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Detection of Gluten in Dishes Offered as Gluten-Free in a Gastronomic Area Located in San José, Costa Rica

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ABSTRACT

Celiac disease, gluten sensitivity and gluten intolerance are health conditions that require consumers to avoid their gluten intake. In Costa Rica, Law 8975 regulates the amount of gluten permitted in prepackaged products labeled as "gluten-free", but the parameters to be followed are not mandated for food service operations. This study investigated whether restaurants with gluten-free (GF) options in a gastronomic area of the city of San José, Costa Rica, achieved the requirements of the legislation that the dishes offered should contain less than 20 ppm gluten. Using data collected from five restaurants offering GF dishes, two provided dishes with quantities of gluten greater than 20 ppm (restaurant A, four samples and restaurant B, three samples); particularly dishes from the category "meat with sauce". Although those dishes are naturally gluten-free, when they are handled in areas of shared production, they are exposed to the risk of cross contact, furthermore the adding of misscellaneous such as: marinades, sauces and condiments, which could contain traces of gluten or hidden gluten, a risk associated with the use of ingredients without certification GF (supplier's practices and label declaration). Therefore improvements in food service procedures should be enforced. The findings of the present study emphasize the need to include restaurant foods in the relevant legislation to ensure that the gluten-intolerant sector of the population remains safe when eating out.

1. INTRODUCTION

In 2014, authorities in Costa Rica published the "National Standard for the care of people with celiac disease", which requires that foods declared gluten-free must contain less than 20 mg of gluten per kilo-

gram of food or 20 parts per million (ppm). This standard was developed in response to requests from people with a diagnosis of celiac disease and other pathologies (non-celiac sensitivity to gluten and allergy to wheat). This disease leads to adverse reactions to the consumption of gluten, a set of proteins composed of prolamines and glutenins, which occur naturally in wheat, barley and rye [1-3].

Celiac disease is an autoimmune illness, which damages the mucosa of the small intestine, with the consumption of more than 20 ppm in a food representing a danger to the health of any celiac [4]. Among the clinical manifestations are diarrhea, vomiting, alterations typical of malabsorption, growth retardation in children, as well as anemia and osteoporosis [5, 6].

Most of the restaurants offering GF dishes in the gastronomic area of the study used shared production areas but had inadequate operational processes to ensure that the dishes offered as GF complied with the provisions of standard N˚. 38514-S, the relevant legislation operating in Costa Rica. This situation represents a potential risk to the health of people with adverse reactions to the consumption of gluten. Therefore, our study aims to quantify the gluten content in preparations offered as "GF" available in a gastronomic area and to use as a reference a restaurant with an exclusive GF offer (non-shared production), located in San José, Costa Rica.

2. MATERIALS AND METHODS

2.1. Sample

The samples were taken directly from the plate and without prior notice (as any other consumer would do in the restaurant). The samples were placed in a sterile container, following the same procedure for each of them. The food samples were taken mostly in triplicate, at random, from six dishes declared as gluten-free (according to the recommendation of the waitress or labeled in the menu) and selected at five restaurants identified as supplying gluten-free dishes in the geographical area of San José, Costa Rica. One of the five restaurants offered dishes which were exclusively gluten-free.

2.2. Taking the Sample

A portion of each food that makes up the dish served at the table was collected in a test tube, using restaurant utensils (knives, spoons, forks, etc.). The samples represented the normal service flow in a restaurant for a food before being consumed by a person with celiac disease. The samples were labeled with the name of the dish and where it was sampled. The process was observed and recorded.

2.3. Transport and Storage of the Samples

The samples were transported to the analysis laboratory in a cooled container then stored in a refrigerator at 4˚C until weighing before analysis.

2.4. Sample Preparation and Weighing

Approximately 5 g of the sample were taken, then crushed and homogenized using an electric mincer. After processing, three amounts of approximately 0.25 g were weighed accurately using an analytical balance.

2.5. Gliadin Extraction and Quantification

The Veratox® kit for Gliadin R5 was used to determine the gluten concentration in the sample. This analysis is a sandwich-type enzyme-linked immunosorbent assay (S-ELISA) [7].

The gliadin was extracted from the samples using an 80% ethanol solution using a shaker. The extract was diluted in Phosphate Buffered Saline (PBS) then added to the R5 antibody-coated wells (capture antibody) where the gliadin binds to the antibody during the incubation period. Any unbound gliadin was then washed away, and gliadin-binding detector antibody was added for another incubation period. After washing away any unbound antibody, a one-step substrate was added. The stop reagent was then added

and the solution color observed. The optical densities of the standards form a curve, so that the optical densities of the samples can be plotted against the curve to calculate the exact concentration of gliadin in parts per million (ppm). This method has a quantitation range from 2.5 to 40 ppm of gliadin (5 - 80 ppm gluten), which was deemed adequate for the present study. It should be noted that the gliadin concentrations obtained were multiplied by 2, because the concentration of gluten in food is calculated on the basis of its two glycoprotein components, gliadin and glutenin [8].

2.6. Statistical Analysis

Excel 2016 (Microsoft Office) was used for the statistical analysis. The average, standard deviation and confidence level ($1 - \alpha = 99\%$) of the gluten concentrations of each dish were calculated using the following equations [9]:

The lower and upper limits of the confidence interval are given by $\bar{x} - z \times \frac{\sigma}{\sqrt{n}}$ and $\bar{x} + z \times \frac{\sigma}{\sqrt{n}}$, respectively, where: $\bar{x}$: average of the same samples; σ: standard deviation of the average; $\sqrt{n}$: square root of the number of samples obtained or each dish; and "$-z$" and "$+z$" refer to the probability associated with the confidence level 99% (1.8856). The proportions of dishes that exhibited gluten concentrations higher than 20 ppm were determined according to restaurant and dish category.

3. RESULTS AND DISCUSSION

During the research period, there were changes in the menu of three of the selected restaurants, for which it was not possible to collect the third sample of four of the dishes. Therefore, the total of samples analyzed corresponds to 86 dishes.

After analysis using the Veratox® Gliadin R5 Kit, based on the ELISA method for gliadin quantification, 4 of the 30 dishes sampled exhibited a gluten content greater than 20 ppm (**Table 1**). Using confidence intervals based on the results from these four dishes, two (dishes A1 and B4) had a high probability (99%) of their gluten content being higher than allowed by Standard 38514-S due to gluten transfer through cross contact. However, the other two dishes (A4 and B6), based on the lower confidence limit, indicated that the samples were indeed gluten-free (<20 ppm).

When comparing the results from the reference restaurant (code E) with the dishes obtained from restaurants using shared production methods [10], suggested that the application of guidelines and operational processes during the different stages of preparation would help to prevent cross contact with gluten. These results and those of Vincentini *et al.* [11] and Villegas *et al.* [12], reaffirm that restaurants using shared production methods represent a potential risk to the health of consumers who require a gluten-free diet.

Gluten was found in amounts higher than that allowed by the standard (>20 ppm) in 7 of the 86 samples analyzed (8.1%). Of the four restaurants where shared production was used (gluten-containing and gluten-free dishes), it was found that two of them had at least three samples with gluten concentrations greater than 20 ppm (**Table 2**). The samples of dishes from restaurant (reference "E") which offered exclusively GF dishes, did not contain gluten at concentrations above 20 ppm.

The findings from previous studies have been widely documented as exposing certain consumers to a potentially dangerous situation, because the presence of traces of gluten can be enough to cause clinical problems for consumers requiring a GF diet, and thus compromise their health [13-16].

When comparing the gluten concentrations of dishes defined by categories "Starters", "Main Course (based on meat)" and "Vegetarian Options", those with more than 20 ppm were identified only in the meat with sauce group (**Table 3**). The statistical analysis and the calculated confidence intervals suggest that, in a selection of 100 main dishes based on meats in these restaurants, 99% of the time the dishes will contain a gluten concentration > 20 ppm (74.41 - 104.63 ppm). This means that the consumer cannot be sure that the dish is GF because the gluten concentration exceeds 20 ppm in the confidence interval even though meat is naturally gluten-free [17].

Table 1. Confidence intervals for the gluten concentrations of each dish sampled from restaurants in the gastronomic area of San José.

Dish code	Category	Numbers of samples	Gluten concentration* (ppm)	Lower limit* (ppm)	Upper limit* (ppm)
A1	Meat with sauce	3	>20	>80	>80
A2	Bread-Wheat flour substitute	3	8.29 ± 4.99	<5	13.72
A3	Pasta	3	<5	<5	<5
A4	Meat with sauce	3	>20	<5	>80
A5	Rice dishes	3	7.46 ± 1.19	6.16	8.76
A6	Cold meat	3	<5	<5	5.21
B1	Sea food	3	<5	<5	5.81
B2	Rice dishes	3	<5	<5	6.95
B3	Salad with meat	3	<5	<5	5.37
B4	Meat with sauce	2	>20	>80	>80
B5	Cold meat	3	<5	<5	6.10
B6	Meat with sauce	2	>20	<5	>80
C1	Corn meal vegetarian	3	<5	<5	<5
C2	Corn meal with meat	3	<5	<5	<5
C3	Corn meal with meat	3	<5	<5	<5
C4	Fry food	3	<5	<5	5.59
C5	Fry food	2	<5	<5	<5
C6	Corn meal with meat	3	<5	<5	<5
D1	Vegetarian with sauce	3	7.56 ± 6.30	<5	14.42
D2	Costa Rican food (without sauce)	3	<5	<5	<5
D3	Sea food	3	<5	<5	<5
D4	Salad (vegetables only)	3	7.85 ± 3.41	<5	11.56
D5	Costa Rican food (without sauce)	3	<5	<5	<5
D6	Costa Rican food (without sauce)	3	<5	<5	<5
E1	Sea food	3	<5	<5	14.42
E2	Rice dishes	3	<5	<5	<5
E3	Fry food	3	<5	<5	<5
E4	Corn meal with meat	2	<5	<5	<5
E5	Costa Rican food (without sauce)	3	<5	<5	<5
E6	Corn meal	3	10.27 ± 6.08	<5	16.89

*Values below 5 ppm and above 80 ppm are not shown due to the scope of the method.

Table 2. **Summary of quantity and proportion of dishes analyzed according to their gluten content in five restaurants in the gastronomic area of San José, Costa Rica.**

Restaurant	Total no. samples	Gluten concentration (ppm)			
		<5	5 - 19	>20	Proporción > 20 ppm
A	18	7	7	4	22.0
B	16	9	4	3	18.75
C	17	16	1	0	0
D	18	14	4	0	0
E	17	14	3	0	0
Total	86	60	19	7	8.14

Table 3. **Confidence intervals for the gluten concentrations for different types of dish analyzed of the gastronomic area of San José, Costa Rica.**

Type of dish	Total no. samples	99% confidence intervals (ppm)	Proportion (%) > 20 ppm
Bread-Wheat flour substitute	3	2.85 - 13.72	0
Cold meat	6	2.78 - 3.49	0
Corn meal	3	3.66 - 16.89	0
Corn meal with vegetables	3	0.39 - 3.31	0
Corn meal with meat	11	0.26 - 1.08	0
Costa Rican food (without sauce)	12	1.07 - 2.53	0
Fry food	8	0.19 - 2.93	0
Meat with sauce	10	74.41 - 104.63	100
Pasta	3	0.58 - 3.57	0
Rice dishes	9	0.90 - 7.75	0
Salad (vegetables only)	3	4.14 - 11.56	0
Salad with meat	3	1.16 - 5.37	0
Sea food	9	0.10 - 3.19	0
Vegetarian with sauce	3	0.70 - 14.42	0

Although those dishes are naturally gluten-free, during the preparation process other miscellaneous are added such as: marinades, sauces and condiments, which ones could contain traces of gluten due its ingredients are not gluten-free certified so that carries an associated risk when using such products (supplier's practices and labeling). The research found a trend among restaurants to offer dishes that were naturally GF, nevertheless the lack of protocols to prevent the risk of gluten cross-contact is still a risk. Authors such as Wieser *et al.* [18], suggests the need to implement improvements in the production and sur-

veillance systems of foodservices, due to the high number of samples reported with a significant concentration of gluten. As well, these suggestions must be implemented in the preparation of packaged foods usually used as miscellaneous during preparation [19].

As mentioned by Wieser *et al.* [18], eating in restaurants, workplaces or outside the home, continues to be a significant risk of involuntary exposure to gluten. Because the information on the label regarding the presence or absence of gluten is not reliable.

For Polanco-Allué [20] and El Khoury *et al.* [21], products declared as GF may present a hidden source of gluten in the diet. Even minor violations can cause symptoms and clinical complications in consumers requiring a gluten-free diet. Therefore, it is recommended that industries and restaurants have guidelines and controls for all their processes when preparing food.

4. CONCLUSIONS

Although some foods are naturally gluten-free, once handled in restaurants with shared production, there is a potential risk of cross contact, because of the shared use of areas, utensils, equipment, spices, condiments, sauces and marinades. As observed in our findings, the dishes which are naturally GF, such as meats, exhibited the highest gluten levels. The findings of this study emphasize the importance of restaurants complying with guidelines that can assure the absence of gluten and prevent the risk of cross contact during their operational processes.

In particular, the use of ingredients or additives without gluten-free certification label and lack of knowledge of food service staff are important factors that compromise the health of consumers.

These findings suggest that there is a lack of research on strategies or practices for preventing cross contact with gluten in shared production food services. The results of the this study also warn about the need to strengthen the existing laws regarding the supply of gluten-free food, by including restaurants in their scope and to improve the supervision of foodservice operational processes in order to prevent cross contact.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest regarding the publication of this paper.

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