Towards the development of wheat extruded snacks with reduced gluten toxicity

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Abstract. The negative effects of gluten on human health are becoming a serious problem for the development of new foods. Gluten-free or very low-gluten labeling is an important factor in FMCG food marketing. An alternative to a gluten-free diet is the use of enzymatic hydrolysis to break down the immunogenic polypeptides of the gliadin and glutenin fractions. Gluten polypeptides that are resistant to gastrointestinal digestion can be hydrolyzed by prolyl endoproteases from Flavobacterium meningosepticum, Sphingomonas capsulate and Myxococcus Xanthus, or enzyme preparations from Aspergillus niger or Aspergillus oryzae. In many studies, the ability of high-temperature short-term extrusion to modify molecular structure of gluten and its fractions and improve enzymatic access to the biopolymers is noted. A method for integrating biocatalysis and extrusion processes to reduce or neutralize the immunogenic effect of gluten epitopes and to obtain extruded ready-to-eat snacks is proposed. The process flow chart includes the use of extrusion as a pretreatment stage for enzymatic hydrolysis of wheat and as a stage of thermal and shaping processing of extruded hydrolyzed wheat into ready-to-eat snacks.

1. Gluten-associated diseases and their causes

Cereals of the Triticeae tribe, such as wheat, rye, barley, as well as oats, are the ingredients and the basis for a large range of food products produced by various industries: bakery, confectionery, snack, baby food, brewing and many others. A serious challenge for the medical community and food science is overcoming the health problems caused by consumption of foods with these cereals due to allergic reactions mediated by immunoglobulin E (IgE) or non-IgE [1]. Health disorders are caused by the group of storage proteins with general definition as gluten including the combined gliadin and glutenin fractions. Genetically determined autoimmune celiac disease is not the only disease caused by gluten intolerance. In addition to celiac disease, gluten fractions cause non-celiac gluten sensitivity, dermatitis herpetiformis, gluten ataxia [2]. Viljamaa et al. showed that the prevalence of autoimmune diseases including insulin-dependent diabetes mellitus, autoimmune thyroid diseases, Addison's disease, primary Sjogren's syndrome, psoriasis was significantly higher in the group of patients with celiac disease [3]. Rewers et al. showed in their review that celiac disease is 16 times more common in the group of patients with type 1 diabetes compared with the main population [4]. Di Sabatino and Corazza also noted that celiac disease is statistically associated with endocrine autoimmune diseases, genomic diseases of Down and Turner syndromes [5].
Both gluten fractions gliadins and glutenins are characterized by a high content of proline (about 15%), glutamine (about 35%), and hydrophobic amino acids (about 19%) [6]. Four fractions α, β, γ, and ω are distinguished in gliadin with the molecular weight of proteins in the range of 28000–55000 [7]. And the main initiator of the immune response is the 33-dimensional polypeptide resistant to hydrolysis by gastric, pancreatic, and intestinal proteases [6]. The resistance of gluten fractions to hydrolysis by these proteases is determined by the presence of structural domains, which contain unique repeating amino acid sequences of glutamine and proline.

The structure-forming properties of wheat gluten in food products historically and technologically caused its widespread use in the food industry as the main and adjuvant ingredient. Moreover, the desire to optimize technological properties such as dough viscoelastic properties determined the selection of wheat varieties in favor of varieties with a high content of hydrolysis-resistant fractions including a 33-dimensional gluten peptide with its negative immunological reactions. The problem is also exacerbated by the widespread use of refined gluten produced by the integrated wet-milling technology.

2. Solutions for food industry
An effective and generally accepted solution to overcome gluten-related digestive diseases is a lifelong gluten-free diet and renunciation of gluten-containing foods. [8].

A new class of gluten-free snacks produced by extrusion cooking technology is developing along with the trends of replacing allergenic cereals with gluten-free ones and using modified starches, proteins and hydrocolloids to maintain the textural and rheological properties of traditional cereals products [9, 10]. The porous structure in such ready-to-eat extruded snacks is formed by starch under conditions of sharp pressure drops during extrusion in contrast to the porous structure of bread formed by gluten proteins during fermentation. In addition, extrusion technology allows the wide use of various non-cereal ingredients in acceptable amounts without noticeable deterioration in the sensory and structural properties of the products. It allows to develop a wide range of ready-to-eat extruded snacks with additional functional properties. A problem for traditional and innovative gluten-free products is the contamination of gluten-free raw materials with gluten during storage in grain silos and on production lines. At the same time, strict control at all stages of the production of gluten-free products from seed preparation and cultivation to storage, processing and packaging significantly increases the cost of such products for the consumer.

A promising direction in the prevention of nutritional diseases caused by gluten is the detoxification of gluten proteins by biotechnological methods aimed at hydrolysis of proline and glutamine containing polypeptides [11].

The hydrolysis of wheat protein fractions in the dough fermentation was investigated by Di Cagno et al. using starter cultures of lactic acid bacteria Lactobacillus alimentarius, Lactobacillus brevis, Lactobacillus sanfranciscensis and Lactobacillus hilgardii [12]. The results of the study showed that fractions of albumin, globulin and gliadin including the 31-43 fragment of A-gliadin were hydrolyzed. Concentrations of free amino acids such as proline, glutamic and aspartic acids increased.

Marti et al. stated the possibility of using prolyl endopeptidases from Flavobacterium meningosepticum as a biocatalyst for gluten hydrolysis and reducing the number of potentially immune stimulating peptides thereby weakening their toxic effect [13]. Caputo et al. conducted a review of researches concerning of enzymes applications for the breakdown of proline-containing peptides with the prospect of their use in oral enzyme therapy. Prolyl oligopeptidases from Flavobacterium meningosepticum, Sphingomonas capsulate, and Myxococcus Xanthus have been shown to be effective [11]. Mitea et al. investigated the effectiveness of prolyl endopeptidase from Aspergillus niger in a system that mimics digestion in vivo [14]. In this research, prolyl endopeptidase accelerated the degradation of gluten. This prevented gluten fragments from moving into the duodenum. Rimareva et al. [15, 16] studied the effect of various experimental enzyme systems from Aspergillus oryzae, Aspergillus foetidus, Bacillus subtilis, as well as the commercial protease from Bacillus licheniformis and papain from Papaya latex on the degree of destruction of triticale cereals proteins. The most effective was the treatment by enzyme preparations from Aspergillus oryzae. The degree of protein hydrolysis was about
90%. About 50% of the total number of amino acids passed into a free state. The free-form proline content in bread from hydrolyzed triticale increased from 0.01 to 1.04 g per 100 g of dry solids of bread [16]. The content increase was also noted for free methionine, valine, isoleucine, leucine, phenylalanine, threonine, tryptophan, and lysine.

The results of hydrolysis of gluten by papain were largely influenced by heat treatment [17] induced the forming of new chemical S-S bonds of glutenin fractions. Solubility and content of fractions with a molecular weight of less than 10 kDa decreased. Treatment within 30 minutes at 80 and 90 °C significantly reduced the content of glutenin extractable by sodium dodecyl sulfate.

3. Extrusion cooking as pretreatment stage for the wheat hydrolysis

High-temperature short-time extrusion is a promising way of physical modification of gluten proteins and their preparation for efficient enzymatic hydrolysis. The proteins of processed materials are being unfolded, rearranged and denatured by extrusion cooking. Secondary, tertiary and quaternary structures of proteins undergo significant changes [18, 19]. The modification of wheat proteins during extrusion is determined by the extrusion modes. Li and Lee showed that the formation of disulfide bonds and hydrophobic interaction play a key role in the aggregation of wheat proteins during the extrusion process, while glutenins and gliadins play a major role in protein polymerization [20]. The molecular weight of proteins increases and their solubility in various solvents (water, 0.01 M sodium hydroxide, 0.5 M sodium chloride, 70% ethanol, 0.1 M, hydrochloric acid, 0.05 M sodium phosphate buffer (pH 7.0 and 8.0), 6 M urea, 1% sodium dodecyl sulfate, 2% mercaptoethanol) decreases. The gliadins with a molecular weight between 45-66.2 kDa slightly modified under the influence of high-temperature extrusion.

Fisher investigation [19] also showed that gliadins are actively involved in intermolecular protein cross-linking with a disulfide bonds. At low and medium temperatures, solubility was lower at low moisture content. An increase in temperature caused an increase in solubility. Temperature variations from 90 to 160 °C during wet extrusion of wheat gluten caused significant changes in protein polymerization [21]. The native structure of proteins has changed as a result of denaturation and changes in molecular structure leading to the formation of soluble and/or insoluble aggregates.

Extrusion can be used for pretreatment of raw materials for enzymatic hydrolysis and as a continuous bioconversion stage mainly for starch-containing raw materials [22]. Cui et al. [18] investigated the effect of extrusion on gluten hydrolysis. Wheat gluten was extruded at a temperature of 150 °C and at a moisture content of 20% using an extruder with an L/D ratio of 27:1 and at screw speed of 135 rpm. Extrusion pretreatment had a significant effect on gluten hydrolysis. The degree of hydrolysis increased by 19.2%, the level of protein recovery of wheat gluten hydrolysates increased by 13.4%. Cui et al. noted that extrusion-induced changes in the secondary structure of the protein led to an increase in the content of free and total amino acids and peptides with a molecular mass below 5 kDa in the extruded gluten hydrolysates.

4. Potential ways to integrate extrusion and biocatalysis in for gluten detoxification

A review of biocatalysis and extrusion processes shows that these processes have a great potential in modifying the molecular structure of wheat glutenins and gliadins fractions. Approach to combine these processes in the technology of ready-to-eat snacks using wheat and other gluten-containing cereals can be promising for the detoxification of gluten. High-temperature short-time extrusion in such technology can be used at two stages: the first is the pretreatment of wheat for enzymatic hydrolysis; the second is a production of ready-to-eat snacks from hydrolyzed wheat with a consumer-acceptable texture formed by extruded biopolymers of wheat starch.

Potential process flow-charts for processing of wheat into ready-to-eat snacks are shown in figure 1. An important challenge for developing such process is a high water content in wheat hydrolysates before extrusion. The problem can be partially solved by adding gluten-free cereals and/or legumes and by using a steam venting systems installed at extruder. Important aspects in further studies in this area of research are the study of effect of extrusion modes on wheat gluten hydrolysis, investigation of effective
enzymatic systems for gluten detoxification and significant increase of the medium solids concentration during enzymatic hydrolysis. Also the use of additional enzymes for hydrolysis of non-protein cereals biopolymers in order to reduce the viscosity of hydrolysates should be investigated.

**Figure 1** Potential process flow charts on the base of extrusion and biocatalysis integration for ready-to-eat snacks production and gluten detoxification.

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