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Gluten Contamination: Prevalence And Risks Associated With Foods Labeled "Gluten-Free"

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Abstract

Gluten is a common protein found in wheat, rye, and barely. It can sometimes be found in products that do not contain these grains. Gluten contamination in naturally gluten-free foods, products processed to remove gluten, and foods made with non-gluten derivatives is of concern for those suffering from an intolerance to gluten, such as Celiac disease. For these individuals, knowing whether or not food products advertised as "gluten-free" are truly so is necessary for their health and safety. The current study was designed to determine the reliability of "gluten-free" food labels in the consumer marketplace. Samples of "gluten-free" foods were collected from a variety of locations in central Ohio, including grocery stores, health-food stores, and restaurants. A total of 168 samples were analyzed using a Nima Gluten Sensor (antibody-based colorimetric assay) to determine if gluten content was <20 ppm, the maximum allowed by US law for a product labeled "gluten-free". Of the samples tested, 98 were certified as "gluten-free" (<10 ppm) by the Gluten-Free Certification Organization (GFCO); only 13 of these (13.3% of samples) were found to exceed 20 ppm of gluten. By comparison, 115 samples were labeled "gluten-free" on the packaging but did not contain certification; of these, 33 (28.7%) were found to actually contain more gluten than allowed by law. Samples obtained from grocery stores (n=146) and health-food stores (n = 19) had relatively few positive results (14.4% and 15.8% respectively), whereas samples obtained from stores whose primary function is not to sell food (n = 41) were much more likely to exceed the federal limit on gluten (41.5% of samples). While results reveal that "certified gluten-free" products may be more trusted in their claim of being "gluten-free", contamination still poses some risk to those who are intolerant of gluten.

Keywords: Gluten, food labeling, Celiac disease

1. Introduction

Celiac disease is a genetically predisposed condition defined as a permanent intolerance to gluten; it affects 1% of the population in western countries[1, 2, 3] and can manifest as subtle or extreme. Celiac disease is characterized by the occurrence of gastrointestinal symptoms, including weight loss and chronic diarrhea[4], and limiting or avoiding gluten completely may reduce the risk for long term complications[5]. Extraintestinal symptoms include neurological disturbances, anemia, dermatitis herpetiformis, and osteoporosis, but in order to avoid the associated symptoms, celiac patients must adhere to a lifelong avoidance of gluten and limit consumption to less than 50 mg/kg of gluten per day[6, 7]. Inflammation can occur if an afflicted individual consumes more than the allotted intake, possibly damaging the mucosal villi lining the intestine. This can lead to a flattened mucosa and result in malabsorption of nutrients and deficiency-related complaints[8]. If the proper precautions are not taken, celiac disease can eventually lead to enteropathy-associated T-cell lymphoma as well as intestinal adenocarcinoma[8]. A similar disease, called non-celiac gluten sensitivity, or NCGS, is characterized by behavioral changes, bone or joint pain, muscle cramps, leg numbness, weight loss, and chronic fatigue. NCGS affects 0.63-6% of the general population in the US[9]. A third disease caused by an intolerance to gluten is gluten ataxia, which causes purkinje fibers to be destroyed upon gluten ingestion; these

fibers normally release neurotransmitters that regulate and coordinate motor movements in the body[10]. Previous research suggests irritable bowel syndrome may also be comparable to celiac disease[11, 12].

Gluten is made up of proteins that are found in wheat, barley, rye, and triticale[13]. On a biochemical level gluten is a mix of several protein components including gliadins, globulins, albumins, and glutenins[14]. In food production, it is used widely in baking as well as for modification of texture and form, as a substitute for animal protein in meat products, and as a filler in some drugs[15, 16]. A wide variety of foods contain gluten. Naturally gluten-free foods such as fruits, vegetables, meat and poultry, fish and seafood, dairy, beans, legumes, nuts, pure wheat and barley grass, and some grains and starch-containing foods[17]. In addition, some alcoholic beverages do not contain gluten, including wines fermented from grapes and other fruits, and distilled spirits made from materials other than gluten-containing grains[18]. Common gluten-containing ingredients found in food are wheat, barley, rye, malt, Brewer's yeast, oats, and triticale (18). Gluten can also be found in other ingredients including some meats, sausages, soups, and ready-to-eat meals[15, 19].

Refraining from gluten ingestion is an important step for patients intolerant to gluten to prevent symptoms, but some of the unaffected population participates in a gluten-free diet as well. People often use a gluten-free diet because they think they will lose weight; however, this is not the case. In fact, the National Health Council recommends that people do not participate in a gluten-free diet unless complications require them to do so[20]. There is no solid evidence of weight loss, decreased toxicity, or improved health from participating in this diet[21]. In fact, not consuming gluten can cause other consumption habits to change as well. For example, there is a decrease in protein consumption as well as an increase in fat intake when consuming gluten-free food[22]. Lastly, the content of zinc as well as potassium is significantly lower in a gluten-free diet compared to a normal diet[23]. Results from a study conducted on gluten-free samples from 12 Austrian supermarkets supported the notion that gluten-free products offer no additional health benefits beyond helping those with intolerance; those without gluten intolerance should seek additional nutrients not provided by the gluten-free diet if they are to participate[23]. 57% of gluten-free consumers purchase products for medical reasons; others say they purchase products for their positive association with health and lifestyle choices[24]. The increased prevalence of gluten-free dieting may be due to the public portrayal that gluten-free food will improve general health[21]; TV and magazine advertisement influence has caused a major increase in participation of a gluten-free lifestyle[5, 21]. Gluten-free products are more expensive than gluten-containing products due to this increased popularity and higher costs of production[25]. However, due to the amount of non-celiac disease customers that purchased gluten-free products, "gluten-free" product sales have risen exponentially over the last decade[26, 27]. The cost of gluten-free products compared to non-gluten free products are 70-510% higher, and this can be attributed to gluten-free food companies marketing their products as creating healthy lifestyles. Another common issue with gluten-free foods is the lack of quality taste in gluten-free snacks[28]. Regardless of the reason for participating in a gluten-free diet, consumers can take comfort in knowing that gluten-free food products in the United States are regulated by the Food and Drug Administration (FDA).

There are several laws that have been put into place that assist consumers by regulating gluten-free foods. The FDA allows foods to be labeled "gluten-free", indicating that the food product must either be naturally gluten free or must comply with several rules. The gluten-free product must have no gluten-containing grains, no foods derived from gluten-containing grains that are processed to remove gluten, and must contain less than 20 ppm of gluten[29]. The criteria for a food product to be labelled "gluten-free" was not always this strict. In 1979, the standard for gluten-free products stated that the total nitrogen content in gluten protein cannot exceed 200 ppm[30]. In 2008, it was proposed that "gluten-free" foods must include less than 20 ppm and "very low gluten" foods must include less than 100 ppm[31]. The rule was finalized in 2013 and is still currently effective[29]. These rules do not apply to the USDA (US Department of Agriculture), alcoholic beverages regulated by the Alcohol Tobacco Tax and Trade Bureau, cosmetics, prescription and non-prescription drugs, and pet food[32]. According to the 2013 FDA ruling, manufacturers are not responsible for testing their final product for gluten after adopting the "gluten-free" label[29]. The FDA, however, does a labeling review and testing of the product prior to it being approved[18, 33]. Companies are still encouraged by some celiac organizations to adopt the cross-grain symbol to represent product certification[34, 18, 33]. Before the FDA had established a definition of "gluten-free," some certification organizations were created in order to establish this definition[33]. Third party organizations conducted their own testing procedures in order to assure consumers that the "gluten-free" advertised foods they are purchasing are indeed gluten-free[29]. Unfortunately, most countries do not have a monitoring process in gluten-free food production, so a global limit of gluten quantification in "gluten-free" food products has yet to be proposed[5, 35].

Although gluten-free products are regulated in most countries, a few studies have demonstrated concerning levels of contamination in labeled "gluten-free" products. In 2013, 16 of 78 labeled "gluten-free" food products purchased from different markets in Moscow, ID (20.5%) were found to contain more than 20ppm of gluten[15]. Similarly, gluten contamination was found in 21.5% of 130 labeled "gluten-free" samples from 25 Brazilian bakeries[5]. In a much larger

study conducted in Spain, consisting of over 4000 samples, gluten contamination was present in almost a third of the "gluten-free" samples[34]. Due to the scarcity of data concerning gluten determination of labeled "gluten-free" foods and the high rate of contamination in previous studies, testing the reliability of products claiming to be gluten-free is imperative to ensure the safety of those intolerant to gluten. This study aims to shed light onto the mislabeling of several foods that are advertised as gluten-free as well as bring awareness to putting proper rules in place to alleviate this mistake.

2. Materials & Methods

Our sample consisted of 222 food samples labeled "gluten-free" as well as one known gluten-containing sample (positive control). These samples were obtained from various retail establishments in 2019-2020 across the United States, including grocery stores, specialty/health-food stores, and stores whose primary sales are not food (such as dollar stores and home improvement stores). A few samples were also obtained from restaurants and bakeries.

A NIMA brand gluten sensor was used to determine the presence or absence of gluten in each sample. Multiple studies have shown that the NIMA device is able to detect gluten at levels greater than or equal to 20ppm, with a reported 99% accuracy rate[36]. This device uses an antibody present in individual capsules to detect gluten at the FDA required level of 20 ppm[37]. Each test gives a gluten-positive or gluten-negative result, rather than revealing specific levels of gluten within the sample, like the more expensive ELISA method. Sample capsules inserted into the NIMA device contain antibodies, a liquid extraction formula, and a test strip that develops and sends a visible result to the NIMA device[38]. The small, hand-held NIMA sensor is marketed for individual use, mostly by individuals who need to know if items they are ordering in a restaurant are gluten-free. Self-reported data from 804 users, and 5,624 tests have been made available by NIMA[39].

Samples for testing are approximately the size of a pea. Samples that are thick or dense require diluting by soaking in 100 mL of distilled water until the sample is soft enough for application into the test capsule. Dry powdered foods also require water to be added to the test capsule. Each sample takes about 1-2 minutes approximately to soften. Following sample application, the test capsule is inserted into the NIMA sensor and twisted until the capsule produces an audible click, indicating it has locked into place. Each sample is tested one time, and positive tests yield a plant icon and the words "gluten found"; a negative sample yields a smile icon. The third option from the sensor is "No Test Result," indicating that the test is inconclusive. These samples were tested again, and if the sample yields another "no result," then the testing proceeds to the next sample.

3. Results

Collectively, 46 of the 222 (20.7%) tested "gluten-free" samples demonstrated gluten levels at or above the FDA limit of 20ppm (*Table 1*). Of these contaminated samples, 13 (28.2%) were certified gluten-free by one of the major certifying organizations, whereas 33 (71.7%) samples were not labeled as certified gluten-free. Although the non-certified positive samples are higher in frequency, there was an almost equivalent number of non-certified and certified samples that tested "gluten-free" or GF. There were 9 non-gluten samples that were "unidentified"; certification status of the products was unknown at the time of sampling.

Table 1. Certification of contaminated samples.

Sample Test Result	C	NC	U	Total
G	13	33	--	**46**
GF	80	76	7	**163**
NR	5	6	2	**13**
Totals	**98**	**115**	**9**	**222**

Table 2. Certification and gluten contamination of samples from each store type.

Store Type	C	NC	U	GF	G	NR
Grocery (n=146)	74	63	9	117	21	8
Special (n=19)	11	8	--	14	3	2
Non-specific (n=41)	13	28	--	21	17	3
Restaurant (n=16)	--	16	--	11	5	--

(C = certified, NC = non-certified, U* = undefined.
G = gluten-containing, GF = gluten-free, NR = no result)

Most of the collected samples were from grocery stores, but the greatest number of certified samples per store type was attributed to special food stores or health food stores; 11 certified samples out of the 19 tested came from these stores (*Table 2*). The only positive samples from health food stores came from certified products (3/19). The store types with the highest rate of positive samples per store were those where food was not their primary product (non-specific) and restaurants, respectively (41.5%, 31.3%). The samples obtained from non-specific stores that tested gluten-positive (5) also contributed to almost half of the overall contamination (41.5%), though the sample size is much smaller than that of the grocery store type. In contrast, the store type that contributed the fewest number of contaminated samples was special food stores or health food stores: 3 out of 46 total contaminated samples (6.5%).

Table 3. Certification and gluten contamination of samples by food category.

Category	C	NC	U	GF	G	NR
Bars (n = 11)	4	5	2	7	2	2
Beans (n = 1)	--	1	--	--	1	--
Bowls (n = 20)	12	8	--	19	1	--
Breads (n = 36)	13	22	1	25	9	2
Cereals (n = 19)	7	10	2	12	3	4
Chips (n = 46)	15	31	--	33	11	2
Cookies (n = 26)	15	11	--	21	5	--
Dairy (n = 1)	1	--	--	1	--	--

Flour (n = 3)	2	--	1	3	--	--
Drink (n = 1)	--	1	--	1	--	--
Grains (n = 12)	3	9	--	10	1	1
Nuts (n = 4)	2	2	--	4	--	--
Pasta (n = 12)	7	3	2	6	6	--
Potatoes (n = 3)	--	3	--	2	1	--
Snacks (n = 27)	17	9	1	19	6	2

Due to large sample sizes and food product variation among the various food groups (*Table 3)*, the 13 food categories were subdivided and are found listed associated with specified acronyms in *Table 4*. The food categories that had the most certified samples were chips and cookies, which included the bulk of the certified samples; samples tested at 15.3% of 98 total samples. Conversely, the flour and nut food categories presented only 2% of the total certified samples. Chip samples also had a high rate of positive results (23.9%) and this contamination was mostly due to tortillas (45.5%) and potato (36.4%) chips. Another food category with a high contamination rate was snacks; there were 6 contaminated samples (22.2%). Half of the contaminated snacks were crackers and a third of them were pretzel-based snacks. A low rate of contamination was observed in frozen food bowls (1/20) and grains (1/12), and samples that did not test positive for gluten are shown in *Table 3*. The samples that tested "No Test Result" or NR were distributed almost evenly aside from an outlier of 4 contaminated samples from the cereals food category. All of the NR samples within the cereals were oat-based (*Table 3).* Specifics concerning the gluten content of designated subcategories (*Table 4)* are displayed in *Figure 1*.

Table 4. Food categories and Subcategories. Abbreviations of subcategories used for analysis in *Figure 1*.

Category	Subcategory (n)	Abbreviation
Bar	Fig (2)	FI
Bar	Granola (3)	GR
Bar	Protein (8)	PN
Bread	Bread (8)	BD
Bread	Breadcrumbs (1)	BDC
Bread	Bread Mix (3)	BM
Bread	Donut (1)	DO
Bread	Muffin (2)	MU
Bread	Nuggets (2)	NU

Bread	Pancake/Waffle (2)	PW
Bread	Pastry (1)	PA
Bread	Pizza (9)	PI
Bread	Tortilla (7)	TB
Cereal	Cereals (14)	CE
Cereal	Oats (5)	OA
Chips	Bean, Rice, Corn (5)	BRC
Chips	General- Popped, Puffed, Crisped, or Other (11)	GE
Chips	Potato (12)	PT
Chips	Tortilla (18)	TC
Cookies	Brownie Mix (1)	BM
Cookies	Cookie Mix (1)	CM
Cookies	Cookies (24)	CO
Grains	Quinoa (3)	QU
Grains	Rice (9)	RI
Snacks	Crackers (12)	CR
Snacks	Fruit Snacks (2)	FS
Snacks	Popcorn (7)	PO
Snacks	Pretzel (5)	PR
Snacks	Ice Cream Cup (1)	ICC

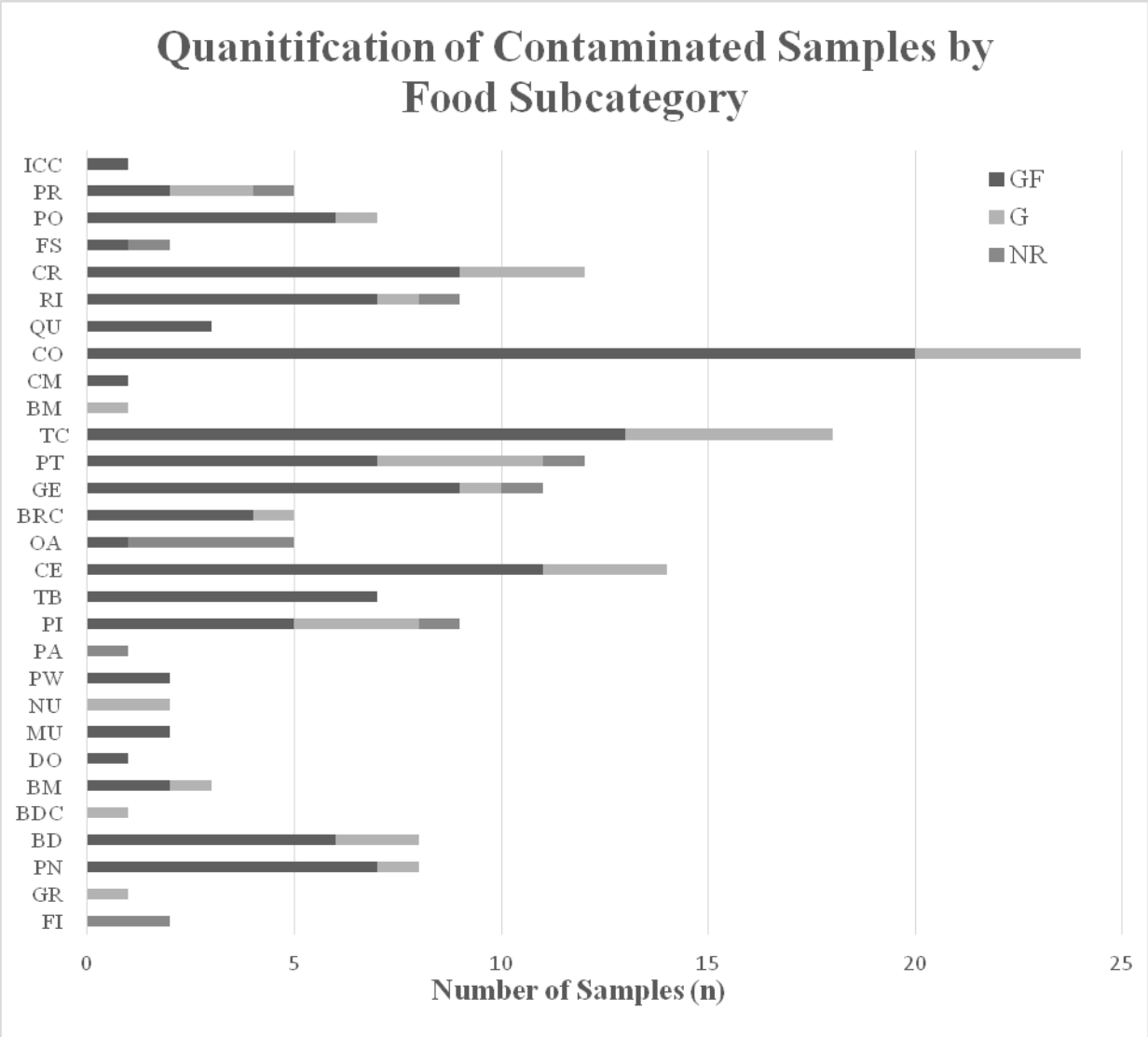

Figure 1. Comparison of tested samples by respective food subcategories.

4. Discussion

Samples that showed contamination of gluten (positive results) were found in both certified gluten-free samples as well as non-certified ones. However, over half of the samples that were found to be contaminated were non-certified foods labeled as gluten-free. For example, a study was performed in Brazil that tested foods that were labeled as gluten free. These samples were not certified gluten-free, and the results determined that 11% of the samples were contaminated despite having a label[16]. The most contaminated food groups in this study were flours and starches as well as cookies and muffins[16]. This aligns with our results, as there were a larger amount of these food groups that were contaminated. Furthermore, certified products are assumed to have a greater likelihood of meeting the standards set for gluten content, since a third-party has assayed them sometime in the past[33]. Since a higher percentage of certified samples came from special/health food stores, it makes sense that people who experience gluten intolerance have a better chance to find certified (and gluten-negative) foods when shopping at special/health food stores, where more of the products meet the rigorous standards. This emphasizes the importance of certification of products.

Due to the high level of foods found to contain gluten (1 in 5 samples), it is not uncommon for an individual practicing a gluten-free diet to experience a certain level of cross-contamination. Initially, gluten can contaminate

supposed gluten-free foods during growth, harvesting, and/or processing. For example, an occurrence of "comingling" of grains in fields is common due to crop rotation; gluten-free strands may be grown in rotation with gluten-containing grains. In addition, cross-contamination can occur from sharing storage facilities, using the same vehicle for transportation of the food products, or using the same equipment/facilities[15]. Not only can gluten contaminate in the initial steps of production, but it can also take place during stockage or manufacturing steps[15]. For example, there are several restaurants that advertise gluten-free items on their menus. The highest rate of contamination during our study was found in samples taken from these restaurants; this is not surprising due to the high risk of contamination that is found in the restaurant setting. If there is more demand for change within restaurants, they may become more aware of the issue and attempt to avoid cross-contamination[40]. Inadequate staff training as well as careless use of tools contribute to a higher risk of contamination in these food establishments compared to the individual's home[41]. It is clear that a celiac disease patient can unintentionally consume gluten due to contamination in food production and handling. However, misleading labels are also a cause of consumption. Gluten can be found in hidden sources because it is used as a thickener, flavor enhancer, filler, emulsifier, and fortification ingredient[34, 42], and labels that include the words "flavorings," "stabilizers," or "hydrolysed vegetable protein" can indicate that the product contains gluten[8]. By paying attention to the ingredients found on the packaging of foods, a person will be able to tell if the product contains gluten or not.

The level of non-certified contaminated foods demonstrates the lack of proper precautions that are put in place when advertising gluten-free foods. Popular media is the primary source that consumers use for health information[43]. By bringing to light the issue of improper labeling of gluten-free foods, consumers may become increasingly demanding for a change. This increasing demand may drive food service staff to put more effort towards avoiding cross-contamination as well as increasing awareness of the problem[40]. The underlying solution to avoiding accidental consumption of gluten is to decrease the amount of mislabeling of products[16]. This can be done by practicing good manufacturing techniques as well as stricter rules for testing of gluten content prior to distribution.

In order to ensure proper labeling of gluten-free foods, different certification organizations label products based on their gluten-content with different types of labels. The FDA labels the products they are tested as "gluten-free" if the gluten content is less than 20 ppm[18]. In addition, there are third-party organizations that certify gluten-free products based on actual content. Gluten-free non-certified products are labeled as such when their gluten content is not necessarily high, but the content level is at a higher level than perhaps it should be[33]. Countries other than the USA often have different certification labels, making it harder for individuals to compare products made abroad. For example, Italy has a crossed-ear symbol like that of the FDA to certify products[41]. The Association of European Celiac Societies mark their products tested with less than 20 ppm gluten content with a certified cross-grain symbol[34]. Even though there are many countries with organizations that certify products as gluten-free, most countries do not have a specific monitoring process to assess the gluten content in gluten-free foods[5]. In addition to labeling, it is important to have proper manufacturing practices. However, adopting these practices can be expensive and will factor into the cost of the product of the consumer. Adopting better practices would include quality control as well as testing samples for gluten content before and after production.

There were a few limitations to conducting this study. Foremost, the test results from NIMA may not be representative of an entire food item, since only a pea-sized sample was used[44] (this may lead to false negative results in some cases). In addition, we only tested a small fraction of all available gluten-free foods (there are thousands on the market). Nonetheless, the percent of sampled foods that tested positive for gluten is in line with previous studies that have been published. A further limitation was the inability of the NIMA device to read samples that were oat-ingredient based. A study in Italy found that gluten-contaminated products most commonly belong to oats, buckwheat, and lentil-based items[41]. This may be why the NIMA device struggled to read our samples that contained oats. Another study done in Canada also tested samples that had oat-based ingredients. This study revealed that eight out of twelve samples were contaminated above 20 ppm[35]. This is an area that requires further research.

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