Journal of Food Protection, Vol. 79, No. 9, 2016, Pages 1642–1646
doi:10.4315/0362-028X.JFP-15-538

Research Note

Risk of Cross-Contact for Gluten-Free Pizzas in Shared-Production Restaurants in Relation to Oven Cooking Procedures

OLIMPIA VINCENTINI,[1] MARTINA IZZO,[2] FRANCESCA MAIALETTI,[1] ELENA GONNELLI,[1] SUSANNA NEUHOLD,[2] AND MARCO SILANO[1]*

[1]*Unit of Human Nutrition and Health, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161Roma, Italy; and*
[2]*Italian Coeliac Association—Associazione Italiana Celiachia, Via Caffaro 10, 16124 Genova, Italy*

MS 15-538: Received 24 November 2015/Accepted 1 May 2016

ABSTRACT

To allow celiac patients to have meals out, a growing number of restaurants and pizzas houses that simultaneously provide gluten-free (GF) pizzas and wheat-based (WB) pizzas have recently been opened in Italy. In these restaurants, GF pizzas are prepared with GF raw materials, following procedures that minimize the risk of gluten cross-contact. Here, we evaluate the risk of gluten cross-contact of GF pizzas in relation to the preparation procedures, thus aiming at identifying a safe procedure for cooking GF pizzas. Our results show that, when specific requirements are complied with, the simultaneous cooking of GF and WB pizzas is a procedure as safe as having an oven dedicated to GF pizzas or the alternate cooking of GF and WB pizzas in the same oven.

Key words: Celiac disease; Cross-contact; Gluten; Gluten-free

Celiac disease (CD) is a permanent inflammatory enteropathy, triggered in predisposed individuals by dietary gluten *(9)*. Gluten is the alcohol-soluble protein fraction of wheat, rye, and barley *(5)*. CD has an estimated worldwide prevalence of roughly 1:100 in the general population *(6)*.

At the moment, the only available therapy for CD is a strict, lifelong withdrawal of gluten from the diet. Fully complying with a gluten-free (GF) diet is difficult; however, a strict adherence to this dietary regimen is necessary not only to obtain the remission of the signs and symptoms associated with CD, but also to prevent the life-threatening complications of this condition *(8)*.

Following a GF diet heavily impacts the social life of celiac patients. To avoid even the smallest amount of dietary gluten, celiac patients often refrain from having meals away from home, if not strictly necessary *(2)*. Thus, to meet the needs of CD-affected patients who dine out, a growing number of restaurants, cafeterias, and pizza houses have been providing GF foods (with a final gluten concentration lower than 20 ppm) simultaneously with providing wheat-containing foods. In these shared-production restaurants, GF foods are prepared with GF raw materials by trained chefs who follow procedures set by law, with the aim of minimizing the risk of gluten cross-contact.

These procedures are based on specific instructions for storage, preparation, cooking, and serving of foods. In Italy, these procedures have been established during the several years of application of the guidelines of the "GF Eating Out" program, developed by the Italian Coeliac Association *(4)*, in cooperation with the national food safety authorities. The "GF Eating Out" program started in 2000, and so far it includes about 4,000 venues throughout Italy.

Shared-production pizza houses are restaurants with a very high risk of gluten cross-contact, because aerosolized wheat flour might spread and contaminate GF pizzas at different steps of the production process.

In these shared-production restaurants, GF raw materials have to be stored in dedicated cabinets and pizzas have to be prepared on a clean surface, avoiding contact with any source of gluten. For the oven-cooking of GF pizzas, a spatial or temporal separation from wheat-based (WB) pizzas is currently suggested. So, GF pizzas may be cooked in a dedicated oven; alternatively, they may be cooked in the same oven used for WB pizzas but not at the same time as WB pizzas. Staff involved in the cooking of GF pizzas must also apply specific procedures when they put GF pizzas in the oven and when they serve it.

The procedures described above are based on a theoretical risk assessment of gluten cross-contact of GF pizzas, and a systematic investigation of the risk of these procedures has not been carried out. Thus, with the aim of identifying safe procedures to prepare GF foods, in this article we have compared the gluten concentration of GF pizzas prepared in different shared-production pizza houses, following different procedures.

MATERIALS AND METHODS

Pizza preparation. The dough for GF pizzas was made with a mix of GF maize, rice, and buckwheat flours specifically formulated for celiac patients, guaranteed to have a final gluten

* Author for correspondence. Tel: 0039-06-49906001; Fax: 0039-06-49387149; E-mail: marco.silano@iss.it.

TABLE 1. *Number of GF pizzas cooked and analyzed according to various procedures in a bakery training school*[a]

Bakery training school	Day 1	Day 2	Day 3	Samples collected
GF pizzas	10 ALT +10 TRAD	10 ALT +10 TRAD + 10 DED	10 ALT +10 TRAD	70 GF pizzas

[a] ALT, alternating; TRAD, traditional shared; DED, dedicated.

concentration below 20 ppm; the dough for WB pizzas was made of wheat flour. As pizza topping, tomato sauce and traditional Italian Mozzarella cheese (both naturally GF) were used. The ingredients used for the preparation of GF pizzas were stored on separate shelves of the refrigerator and in a separate cabinet from those ingredients used for WB pizzas. GF and WB pizzas were prepared on different, separated benches. The personnel in charge of GF pizza preparation wore disposable uniforms and carefully washed their hands with soap each time they were in contact with wheat flour and other gluten-containing ingredients. One pizza paddle was dedicated to putting GF pizzas into the oven and another was used for WB pizzas. In the first stage of the study, GF pizzas and wheat pizzas were prepared in the training kitchen of a traditional bakery school in Genova, Italy, where a trained chef prepared wheat-based (WB) pizzas and GF pizzas, following three different oven-cooking procedures.

In procedure one (TRAD), WB and GF pizzas were cooked simultaneously in the same oven. Before the GF pizzas were placed in the oven, they were put in a pan with 2.5-cm-high edges, to further minimize the risk of gluten cross-contact. The cooking surface of the oven was equally divided for the two types of pizzas, one or two GF pizzas on the left and one or two WB pizzas on the right. In the second procedure (ALT), WB and GF pizzas were cooked alternately in the same oven, with a batch of GF pizzas followed by a batch of WB pizzas. In the third procedure (DED), WB and GF pizzas were cooked in two different ovens, one dedicated to GF pizzas and one dedicated to WB pizzas.

The training kitchen surface used for WB pizzas was not cleaned after a normal traditional bakery lesson, to simulate a real pizza house during opening hours. The surface where GF pizzas were prepared was carefully cleaned with soap. Electric ovens were used (124 by 80 by 25 cm; model 4T-60/40, Mondial Forni S.p.A., Verona, Italy). For procedures ALT and TRAD, 10 GF and 10 WB pizzas were prepared and cooked per day on three different days. For the DED procedure, 10 GF pizzas were prepared and cooked (see Table 1).

In the second stage of this study, we collected GF pizzas from five different pizza houses during working days, when the restaurants were open to customers, to evaluate the possible contamination under real working conditions. All the pizza houses served both GF and WB pizzas. The staffs of the pizza houses were specifically trained in the correct procedures to avoid gluten cross-contact. Three pizza houses used traditional electric ovens: pizza house 1 (80 by 60 by 25 cm; Moretti S.p.A., Mondolfo, Italy), pizza house 3 (40 by 35 by 25 cm; double-chamber P234H vented, Effeuno S.r.l., Padova, Italy), and pizza house 4 (62 by 50 by 12 cm; basic model 2/50/V - N. 2 chamber with glass, AllForFood, Fano, Italy). Pizza house 2 used a wood fire oven (134 by 110 by 50 cm), and pizza house 5 used an electric oven with high forced air ventilation (135 by 100 by 18 cm; FRV 100, Morello Forni S.a.s., Santa Marta, Italy). Ovens with forced air ventilation are usually used to reduce the cooking time of pizzas, because the air ventilation helps to maintain high temperatures (Table 2). We collected GF samples using the TRAD, ALT, and DED procedures as described above.

Usually, before rolling out pizza, a small amount of flour is spread on the table to prevent the pizza from sticking to the bench surface. This procedure causes a significant amount of aerosolized flour to be present in the kitchen, which can cross-contaminate GF pizzas. Thus, in the shared-production pizza houses (but not in the bakery school), on a specific day we carried out the different procedures (DED, TRAD, and ALT) using GF flour to roll out both GF and WB pizzas, to evaluate whether the use of GF flour as rolling flour could decrease the risk of cross-contact in the pizza

TABLE 2. *Number of GF pizzas cooked and analyzed according to various procedures in five pizza houses*[a]

Pizza house	Training on GF production	Oven	Days	Procedures applied	GF pizza samples collected
1	Formal and on-site	Electric oven	2	6 TRAD + 6 ALT + 6 DED	18
2	Formal and on-site	Wood fire oven	4	6 TRAD + 6 ALT + 6 DED + 3 ALT with GF rolling flour also for WB pizzas + 3 TRAD with GF rolling flour also for WB pizzas	24
3	Formal and on-site	Electric oven	2	6 TRAD with GF rolling flour also for WB pizzas + 6 ALT with GF rolling flour also for WB pizzas + 6 DED with GF rolling flour also for WB pizzas	18
4	On-site	Electric oven	2	3 ALT + 3 TRAD + 3 ALT with GF rolling flour also for WB pizzas + 3 TRAD with GF rolling flour also for WB pizzas	12
5	On-site	Electric oven with forced air ventilation	2	3 ALT + 3 TRAD + 3 ALT with GF rolling flour also for WB pizzas + 3 TRAD with GF rolling flour also for WB pizzas	12
Total					84

[a] TRAD, traditional shared; ALT, alternating; DED, dedicated. Wheat flour was used to roll out WB pizzas, except when indicated "with GF rolling flour also for WB pizzas."

TABLE 3. *Concentration of gluten in GF pizzas cooked in the bakery school*[a]

	Gluten concn (ppm)		
	Day 1	Day 2	Day 3
ALT	BLQ (×3)	BLQ (×3)	BLQ (×3)
	BLQ (×3)	BLQ (×3)	BLQ (×3)
	6.1; BLQ (×2)	BLQ (×3)	BLQ (×3)
	BLQ (×3)	7.1; BLQ (×2)	BLQ (×3)
	BLQ (×3)	BLQ (×3)	BLQ (×3)
	BLQ (×3)	BLQ (×3)	BLQ (×3)
	BLQ (×3)	BLQ (×3)	BLQ (×3)
	9.4; BLQ (×2)	BLQ (×3)	BLQ (×3)
	BLQ (×3)	BLQ (×3)	BLQ (×3)
	BLQ (×3)	BLQ (×3)	BLQ (×3)
TRAD	11.4; BLQ (×2)	BLQ (×3)	BLQ (×3)
	14.0; BLQ (×2)	BLQ (×3)	BLQ (×3)
	BLQ (×3)	BLQ (×3)	BLQ (×3)
	12.7; BLQ (×2)	BLQ (×3)	BLQ (×3)
	5.5; BLQ (×2)	12.6; BLQ (×2)	BLQ (×3)
	5.7; BLQ (×2)	**23.6;** 12.1; 7.6	BLQ (×3)
	BLQ (×3)	BLQ (×3)	BLQ (×3)
	BLQ (×3)	BLQ (×3)	BLQ (×3)
	BLQ (×3)	BLQ (×3)	BLQ (×3)
	BLQ (×3)	7.2; BLQ (×2)	BLQ (×3)
DED		BLQ (×3)	
		BLQ (×3)	
		BLQ (×3)	
		BLQ (×3)	
		BLQ (×3)	
		BLQ (×3)	
		BLQ (×3)	
		BLQ (×3)	
		5.5; BLQ (×2)	
		5.4; BLQ (×2)	

[a] ALT, alternating; BLQ, below limit of quantification; TRAD, traditional shared; DED, dedicated. Three samples were obtained from each slice homogenate and were analyzed for gluten content by R5 ELISA. Each value of these three measurements is reported. Values higher than 5 ppm are reported in the table; results below the ELISA limit of quantification (5 ppm according to manufacturer's instruction) are shown as BLQ. The one result greater than 20 ppm is shown in boldface.

houses. On the other days, we carried out the same procedures using wheat flour to roll out WB pizzas, as is usually done in pizza houses. All GF pizzas were cooked in pans with 2.5-cm-high edges.

Determination of gluten concentration in the pizzas. Each GF pizza was cut in four equal slices. One of these slices was homogenized using a 600-W six-blade blender (Moulinex, Seb Group, Ecully, France) at the maximum rpm for 10 min. The resulting homogenate was stirred for 15 min at 4,000 rpm with a magnetic stirrer (Biosan, Riga, Latvia) to ensure an even distribution of gluten. Three 25-mg samples were taken from the homogenate of each pizza, and the gluten was extracted using the RIDA cocktail solution, according to the manufacturer's instructions (R-Biopharm AG, Darmstadt, Germany). Briefly, for each 0.25-ml portion of homogenate, we added 2.5 ml of cocktail solution; then, after a 40-min incubation, we added 7.5 ml of an 80% ethanol aqueous solution, followed by incubation at room temperature for 45 min. Then the samples were centrifuged for 10 min at 2,500 rpm, and a 100-μl aliquot of each sample was analyzed. The extracts were analyzed for gluten content (expressed in ppm) by means of RIDASCREEN gliadin, an enzyme-linked immunosorbent assay (ELISA) sandwich kit, based on the R5 antibody (R-Biopharm AG). This ELISA kit has a limit of detection of 3 ppm of gluten and a limit of quantification (LOQ) of 5 ppm. Therefore, all results below 5 ppm of gluten are reported as "below the LOQ." Results of each gluten determination are reported in ppm.

RESULTS

Tables 1 and 2 describe the number of pizzas cooked for our analysis, according to the cooking procedure. In the first part of the experimental procedure (Table 1), we studied the risk of gluten cross-contact of GF pizzas cooked following the three different procedures in a school kitchen, where we mimicked the conditions of a shared-production restaurant that simultaneously provides pizzas specially formulated for CD patients and WB pizzas. We report the results as gluten concentration of each analysis, because the means of the three determinations of gluten for each slice would have been impossible to calculate in the presence of values below the LOQ. Table 3 reports the gluten content of the GF pizzas. All the results, except one related to a pizza cooked

TABLE 4. *Concentration of gluten in GF pizzas cooked in shared-production pizza houses*[a]

	Gluten concn (ppm)				
	Pizza house 1	Pizza house 2	Pizza house 3	Pizza house 4	Pizza house 5
ALT	6.4; BLQ (×2)	BLQ (×3)	BLQ (×3)[b]	BLQ (×3)	BLQ (×3)
	BLQ (×3)	BLQ (×3)	BLQ (×3)[b]	BLQ (×3)	BLQ (×3)
	BLQ (×3)	5.0; BLQ (×2)	BLQ (×3)[b]	BLQ (×3)	BLQ (×3)
	8.2; BLQ (×2)	BLQ (×3)	BLQ (×3)[b]	BLQ (×3)	5.0; BLQ (×2)[b]
	8.3; BLQ (×2)	BLQ (×3)	BLQ (×3)[b]	BLQ (×3)	8.5; BLQ (×2)[b]
	15.6; 6.1; BLQ	BLQ (×3)	BLQ (×3)[b]	BLQ (×3)	BLQ (×3)[b]
		BLQ (×3)[b]			
		BLQ (×3)[b]			
		BLQ (×3)[b]			
TRAD	BLQ (×3)	BLQ (×3)	BLQ (×3)[b]	BLQ (×3)	BLQ (×3)
	BLQ (×3)	BLQ (×3)	BLQ (×3)[b]	BLQ (×3)	BLQ (×3)
	BLQ (×3)	BLQ (×3)	BLQ (×3)[b]	BLQ (×3)	BLQ (×3)
	11.0; BLQ (×2)	BLQ (×3)	BLQ (×3)[b]	BLQ (×3)	BLQ (×3)[b]
	BLQ (×3)	BLQ (×3)	BLQ (×3)[b]	BLQ (×3)	BLQ (×3)[b]
	BLQ (×3)	BLQ (×3)	BLQ (×3)[b]	BLQ (×3)	BLQ (×3)[b]
		BLQ (×3)[b]			
		BLQ (×3)[b]			
		BLQ (×3)[b]			
DED	BLQ (×3)	BLQ (×3)	BLQ (×3)[b]		
	BLQ (×3)	BLQ (×3)	BLQ (×3)[b]		
	BLQ (×3)	BLQ (×3)	BLQ (×3)[b]		
	17.6; 5.6; BLQ	BLQ (×3)	BLQ (×3)[b]		
	BLQ (×3)	BLQ (×3)	BLQ (×3)[b]		
	BLQ (×3)	BLQ (×3)	BLQ (×3)[b]		

[a] ALT, alternating; BLQ, below limit of quantification; TRAD, traditional shared; DED, dedicated. Three samples were obtained from each slice homogenate and were analyzed for gluten content by R5 ELISA. Each value of these three measurements is reported. Values higher than 5 ppm are reported in the table; results below the ELISA limit of quantification (5 ppm according to manufacturer's instruction) are shown as BLQ.

[b] Wheat flour was used to roll out wheat pizzas.

according to the TRAD procedure, show a gluten concentration in pizzas below 20 ppm.

To confirm the results obtained in the first stage of the study, we evaluated the possible contamination of pizzas cooked during operating hours in five shared-production restaurants that simultaneously serve GF and wheat-containing foods (Table 2). The personnel of all the pizza houses involved in the study received specific training on GF food storage and production. All the samples taken from pizzas prepared in those "real" settings had gluten concentrations below 20 ppm (Table 4).

DISCUSSION

Hidden gluten cross-contact of GF foods is a serious concern for celiac patients *(3)*. This cross-contamination is thought to explain why roughly 50% of adult CD patients still have mucosal atrophy after 2 years of a self-reported strict GF diet *(7)*. Thus, shared-production restaurants and cafeterias that simultaneously provide GF and gluten-containing foods must follow strict cooking and handling procedures that minimize the risk of cross-contact *(1)*.

To our knowledge, this is the first study to systematically evaluate the risk of gluten cross-contact of GF foods in relation to preparation procedures, with the aim of not only identifying safe procedures, but also taking into account the sustainability and the costs of those procedures. We chose to set our study in pizza houses, because this type of restaurant is at a very high risk of gluten cross-contact, owing to aerosolized wheat flour that can contaminate GF pizzas and GF raw materials at several stages of the production and cooking process.

Strictly speaking, gluten cross-contact risk assessment for CD patients should take into account only the limit of 20 ppm; European Regulations 41/2009 and 828/2014 establish that foods specifically formulated for celiac patients should have a maximum gluten concentration of 20 ppm. Thus, GF food providers are only required to ensure that this gluten threshold is not exceeded. One sample of GF pizzas cooked in the bakery school following the TRAD procedure showed gluten cross-contact, with one of three values above 20 ppm (23 ppm). Although the TRAD procedure was not associated with cross-contact when it was tested in actual shared-production restaurants, which are more challenging settings, the procedure requires higher vigilance by the operators; we advise the use of GF flour for rolling out both types of pizza when this procedure is used, to reduce aerosolized wheat. As seen by the general trend of results for each of the cooking procedures tested in the bakery school, the TRAD procedure has an increased risk of cross-contact, and, as expected, the DED procedure showed the lowest trend of risk. However, these results differ from those obtained in the real shared-production pizza houses. Note that results varied among

pizza houses: several pizza samples from pizza house 1 had gluten content above the LOQ of the ELISA R5 kit, with two samples having between 15 and 20 ppm of gluten; in all samples from two pizza houses, all gluten content values were below the LOQ; and samples from the other two pizza houses had only one value above the LOQ. So, the adherence to the procedures may vary among the restaurants involved, and this aspect might be a limitation of our study.

Our results do not allow us to make any definitive conclusion about the impact that the use of wheat flour for rolling out WB pizzas has on the gluten cross-contact of GF pizzas, because we did not perform a systematic case-control evaluation of this aspect. However, rolling out the WB pizzas with wheat flour resulted in a gluten determination above 20 ppm in a pizza cooked according to the TRAD procedure, which strongly suggests that GF flour should also be used for rolling out WB pizzas, when using this procedure. Also, although rolling out the WB pizzas with wheat flour did not result in any gluten determination above 20 ppm in the DED and ALT procedures, two pizza samples cooked according to the ALT procedure showed a trend toward a higher gluten concentration.

In our study, we homogenated just a slice of each pizza, and not the whole pizza, because our aim was to compare the risk of cross-contact associated with the different cooking procedures and not to measure the whole gluten content of each pizza. We assumed that each slice of pizza had the same probability of being in contact with gluten during the cooking in the oven and that the gluten from cross-contact had a homogeneous distribution in the pizzas.

The main question is whether and how these study results should affect the cooking procedures in shared-production restaurants. Is an oven specifically dedicated to GF pizzas necessary? Our results indicate that a dedicated oven is not necessary, as long as all the shared procedures are carried out carefully and the personnel are aware of the risk of cross-contact.

Keeping the procedures and the facilities required for shared-production restaurants to provide GF foods as simple and cheap as possible could have advantages not only for the chefs and operators, but also for CD patients, who may have a wider choice of safe places for dining out.

ACKNOWLEDGMENT

This work was supported by a grant from AIC—Italian Celiac Association.

REFERENCES

1. 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.
2. Gibert, A., A. G. Kruizinga, S. Neuhold, G. F. Houben, M. A. Canela, A. Fasano, and C. Catassi. 2013. Might gluten traces in wheat substitutes pose a risk in patients with celiac disease? A population-based probabilistic approach to risk estimation. *Am. J. Clin. Nutr.* 97:109–116.
3. 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.
4. Italian Coeliac Association. "GF Eating Out" program. Available at: http://www.celiachia.it/dieta/Dieta.aspx?SS=95. Accessed 29 April 2016.
5. Jabri, B., D. D. Kasarda, and P. H. Green. 2005. Innate and adaptive immunity: the yin and yang of celiac disease. *Immunol. Rev.* 206:219–231.
6. Lionetti, E., S. Gatti, A. Pulvirenti, and C. Catassi. 2015. Celiac disease from a global perspective. *Best Pract. Res. Clin. Gastroenterol.* 29:365–379.
7. Rubio-Tapia, A., M. W. Rahim, J. A. See, B. D. Lahr, T. T. Wu, and J. A. Murray. 2010. Mucosal recovery and mortality in adults with celiac disease after treatment with a gluten-free diet. *Am. J. Gastroenterol.* 105:1412–1420.
8. Silano, M., U. Volta, A. M. Mecchia, M. Dessì, R. Di Benedetto, M. De Vincenzi, and the collaborating centers of the Italian registry of the complications of coeliac disease. 2007. Delayed diagnosis of coeliac disease increases cancer risk. *BMC Gastroenterol.* 9:8.
9. Sollid, L. M., and B. Jabri. 2013. Triggers and drivers of autoimmunity: lessons from coeliac disease. *Nat. Rev. Immunol.* 13:294–302.