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Enzyme-Linked Immunosorbent Assay Gliadin Assessment in Processed Food Products Available for Persons With Celiac Disease: A Feasibility Study for Developing a Gluten-Free Food Database

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Inappropriate food labeling and unwillingness of food companies to officially register their own gluten-free products in the Greek National Food Intolerance Database (NFID) result in a limited range of processed food products available for persons with celiac disease (CDP). The objective of the study was to evaluate the feasibility of developing a gluten-free food product database based on the assessment of the gluten content in processed foods available for CDP. Gluten was assessed in 41 processed food products available for CDP. Group A consisted of 26 products for CDP included in the NFID, and group B contained 15 food products for CDP not registered in the NFID but listed in the safe lists of the local Celiac Association (CA). High-sensitivity ω -gliadin enzyme-linked immunosorbent assay (ELISA) was used for analysis. Gluten was lower than 20 ppm

in 37 of 41 analyzed products (90.2%); in 24 of 26 (92.3%) products in group A and in 13 of 15 (86.7%) products in group B ($P = .61$). No significant difference was found between the 2 groups regarding gluten content. No product in either group contained gluten in excess of 100 ppm. Most of the analyzed products included in the Greek NFID or listed in the lists of the local CA, even those not officially labeled "gluten free," can be safely consumed by CDP. The use of commercially available ω -gliadin ELISA is able to identify those products that contain inappropriate levels of gluten, making feasible it to develop an integrated gluten-free processed food database. (*Nutr Clin Pract.* 2011;26:695-699)

Keywords: celiac disease; glutens; diet, gluten-free

Celiac disease is an autoimmune disease characterized by sensitivity to certain sequences of amino acids found in the prolamin fraction of wheat, barley, and rye. These prolamins or storage proteins (gliadin, hordein, and secalin, respectively) cause mucosal changes to the small intestine. The only treatment with

proven efficacy for celiac disease is strict and lifelong adherence to a gluten-free diet as even small amounts can be harmful.¹⁻³ Great improvement in food labeling has been seen on a global basis over the past 10 years. However, considerable controversy exists regarding the definition of gluten-free food products. The term *gluten free* varies from country to country. Today, there is no official Food and Drug Administration (FDA) accepted definition for gluten free.⁴ In 2008, the World Health Organization (WHO)/Food and Agriculture Organization (FAO) proposed a revision of the Codex Alimentarius, stating that 20 ppm is the maximum acceptable level of gluten allowed for a product to be labeled gluten free, stating that the qualitative analysis that indicates the presence of gluten shall be based on relevant methods (eg, enzyme-linked immunosorbent assay [ELISA]-based methods, DNA methods).⁵ The same limit

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has been set by the US FDA.⁶ In addition, the Codex Alimentarius Commission (2008 revision) states that foods especially processed to reduce gluten content to a level above 20 mg/kg and up to 100 mg/kg (20–100 ppm gluten) cannot be considered gluten free, and the decisions on the marketing may be determined at the national level. Nevertheless, according to the Canadian Celiac Association, “gluten-free in Canada means that the food does not contain wheat, spelt, kamut, rye, barley, oats or triticale, or any parts thereof.”⁷ In Australia and New Zealand, strict food labeling laws suggest that to be labeled gluten free, a food must contain “no detectable gluten” by the most sensitive universally accepted test method.

Information and support resources such as gluten-free food databases are valuable for persons with celiac disease (CDP). In the United States, a couple of paid online services provide this kind of information and support, but their worth to cost is debatable, as is the accuracy of the information because it is based on the information given on a voluntary basis by the manufacturer. In Greece, the Greek section of the European Food Intolerance Database prepared lists of “gluten-free” products based on food companies’ signed statement upon submission of the products to be registered. The information is disseminated through the European Food Intolerance Database network as well as through the Greek Celiac Association (GCA), which also has its own safe list. In Greece, the gluten-free food database is part of the National Food Intolerance Database (NFID), which is part of the European Food Intolerance Database. It has been updated every year based on information provided by the food companies. The major problem in constructing and updating this database is the unwillingness of food companies to submit information about their own products, feeling uncertain about their gliadin content because they do not have full access to the control of the raw materials. As a result, the NFID includes only a limited range of products, and its adequacy in supporting CDP is debatable. On the other hand, CDP cannot simply rely on manufacturers’ statements—even written and signed—that a product is gluten free without any other confirmation.

The European Legislation on Food Labeling (89/2003) currently requires that specified allergenic ingredients used in prepacked foods (gluten among them) are clearly declared on the “allergy advice panel” on the packaging. In Greece, the food labeling legislation has been in accordance with the European Legislation on Food Labeling since 2005. In this context, the processed food products available for CDP in Greece include 4 different types of products: (1) products labeled with the specific gluten-free label; (2) products not labeled but submitted by manufacturers for registration to the NFID; (3) products not labeled, not submitted to the NFID, but listed in the local Celiac Association (CA) lists; and (4) products not labeled, not submitted to the NFID, not listed in the

GCA lists, but consumed by CDP on the basis that gluten is not listed in their “allergy advice panels” on the packaging. Further confusion is created because food companies prefer the misleading warning “may contain traces of gluten” in the allergy advice panels of many foods of the last category, instead of providing accurate information based on specific assessment. In the United States, the paid database services are similarly confusing because the gluten-free status of a product is expressed in safety categories, including (1) “labeled as gluten free,” (2) “appears to be gluten free,” and (3) “may contain gluten.” In addition, they include advisory labels such as “shared equipment” and “shared facility.” Thus, CDP do not have any guidance about the interpretation of ambiguous warnings—which, after all, are not currently regulated by the Food Allergen Labeling and Consumer Protection Act (FALCPA)⁸—and what their expected attitude toward those products would be. They just believe that their choices are further limited in an unacceptable way.

To overcome this confusion, an alternative approach was suggested: to develop a gluten-free food database with processed food products that would make the difference for CDP by certifying their safety by assessing their gluten content. This effort consists of 3 steps: (1) gluten assessment in processed foods available for CDP already included in the NFID, (2) gluten assessment in foods listed in the GCA lists, and (3) gluten assessment in processed foods that CDP would like to consume on the basis of their “allergy advice panels” on the packaging, either not listing gluten or including advisory terms such as “may contain traces of gluten.”

The objective of the present study was to evaluate the feasibility of developing a gluten-free processed food product database based on the assessment of the gluten content in a number of processed foods available for CDP already included in the NFID of Greece as well as in those not submitted by the companies to be registered in the NFID but listed in the lists of the local CA, by a commercially available ELISA kit.

Material and Methods

Gluten content was determined in 41 processed food products available for CDP (flours, dairy products, cereals, pasta, sweets, processed meat, sausage, cakes, and tomato sauce) that were chosen according to the preference of the consumers (mainly children). Group A consisted of 26 food products available for CDP included in the NFID, and group B consisted of 15 food products available for CDP not registered in the NFID but listed in the safe lists of the local CA because of manufacturers’ unwillingness to officially submit their products to the NFID. Three non-gluten-free processed products served as positive controls.

Table 1. Gluten Content in Groups of Similar Items of the Processed Food Products Available for Persons With Celiac Disease

Processed Food Products	Group A (NFID Registered)		Group B (NFID Nonregistered)	
	No. Samples	Gluten Content, ppm, Mean $\pm$ SD (Range)	No. Samples	Gluten Content, ppm, Mean $\pm$ SD (Range)
Flours—cereals	10	8.8 $\pm$ 6.5 (0–18.1)	6	15.1 $\pm$ 13.0 (0.55–35.7)
Dairy ^a	9	9.2 $\pm$ 13.7 (0–39.0)	8	4.9 $\pm$ 6.2 (0–17.8)
Sweets	3	3.9 $\pm$ 6.8 (0–11.7)	—	
Miscellaneous ^b	4	7.7 $\pm$ 9.1 (0–17.5)	1	12.95
Total	26	8.7 $\pm$ 9.8 (0–39.0)	15	10.8 $\pm$ 10.6 (0–35.7)

NFID, National Food Intolerance Database.

^aMilk drinks, yogurt, ice cream, and cheese.

^b"Gluten-free" processed meat, pasta, and tomato sauce.

Samples were analyzed using a commercially available ELISA kit (Transia Plate Gluten, GL0301; Diffchamb SA, Lyon, France). The assay is a sandwich ELISA based on a highly specific ω -gliadin monoclonal antibody that detects the ω -gliadin fraction of gliadin in foods containing both native and heated protein. This method was developed by Skeritt and Hill⁹ and verified in a collaborative study. Sample preparation and gliadin assessment were performed according to the manufacturer's instructions. In brief, prolamines were extracted with 60% ethanol and centrifuged. Three dilutions of supernatant (1/50, 1/250, 1/1000) were tested for each product. An amount of 100 μ L of the supernatant (antigen) was incubated with monoclonal antibody immobilized onto the microwell strip for 30 minutes at room temperature to form a gliadin antigen-antibody complex, which was then incubated with enzyme-labeled antibody. Gliadin forms a complex sandwiched between antibody attached to the well and antibody labeled with enzyme. The amount of gliadin was determined by adding chromogenic substrate. Response was compared with that observed with gliadin standard and blank. All samples were analyzed in duplicate. According to the manufacturer's specification, sensitivity of the assay is 5 ppm gliadin, corresponding to about 10 ppm gluten. Results are expressed as gluten values.

Results

The amounts of gluten contained in the foods available for CDP that were analyzed did not differ significantly between the 2 groups ($P = .52$; Table 1). No product in either group contained >100 ppm of gluten. Gluten amounts below 20 ppm were documented in comparable proportions of foods in groups A and B (92.3% vs 86.7%, $P = .61$; Figure 1). The amount of gluten ranged between 20 and 100 ppm in 2 products of group A (20.75 ppm and 39 ppm, respectively)

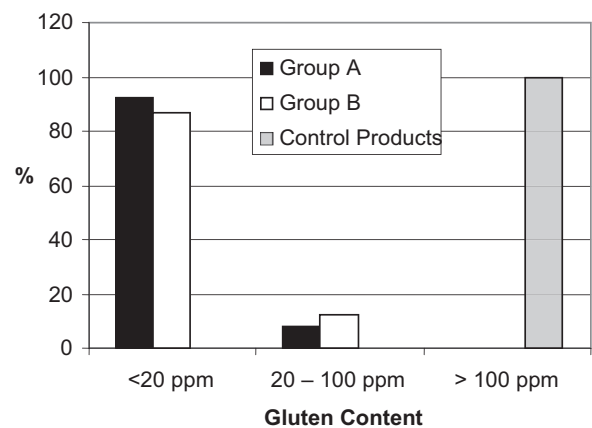


Figure 1. Percentage of processed food products with gluten content (<20 ppm, 20–100 ppm, and >100 ppm) in the 2 groups of food available for persons with celiac disease.

and in 2 products of group B (22 ppm and 36 ppm, respectively). Gluten was undetectable in 10 of 26 (38.5%) and 3 of 15 (20%) products in groups A and B, respectively. Gluten was also undetectable in a homemade cake prepared using gluten-free products.

Gluten content in the positive control products—namely, concentrated vegetable cubes, processed meat, and cereals—ranged between 220 and 244 ppm.

Discussion

Most of the processed food products available for CDP included in the NFID that were analyzed in the present study contained gluten at a level <20 ppm that was set as an upper limit for foods labeled "gluten free".^{5,6} Only 2 products in this group of foods contained gluten in excess of this limit. On the other hand, a similar proportion of the analyzed products available for CDP not submitted by

the companies to be registered in the NFID but in the lists of the local CA also contained gluten at levels lower than 20 ppm.

Although our results reflect a random sample, they indicate that most processed food products for CDP included in the lists of CA either registered or not in the Greek NFID can be consumed safely by CDP. Furthermore, they indicate that CDP are justifiably reluctant to consume products about which they do not have accurate information because in 4 products, the gluten level ranged between 20 and 40 ppm (although not higher than 100 ppm). It is important to identify which of the processed food products available are really suitable for CDP by further analyzing larger numbers of them, a task facilitated by the commercially available high-sensitivity assays for quantitative determination of gluten, and introduce an integrated database. The sandwich ELISA using ω -gliadin monoclonal antibody is a commonly used assay for measurement of gliadin and related prolamins in raw, cooked, or processed foods.^{9,10} Previous studies confirmed the results obtained by ω -gliadin ELISA by using Western blot and found no statistically significant differences between the 2 methods.¹¹ This assay, which was also used in our study, is based on the use of monoclonal antibodies against heat-stable ω -gliadins and related prolamins from wheat, rye, and barley. The sensitivity of the method is satisfactory as it can detect concentrations of gluten as low as 10 ppm, which is far below the suggested limit for gluten-free processed foods. The method is cost-effective and gives results within 2 hours of sample preparation. The main criticism concerns the ability of the assay to accurately detect and quantify barley prolamins, as it may underestimate gluten content, leading to false-negative results if the threshold of 200 ppm is used for gluten contamination.^{12,13} However, this limitation of the method cannot be considered a weakness of our study because the amount of gluten measured in the processed food products available for CDP included in both lists was far below that limit. Actually, the gluten level was lower than the detection limits of the method (10 ppm) in 37 of 41 (90.2%) analyzed foods. Thus, even if the actual amount of gluten was underestimated, it cannot possibly exceed the limit of 20 ppm, which is double the detection limit.

In 2006, the Codex Committee on Methods of Analysis and Sampling endorsed the sandwich R5 ELISA developed by Méndez et al¹⁴ for determination of gluten content in gluten-free foods with a detection limit of 3 ppm.⁴ The R5 ELISA is based on monoclonal antibodies, which target immunogenic peptides. This assay, which recognizes all fractions of wheat gliadin as well as barley hordeins and rye secalins, is not devoid of weaknesses either because it may overestimate barley hordein in barley-contaminated foods, depending on the standard used.^{11,12} Ideally, the combination of ELISA with another

method (ie, Western blot) would increase the ability to detect foods that are really gluten free.¹³ However, the use of such a methodology in everyday practice for analyzing large numbers of products is not a practical and cost-effective approach.

Many issues concerning cutoffs, analysis, and sampling methods are expected to be regulated in the coming years. However, an issue that has not yet been addressed is whether the gluten-free certification should be provided by the manufacturer using on-site techniques of analysis or an independent lab using the above suggested by Codex Standard techniques. Because the literature reflects justifiable skepticism and even suspicion from consumers,^{7,15} we recommend that the certification be centrally regulated and managed. Appropriately accredited food safety labs should be commissioned to perform this kind of assessment using standard techniques. The use of specific and general best practices should be certified as well, with a food safety management system consistent with the standard. As an example, the Canadian Celiac Association Gluten-free Certification Program is currently in partnership with a quality management certification service to provide annual audits and certify products that are manufactured for CDP.⁷ These local initiatives are valuable to CDP as they wait for unanimously accepted gluten-free terminology and assessment methodology. In this context, our feasibility study provides the basis to develop an integrative "open-access" gluten-free database in our country, by further assessing the gluten content of all products in the 3 different categories of processed food products available for CDP. This kind of database, providing access to information about products certified safe for CDP, instead of misleading advisory labels, would make the difference.

In conclusion, most processed food products available for CDP in the Greek NFID as well as in the local CA lists that were analyzed in the present study, although not bearing the "gluten-free" label, can be safely consumed by CDP. A high-sensitivity commercially available ELISA is able to discriminate processed food products available for CDP that contain inappropriate levels of gluten, making it feasible to develop an integrated gluten-free processed food database.

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