

## Research Note

# Gluten Contamination in Foods Labeled as “Gluten Free” in the United States

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### ABSTRACT

Gluten is the main storage protein in grains and consists of gliadin and glutenin occurring in the same ratio. Persons suffering from intolerances, including celiac disease, must avoid foods containing gluten or products containing wheat, barley, and rye. Accordingly, gluten detection is of high interest for the food safety of celiac patients. This study was designed to determine the concentrations of gluten in foods labeled “gluten free” available in the United States. Seventy-eight samples labeled gluten free were collected and analyzed using a gliadin competitive enzyme-linked immunosorbent assay. The gluten content was calculated based on the assumption of the same ratio between gliadin and glutenin. Forty-eight (61.5%) of the 78 samples contained less than the limit of quantification of 10 mg/kg for gluten. In addition, 14 (17.9%) of the 78 samples labeled gluten free contained less gluten than the guidelines established by the Codex Alimentarius for gluten-free labeling (20 mg/kg). However, 16 samples (20.5%) did contain gluten levels of  $\geq 20$  mg/kg, ranging from 20.3 to 60.3 mg/kg. In particular, five of eight breakfast cereal samples showed gluten contents higher than 20 mg/kg. These results may be of concern, as gluten sensitivity is known to vary among celiac disease patients.

Celiac disease (CD) is also known as gluten-sensitive enteropathy and now affects about 1% of the population in regions such as Northern Europe and the United States (9). Typical symptoms of the disease occur in susceptible patients after ingestion of gluten from wheat and of similar prolamin proteins of related cereals. These symptoms can include abdominal pain, diarrhea, nausea, constipation, and loss of weight and can be characterized by small intestinal inflammation, villous atrophy, and crypt hyperplasia. The mucosal lesion recovers when the gluten-containing cereals, e.g., wheat (gliadins), rye (secalins), and barley (hordeins), are withdrawn from the diet. Therefore, people diagnosed with CD must adhere to a gluten-free diet (5).

Gluten is a generic name for a protein mixture used for protein storage by certain cereal grains that contains gliadins (usually estimated as 50% of gluten) and glutenins. Gliadins are monomers with molecular masses between 30 and 70 kDa and are responsible for triggering the production of autoantibodies by the immune system (19). Gluten has long been appreciated by bakers for its ability to retain leavening gases and provide structure in bakery products. Gluten is found not only in all products made with wheat, rye, and barley but also as an ingredient in foods including meat, sausages, soups, and ready-to-eat meals (7). Due to its physicochemical characteristics, gluten is used in food

products to modify both texture, e.g., as a thickener to improve texture and water or fat retention, and form, e.g., to increase the extensibility. Gluten can also be used as an animal protein substitute in meat products to reduce manufacturing costs. Furthermore, gluten and wheat starch are found in some drugs as a filler.

To comply with the Codex Alimentarius standard and labeling regulations, the absence or reduction of gluten in gluten-free products must include prolamin fractions from rye, barley, and wheat. To be labeled “gluten free,” products must contain less than 20 mg/kg gluten, i.e., equivalent to 10 mg/kg gliadin, while foods labeled as “foods specially processed to reduce gluten content” or “very low gluten” must comply with levels between 20 and 100 mg/kg (4). In October 2013, the U.S. Food and Drug Administration (FDA) issued a final rule to define the term “gluten free” for voluntary use in the labeling of foods (17). According to the final rule, gluten free means that the food bearing the claim does not contain (i) an ingredient that is a gluten-containing grain (e.g., spelt wheat), (ii) an ingredient that is derived from a gluten-containing grain and has not been processed to remove gluten (e.g., wheat flour), or (iii) an ingredient that is derived from a gluten-containing grain and has been processed to remove gluten (e.g., wheat starch) if the use of that ingredient results in the presence of 20 mg/kg or more gluten in the food, or it means that the food (iv) inherently does not contain gluten, and food with any unavoidable presence of gluten that is below 20 mg/kg gluten can be labeled as gluten free.

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Foods typically made from wheat, such as bread and pasta, are replaced with products specially formulated to be gluten free by using inherently gluten-free grains. Gluten may be present in gluten-free foods mainly due to (i) the use in the manufacturing of the food of an ingredient containing small amounts of gluten (e.g., wheat starch) or (ii) contamination or cross-contact with gluten-containing grain (e.g., wheat). Consequently, gluten-free foods will be considered misbranded if they contain 20 mg/kg or more gluten by cross-contamination. Over the years, the threshold for gluten contamination in food has been a controversial question. Reports on the levels of gluten in food products intended for CD patients are scarce. A few small studies have shown that contamination may occur in gluten-free foods or inherently gluten-free grains and their milled fractions, such as oats, millet flour, and sorghum flour (8, 11, 14–16). In addition, gluten has been detected in rice-, corn-, oat-, and buckwheat-based foods with or without the gluten-free label (8). Hence, the aim of the present study was to analyze foods in the U.S. market labeled gluten free for gluten contamination.

## MATERIALS AND METHODS

**Sampling and preparation.** A total of 78 commercially available samples labeled gluten free were randomly collected from different local markets in Moscow, ID, in May 2013. All samples were ground to a fine powder using a household blender so that 75% would pass through a 20-mesh sieve and then thoroughly mixed to yield subsamples of 5 g. Between samples, the blender parts were removed and cleaned with alkaline-enzyme detergent, rinsed with 70% ethanol, and dried.

**Determination of gluten.** All samples were homogenized and tested in duplicate using the Ridascreen Gliadin sandwich R5 enzyme-linked immunosorbent assay (R-biopharm, Darmstadt, Germany), following the manufacturer's instruction. In brief, each sample was weighed into a 50-ml centrifuge tube and 10 ml of 60% aqueous ethanol solution was added. The samples were then mixed for 10 min with a wrist-action shaker, followed by centrifugation ( $1,200 \times g$ ) at room temperature. A measured aliquot of the supernatant was removed and diluted 50 times, and then these solutions were used in the assay. Standards and samples were added into duplicate wells on the plate. Enzyme conjugate was added to each well, and the plate was incubated for 30 min, followed by an additional five washing steps using diluted washing buffer. At this point, substrate and Chromogen were added to each well and allowed to react for 10 min, followed by the addition of stop reagent. The absorbance was read at 450 nm, and the data analyzed to determine the gluten concentration. All samples that showed an absorbance value above the highest standard were further diluted until the results were within the calibration range. The gluten content was calculated by the value of gliadins in parts per million multiplied by a factor of 2. The limit of quantification of this commercial kit was 5 mg/kg.

## RESULTS AND DISCUSSION

Table 1 presents the gluten levels measured in foods labeled gluten free that were analyzed in this study. Based on the gluten levels of samples, 48 of the 78 (61.5%) products contained gluten below the limit of quantification (less than 10 mg/kg gluten). Fourteen of the 78 (17.9%)

products contained a detectable amount of gluten ranging from 10.9 to 18.7 mg/kg. Sixteen (20.5%) of the 78 would not be considered gluten free under the proposed FDA rules for gluten-free labeling. Among other parameters, foods labeled gluten free must contain  $<20$  ppm gluten to be labeled gluten free (17). The gluten contamination frequency was highest in breakfast cereal (62.5%), followed by bread (37.5%), pasta (23.1%), snack food (13.3%), and baking mix (11.1%). Being the most popular ingredients in gluten-free products, rice and corn might be considered to be safer cereal-based foods for CD patients (8). According to Table 1, of the 16 gluten-contaminated samples, the most contaminated gluten-free food samples were made with rice, corn, or mixed grains, including seven rice-based foods, three corn-based foods, and six mixed-grain-based foods. Moreover, all of 6 mixed-grain-based samples included rice flour. According to our data, the most contaminated samples labeled gluten free were made from rice or corn and the levels of contamination were less than 50 mg/kg gluten.

A few previous studies have examined gluten in gluten-free foods and reported cross-contamination of 14 to 22% in inherently gluten-free foods and 46% in products based on gluten-free wheat starch produced by a deglutination process (5, 8, 14). According to Valdés et al. (18), a study of more than 3,000 gluten-free foods in Europe showed that one third had gluten levels higher than 20 mg/kg, which is above the gluten-free threshold. Another study reported that 5% of 1,583 different products labeled as gluten free contained gluten (4). In a study of Canadian cereal foods, about 10% of the 77 gluten-free foods were contaminated with gluten (8).

Products made from inherently gluten-free crops that are labeled gluten free but are not tested to be gluten free may be deemed misbranded if the label implies that all inherently gluten-free crops are free of gluten, since these inherently gluten-free grains, such as rice, corn, and buckwheat, can be contaminated with gluten (8, 14–16). Cross-contamination of inherently gluten-free foods can occur at all stages of the food chain, including when they are grown, harvested, and/or processed. Comingling of grain in the field can occur because of crop rotation with wheat, barley, or rye if they are grown next to or in rotation with these grains. It is possible that seeds of the gluten-containing grains will linger in the soil and, as a result, some of the gluten-containing grain may be collected during the same harvest with the inherently gluten-free grain. Sharing of storage facilities where relevant, such as in grain elevators, can result in comingling of grains. Further, using the same transportation vehicles for moving the grains to the processing site and sharing of processing facilities and equipment within those facilities can also result in cross-contamination. The presence of wheat in oats is a good example of on-farm cross-contamination (15). All specially formulated gluten-free foods, such as breakfast cereal and bread, can become contaminated during processing, since food manufacturers often use shared equipment and facilities for the production of gluten-free foods and gluten-containing foods and current good manufacturing practices may not be followed. If cross-contamination

TABLE 1. Incidence and range of gliadin determined for each type of foodstuff labeled gluten free<sup>a</sup>

Product type	Base ingredient	No. of samples	Incidence (%) of <10 mg/kg gluten	<20 mg/kg gluten		≥20 mg/kg gluten		
				Incidence (%)	Range (mg/kg)	Incidence (%)	Range (mg/kg)	
Breakfast cereal	Corn	4	—	2 (50.0)	10.9–18.4	2 (50.0)	20.3–37.5	
	Rice	2	1 (50.0)	—	—	1 (50.0)	40.9	
	Mixed	2	—	—	—	2 (100)	20.8–33.2	
	Total	8	1 (12.5)	2 (25.0)	10.9–18.4	5 (62.5)	20.3–40.9	
Pasta	Rice	12	6 (50.0)	3 (25.0)	14.8–16.7	3 (25.0)	25.5–37.7	
	Mixed	1	1 (100)	—	—	—	—	
	Total	13	7 (53.8)	3 (23.1)	14.8–16.7	3 (23.1)	25.5–37.7	
Bread	Corn	1	—	1 (100)	12.8	—	—	
	Rice	4	2 (50.0)	—	—	2 (50.0)	28.5–31.3	
	Mixed	3	1 (33.3)	1 (33.3)	15.5	1 (33.3)	33.2	
	Total	8	3 (37.5)	2 (25.0)	12.8–15.5	3 (37.5)	28.5–33.2	
Tortilla	Rice	1	—	1 (100)	16.9	—	—	
Nutrition bar	Corn	1	1 (100)	—	—	—	—	
	Oat	1	—	1 (100)	14.9	—	—	
	Rice	2	2 (100)	—	—	—	—	
	Mixed	3	3 (100)	—	—	—	—	
	Chick pea	1	—	1 (100)	18.7	—	—	
	Soybean	1	1 (100)	—	—	—	—	
	Total	9	7 (77.8)	2 (22.2)	14.9–18.7	—	—	
	Snack food	Corn	4	2 (50)	2 (50)	11.0–11.5	—	—
Snack food	Oat	1	1 (100)	—	—	—	—	
	Potato	3	3 (100)	—	—	—	—	
	Rice	10	9 (90.0)	—	—	1 (10.0)	41.2	
	Mixed	11	6 (54.5)	2 (18.2)	11.7–16.7	3 (27.3)	30.2–60.3	
	Quinoa	1	1 (100)	—	—	—	—	
	Total	30	22 (73.3)	4 (13.3)	11.0–16.7	4 (13.3)	30.2–60.3	
	Baking mix	Corn	1	—	—	—	1 (100)	27.3
		Rice	6	6 (100)	—	—	—	—
Mixed		1	1 (100)	—	—	—	—	
Garbanzo bean		1	1 (100)	—	—	—	—	
Total		9	8 (88.9)	—	—	1 (11.1)	27.3	
Total		78	48 (61.5)	14 (17.9)	10.9–18.7	16 (20.5)	20.3–60.3	

<sup>a</sup> The incidence is the number of samples with the indicated concentration of gluten. —, no sample had the indicated level of contamination.

occurs at any stage in the food chain, undeclared glutes can end up in the processed food products.

It has been debated what amount of gluten would be harmful to the small intestinal mucosa, and the potential toxicity of trace amounts of gluten is still unclear (3, 12). Even though some researchers report that a single intake of small amounts of gluten may not damage the mucosa, care should be taken when gluten ingestion becomes more frequent (2, 10, 13). A recent clinical trial has concluded that CD patients can tolerate gluten levels lower than 20 to 50 mg per day (1, 6). However, frequent or daily consumption of cross-contaminated products could cause symptoms and damage the small intestinal mucosa, with increased risk of complications.

Greater attention to cross-contamination in all processed foods might be necessary among regulators and food manufacturers. Under the proposed FDA rule for labeling of foods as gluten free, manufacturers who voluntarily choose to label their single-ingredient grain products as gluten free will have to imply to consumers that since all inherently gluten-free grains, such as rice, corn, millet, buckwheat, and sorghum, are gluten free by

nature, their products using these grains are gluten free; this does not guarantee, though, that there will be no gluten contamination. The use of inherently gluten-free grain may still be a problem owing to possible cross-contamination with gluten-containing grains or in the manufacturing facilities. Some manufacturers that do not label their products as gluten free might not test their products to ensure they contain <20 mg/kg gluten. Statements such as “all millet is gluten free” can be misleading and potentially harmful to the consumer with CD who requires a strict gluten-free diet. Therefore, the determination of gluten in all grain-based products, including those made with inherently gluten-free grains or ingredients, is recommended. This study shows that there is no guarantee that products labeled gluten free are in fact gluten free, which could be harmful for patients with CD.

#### ACKNOWLEDGMENT

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**ERRATUM**

In the article “Gluten Contamination in Foods Labeled as “Gluten Free” in the United States” by H. J. Lee, Z. Anderson, and D. Ryu, *Journal of Food Protection* 77(10):1830–1833, 2014, the first and last sentences of the second paragraph in “Materials and Methods,” first column, on p. 1831 should read as the following:

**Determination of gluten.** All samples were homogenized and tested in duplicate using the Ridascreen Gliadin competitive R5 enzyme-linked immunosorbent assay (R-biopharm, Darmstadt, Germany), following the manufacturer’s instruction.

The limit of quantification of this commercial kit was 5 mg of **gliadin** per kg.