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## EVALUATION OF GLUTEN CONTAMINATION IN GLUTEN-FREE PRODUCTS IN THE REPUBLIC OF MOLDOVA

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**Abstract.** Celiac disease (CD) is an autoimmune condition, triggered by gluten ingestion, which affects the small intestine, destroying the villi. It is considered that in recent years CD has undergone a real "metamorphosis" due to the constant increase in diagnosed cases. The only treatment available for BC is to stick to a gluten-free diet throughout your life. Adherence to a GFD requires strict elimination of gluten-containing products, as patients with CD are very sensitive to the toxic effect of gluten. The purpose of the research is to evaluate whether the products marketed on the territory of the Republic of Moldova, labeled as gluten-free products, are safe for people with celiac disease, from the perspective of their gluten content. The identification of gluten in GF products sold in supermarkets in the capital was done using the GlutenToxPro gluten detection kit for food, beverages, and work surfaces (AOAC-RI). Research has shown that both gluten-free products imported with the Crossed Grain logo and those just labeled gluten-free (both imported and local), pose no risk to people with gluten-related disorders: the gluten content of all samples was up to 20 ppm. Local, non-packaged GF products (developed and made available to consumers by the supermarkets concerned) pose an increased risk of contamination.

**Keywords:** *celiac disease, certified products, Crossed Grain logo, food safety, food labeling, gluten free.*

**Rezumat.** Boala celiacă (BC) este o afecțiune autoimună, declanșată de ingestia la gluten, care afectează intestinul subțire, cu distrugerea vilozităților. Se consideră că în ultimii ani BC a suferit o adevărată „metamorfoză” din cauza creșterii constante a cazurilor diagnosticate. Singurul tratament disponibil pentru BC este aderarea pe tot parcursul vieții la o dietă fără gluten. Aderarea la un GFD impune eliminarea strictă a produselor care conțin gluten, deoarece pacienții cu BC sunt foarte sensibili la efectul toxic al glutenului. Scopul cercetării constă a evalua dacă produsele comercializate pe teritoriul Republicii Moldova, etichetate ca produse fără gluten, sunt sigure pentru persoanele cu maladia celiacă, din perspectiva conținutului de gluten în ele. Identificarea glutenului în produsele GF comercializate în supermarketurile din capitală s-a realizat cu ajutorul kitului de detecție a glutenului pentru alimente, băuturi și suprafețe de lucru GlutenToxPro (AOAC-RI). Cercetările au arătat că atât produsele fără gluten importate cu sigla Crossed Grain, cât și cele doar etichetate fără gluten

(atât de import cât și locale), nu prezintă niciun risc pentru persoanele cu tulburări legate de gluten: conținutul de gluten din toate probele a fost de până la 20 ppm. Produsele GF locale, neambalate (elaborate și puse la dispoziția consumatorilor de către supermarketurile în cauză) reprezintă risc sporit de contaminare.

**Cuvinte cheie:** *etichetarea alimentelor, fără gluten, maladia celiacă, produse certificate, siguranța alimentelor, sigla spicul tăiat.*

## 1. Introduction

Celiac disease (CD) is an autoimmune condition, triggered by gluten ingestion, which affects the small intestine, destroying the villi. It is considered that in recent years CD has undergone a real "metamorphosis", largely due to the constant increase in diagnosed cases [1,2]. Some theories suggest that this is due not only to the high availability of screening tests, but also to globalization, including the consumption of large amounts of gluten (up to 20 g / day), which has led to the increased prevalence and incidence of MC [3]. It is estimated that over 70% of people with celiac disease remain undiagnosed, which means that they become chronic patients who experience a decrease in quality of life associated with various gluten-related health problems that occur over time [4,5].

The incidence of MC in the world is about 1%. People with MC may experience a wide variety of gastrointestinal and malabsorption symptoms or extra-intestinal symptoms. The only treatment available for celiac disease is to stick to a gluten-free diet throughout your life. Adherence to a GFD refers to the strict elimination of gluten-containing products [6,7]. Gluten is made up of protein fractions, which are found mostly in the endosperm of grains, such as wheat, barley, rye, and foods that are derived from grains (e.g., semolina, durum, spelled, triticale, kamut, and malt) [8–11].

Gliadin, a glycoprotein, alcohol-soluble fraction of gluten, is thought to be responsible for the immune reaction to gluten consumption. In general, gliadin contains both toxic and immunogenic peptides.

- Toxic peptides can affect tissues, causing damage to the intestinal mucosa, justifying the activity of T-helper lymphocytes, by activating an innate immune reaction [12].
- Immunogenic peptides can activate the acquired immune response by specifically stimulating HLA-DQ2/DQ8 T lymphocytes and B lymphocytes [13].

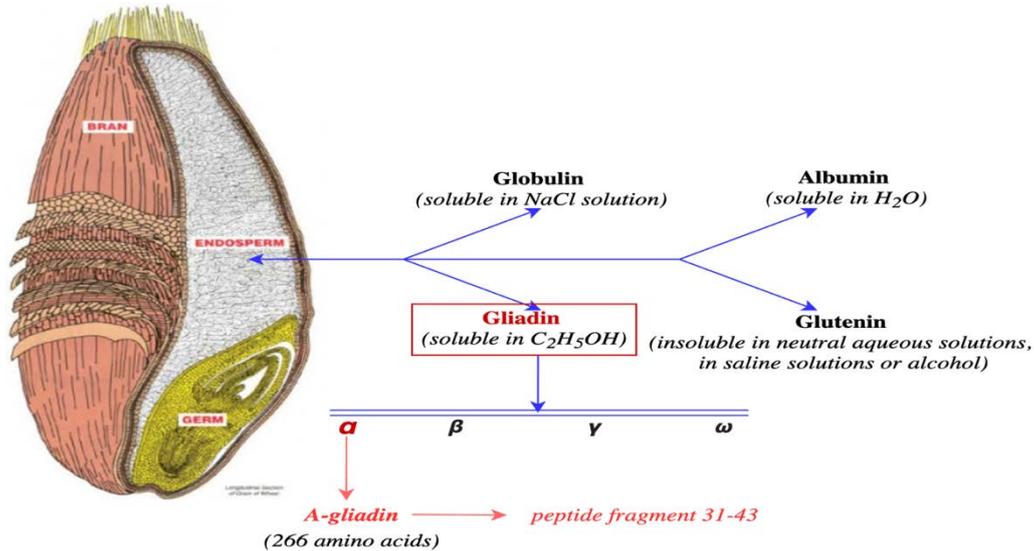
Due to the chemical complexity, the digestibility of gliadins is quite low. Fragments obtained from the partial digestion of proline and glutamine cause inflammation and destruction of intestinal epithelial cells. According to the electrophoretic mobility of prolamins, 4 fractions were identified:  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\omega$ .

The  $\alpha$ -gliadin fraction, with 266 amino acids, is considered to be the most toxic, containing the most active epitopes for the immune system [14, 15].

In people with CD, the sequence 31-34 (A-gliadin) has no immunological activity against T-helper lymphocytes and is transported across the mucosa of celiac patients in double amounts compared to healthy individuals (Figure 1).

The main protein fractions are prolamins and glutelins and are responsible for the development of symptoms in celiac disease [2].

According to European legislation and Codex Alimentarius, a food can only be labeled "gluten free" if it contains less than 20 ppm gluten (20 mg /kg) in the final product [12–14].



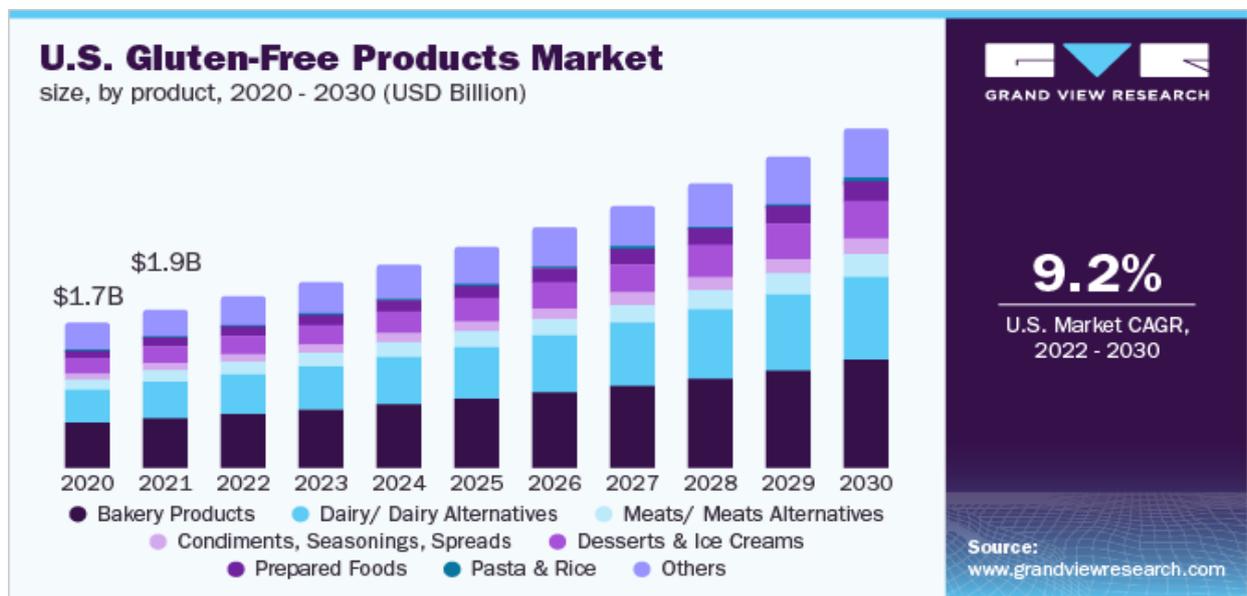
**Figure 1.** Protein fractions of cereals and subfractions of gliadin.

Also, a food labeled “very low gluten” may be labeled as such if it contains less than 100 ppm gluten (100 mg/kg) in the final product [12,14,15]. There are studies that have shown that prolonged ingestion of even traces of gluten (10–50 mg/day) can affect the integrity of the intestinal mucosa, an increased number of IELs being the first marker of mucosal damage [16].

The most significant long-term risk factor is inadequate adherence to a gluten-free diet [5,6,17]. A strict gluten-free diet normalizes the levels of antibodies associated with celiac disease and, respectively, the recovery of the affected tissues takes place, which can often take several months [4,12,17]. Carefully performed and monitored by a specialist, the GF diet will positively influence the health of the patient with gluten-related disorders [2].

## 2. The market for gluten-free products

The size of the global gluten-free market has been estimated at \$ 5.9 billion in 2021 and is expected to expand at an annual growth rate (CAGR) of 9.8% from 2022 to 2030 (Figure 2).



**Figure 2.** The global market for gluten-free products [18].

The bakery market accounted for the largest share of revenue of almost 29.0% in 2021 and is expected to maintain its dominance over the forecast period. The growing prevalence of gluten-related disorders stimulates the demand for gluten-free products. The COVID-19 pandemic has also exponentially affected the use of gluten-free products due to growing concerns about health and well-being among consumers. Inspired by those who encourage gluten-free diets for better health, many consumers have joined the diet of people for whom gluten-free consumption is a mandatory medical necessity [18,19]. Studies in the field have shown that the rate of adherence to a GFD varies from 44 to 90% in patients with MC. Most often the adherence to a GF diet is restricted by the high cost of these products, compared to the classic products.

A review of the cost of gluten-free products in the United States found that the total cost of gluten-free products is 183% higher than their wheat-based counterparts. Gluten-free biscuits have been found to be 270% more expensive than those made with gluten-containing flours. Commodities such as bread and pasta were also significantly more expensive - by 229% and 227%, respectively. In a survey conducted by GIG in 2021, 78% of respondents - followers of a gluten-free diet said that the cost of gluten-free foods was the biggest challenge they faced. 38% said access to gluten-free foods in general is a challenge [20].

The food safety of people following a GF diet is disturbed by other factors such as: limited availability of gluten-free products, insufficient labeling, risk of cross-contamination, lack of certification policies for GF products, etc. [6,17,21–24]. Patients with CD are very sensitive to the toxic effects of gluten.

The purpose of the research is to evaluate whether the products marketed in the territory of the Republic of Moldova, labeled as gluten-free products, are safe for people with celiac disease, from the perspective of their gluten content.

### 3. Materials and methods

During the month of May, this year, the supermarkets and small (corner) stores in the capital were visited to identify if they have GF products and to evaluate their assortment. List of supermarkets and subsidiaries under investigation:

1. Nr. 1.
4. Fourchette.
5. Linella.
6. Metro.
7. Kaufland.

A total of 13 supermarkets and 23 small (corner) stores were visited. GF products were purchased to assess whether they were contaminated with gluten, as well as the degree of contamination.

#### 3.1. Materials

The gluten-free products (48 products) purchased were classified into 3 categories:

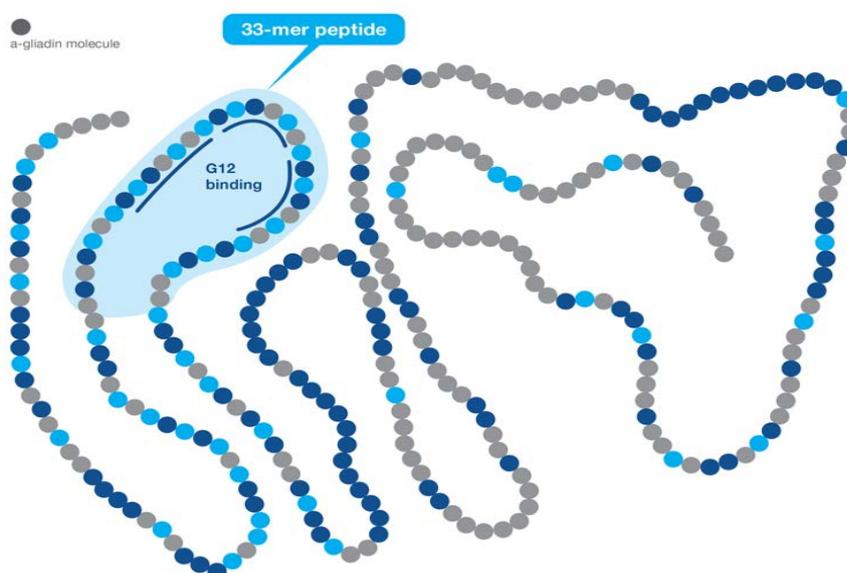
- Imported products, labeled *gluten free*.
- Imported products, labeled *gluten-free*, with the logo of the *Crossed grain*.
- Gluten-free local products (non-certified) - 17 products.

GlutenToxPro Kit (AOAC-RI).

### 3.2. Methods

In order to achieve this goal, imported and domestic GF packaged products were purchased from the supermarkets in the capital. A total of 45 products were purchased: bread, pasta, flour and flour mixes, pastries. Products were tested for gluten using the GlutenToxPro Kit (AOAC-RI) (Gluten Detection Kit for Food, Beverage, and Workspaces) [25].

GlutenToxPro is an immuno-chromatographic test used to detect gluten in foods with different levels of processing. The test is used in routine gluten monitoring and to ensure that products are HACCP compliant and properly labeled. Tests also allow decisions and corrective action to be taken quickly if there is a risk of contamination along the production chain. The kit contains the G12 antibody that specifically recognizes the 33-mer peptide of the  $\alpha$ -gliadin protein that induces celiac disease [26], (Figure 3). This recognition sequence is repeated three times within the gliadin 33-mer peptide. The G12 antibody recognizes immunotoxic prolamins from wheat, barley, rye and also from some varieties of oat [27]. The G12 antibody is also capable of reacting to other epitopes that are found in other toxic prolamins [27,28].



**Figure 3.** The 33-mer peptide and G12 antibody binding.

**The principle of the method.** The method consists in the reaction of immunotoxic peptides, like peptide 33 in the sample, with colored conjugates, previously fixed on the stick. That complex has capillary spread on the test strip. In the case of a positive result, a red line appears in the test area of the strip. The result is negative if the red line is missing.

If the test has been performed correctly, a blue line (the control line) will appear on the test strip, regardless of whether or not there is gluten in the tested sample. The detection threshold is conditioned by the number of drops (1, 2, 4 or 10 drops) added to the blue cap dilution bottle (Table 1).

*Table 1*

		Interpretation of results			
		Detection threshold			
		10 drops	4 drops	2 drops	1 drop
Test result	Positive	>5 ppm	> 10 ppm	> 20 ppm	> 40 ppm
	negative	< 5 ppm	< 10 ppm	< 20 ppm	< 40 ppm

**Warning and limitations.** The efficiency of gluten extraction depends on the type of test samples and in such cases, involves conditions of maximum sensitivity (for a detection limit of at least 10 ppm, 10 drops of extract are required in the dilution bottle with a blue cap).

This product category includes:

- Food with ingredients (up to 70%) containing polyphenols or tannins in high concentrations (coffee, black tea, chocolate, wine, berries, legumes, etc.).
- Foods rich in antioxidants (vitamin A, E and C).
- Heat-treated foods with temperatures above 180 °C.

The gluten content could be underestimated in samples subjected to intense hydrolysis processes (beer, sourdough, syrups) [33].

The tests were performed in May 2022, at the Technical University of Moldova, Department of Food and Nutrition.

#### 4. Results and Discussion

Complete avoidance of gluten in the diet is difficult to maintain. A "pervasive" nutrient, gluten can contaminate gluten-free items along the production chain, from the field to the grinding, storage, and manufacturing stages [16]. Most supermarkets had a (common) section for special purpose products (diabetic products, vegan products, organic products, gluten-free products, etc.). Of the 13 supermarkets, only eight had a higher availability of gluten-free foods during that period. Probably because of the Covid 19 pandemic [29], but also of the war in Ukraine, the range of GF products was quite limited. Small (corner) stores did not have gluten-free products.

A total of 48 GF foods were analyzed. The selected products included different brands of bakery and pastry products, flours and mixes of flour and pasta: 13 imported products, labeled GF; 18 imported products, with the *Crossed Grain* logo; 15 local packaged products and 2 local products for use current (unpackaged). The results of the research are presented in Table 2.

Table 2

**Level of gluten contamination in the products examined**

Tested product category (GF)	Number of products	Gluten content (GC)		
		> 20 ppm	> 10 ppm	< 10 ppm
Imported products, labeled GF	n = 13	-	1 (8%)	12 (92%)
Imported products with the Crossed grain logo	n = 18	-	-	18 (100%)
Packaged local products	n = 15	-	5 (33%)	10 (67%)
Unpackaged local (current) products	n = 2	2 (100%)		

The European License System (ELS) is the standard by which gluten-free products are certified. It refers to those products that carry the Crossed Grain logo on the packaging [13]. The products, which had the Crossed Grain on their packaging, have confirmed their safety and integrity, and continue to be seen as the most effective means of communicating to consumers that these products are safe for people with MC [12,15,30]. All 18 products tested, with the Crossed Grain logo on the package, contained less than 10 ppm gluten.

Another 13 imported products, which had the label gluten free but did not have the Crossed Grain logo, confirmed their safety for consumers with disorders related to gluten consumption: in 12 of them the gluten content was below 10 ppm and only in one product, the gluten content was between 10 ppm and 20 ppm. What characterizes these products as gluten-free products.

The packaged local products under test were products that were naturally gluten-free. Namely cereal derivatives (sorghum flour, corn, legumes, from different producers). It should be noted that the list of local products, labeled gluten-free, contained: flour, semolina and sorghum groats, corn flakes, corn flour, chickpea flour, bean flour, potato flour and flour mixes. The list did not include any products such as bread, biscuits, pasta, snacks, etc. The results showed that even local products, labeled as gluten-free products, do not pose risks for people with disorders related to gluten consumption: in 10 of the 15 local products tested, the gluten content was below 10 ppm, and in 5, between 10 ppm and 20 ppm.

Only one chain of supermarkets in Chisinau offered consumers gluten-free products for current consumption, namely bread made from legumes and pseudocereals. These products were developed in the bakery sections of the supermarkets involved. Those products had the list of ingredients listed on the label and were marketed as gluten-free products. In both products, the gluten content exceeded 20 ppm, which constitutes a risk for the safety of people with celiac disease.

Research has shown that both gluten-free products imported with the Crossed Grain logo and those just labeled gluten-free pose no risk to people with gluten-related disorders: the gluten content of all samples was up to 20 ppm.

Local products labeled gluten-free, according to the results obtained, are just as safe. We just have to keep in mind that when we refer to local GF products, we usually refer to grains, legumes and their derivatives. In the Republic of Moldova, gluten-free products such as: bread, pasta, croissants, pizza, puff pastry, etc. are not currently produced.

## **5. Conclusions**

- Gluten-free products are an example of reverse functional foods, in which gluten is eliminated and not included, like other constituents. These are currently an area in full swing.
- According to the results obtained, GF products with the Crossed grain logo as well as those certified GF continue to be seen as the most effective means of communicating to consumers about their safety for people with MC.
- The assortment of gluten-free foods in the Republic of Moldova is extremely low, and this makes the food security of these categories of people vulnerable. However, research results have shown that local products (cereals, legumes, and their derivatives, etc.) labeled as gluten-free products do not pose risks for coeliacs.
- Unpackaged local GF products (developed and made available to consumers by the supermarkets concerned) pose an increased risk of contamination.
- The lack of certification policies for this product category reduces confidence in the safety of local GF products.
- Food education, informatization of the population, especially those in the food industry, with reference to gluten sources, workplace hygiene, sources, and consequences of cross-contamination with gluten, would contribute to a better

safety of these products and increase nutritional security of people with disorders related to gluten consumption in the Republic of Moldova.

- Launching a systematic gluten-free food sampling program would help to quickly identify risky products, ensure the safety of available products, and ultimately improve the long-term well-being of people with CD or other gluten-related disorders.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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