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Gluten Contamination of Grains, Seeds, and Flours in the United States: A Pilot Study

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ABSTRACT

Under the Food Allergen and Consumer Protection Act, the Food and Drug Administration (FDA) must issue a rule for the voluntary labeling of food as gluten-free. In the proposed rule, many single-ingredient foods, such as millet, are considered inherently free of gluten. Inherently gluten-free grains will be considered misbranded if they carry a gluten-free label and do not also state that all foods of the same type are gluten-free (eg, "all millet is gluten free"). Twenty-two inherently gluten-free grains, seeds, and flours not labeled gluten-free were purchased in June 2009 and sent unopened to a company who specializes in gluten analysis. All samples were homogenized and tested in duplicate using the Ridascreen Gliadin sandwich R5 enzyme-linked immunosorbent assay with cocktail extraction. Thirteen of 22 (59%) samples contained less than the limit of quantification of 5 parts per million (ppm) for gluten. Nine of 22 (41%) samples contained more than the limit of quantification, with mean gluten levels ranging from 8.5 to 2,925.0 ppm. Seven of 22 samples (32%) contained mean gluten levels ≥ 20 ppm and would not be considered gluten-free under the proposed FDA rule for gluten-free labeling. Gluten contamination of inherently gluten-free grains, seeds, and flours not labeled gluten-free is a legitimate concern. The FDA may want to modify their proposed rule for labeling of food as gluten-free, removing the requirement that gluten-free manufacturers of inherently gluten-free grains, seeds, and flours must state on product labels that all foods of that type are gluten-free.

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As part of the Food Allergen Labeling and Consumer Protection Act, the Food and Drug Administration (FDA) must issue a rule for the voluntary labeling of food as gluten-free (1). In the FDA's proposed rule, single-ingredient grain foods, such as corn, rice, and millet, are

considered inherently free of gluten (2). However, these grains will be considered misbranded if they carry a gluten-free label that does not also state that all foods of the same type are gluten-free (eg, "all millet is gluten-free" or "millet, a gluten-free grain"). They also will be considered misbranded if they contain 20 parts per million (ppm) or more of gluten.

Oats are the one grain that will not be considered misbranded if they carry a gluten-free label and do not also state that all foods of the same type are gluten free (2). Although oats are considered an inherently gluten-free food, research suggests that commercially available oats may be contaminated with the gluten-containing grains wheat, barley, and rye while being grown, harvested, and/or processed (3). As a result, oats labeled gluten-free will be deemed misbranded if the label implies that all oats are free of gluten (2).

Oats might not be the only grain contaminated with gluten. Any inherently gluten-free grain, seed, and flour can become contaminated with wheat, barley, and/or rye while being harvested, transported, and/or processed (4). Under the proposed FDA rule for gluten-free labeling, manufacturers who voluntarily chose to label their single-ingredient grain products gluten-free will have to imply to consumers that all inherently gluten-free grains and seeds, such as millet, flax, buckwheat, and sorghum, are gluten-free, even if sold by manufacturers who do not label their single-ingredient products gluten-free. Unlike manufacturers who do label their products gluten-free, some manufacturers that do not might not test their products to ensure they contain <20 ppm gluten. Therefore, to determine whether single-ingredient grain foods other than oats might be contaminated with gluten, 22 inherently gluten-free grains, seeds, and flours not labeled gluten-free were assessed for gluten contamination.

METHODS

Food Samples

A convenience sample (ie, ready-available products) of 22 single-ingredient inherently gluten-free grains, seeds, and flours were purchased in Massachusetts or mail-ordered in June 2009. Grains, seeds, and flours were selected for testing because they are eaten by individuals with celiac disease who must follow a gluten-free diet. By design, none of the products chosen for this study were labeled gluten-free. As products meeting this criteria were found in grocery stores and online, they were purchased for the study. No additional criterion other than availability was used for selecting products. Purchased products included white rice, brown rice, white rice flour, corn meal, polenta, buckwheat, buckwheat flour, amaranth flour, amaranth seed, flax seed, millet flour, millet

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grain, sorghum flour, and soy flour. In some cases, two different brands of the same grain, flour, or seed were purchased. Seven of the 22 products contained a voluntary allergen advisory statement pertaining to wheat. This study protocol is exempt from Institutional Review Board approval.

Analysis

Products were sent unopened to a company that specializes in gluten analysis (Bia Diagnostics, Burlington, VT). All samples were homogenized and tested in duplicate using the Ridascreen Gliadin sandwich R5 enzyme-linked immunosorbent assay (ELISA) with cocktail extraction (R-biopharm, Darmstadt, Germany). The R5 ELISA was validated in a collaborative trial and uses the gliadin standard developed by the Prolamin Working Group (5-7). The R5 ELISA is based on the R5 monoclonal antibody to the antibody binding site QQFPF (glutamine-glutamine-proline-phenylalanine, proline) and other closely related amino acid sequences that are present in the gluten proteins of wheat, barley, and rye (8-11). This antibody does not cross-react with proteins from inherently gluten-free grains, such as oats, corn, rice, millet, teff, buckwheat, amaranth, and quinoa (12). The R5 ELISA has been endorsed by the Codex Committee on Methods of Analysis and Sampling as a type 1 method for determination of the gluten content in gluten-free foods and is the method for determination of gluten in Codex Standard 118-1979 (Codex Standard for Foods for Special Dietary Use for Persons Intolerant to Gluten) (13,14). In its proposed rule for labeling of food as gluten-free, the FDA is tentatively considering using the sandwich R5 ELISA for gluten determination (2). The limit of quantification (LOQ) for this assay is 5 parts per million of gluten (12).

Sampling

Seven or more well-representative aliquots from each submitted product were taken to make between 100 and 200 g (about 400 cm³) of each sample. Samples were ground in a Kitchen-Aid BCG 100ER1 processor (Kitchen Aid Inc, St Josephs, MI) to a fine powder (all parts were removed and cleaned with alkaline-enzyme detergent, rinsed with 60% EtOH, and dried between samples). From each ground sample, several aliquots were obtained and weighed on a Mettler AK 160 Analytical Balance (Mettler Instruments, Hightstown, NJ) to make each of the 0.50-g samples for analysis (± 0.001 g). Although the kit manufacturer recommends a 0.25-g sample for analysis, this amount was felt to be too small for reproducible results.

Extraction

Samples were then put into a 50-mL sterile centrifuge tube, and 5 mL 2-mercaptoethanol Guanidine Hydrochloride Cocktail solution (R21/22 S26-36/37) was added per instructions for a 1/10 dilution. Samples were then incubated at 60°C for 40 minutes on a horizontal shaker as prescribed by the kit instructions. After incubation samples were allowed to cool; 80% EtOH was added and further shaken for 60 minutes at 22°C. Samples were

Table. Gluten content of inherently gluten-free grains, flours, and seeds not labeled gluten-free

Product	Allergen advisory statement	Extraction 1 ^a ppm ^b gluten	Extraction 2 ppm gluten	Mean ppm
Millet flour	Yes	308.0	302.0	305.0
Millet flour	Yes	310.0	344.0	327.0
Millet grain	No	22.0	6.0	14.0
Millet grain	No	10.0	40.0	25.0
White rice flour	Yes	9.0	8.0	8.5
Buckwheat flour	No	66.0	64.0	65.0
Sorghum flour	Yes	238.0	230.0	234.0
Soy flour	No	3,000.0	2,850.0	2,925.0
Soy flour	No	96.0	88.0	92.0
Basmati rice	No	<5 ^c	<5	<5
Long-grain brown rice	No	<5	<5	<5
Enriched corn meal	No	<5	<5	<5
Instant polenta	No	<5	<5	<5
Rice flour	No	<5	<5	<5
Hulled buckwheat	No	<5	<5	<5
Buckwheat groats	Yes	<5	<5	<5
Amaranth flour	Yes	<5	<5	<5
Amaranth flour	No	<5	<5	<5
Flax seed	Yes	<5	<5	<5
Flax seed	No	<5	<5	<5
Amaranth seed	No	<5	<5	<5
Amaranth seed	No	<5	<5	<5

^aAssay used: Sandwich R5 enzyme-linked immunosorbent assay with cocktail extraction.

^bppm=parts per million.

^c5 ppm gluten is the lower limit of quantification for this assay.

then centrifuged at 2,500g in a Beckman TJ-6 Refrigerated swinging bucket centrifuge (Beckman Coulter Inc., Brea, CA) for 10 minutes to pellet residue.

Per kit instructions, 80 μ L of each duplicate sample supernatant was diluted into the 920 μ L of diluted running buffer and analyzed according to kit instructions. All samples were read on a Bio-Tek EL340 automated microplate reader (Bio-Tek Instruments, Winooski, VT) at 450 nm and gluten calculations were made using a 4p fit logarithm program (KC-JR) provide by Bio-Tek Instruments (Bio-Tek Instruments, Winooski, VT).

RESULTS AND DISCUSSION

Based on the mean gluten level of two extractions, 13 of 22 (59%) products contained below the LOQ (5 ppm) for gluten (Table). Of these 13 products, three contained a voluntary allergen advisory statement for wheat (Table). Nine of 22 (41%) products contained more than the LOQ for gluten, with mean gluten levels ranging from 8.5 to 2,925.0 ppm (Table). Of these nine products, four contained a voluntary allergen advisory statement for wheat

(Table). Seven of 22 products (32%) would not be considered gluten-free under the proposed FDA rule for gluten-free labeling. Among other parameters, foods labeled gluten-free must contain <20 ppm gluten to be labeled gluten-free (2).

Results of this analysis strongly suggest that not all inherently gluten-free grains, seeds, and flours are indeed gluten-free when they are market-ready for the consumer. Inherently gluten-free grains and seeds can become contaminated with wheat, barley, or rye anywhere from the field to the packaging plant. Comingling of grain and seed can occur because of crop rotation—during one harvest, wheat, barley, or rye is grown and during the next, a gluten-free grain is grown. It is likely that errant wheat, barley, or rye seed(s) will remain in the soil. As a result, the gluten-containing grain will be harvested right along with the gluten-free grain. Foreign grain also can be found growing among gluten-free grain as a result of different grains being grown in close proximity. Comingling of grain also can occur because of the use of shared harvesting, transporting, and processing equipment (3). In fact, under the US Grain Standards Act, products such as corn, flaxseed, sorghum, soybeans, and oats are allowed to contain a certain percentage of other grains, including wheat, barley, rye, and triticale (15).

The findings of this analysis also suggest that consumers cannot rely on voluntary advisory allergen labeling to make decisions on which inherently gluten-free grains, seeds, and flours are free of gluten contamination. Four of seven products containing ≥ 20 ppm gluten did not contain an allergen advisory statement. Three of the 13 products that contained less than the LOQ for gluten had an advisory statement for wheat on the product label. Allergen advisory labeling is used by some manufacturers to provide information to consumers about processing procedures. Labels might read, “processed in a facility that also packages products containing wheat” or “this product was packaged using equipment that also handles wheat.” Currently, these statements are voluntary and there are no government guidelines in place regarding their use (16).

Inherently gluten-free grains, seeds, and flours not labeled gluten-free were purposefully chosen for this study to be assessed for gluten content. Manufacturers of labeled gluten-free products presumably have several controls in place to ensure their products meet the FDA’s proposed gluten-free rule of <20 ppm gluten. These safety measures might include very carefully controlled growing, harvesting, transporting, and processing procedures. Manufacturers of labeled gluten-free foods also test their products to ensure the reliability of their gluten-free label.

Under the proposed FDA rule for labeling of foods as gluten-free, if a manufacturer wants to place a gluten-free label on a package of a single-ingredient food that is inherently gluten-free (eg, millet), the label would have to state that all such products were gluten-free (2). Statements such as “all millet is gluten-free” can be misleading and potentially harmful to the consumer with celiac disease who requires a strict gluten-free diet.

It has been described in the literature that products labeled gluten-free are more costly than their wheat-based counterparts (17). Based on a literature search of

PubMed, there are no published cost studies on inherently gluten-free grains labeled gluten-free vs those that are not labeled gluten-free. Nonetheless, it may be the case that products labeled gluten-free are more expensive because of increased production costs. The cost differential between items labeled gluten-free and those that are not might impact a consumer’s choice of product. Although the products not labeled gluten-free might be a better economic choice, the potential for contamination is a risk, as indicated by the findings in this study.

Based on a literature search of PubMed, there are no other published studies on the gluten contamination of inherently gluten-free single-ingredient foods, such as grains, seeds, and flours (with the exception of oats). A Canadian study did assess the gluten content of processed food products available in the Montreal area that appeared to be gluten-free (based on researchers’ assessment of food labels), but were not labeled gluten-free (18). Sixteen of 70 products tested contained >20 ppm gluten, including three buckwheat flours.

This study does have some limitations. Although this analysis indicates that there is some degree of contamination of inherently gluten-free grains, seeds, and flours not labeled gluten-free in the United States, sampling was not large enough to allow any inferences to be made on the overall percentage of contaminated product. In addition, no inferences can be drawn on the specific grains, seeds, and flours likely or unlikely to be contaminated. A much larger study involving multiple lots of numerous brands of grain would have to be done to achieve any degree of statistical significance.

CONCLUSION

The findings of the current study indicate that some inherently gluten-free grains, seeds, and flours not labeled gluten-free are contaminated with gluten. This potential risk of contamination is a health concern for people with celiac disease, who must follow a gluten-free diet. The consumption of these products can lead to inadvertent gluten intake. A much larger study will be necessary to determine whether certain grains and seeds are more likely than others to be contaminated. The FDA might want to modify their proposed rule for labeling of food as gluten-free, removing the requirement that gluten-free manufacturers of inherently gluten-free grains, seeds, and flours must state on product labels that all foods of that type are gluten-free.

STATEMENT OF POTENTIAL CONFLICT OF INTEREST:

Anne Roland Lee is the Director of Nutrition Services for Schar USA. Schar USA partially funded this study, including reimbursing the cost of purchased grains, flours, and seeds, as well as the cost of ELISA kits, necessary reagents, and equipment. Researcher time for Tricia Thompson also was partially covered by Schar USA. Thomas Grace is the CEO and Director of Bia Diagnostics, the company responsible for conducting the gluten testing on the product samples for this study. Although Schar USA has a commercial interest in gluten-free foods, none of the products assessed in this study (ie, single-ingredient grains, flours, and seeds) are in direct competition with products manufactured by Schar USA (ie, ready-to-eat packaged breads, pastas, crackers, and cookies).

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