

Research Note

Cross-Contamination with Gluten by Using Kitchen Utensils: Fact or Fiction?

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ABSTRACT

A risk of cross-contamination exists when preparing a gluten-free (GF) meal in kitchen facilities that usually handle gluten-containing (GC) foods. Cross-contamination with gluten may occur during the preparation or cooking process; however, published data are lacking on gluten cross-contamination from kitchenware. This study was conducted to determine whether cross-contamination occurs through shared domestic kitchenware and, if so, which cleaning method is most reliable for avoiding this cross-contamination. Kitchenware (wooden spoon, colander, ladle, and knife) previously used to cook and/or prepare GC foods was used for the preparation of GF foods (bread and pasta). The gluten concentration of the GF foods was then determined using an established enzyme-linked immunosorbent assay. A PCR assay was also used to detect the presence of wheat ω -gliadin DNA in the food samples. Three cleaning methods were assessed to determine the concentrations of gluten and wheat DNA in GF foods cooked with utensils cleaned directly after the preparation of GC foods. Contrary to our expectations, gluten was not detected in relevant and quantifiable amounts in our samples (<20 mg/kg). The cleaning method used did not influence gluten concentrations: all samples contained <10 mg/kg. Based on PCR analyses, the only sample with lower cycle threshold (C_T) values (i.e., higher concentration of wheat DNA) was from the contaminated ladle used to serve GF pasta. This outcome led to the hypothesis that shared ladles pose a higher risk for contamination of GF foods than do shared wooden spoons, colanders, or knives. Cross-contamination with gluten in a kitchen environment may occur, but kitchen utensils used for preparing GC pasta and for cutting GC bread should not pose a relevant problem to patients with celiac disease, at least in a domestic environment.

Key words: Celiac disease; Cleaning methods; Contaminated kitchenware; Cross-contamination with gluten

Celiac disease (CeD) is a chronic immune-mediated disease with intestinal and systemic manifestations that are triggered by gluten ingestion in persons with a genetic susceptibility (5). When CeD patients remain on a diet containing gluten, significant health complications may occur. A diet with zero gluten is virtually impossible, because traces of gluten are found even in specially processed certified gluten-free (GF) products (4). However, GF products are not allowed to contain more than 20 mg/kg (ppm), and the daily consumption of gluten in GF goods is estimated to be well below 50 mg (13). In a recent review of the relevant literature, the authors determined that a daily gluten intake of <10 mg is unlikely to cause significant histological abnormalities for most CeD patients (1). In a prospective, double-blind, placebo-controlled trial in which the toxicity of these gluten traces was investigated, the authors found a large interpatient variability in sensitivity. A clear deterioration of the intestinal morphometry was seen in some patients after ingesting only 10 mg of gluten daily, whereas none of the individuals receiving 50 mg/day had

clinical evidence of a relapse, although a significant decrease in the villous height–crypt depth index was noted in this group (3). This wide range in individual responses to gluten suggests that a gray area between 10 and 50 mg exists, and a 50-mg/day gluten challenge can cause deterioration in intestinal morphometry.

This threshold should not be exceeded and cross-contamination should be avoided during food preparation and cooking. See et al. (11) described four common sources of cross-contamination: the field, factory or retail premises, restaurants, and the home. Cross-contamination at home can occur through shared kitchenware, countertops, cupboards, and spreads commonly used with other gluten-containing (GC) foods (e.g., butter or jam). However, evidence for the efficacy of using separate kitchen equipment for the preparation of GF foods is sparse, and recommendations by See et al. were not based on empirical studies. Only two studies were designed to systematically examine cross-contamination in GF food preparation. Miller et al. (9) studied the conditions required to produce a GF meal in a commercial kitchen when wheat flour is simultaneously used. These authors found that a minimum distance of 2 m from the place where wheat flour was used is required when

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standard hygiene procedures are followed (i.e., clean surfaces, utensils, and equipment). Vincentini et al. (13) found that when specific requirements are met, the simultaneous cooking of GF and GC pizzas is safe. Both studies included evaluation of the risk of gluten cross-contamination with flour. However, empirical data are lacking for the gluten concentration on kitchenware cross-contaminated with solid GC food (other than flour). Our primary aim was to determine the degree to which gluten cross-contamination through shared domestic kitchenware occurs. We also assessed which cleaning methods are most effective for avoiding contamination. We hypothesized that gluten cross-contamination occurs at ≥ 20 mg/kg and will vary depending on the kitchen utensils used. We expected the degree of cross-contamination to be strongly correlated with the cleaning method, particularly using a cloth and a towel for kitchenware that were exposed to GC food.

MATERIALS AND METHODS

We performed 10 standardized experiments to measure the amount of gluten cross-contamination coming from kitchen utensils using as a model two GC foods: pasta and bread. The study was carried out by two registered dietitians with newly bought and dishwasher-cleaned utensils. We divided the GF kitchen into a GF and a GC area with a 4-m space between the two areas to prevent cross-contamination during the study. We replicated each experiment three times and used identical and new kitchenware (i.e., knife, colander, spoon, and ladle) for each repetition to prevent contamination during the experiments. We also used the same type of GC penne pasta (semolina wheat flour) and GF penne pasta (cornflour, rice flour, mono- and diglycerides; guaranteed to have a final gluten concentration of < 20 ppm) for all experiments. We performed the following cooking and handling experiments.

(i) Wooden spoon in pan. We placed a wooden spoon in the cooking pot during the entire cooking time for the GF pasta. The same spoon was then placed in the pot during the entire cooking time for the GC pasta. We used three identical spoons that were all used for about 2 years in a GC household and cleaned using a standard dishwasher before the experiments.

(ii) Unwashed colander. We cooked 100 g of GC pasta according to the package instructions for 10 min in 1 L of salted water. We drained the pasta in a stainless steel colander and allowed the excess water to drain for 10 s. The GC pasta was removed, and GF pasta cooked the same way was drained immediately in the unwashed colander. After 10 s, the GF pasta was transferred to a plastic bag and allowed to cool for 30 min, and the bag was sealed.

(iii) Ladle. In the GC kitchen area, we used a ladle to place 100 g of cooked GC pasta on a plate and then transferred the unwashed ladle to the GF area and used it to place the same amount of cooked GF pasta on another plate.

(iv) Cutting bread. The GF bread was a 80-g bun (corn starch, water, tapioca starch, rice flour, sourdough [quinoa and rice], yeast, psyllium, salt, and inulin; Huttwiler Glutenfree, Volketswil, Switzerland) guaranteed by the manufacturer to have a final gluten concentration of < 20 ppm, and the GC bread was a wheat bread (wheat flour, water, yeast, salt, malt flour, and acerola

powder) from a local bakery. One slice (80 g) of the GC bread was cut in the GC area. The unwashed knife was then transferred to the GF area and used to cut the GF bun in half. We used separate cutting boards to prevent contamination with flour and/or breadcrumbs.

For the four cleaning methods used with the colander (experiments v through viii), the same process was used as that for experiment ii: GC pasta was drained using the test colander, which was then cleaned using each of these four methods before being used to drain the GF pasta.

(v) Cleaning the colander with cold water. The colander was rinsed thoroughly with cold water for 10 s.

(vi) Cleaning the colander with warm water. The colander was rinsed thoroughly with warm (50°C) water for 10 s.

(vii) Cleaning the colander with a clean cloth and towel. The colander was wiped with the cloth, rinsed thoroughly with lukewarm water for 10 s, and dried with a towel. Both the cloth and towel were new and had been washed before using.

(viii) Cleaning the colander with a GC cloth and towel. We contaminated a newly bought and washed cloth and towel with 5 g of wheat flour each in a GC kitchen. The dry towel was spread on a table, sprinkled evenly with the flour (in a flour sieve), folded, placed into a plastic bag, and shaken. The cloth was damp before it was sprinkled with flour but was otherwise handled in the same way. The cloth and towel were shaken once to remove any residual flour before these items were used in the cleaning process. This source of contamination represents a worst-case scenario in any kitchen involving the use of a cloth or towel to clean a surface with flour. The colander was cleaned using the same procedure as used in experiment vii but with the contaminated cloth and towel.

To evaluate knife cleaning methods, the knife used to cut GC bread and then to cut GF bread was cleaned between uses according to the methods outlined in experiments vii and viii. Both the cloth and towel were new and washed before using.

(ix) Cleaning the knife with a clean cloth and towel. One slice (80 g) of the GC bread was cut in the GC area. The unwashed knife was then transferred to the GF area (separate cutting boards were used to prevent contamination with flour and/or breadcrumbs). Before cutting the GF bread, the knife was wiped with the clean cloth, rinsed thoroughly with lukewarm water for 10 s, and dried with the towel.

(x) Cleaning the knife with a GC cloth and towel. We contaminated a newly bought and washed cloth and towel with 5 g of wheat flour each in a GC kitchen. The cloth and towel were shaken once to remove any residual flour before using in the cleaning process. After cutting the GC bread and before cutting the GF bread, the knife was wiped with the cloth, rinsed thoroughly with lukewarm water for 10 s, and dried with the towel.

The resultant 30 food samples and a noncontaminated ("clean") GF pasta sample were transferred to plastic bags, sealed, marked, and stored in a refrigerator until transport to the State Laboratory within 4 h after preparation.

Homogenization. All samples were homogenized (the entire batch of pasta or the entire bun) with a knife mill (Grindomix, Retsch, Haan, Germany), dried in a drying cabinet at 102°C (pasta

for 4.5 h, bread for 3.5 h), and mixed again using the knife mill to attain optimal homogeneity. This procedure was necessary because a nonhomogeneous sample cannot be analyzed correctly. Unlike dry product contaminations, a mass of wet GF pasta mixed with wet, sticky GC pasta cannot be expected to be homogeneously contaminated, even after mixing (without drying). The extraction method for the R5 enzyme-linked immunosorbent assay (ELISA), which has been validated, is compliant with Codex Alimentarius Commission regulations (7), and has been routinely used by food control laboratories, requires only a small portion (0.25 g) of the food sample. The risk of false-negative results is high when the homogenization is insufficient and gluten is not evenly distributed throughout the sample. Gluten recovery also can be affected by the handling protocol (milling, drying, and milling again).

ELISA and real-time PCR analysis. Following homogenization, two samples (0.25 g each) from each replicate were analyzed by ELISA and PCR. After extraction of each sample twice with a patented Ridascreen Fast Gliadin cocktail solution (r-Biopharm AG, Darmstadt, Germany), the amount of prolamin (milligrams per kilogram) in the food samples was measured using the Ridascreen Fast Gliadin R5-ELISA (r-Biopharm AG), which has been successfully validated for raw and processed food (8) and is officially recommended for determining the gluten concentration in foods by the Codex Alimentarius Commission (7). The gliadin limit of detection (LOD) for this method is 2 mg/kg (ppm), and the limit of quantification (LOQ) is 5 mg/kg (ppm), which correspond to gluten concentrations of 4 and 10 mg/kg (ppm), respectively.

From each homogenized sample, DNA was extracted twice (Wizard, Promega, Madison, WI). The wheat DNA as wheat ω -gliadin was detected with a real-time PCR assay according to a modification of the method of Sandberg et al. (10). All amplifications were performed with 100 ng of total DNA using the SensiFast No-Rox Master Mix (Bioline, London, UK) with 400 nM of each primer and 200 nM of probe labeled at the 5'-end with the fluorescent reporter dye FAM (6-carboxy-fluorescein). The Black Hole Quencher 1 was located at the 3'-end of the probe. The following thermal cycling profile was used for the PCRs: 1 cycle of 15 min at 95°C and 50 cycles of 15 s at 95°C, 60 s at 60°C, and 60 s at 72°C. The LOD for the PCR method was 0.01% wheat DNA in maize DNA, corresponding to about 10 mg/kg gluten. Each real-time PCR assay was duplicated.

Two completely different methods for analyzing the rate of contamination in our samples were used. Compared with the ELISA, the real-time PCR assay is a highly sensitive test that does not quantify the actual gluten concentration but detects wheat DNA. Hence, the PCR assay was performed to supplement the ELISA results.

Quality controls. In the final analysis, we used quality controls for the sample extraction, ELISA, and PCR assay by titrating small amounts of cooked GC pasta in cooked GF pasta. Samples of 200 g of GF pasta were spiked with known concentrations of 0.01, 0.02, 0.1, 0.2, and 1% GC pasta. Wheat semolina contains approximately 90 g/kg gluten (9); therefore, a GF sample with 0.01% GC pasta should have a calculated gluten concentration of approximately 9 mg/kg, a sample spiked with 0.02% should have 18 mg/kg gluten, one spiked with 0.1% should have 90 mg/kg gluten, one spiked with 0.2% should have 180 mg/kg gluten, and one spiked with 1% should have 900 mg/kg gluten.

We use the same sample protocol as used for the experimental samples (homogenization, drying, and second homogenization) and repeated the experiments as follows: two spiking experiments for each concentration, two protein extractions and two DNA

extractions from each spiking sample, one ELISA, and two PCR assay.

RESULTS

Figure 1 shows the gluten concentration of the 30 food samples, a noncontaminated ("clean") GF pasta sample, and the quality controls. The gluten concentration of all systematically contaminated samples as measured with the ELISA was below the LOQ of 5 mg/kg gliadin or 10 mg/kg gluten. In the experiment in which GF pasta was scooped with a contaminated ladle (experiment iii), we found a weak background signal in the ELISA (optical density of ca. 0.14 instead of ca. 0.06 as in the other experiments), although the gluten concentration was below the LOQ of 10 mg/kg.

The C_T values (number of cycles required for the fluorescent signal to cross the threshold; inversely proportional to the amount of target nucleic acid) from the PCR assay fluctuated widely. Most samples were negative several times. The PCR assay for experiment iii (GF pasta scooped with a contaminated ladle) produced lower C_T values than did all other samples and no negative PCR results, indicating a higher concentration of wheat DNA in these samples. The C_T values in the experiment in which GF pasta was scooped with a contaminated ladle corresponded to 0.01 to 0.1% semolina pasta in the GF pasta. The results of the ELISA and the PCR assay indicate that the ladle poses the highest risk for a contamination.

Both tested cleaning methods (clean versus contaminated cloths and towels) did not result in relevant contamination; all samples from both methods had gluten concentrations of <10 ppm.

To confirm the results obtained in the first stage of the study, we conducted quality controls for the sample gluten extraction method by titrating certain tiny amounts of cooked semolina pasta in cooked GF pasta. The quality control findings were correlated with spiking level, although the concentrations were lower than those theoretically calculated. Cooked pasta, which was dried at 102°C and milled for homogeneity, had a lower gluten concentration than that theoretically calculated (reference gluten concentration of wheat flour). The recovery of gluten in the spiked cooked samples was calculated as 25 to 30%.

DISCUSSION

Cross-contamination with gluten is a serious concern for CeD patients (6) and a possible explanation for mucosal atrophy and symptoms of CeD patients on a self-reported strict GF diet. The results of the present study indicate that although gluten cross-contamination from kitchen utensils is theoretically possible, the investigated kitchen handling methods seem not to pose a relevant risk for CeD patients compared with ingestion of wheat flour. Apart from the GF pasta handled with the contaminated ladle, all tested samples had <0.02% semolina pasta in GF pasta, corresponding to <20 ppm of gluten.

Our experimental samples from the contaminated ladle had the highest C_T values (highest wheat DNA concentration) of all samples, corresponding to 0.01 to 0.1% gluten in GF pasta. In this range of contamination, a gluten analysis

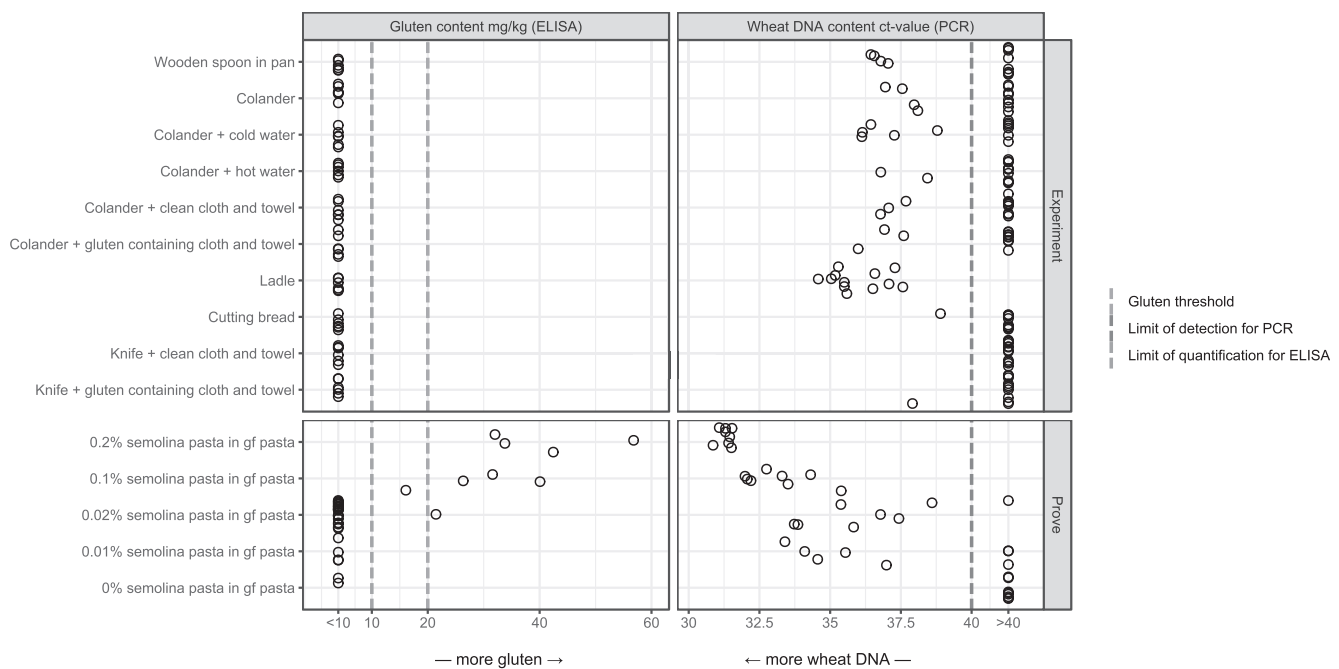


FIGURE 1. Gluten and wheat DNA concentrations in 30 collected samples and in quality controls used for the sample gluten extraction method.

method with an LOD of <4 mg/kg would have been helpful. However, because we predicted that significantly higher contamination would occur, we used the well-validated R5-ELISA recommended by the Codex Alimentarius Commission (7).

We hypothesized that use of contaminated cleaning utensils to wipe kitchen utensils during the preparation of GF foods results in quantifiable increases in gluten concentration through cross-contamination. However, when the kitchenware was cleaned with a cloth and towel visibly contaminated with wheat flour, the gluten concentration of the tested samples was not increased and was well below the LOQ, and further, the C_T value indicated no contamination. Therefore, our hypothesis was not supported.

Measurement inaccuracy occurred in this study, as indicated by the reduced analytical recovery maximum of 30% for our samples. Unlike gluten in flour dust, in wet ingredients containing low concentrations of gluten, the gluten is not equally distributed throughout the food, even after thorough mixing. Thus, false-negative results can be obtained from nonhomogeneous samples, even though theoretical the analytical recovery should be higher because the samples were not processed.

We did our best to optimize homogeneity by drying, which reduced the sample mass, but only small samples (0.25 g) are needed for extraction for the R5-ELISA. However, during homogenization of the samples with a knife mill, we noticed that wet pasta did not form a homogeneous, fine mixture as did bread or other baked goods, and we suspected that the gluten traces in the sample may not have been evenly distributed. Therefore, we dried all samples in a drying cabinet at 102°C and repeated the

milling for homogenization, which produced a finely ground mixture.

Our quality control samples had lower gluten concentrations than theoretically calculated, but homogeneity is more important than a high analytical recovery because a nonhomogeneous sample cannot be analyzed correctly. Compared with the artificial quality control system, experimental gluten contamination at relevant concentrations would be detectable because the gluten concentrations obtained with the ELISA and the gliadin DNA concentrations obtained with the PCR assay would increase continuously with increasing contamination.

The detection of minuscule amounts of gluten is technically limited (due to the LOQ and homogenization issues), and it is nearly impossible to test all conceivable possible contamination routes. Therefore, we do not have any reference point to determine whether surfaces (e.g., stainless steel versus plastic versus ceramic) have an impact on contamination. During design of the study, we discussed possible surface tests but decided that such tests would not be helpful because we wanted to quantify the amount of gluten coming from the utensils and the available surface tests did not allow such quantification and were useful only for process controls.

Despite the limitations of the study, our data suggest that cross-contamination via domestic kitchen utensils during the preparation of GF meals is less critical than the previously investigated cross-contamination directly from wheat flour (9). Recommendations for avoiding cross-contamination with gluten are often not based on empirical data. Those following such recommendations may expend great effort and time preparing GF meals. Whitaker et al. (14) found that dietary restriction (which included avoiding

cross-contamination) affected the enjoyment of food in 46% of the surveyed persons in their study. Overly restrictive recommendations for avoiding cross-contamination that are not based on evidence do not seem helpful for CeD patients. These patients need to be informed that domestic kitchen utensils, when properly cleaned, do not pose a health risk from gluten. Purchase of new kitchen utensils or cooking GF foods in separate kitchen does not seem to be necessary. Miller et al. (9) found that relevant cross-contamination to GF foods from wheat flour in the kitchen is possible and can be avoided by preparing meals at a distance of 2 m from where wheat flour is used; the detected gluten concentrations found by Miller et al. were much higher than the concentrations in our experiments. We assume that these deviations are caused by the lower particle size and better adhesion of flour.

Because of the difference between wet and probably sticky GC foods and fine flour, we assume that wheat flour poses relevant risks for cross-contamination in kitchens, but our findings revealed that such a risk is not necessarily associated with kitchen utensils in general. Recommendations that are too restrictive, maybe out of ignorance and a lack of data in this field, are not helpful to either CeD patients or to chefs and caterers.

A single cross-contamination event as a consequence of negligence during the cooking process is considered harmless for CeD patients (12). As we found, the resulting contamination of pasta by GC wooden spoons or GC colanders and the contamination of GF bread by a GC knife is clearly below the threshold level for GF labeling (20 mg/kg gluten). Although a small percentage of CeD patients have reported suspected reactions to trace amounts of gluten cross-contamination (12), the gluten concentrations found in our samples were almost non-detectable and reactions to such amounts have not been found in previous studies. Unfortunately, few studies have focused on the reactions of CeD patients after the ingestion of gluten traces (2). Two studies were conducted using different endpoints and did not include the types of symptoms suffered by patients directly after the ingestion of trace gluten (3). To date, data are lacking on the reactions of CeD patients after ingestion of a single gluten dose of <20 ppm. Nevertheless, we assume that CeD patients who do react to trace amounts of gluten through cross-contamination may suffer at the same time from a wheat allergy and that their symptoms are caused by the allergy and not CeD.

Our study has several strengths and limitations. One of the most important limitations was the inability to investigate all of the innumerable possible scenarios under which gluten contamination can occur. Cross-contamination can happen during every preparation and cooking step, and we focused on a few of the common and relevant situations.

In addition to measuring the concentration of gluten in samples, a study could be conducted to obtain data concerning symptoms and reactions of CeD patients exposed to food samples with quantified gluten contamination. We attempted to conduct an experimental trial with a systematic and carefully chosen approach including optimal

homogenization of the samples, two sensitive and well-established methods of determining gluten or wheat concentrations in samples, and a quality control aspect for the sample extraction methods with proof of the analytical recovery rate.

Cross-contamination with gluten may occur with domestic kitchen utensils but does not seem as relevant as previously thought. Kitchen equipment and utensils used for GC food may not pose such a high risk for CeD patients as do wheat flour and bread crumbs. Nevertheless, cross-contamination during the preparation of food or cooking process should be avoided by cleaning utensils and washing hands and surfaces regularly. However, overly restrictive recommendations and scaremongering is inappropriate. We do not propose here a general recommendation for the safe handling of contaminated utensils, and the findings apply only to the tested scenarios.

Further research on this topic is clearly necessary. We propose further investigation to determine whether shared toasters, ovens, cutting boards, deep fryers, and spreads (e.g., butter, peanut butter, and honey) pose risks for relevant gluten cross-contamination.

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REFERENCES

1. Akobeng, A. K., and A. G. Thomas. 2008. Systematic review: tolerable amount of gluten for people with coeliac disease. *Aliment. Pharmacol. Ther.* 27:1044–1052.
2. Brottveit, M., A. C. Beitnes, S. Tollefsen, J. E. Bratlie, F. L. Jahnsen, F. E. Johansen, L. M. Sollid, and K. E. Lundin. 2013. Mucosal cytokine response after short-term gluten challenge in celiac disease and non-celiac gluten sensitivity. *Am. J. Gastroenterol.* 108:842–850.
3. Catassi, C., E. Fabiani, G. Iacono, C. D'Agate, R. Francavilla, F. Biagi, U. Volta, S. Accomando, A. Picarelli, I. De Vitis, G. Pianelli, R. Gesuita, F. Carle, A. Mandolesi, I. Bearzi, and A. Fasano. 2007. A prospective, double-blind, placebo-controlled trial to establish a safe gluten threshold for patients with celiac disease. *Am. J. Clin. Nutr.* 85:160–166.
4. Catassi, C., and A. Fasano. 2008. Celiac disease. *Curr. Opin. Gastroenterol.* 24:687–691.
5. Fasano, A., and C. Catassi. 2012. Clinical practice. Celiac disease. *N. Engl. J. Med.* 367:2419–2426.
6. Hollon, J. R., P. A. Cureton, M. L. Martin, E. L. Puppa, and A. Fasano. 2013. Trace gluten contamination may play a role in mucosal and clinical recovery in a subgroup of diet-adherent non-responsive celiac disease patients. *BMC Gastroenterol.* 13:40.
7. Joint Food and Agriculture Organization of the United Nations and World Health Organization. 1999. Codex Alimentarius standard for foods for special dietary use for persons intolerant to gluten. Standard 118-1979. Codex Alimentarius Commission, Rome.
8. Mendez, E., C. Vela, U. Immer, and F. W. Janssen. 2005. Report of a collaborative trial to investigate the performance of the R5 enzyme linked immunoassay to determine gliadin in gluten-free food. *Eur. J. Gastroenterol. Hepatol.* 17:1053–1063.
9. Miller, K., N. McGough, and H. Urwin. 2016. Catering gluten-free when simultaneously using wheat flour. *J. Food Prot.* 79:282–287.
10. Sandberg, M., L. Lundberg, M. Fern, and I. M. Yman. 2003. Real time PCR for the detection and discrimination of cereal contamination in gluten free foods. *Eur. Food Res. Technol.* 217:344–349.

11. See, J. A., K. Kaukinen, G. K. Makharia, P. R. Gibson, and J. A. Murray. 2015. Practical insights into gluten-free diets. *Nat. Rev. Gastroenterol. Hepatol.* 12:580–591.
12. Silvester, J. A., L. A. Graff, L. Rigaux, J. R. Walker, and D. R. Duerksen. 2016. Symptomatic suspected gluten exposure is common among patients with coeliac disease on a gluten-free diet. *Aliment. Pharmacol. Ther.* 44:612–619.
13. Vincentini, O., M. Izzo, F. Maialetti, E. Gonnelli, S. Neuhold, and M. Silano. 2016. Risk of cross-contact for gluten-free pizzas in shared-production restaurants in relation to oven cooking procedures. *J. Food Prot.* 79:1642–1646.
14. Whitaker, J. K., J. West, G. K. Holmes, and R. F. Logan. 2009. Patient perceptions of the burden of coeliac disease and its treatment in the UK. *Aliment. Pharmacol. Ther.* 29:1131–1136.