     | 0.150±| 0.119±| 0.078±| 0.064±| 0.049±|
|         |                                     | 0.002 | 0.007 | 0.001 | 0.001 | 0.003 |

Pictures with traces of residue gluten after washing every hour of enzymatic hydrolysis are shown in Figure 4.

![Picture](image)

**Figure 4.** Traces of residual gluten after enzymatic hydrolysis during 5 hours.

It can be seen that after the first hour of hydrolysis the quantitative content of crude gluten in the rest samples did not change. Further studies were carried out every 10 minutes during one hour. The results are presented in Figure 5.

![Graph](image)

**Figure 5.** Results of enzymatic hydrolysis of samples every 10 minutes.
4. Conclusions
It can be concluded that the protein content in the reaction, the enzyme-substrate ratio, pH, the temperature and the time of the reaction are the most important parameters for carrying out enzymatic hydrolysis. Based on the results of the research done the enzyme-substrate ratio of 1:1.962 was determined and the mechanochemical treatment of wheat flour of different grades was carried out. Enzymatic hydrolysis was completed by using the Feedbest-VGPro complex enzyme preparation. As a result wheat protein which is the key factor in gluten intolerance was degraded during 10 minutes into low molecular weight compounds.

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