SPECIAL GUEST EDITOR SECTION: FOOD ALLERGENS MANAGEMENT

The Use of Visual Examination for Determining the Presence of Gluten-Containing Grains in Gluten Free Oats and Other Grains, Seeds, Beans, Pulses, and Legumes

LAURA K. ALLRED, CYNTHIA KUPPER, and CHANNON QUINN
Gluten Intolerance Group of North America, 31214 124th Ave SE, Auburn, WA 98092

Obtaining representative test samples for antibody-based testing is challenging when analyzing whole grains for gluten. When whole grains are ground into flour for testing, confocal microscopy studies have shown that gluten tends to exist as aggregates within the starch background, making single-sample testing inaccurate and complicating the ability to arrive at an accurate average from multiple samples. In addition, whole-grain products present a unique risk to gluten free consumers, in that any contamination is localized to specific servings rather than being distributed across the product lot. This makes parts-per-million values less relevant for whole-grain products. Intact grains, seeds, beans, pulses, and legumes offer an alternative opportunity for gluten detection, in that contaminating gluten-containing grains (GCGs) are visible and identifiable to the trained eye or properly calibrated optical sorting equipment. The purpose of the current study was to determine a Gluten Free Certification Organization threshold level for the maximum number of GCGs within a kilogram of nongluten grains sold as specially processed gluten free product and to determine the feasibility of this threshold by evaluating visual examination data from two major oat processors.

Wheat, rye, and barley and their related grains and hybrids (emmer, spelt, triticale, durum, and kamut) are avoided by those consuming a gluten free diet. The adventitious presence of these gluten-containing grains (GCGs) in other cereals, beans, pulses, legumes, and seeds presents a major risk for manufacturers and consumers of gluten free foods. Grains, seeds, pulses, beans, and legumes can share many steps of the supply chain with GCGs, including being grown in the same fields, harvested with the same equipment, transported on the same vehicles, stored in the same facilities, and processed in the same mills. Because of this, most countries have allowances for the percentage of "other" crops that can be present in any defined commodity (1–5). As shown in Table 1, these allowances can range from 0.05 to >10%, which can be equivalent to between 50 and more than 10 000 ppm gluten.

These allowances were developed as quality standards for grain sales and trading and were not intended to provide guarantees of safety for those who have food allergies, intolerances, or sensitivities. Processors who intentionally produce gluten free grain ingredients for food manufacturers must take additional steps to ensure that their products are appropriate for gluten free consumers. These steps can include controls during growing, harvesting, transport, storage, and processing. Once these additional steps are taken, the processor must be able to demonstrate that the resulting ingredient meets the requirements for gluten free labeling according to the country of sale or other applicable regulations.

Oats are a cereal grain with a very high risk of contamination from GCGs, particularly wheat, rye, and barley. In the United States (Figure 1) and Canada, this heightened risk occurs because oats, barley, hard red spring wheat, and durum wheat have overlapping growing regions; are all seeded between April and June; and are all harvested between July and October. The prevalence of gluten contamination in oats has been significant enough that oats are listed as a possible source of gluten by Health Canada regulations (6) and Codex Alimentarius standards (7), although, in May of 2015, Health Canada issued a Market Authorization (8) allowing oats that are not contaminated with gluten grains to be sold as gluten free. It is now generally accepted that pure oats uncontaminated with GCGs can be safe for persons who have celiac disease (9), and many oat suppliers now make gluten free labeling claims based on their ability to control GCG cross-contamination. These claims are usually supported by results from antibody-based lateral-flow devices or ELISA testing as proof that the product meets the definition of gluten free.

Obtaining representative test samples for antibody-based testing is challenging when analyzing whole grains. Because of their large particle size, increased sample volumes and sample numbers are required to obtain representative data, and the mass differences between different grain types can mean that GCGs may be predominantly found toward the top or bottom of a grain container (10). Even if a representative sample is obtained and milled into flour for measurement, any gluten within the sample may not be uniformly distributed. Confocal microscopy of flour and dough prepared from wheat show that gluten tends to be distributed in aggregates within the background of the flour starch (11), which may make it more difficult to take a representative subsample for testing (typically 0.25–1 g for antibody-based gluten assays). An analysis of repeated sampling of milled oat products has indicated that the distribution of gluten contamination follows a log-normal

Guest edited as a special report on "A Global Reflection on Food Allergen Regulations, Management, and Analysis" by Carmen Diaz-Amigo and Bert Popping.
Corresponding author's e-mail: laura.allred@gluten.org
DOI: https://doi.org/10.5740/jaoacint.170414

Table 1. Allowed other grains/seeds/pulses/oilseeds under different grain standards

Crop	U.S. grain standard[1]	Canadian grain standard[2]	Codex
Canola	1–2%, depending on grade	1–2%, depending on grade	—
Corn	2–7%, depending on grade	2–12%, depending on grade	2%[3]
Flaxseed	20%	1–2%, depending on grade	—
Oats	3–20%, depending on grade	Barley and wheat: 0.75–8% depending on gradeother grains: 1–8% depending on grade	3%[4]
Sorghum	1–4%, depending on grade	—	—
Soybeans	1–5%, depending on grade	1–8%, depending on grade	—
Safflower seed	—	2.5%	—
Sunflower seed	10%	2.5%	—
Brown rice	10–150 seeds per 500 g, depending on grade	—	—
Milled rice	2–75 seeds per 500 g, depending on grade	—	0.5%[5]
Peas	0.1–0.5%, depending on grade	0.1–0.5%, depending on grade	—
Lentils	0.2–0.5%, depending on grade	0.2–1%, depending on grade	—
Beans	0.5–1.5%, depending on grade	0.05–0.5%, depending on grade	—
Large lima beans	0.5–1%, depending on grade	—	—
Mustard	—	0.3–3%, depending on grade	—
Buckwheat	—	1–5%, depending on grade	—
Faba beans	—	0.2–2%, depending on grade	—
Chickpeas	—	0.1–0.2%, depending on grade	—

pattern rather than a normal distribution, making single-sample testing inaccurate and complicating the ability to arrive at an accurate average result from multiple test samples (12).

The non-normal distribution of gluten may increase the error introduced by sampling when testing any raw material or food product for gluten, but in most cases, laboratory analysis is the only option for detecting and quantitating gluten in these matrixes. Whole grains, seeds, beans, pulses, and legumes offer an alternative opportunity for gluten detection, in that contaminating GCGs are visible and identifiable to the trained eye or properly calibrated optical sorting equipment. In fact, visual scanning and "picking" of grain samples is how the percentage of "other grains" and foreign materials is determined when grading grains under international grain standards (13).

Accurate gluten detection and quantitation is essential for providing properly labeled gluten free foods for consumers. The Gluten Intolerance Group of North America operates an independent certification program for gluten free products, the Gluten Free Certification Organization (GFCO). GFCO enforces a 10 ppm gluten threshold for both ingredients and finished products and has an interest in ensuring a safe supply of grains, legumes, beans, pulses, and seeds for use in gluten free products. GFCO does not regulate the practices of food ingredient processors, but does have a responsibility to set acceptance criteria for the materials it allows to be used in GFCO-certified products. The purpose of the current study was to determine (*1*) a GFCO threshold level for the maximum number of GCGs within a kilogram of nongluten grains ready for sale as a specially processed gluten free product; and (*2*) whether the two oat processors observed here are able to meet this threshold.

Threshold for Adventitious Gluten-Containing Grains (GCGs)

GCGs can be detected visually or optically within nongluten grains, but determining the level of gluten risk that one or more GCGs presents requires some assumptions. Because GFCO certifies products for gluten free consumers, the level of risk presented by any one GCG was estimated at the high end when determining a threshold, in terms of grain weight, grain protein percentage, and percentage of grain protein that is gluten. Approximate ranges for each of these factors are shown in Table 2 for wheat, rye, and barley (14–18).

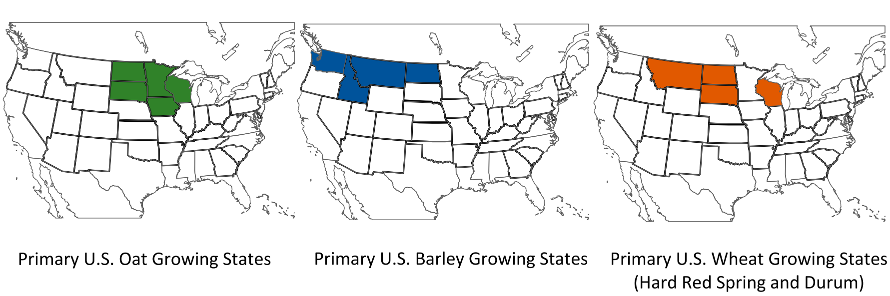

Figure 1. Primary U.S. states for wheat, barley, and oat production (25).

When determining an allowable number of GCGs per kilogram of nongluten grains, the following assumptions were used based on the upper limit of the published ranges:

- a single grain weight of 50 mg;
- 21% grain protein; and
- gluten content as 90% of the protein content of the grain.

Based on these assumptions, in the worst-case scenario, one contaminating GCG would contain 10.5 mg protein, 9.45 mg of which would be gluten. Therefore, to stay below the GFCO threshold of 10 mg/kg (10 ppm) gluten, 1 GCG/kg would be the highest level of gluten contamination that would be acceptable to consumers. In order to reduce the risk of a consumer purchasing a grain product with 1 or more GCGs/kg to <1%, GFCO recommends a threshold of 0.25 GCG/kg for visual examination.

Following the theoretical determination of this threshold, it was essential to determine whether this was an attainable goal for processors of nongluten grains. With the goal of evaluating the grain with the highest risk of gluten grain contamination, oats from two major gluten free oat-processing plants were examined by visual means to determine whether they complied with the proposed threshold.

METHODS

Visual examination data of GCG counts across multiple batches and crop years of oats from Cream Hill Estates Ltd (LaSalle, Quebec) and Grain Millers, Inc. (Yorkton, SK, Canada) were analyzed to determine whether these processors would have been able to meet the proposed threshold. The Cream Hill Estates Ltd and Grain Millers, Inc. facilities were chosen for evaluation because they are both dedicated oat facilities, but use different methods to achieve gluten free product. The oats evaluated by Cream Hill Estates Ltd were grown and processed under the Purity Protocol (19), whereas Grain Millers, Inc. uses proprietary sorting equipment to produce gluten free oats.

In addition to these evaluations of processed oats, the performance of the optical sorting equipment used for QC at the Grain Millers, Inc. Yorkton plant was verified for its ability to detect hard red spring wheat, two-row barley, and rye, the most common contaminants in the region.

Sampling

At the Grain Millers, Inc. Yorkton facility, samples were taken via an autosampler every 3 min during the hour from a chute as the groats travel, via gravity, into bins prior to final processing (rolling, flaking, milling, etc.). The chute is approximately 4 in. in diameter, allowing the entire stream to be accessed with a stainless steel cup. The groats collected were split using a Boerner-type sample divider to obtain analytical subsamples of 350 g each (approximately 10 000 groats).

Table 2. Approximate grain weight, protein, and gluten percentages for wheat, rye, and barley

Grain	Weight, mg[15]	Protein, %[16]	Gluten, %
Wheat	30–50	15–17	70–90[12]
Rye	30–35	6.5–14.5	75[13]
Barley	30–50	8.5–21	50–80[14]

At the Cream Hill Estates Ltd facility, samples were taken from the top of wagons or trucks using a manual bin probe (Deep Bin Probe; Hoffman Manufacturing, Jefferson, OR) or were taken from the output flow from wagons or dump trucks during bin filling using a can. Sample sizes were variable and ranged from 350 g to 10 kg, with a mean of 1600 g. The samples were not split, and each entire sample was visually examined by a Canadian Food Inspection Agency (CFIA)-accredited seed laboratory.

Visual Examination for GCGs

At the Grain Millers, Inc. Yorkton facility, each 350 g sample was run through the facility's optical sorter in order to separate typical oat groats from any other grains or abnormal groats. The grains that were rejected from the normal groat stream were then visually examined for the presence of GCGs. Examples of local wheat, rye, barley, and oat/groat grains were available to staff for comparison during visual examination.

At Cream Hill Estates Ltd, the collected oat samples were submitted to Discovery Seed Laboratories (Saskatoon, SK, Canada) and/or Kent Agri Laboratory Ltd (Tupperville, ON, Canada) for visual examination. The process used to perform the visual examination was to take small increments of the sample, approximately 200–300 grains, and spread them out on a blue or gray surface to single-grain thickness to allow the visual detection of GCGs. The entire sample was examined in this fashion, and the number of GCGs in each sample was recorded.

Optical Sorter-Assisted Visual Examination

The Grain Millers, Inc. Yorkton facility uses proprietary optical sorting equipment to aid in QC inspections for GCGs in their mechanically sorted gluten free groats. The optical sorter does not identify or count nongroat materials. Rather, it is set to separate out any material that does not meet its preset parameters for size, shape, length, width, and color. When a sample has been run through the sorter, it is separated into three streams: the main portion that meets the parameter requirements and two separate streams of rejects. Because of the tight parameters set around what the sorter selects as acceptable, a large number of groats are also fed into the rejected streams. These rejected streams are much smaller than the total sample (perhaps 200–500 grains total, compared with approximately 10 000 grains in a 350 g sample), making final confirmation of the presence of GCGs by trained staff much easier. All of the data generated at the Yorkton facility was the result of optical sorter-assisted visual examination.

In order to verify that the sorter was able to consistently divert GCGs into one of the rejected streams for visual confirmation during our study, 350 g samples of pure oats (confirmed visually) were spiked with one or two grains of either wheat, rye, or barley and sorted 30 times for each condition, resulting in a total of 180 sorting runs. Because the sorter can cause some chipping or breaking of the grains, and in order to make sure that the sorter could detect various examples of each grain, wheat, rye, and barley spikes were switched out every fifth run.

Statistical Analysis

The mean number of GCGs per kilogram was calculated for the across-lot data at each facility.

For the validation of the optical sorter used to assist with quality checks at Grain Millers, Inc., the sort was considered successful if it diverted all of the GCGs present into one of the rejected streams. The probability of detection (rejection) of either one or two spiked wheat, rye, and barley grains was calculated (20).

The distribution of GCGs within specially processed oats follows the Poisson distribution, with the levels of concern being very rare events. In oats, 1 GCG/kg is equal to approximately 1 GCG in 30 000 groats, whereas 0.25 GCG/kg would be equal to approximately 1 GCG in 120 000 groats. Given the large population (grains/lot) sizes for whole-grain commodities and the large number of grains analyzed within each lot, the operating characteristic (OC) curves used here could be calculated using either the Poisson or binomial distribution. Because both distributions gave equivalent probabilities for lot acceptance, the binomial distribution was used with the following formula:

$$P_a = P(d \leq c) = \sum_{d=0}^{c} \frac{n!}{d!(n-d)!} p^d (1-p)^{n-d}$$

where P_a = probability of acceptance; d = number of defectives (i.e., the number of GCGs); c = acceptable number of GCGs; n = number of items examined; and p = probability (fraction) of defective items in the lot.

Results

Across-Lot Analysis

The results of visual examination across multiple lots for each processor are shown in Table 3. Data from several harvests were analyzed for both facilities, and the GCGs per kilogram for each facility were below the threshold of 0.25 GCG/kg.

Optical Sorter Verification

Samples containing one or two wheat, rye, or barley grains were run through the optical sorter at the Grain Millers, Inc. facility 30 times each (n = 180). In every instance, the sorter diverted the GCGs into the smaller stream of rejected material. The calculated probability of detection, or in this case probability of rejection from the oat sample for all three grain types, was 1.00, with a 95% confidence interval of 0.96–1.00.

Discussion

Whole-grain products present a unique hazard for persons who have celiac disease, in that any one GCG that makes it into a final product, such as oatmeal, is going to be consumed in one serving rather than being dispersed within the product lot. This additional hazard was taken into consideration when setting the GFCO threshold at 0.25 GCG/kg. Assuming a typical serving size of 40 g for a product like oatmeal (21), this threshold would result in 1 GCG per every 100 servings. This may still seem high, but it should be recognized that the calculation used to set the unacceptable level at 1 GCG/kg was a worst-case scenario, with numbers at the high extreme for grain size, percent protein, and percent gluten. This calculation does not indicate that 1 GCG/kg is equal to 10 ppm; only that, in an extreme situation, 1 GCG might contain as much as 9.45 mg gluten. A more representative grain that weighed 30 mg with 15% protein would contain a total of 4.05 mg gluten, assuming gluten made up 90% of the total seed protein. But even in the worst-case scenario, the gluten load presented by 1 GCG would not exceed the 10 mg/day level that has been shown to be tolerated by persons who have celiac disease, with no evidence of histological changes to the bowel (22, 23).

This study has attempted to demonstrate that a gluten grain threshold of 0.25 GCG/kg is achievable in oats (Figures 2 and 3) and is, therefore, very likely to be achievable in other cereals, beans, pulses, legumes, and seeds that have a lower risk of contamination with GCGs. The ability to conclude that this threshold is achievable is dependent on the quality of the data presented and the assumption that the false-negative rate was low. This assumption is warranted by the verification of the performance of the optical sorting equipment used by Grain Millers, Inc. and by the integrity of Discovery Seed Laboratories and Kent Agri Laboratory Ltd, which are CFIA-accredited seed-testing facilities. This study does not serve as a validation for either the Purity Protocol or the mechanical sorting method of producing gluten free grains, but rather demonstrates that achieving the proposed threshold is possible under both

Table 3. Across-lot data for Grain Millers, Inc. and Cream Hill Estates Ltd

Parameter	Grain Millers, Inc. Mechanically sorted groats Optical/visual inspection	Cream Hill Estates Ltd Purity protocol groats Visual inspection
No. of samples	n = 859	n = 265
Total weight of product analyzed, kg	300.65	377.59
No. of wheat grains detected	0	36
No. of rye grains detected	0	37
No. of barley grains detected	4	0
Total No. of gluten grains	4	73
No. of GCGs/kg	0.01	0.19
Acceptance level (No. of GCGs/kg)	0.25	0.25

systems. Because the starting material can vary widely from season to season and even truckload to truckload, no method for generating gluten free grains, pulses, seeds, beans, or legumes can ever be considered validated, and examination of each lot from beginning to end is necessary.

The most critical aspect of using visual examination as part of a quality program is employee training, including regular performance checks. It is essential that companies regularly evaluate the performance of employees who perform visual examinations by inserting check samples, with a known number of GCGs, into their normal workflow. These check samples should be inserted at least daily and at random intervals and must be unidentifiable to the employee in order to avoid their being handled or counted differently from routine samples. For companies that have access to optical sorting equipment, employee performance can also be checked by running the batch of material they have accepted through the sorter to determine whether any GCGs have been missed. Employees who do not accurately detect the GCGs in these samples must be retrained and monitored to ensure accuracy. Even with well-trained personnel, hand picking for grading has shown accuracy in the range of 86–90% (24), and we have assumed a 14% nondetection rate with the proposed sampling plan presented (Figure 4).

Gluten-Free Grains per Kg of the Commodity	30,000
Size of each representative sample examined, in kg	0.35
Number of Representative Samples Examined from the Lot	859
Total Number of GCG Seen in ALL Samples	4
Mean GCG per kg	0.01

Sampling Plan	
Sample size (n) =	9,019,500
GCG allowed in all samples (0.25 GCG/kg) (c) =	75.16
Percent Chance of Acceptance (Binomial Distribution)	**100.0**

Given	
Threshold # of GCG per kg ≤	0.25
Unacceptable level of GCG per kg =	1.0
Producer's risk = 1-P_a(Mean GCG/kg)	0.00
Consumer's risk = P_a(1.00) =	0.00

OC-Curve Probabilities

# GCG/kg	P_a
0.00	**100.00**
0.05	**100.00**
0.10	**100.00**
0.15	**100.00**
0.20	**97.30**
0.25	**52.32**
0.30	**5.77**
0.35	**0.12**
0.40	**0.00**
0.45	**0.00**
0.50	**0.00**
0.55	**0.00**
0.60	**0.00**
0.65	**0.00**
0.70	**0.00**
0.75	**0.00**
0.80	**0.00**
0.85	**0.00**
0.90	**0.00**
0.95	**0.00**
1.00	**0.00**
1.25	**0.00**
1.50	**0.00**
1.75	**0.00**
2.00	**0.00**

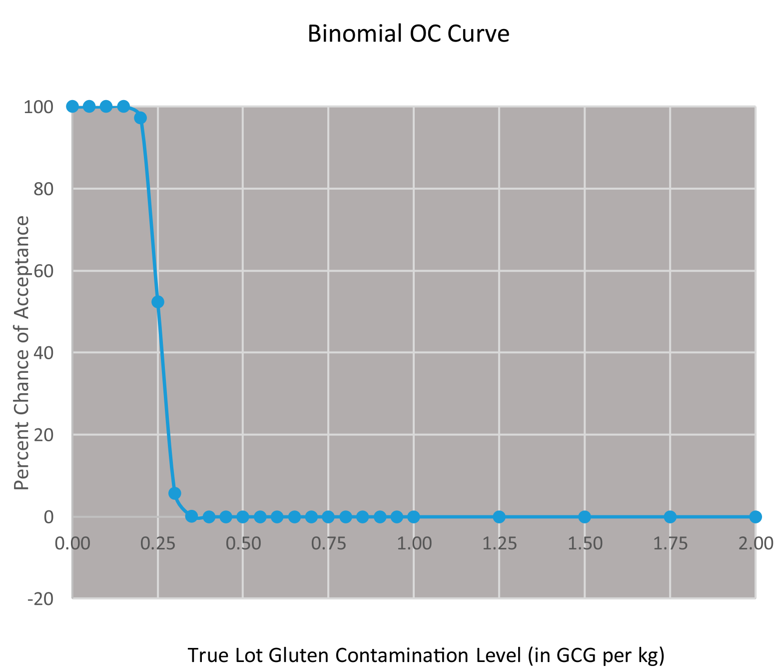

Figure 2. OC curve for Grain Millers, Inc. across-lot data on mechanically sorted groats.

Gluten-Free Grains per Kg of the Commodity	38,500
Size of each representative sample examined, in kg	1.42
Number of Representative Samples Examined from the Lot	265
Total Number of GCG Seen in ALL Samples	73
Mean GCG per kg	0.19

Sampling Plan	
Sample size (n) =	14,537,226
GCG allowed in all samples (0.25 GCG/kg) (c) =	94.40
Percent Chance of Acceptance (Binomial Distribution)	**99.2**

Given	
Threshold # of GCG per kg ≤	0.25
Unacceptable level of GCG per kg =	1.0
Producer's risk = 1-P_a(Mean GCG/kg)	0.01
Consumer's risk = P_a(1.00) =	0.00

OC-Curve Probabilities

# GCG/kg	P_a
0.00	**100.00**
0.05	**100.00**
0.10	**100.00**
0.15	**100.00**
0.20	**98.30**
0.25	**51.11**
0.30	**3.59**
0.35	**0.03**
0.40	**0.00**
0.45	**0.00**
0.50	**0.00**
0.55	**0.00**
0.60	**0.00**
0.65	**0.00**
0.70	**0.00**
0.75	**0.00**
0.80	**0.00**
0.85	**0.00**
0.90	**0.00**
0.95	**0.00**
1.00	**0.00**
1.25	**0.00**
1.50	**0.00**
1.75	**0.00**
2.00	**0.00**

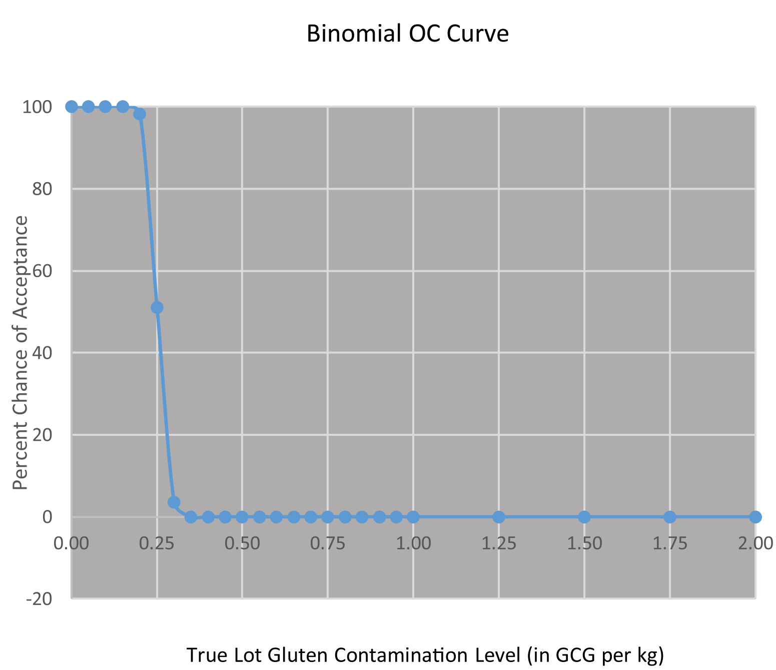

Figure 3. OC curve for Cream Hill Estates Ltd across-lot data on purity protocol groats.

Another crucial component of a program of visual examination is appropriate sampling. Samples must be taken from throughout the height and width of the product, which means that samples should be taken from product in motion in a relatively narrow stream, such as on a conveyor belt or in tubing. This is best accomplished when product is being moved upon receipt from growers or when it is being transferred after dehulling or after removing any fine particles and large contaminants (cleaning). When sampling is performed on product in motion, samples must be taken temporally throughout the lot, however defined. Alternately, manual or automated sampling probes may be used, as long as they are able to access the full depth of stationary product, with samples taken from multiple locations. Grab samples taken from the tops of containers or samples taken from only one area of the product stream or from one point in time in a continuous system will not provide accurate results.

Visual examination for GCGs should only be done on intact, whole, dry commodities. Although it is possible to perform visual examination of rolled grains, these will contain a small percentage of broken and fragmented grains that cannot be accurately assessed. Although these fragments may make up a minute portion of the overall sample, examining whole intact material will provide more accurate results.

OC-Curve Calculator for Visual Examination of Gluten Containing Grains (GCG) in Gluten-Free Grains

Gluten-Free Grains per Kg of the Commodity	30000
Size of each representative sample examined, in kg	0.5
Number of Representative Samples Examined from the Lot	20
Total Number of GCG Seen in ALL Samples	1
Mean GCG per kg	0.10

Sampling Plan	
Sample size (n) =	300000
GCG allowed in all samples (0.25 GCG/kg) (c) =	2.5
Percent Chance of Acceptance (Binomial Distribution)	**94.36**

Given	
Threshold # of GCG per kg ≤	0.25
Unacceptable level of GCG per kg =	1.0
Producer's risk = 1-P_a(Mean GCG/kg)	0.06
Consumer's risk = P_a(1.00) =	0.01

OC-Curve Probabilities

# GCG/kg	P_a	
0.00	**100.00**	
0.05	**99.04**	
0.10	**94.36**	1 GCG
0.15	**85.94**	
0.20	**75.19**	2 GCG
0.25	**63.61**	
0.30	**52.35**	3 GCG
0.35	**42.10**	
0.40	**33.21**	
0.45	**25.78**	
0.50	**19.74**	
0.55	**14.93**	
0.60	**11.18**	
0.65	**8.30**	
0.70	**6.11**	
0.75	**4.47**	
0.80	**3.24**	
0.85	**2.34**	
0.90	**1.68**	
0.95	**1.20**	
1.00	**0.86**	
1.25	**0.15**	
1.50	**0.02**	
1.75	**0.00**	
2.00	**0.00**	

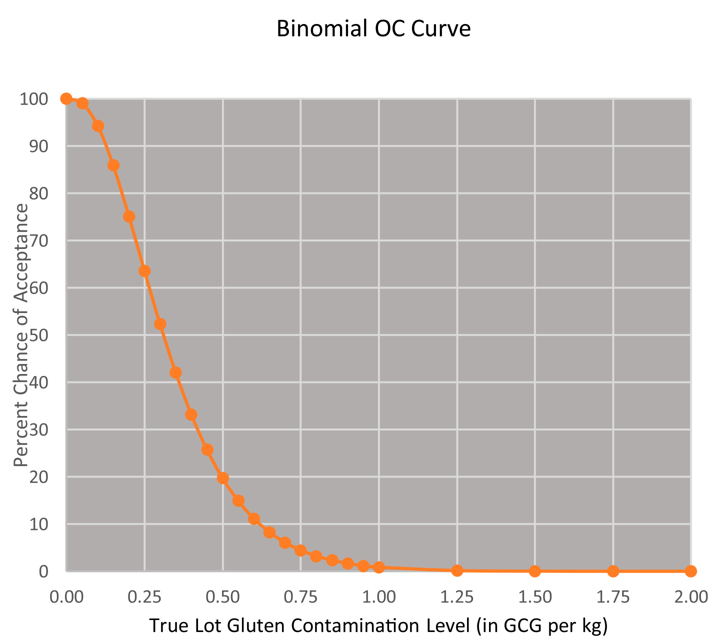

Figure 4. OC curve for the proposed sampling plan. The probabilities for accepting the lot if 1, 2, or 3 GCGs are found in all 20 samples are indicated by the top three arrows in the P_a column. The probability of accepting the lot if it is found to contain 1 GCG/kg is indicated by the last arrow in the P_a column. This curve assumes a 14% nondetection rate.

Properly calibrated optical sorting systems can improve the detection accuracy of GCGs by decreasing the volume of sample being examined visually, while also reducing the time required to examine each sample. The verification performed as part of this study demonstrated that optical sorting systems that are appropriately designed and calibrated can have a high probability of rejection of GCGs, allowing for rapid visual confirmation. Facilities using optical sorters to aid with quality checks must ensure that they are running controls and check samples with sufficient regularity to verify the performance of their equipment.

Although this paper sets a threshold for GCGs per kilogram by visual examination, gluten contamination in these materials can also occur in the form of very small fragments or dust. Therefore, the use of a combination of visual examination and antibody-based testing is necessary to determine the safety of these commodities for gluten free consumers when using appropriate procedures for both.

The following is an example of a sampling plan that would be acceptable to GFCO for the purpose of determining whether a defined lot/batch of any whole agricultural commodity has a less than 1% chance of containing 1 or more GCGs/kg while meeting the 0.25 GCG/kg threshold. Each facility should develop written procedures for sampling from each lot based on its own processes.

Sampling Plan

The initial step in analyzing grains/seeds/beans/pulses/legumes for the presence of GCGs is to develop a strict definition of a lot/batch. This may differ across processors or even across different commodities or activities within the same operation. For example, if a grain processor uses visual examination for GCGs to accept or reject shipments from their growers, the processor may consider each complete shipment from a grower as one batch. If that processor also conducts visual examination of the whole grain once it is dehulled and cleaned prior to milling, the processor may define a batch as the amount of grain that can be process in an 8 h shift or as the total volume of an order from a client. The lot/batch is not defined by grain weight, but processors must balance the time and personnel investment in visual examination versus the financial cost of rejecting large amounts of product. Doing a complete visual examination of every 1 metric ton (approximately 2200 lb) tote might be costly and time-consuming, but spreading the samples over 10 rail cars might mean running the risk of rejecting 1000 metric tons of product due to the detection of 3 GCGs. Once the lot size has been defined, proceed as follows:

- Take 20 samples from lot/batch while it is in motion and can be sampled across the complete width and depth of the grain stream or with the aid of an autosampler that can probe the full depth of the container.
- Take samples at uniformly distributed time points from the beginning to the end of the lot/batch or from throughout the container. Include samples from all containers that make up the lot/batch.
  - If there are more than 20 containers in the lot/batch, take at least one sample from each container.
- Clean each sample to remove fine materials and large contaminants (rocks, stems, etc.) using the same process that would be used prior to examination for foreign grains in the commodity.
- Reduce each sample to 500 g using an appropriate sample splitter (for example, a Boerner-style splitter or a sectorial splitter).
  - If the samples being examined have hulls, shells, or casings that will be removed during processing, this must be taken into account, and the sample size must be increased accordingly. For example, if whole oats are 70% seed and 30% hull, divide the desired sample size (500 g) by 70% (0.70), and take samples of 500/0.70 = 714.3, which can be rounded to 715 g. Each of these 715 g samples would represent one 500 g sample of groats.
- Visually examine each sample for the presence of GCGs using personnel who have been trained and found competent for this task.
- Record the total number of GCGs found in all of the samples and calculate the GCGs per kilogram.
  - Twenty samples of 500 g each make up 10 kg total product examined, so the total number of GCGs in a set of 20 samples can be divided by 10 to obtain the number of GCGs per kilogram.
- Make the decision to accept or reject the lot:
  - If there are no GCGs detected, the GCGs per kilogram will be zero and the lot/batch may be accepted.
  - One grain across all 20 samples would result in 0.1 GCG/kg, and the lot may be accepted.
  - Two grains across all 20 samples would result in 0.2 GCG/kg, and the lot may be accepted.
  - Three GCG across all 20 samples would result in 0.3 GCG/kg, and this would result in rejection of the lot based on the threshold of 0.25 GCG/kg.

The OC curve for this sample plan is shown in Figure 4. Twenty samples allow the consumer risk of a lot containing 1 GCG/kg to be below 1%, taking into account a 14% chance of nondetection of a GCG, as described by previous hand-picking accuracy measurements (24).

This plan and the acceptance probabilities remain constant across a wide grains-per-kilogram range—from corn, at approximately 2600 seeds/kg, to flax, at approximately 176 000 grains/kg (17) —because all of these result in a very large sample size (*n*) in relation to the acceptable number of GCGs (*c*). Therefore, this plan could be applied across most grains, seeds, beans, pulses, and legumes. An Excel calculator for this sampling plan is available by contacting us.

References

(1) U.S. Department of Agriculture, Grain Inspectors, Packers and Stockyard Administration (2015) Official U.S. Standards, U.S. Standards for Grain, https://www.gipsa.usda.gov/fgis/usstandards.aspx (accessed on November 17, 2017)
(2) Canadian Grain Commission (2016) Official Grain Grading Guide, https://www.grainscanada.gc.ca/oggg-gocg/ggg-gcg-eng.htm (accessed on November 17, 2017)
(3) CODEX (1995) Codex Alimentarius Commission, Food and Agriculture Organization of the United Nations and World Health Organization, Rome, Italy, STAN 153-1985, http://www.fao.org/fao-who-codexalimentarius/standards/list-of-standards/en/
(4) CODEX (1995) Codex Alimentarius Commission, Food and Agriculture Organization of the United Nations and World Health Organization, Rome, Italy, STAN 201-1995, http://www.fao.org/fao-who-codexalimentarius/standards/list-of-standards/en/
(5) CODEX (1995) Codex Alimentarius Commission, Food and Agriculture Organization of the United Nations and World Health Organization, Rome, Italy, STAN 198-1995, http://www.fao.org/fao-who-codexalimentarius/standards/list-of-standards/en/
(6) Health Canada (2012) Justice Laws Website, Food and Drug Regulations, Subsection B.01.010.1, http://laws-lois.justice.gc.ca/eng/regulations/c.r.c.,_c._870/
(7) CODEX (2015) Codex Alimentarius Commission, Food and Agriculture Organization of the United Nations and World Health Organization, Rome, Italy, STAN 118-1979, http://www.fao.org/fao-who-codexalimentarius/standards/list-of-standards/en/
(8) Ambrose, A. (2015) Market Authorization for Gluten-free Oats and Foods Containing Gluten-Free Oats, *Canada Gazette*, Vol. **149**, http://www.gazette.gc.ca/rp-pr/p2/2015/2015-06-03/html/sor-dors114-eng.php
(9) Gilissen, L.J.W.J., van der Meer, I.M., & Smulders, M.J.M. (2016) *Med. Sci.* **4**, 21. doi:10.3390/medsci4040021

(10) Esbensen, K.H. (2015) *J. AOAC Int.* **98**, 269–274, doi:10.5740/jaoacint.14-234

(11) Vodovotz, Y., & Chinachoti, P. (1998) *New Techniques in the Analysis of Foods*, M.H. Tunick, S.A. Palumbo, & P. M. Fratamico (Eds), Klewer Academic/Plenum Publishers, New York, NY, pp 9–17

(12) Fritz, R., Chen, Y., & Contreras, V. (2016) *Food Chem.* **216**, 170–175. doi:10.1016/j.foodchem.2016.08.031

(13) U.S. Department of Agriculture, Grain Inspectors, Packers and Stockyard Administration (2014) Grain Inspection Handbook – Book II: Grain Grading Procedures, Revision May, 1, 2014https://www.gipsa.usda.gov/fgis/handbook/gihbk2_inspec.aspx (accessed on May 27, 2016)

(14) DuPont, F.M., Chan, R., Lopez, R., & Vensel, W.H. (2005) *J. Agric. Food Chem.* **53**, 1575–1584, doi:10.1021/jf048697l

(15) Gellrich, C., Schieberle, P., & Wieser, H. (2003) *Cereal Chem.* **80**, 102–109, doi:10.1094/CCHEM.2003.80.1.102

(16) El-Negoumy, A.M., Newman, C.W., & Moss, B.R. (1979) *Cereal Chem.* **56**, 468–473

(17) Government of Alberta, Canada, Department of Agriculture and Forestry (2007) Using 1,000 Kernel Weight for Calculating Seeding Rates and Harvest Losses, *Agri-Facts*, Agdex 100/22-1, http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/agdex81/$file/100_22-1.pdf (accessed on June 1, 2016)

(18) Shewry, P.R. (2006) *Impacts of Agriculture on Human Health and Nutrition*, I. Çakmak, & R.M. Welch (Eds), Encyclopedia of Life Support Systems (EOLSS), Developed Under the Auspices of the UNESCO, Oxford, United Kingdom, pp 118–137. http://www.eolss.net

(19) Allred, L.K., Kupper, C., Iverson, G., Perry, T.B., Smith, S., & Stephen, R. (2017) *Cereal Chem.* **94**(3): 377–379

(20) Wehling, P., LaBudde, R.A., Brunelle, S.L., & Nelson, M.T. (2011) *J. AOAC Int.* **94**, 335–347

(21) U.S. Food and Drug Administration, CFR - Code of Federal Regulations Title 21, Vol. 2, Revision of April 1, 2016, https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=101.12 accessed on November 17, 2017

(22) Catassi, C., Fabiani, E., Lacono, G., D'Agate, C., Francavilla, R., Biagi, F., Voolta, U., Accomando, S., Picarelli, A., & de Vitis, I. (2007) *Am. J. Clin. Nutr.* **85**, 160–166

(23) Akobeng, A.K., & Thomas, A.G. (2008) *Aliment. Pharmacol. Ther.* **27**, 1044–1052, doi:10.1111/j.1365-2036.2008.03669.x

(24) Bevilacqua, R.T. (1987) Grading of Grains and Oilseeds in Canada, The 22nd International Grain Industry Course, Lecture, Canadian International Grain Institute, Winnipeg, MB, Canada

(25) U.S. Department of Agriculture, National Agricultural Statistics Service (2013) Statistics by Subject, http://www.nass.usda.gov/Statistics_by_Subject/index.php (accessed on November 17, 2017)