

Hold the gluten!

Can coeliac consumers enjoy risk-free dining?



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Acknowledgements

This project was designed to assess awareness of coeliac condition and to highlight the importance of providing gluten-free food. The survey was conducted in two parts: first, our samplers purchased gluten-free meals from restaurants, and then they completed a questionnaire. This asked questions relating to their purchasing experience and the level of knowledge displayed by restaurant staff. Their whole meal was then dispatched to the laboratory for analysis of the gluten content.

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Executive Summary

Our samplers found that they were able to identify and purchase a gluten-free meal from the restaurants that they visited. While some were supplied with meals that actually contained gluten, the level of gluten in those meals varied considerably. Our samplers ordered 260 meals. Sixteen per cent of these contained gluten, and six and a half per cent were deemed unsatisfactory (containing more than 200mg/kg of gluten). Some of these samples could produce acute illness in coeliac customers. It is likely that the samples with low levels of gluten had been cross-contaminated, while those containing higher levels are more likely to contain some form of gluten in the ingredients. Food service staff rely on advice and input from the chef or manager to recommend and serve a gluten-free meal.

Confident staff and well-signposted menu choices do not guarantee risk-free dining for coeliac customers. The survey result supports our view that robust training is required to improve the knowledge of chefs, managers and staff, and help them to manage this hazard. To enable coeliacs to eat out safely and enjoyably, we make the following recommendations.

Recommendations

1. All service staff should receive training that addresses food allergy and intolerance (including coeliac condition) and the control of food allergens in a catering setting. **safefood** continues to promote best practice in food allergen management in the catering industry*.
2. Resource materials for the catering industry should be developed and should contain information about hazard control practices for gluten and other allergens**.

* The **safefood** training programme in food allergen control 2007-2009 was implemented to address this need. Over 550 environmental health officers and third level catering lecturers have been trained and empowered to cascade best practice food allergen management to the catering industry during routine inspections and to catering course students who will subsequently work in the industry.

** The Safe Catering and other FSAI publications and the **safefood** 'Food Allergy and Intolerance Guidance for the Catering Industry' will contribute substantially to this requirement.

3. Establishments serving coeliac customers should ensure that their staff understand which foods contain gluten. Help is available, from the Gluten-free Catering Booklet, available from the Coeliac Society of Ireland.
4. Openness of communication is vital. Caterers must communicate any doubts to their coeliac customer, who can then make an informed decision about their purchase.
5. It is important for coeliacs to initiate communication and be clear about their requirements, even in establishments which are 'coeliac-friendly' or have gluten-free menu choices.

Food service staff rely on advice and input from the chef or manager to recommend and serve a gluten-free meal.



Introduction

Project basis

This project was designed to find out if coeliacs are being properly served by the catering industry – from restaurants to bakeries and coffee shops. We surveyed places with advertised gluten-free options, as well as those where our samplers had to ask for options. The project was initiated by **safefood** and the Health Service Executive (HSE) on foot of a report on gluten by the Food Safety Authority of Ireland (FSAI) which suggested that catering staff need specialist training to ensure that they are aware of

- (a) the risks posed by gluten and
- (b) the appropriate control measures.

The report also recommended the development of guidelines for the avoidance of cross-contamination in manufacturing premises, and also in catering and retail establishments. Premises could then be assessed to ensure that they were meeting these guidelines. This in turn would necessitate training for enforcement staff and the trade. The FSAI working group also recommended a continuation of the surveillance programme for gluten-free foods.

It also asked **safefood** to progress the recommendations of the report. As a result, we will be working with the Coeliac Society of Ireland (which has endorsed the project) and with researchers involved in the study of coeliac condition.

What is the coeliac condition?

Coeliac condition (coeliac disease) is a genetically-determined disorder which causes people to have an abnormal response to gluten. Gluten is a water-insoluble, complex mixture of cereal proteins, called prolamins and glutenins, and other constituents. These are found in the seeds of cereal grains. It is usually the prolamins that trigger a reaction in coeliacs. The prolamins of wheat, barley, rye and oats are gliadins, hordeins, secalins and avenins, respectively. Prolamins trigger inflammation in the mucosal lining of the small intestine. This can prevent the absorption of nutrients, minerals and the fat-soluble vitamins A, D, E and K. For a coeliac, eating these proteins can cause symptoms including malnutrition, diarrhoea, anaemia, and (occasionally) lactose intolerance. Chronic exposure carries a risk of osteoporosis and intestinal cancer. The way to prevent these symptoms for a person with coeliac condition is by following a gluten-free diet. This strictly regimented dietary management is a part of life for between five and ten people per 1000 of the Irish population.

Fortunately, they are able to choose from an increasing number of gluten-free foods on the retail market which are routinely monitored for gluten content by the Public Analyst's Laboratories in conjunction with the Environmental Health Service. When it comes to dining out, there is an increasing number and variety of gluten-free menu options in restaurants. Coeliacs can also request a menu option in gluten-free form which can be achieved by serving without sauce or other sources of gluten.

However, the customer does not have complete control over their meal, and so it is probably the restaurant setting that poses the greatest risk to the coeliac. Restaurant food is prepared in a non-gluten-free environment – unlike manufactured gluten-free foods, which are often produced in explicitly gluten-free facilities – so it is more likely to become contaminated.

...strictly regimented dietary management is a part of life for between five and ten people per 1000 of the Irish population



Current labelling requirements for gluten-free foods: the Codex Standard

EU legislation (Directive 2009/39/EC) concerns foods intended for people with particular nutritional requirements, such as gluten-free food.

There is a legal obligation for producers of gluten-free food to treat cross-contamination as a hazard under Hazard Analysis Critical Control Point food safety management systems. This applies to producers of pre-packaged, labelled foods, and not to freshly prepared foods provided by caterers.

Gluten-free foods are either naturally gluten-free (for example, made from rice, which, unlike Triticum species such as wheat, rye and barley, does not contain prolamins) or are made gluten-free by a deglutinisation process. The complete removal of gluten from grains such as wheat and barley is technically and economically challenging, which makes the manufacture of totally gluten-free foods difficult. As a result, many foods labelled 'gluten-free' might actually contain low amounts of gluten.

There are 12 allergens that must be declared on the label of pre-packaged food if they are used as ingredients (Directive 2003/89/EC). This list includes 'cereals containing gluten'. However, if a coeliac took a 'zero tolerance' approach, they would find their choices severely limited.

To address this, in 1983 Codex established a new standard for labelling food products which were specially prepared for coeliacs. Foods could be labelled as 'gluten-free' if there was less than 200 milligram (mg)/kilogram (kg) gluten in the finished product.

In July 2008, this standard was revised. It came into effect via Commission Regulation (EC) No 41/2009.

The key changes to this regulation are:-

1. Foods made for people intolerant to gluten, which contain one or more ingredients made from wheat, rye, barley, oats, or their crossbred varieties, shall contain a level of gluten not exceeding 100 mg/kg in the food as sold to the final consumer. (This is based on the scientific consensus that 100 mg/kg gluten does not cause ill-health in coeliacs.)
2. These products can be labelled and advertised as 'very low gluten'. They may be labelled 'gluten-free' if the gluten content does not exceed 20 mg/kg in the food as sold to the final consumer.
3. Oats in foods made for coeliacs must have been specially produced, prepared and/or processed in a way to avoid contamination by wheat, rye, barley, or their crossbred varieties. The gluten content of such oats must not exceed 20 mg/kg.
4. Foods containing one or more ingredients which substitute wheat, rye, barley, oats, or their crossbred varieties shall contain a level of gluten not exceeding 20 mg/kg in the food as sold to the final consumer. These products may be labelled 'gluten-free'.

NOTE: Where foods contain ingredients which substitute wheat, rye, barley, oats, or their crossbred varieties, AND ingredients made from wheat, rye, barley, oats, or their crossbred varieties, points 1, 2, and 3 apply and point 4 shall not apply.

5. The terms 'very low gluten' or 'gluten-free' should appear close to the name of the food.
6. Slightly different rules apply to other foods that are not produced specifically for coeliacs. They may be labelled as 'gluten-free' if the gluten content does not exceed 20 mg/kg in the food as sold to the final consumer. However, the term 'very low gluten' cannot be applied to these foods, even if the gluten content is between 20 and 100 mg/kg. This term is reserved for foods specially processed to meet the dietary needs of coeliacs.

The Commission Regulation (EC) came into force on 10 February 2009 but will not come into effect until 1 January 2012. The purpose of this delay is to give food businesses enough time to adopt the new labelling requirements. Until January 2012, food businesses will be able to sell food products using both the old and new labelling requirements. Food products that do not comply with the new labelling requirements will become illegal on 1 January 2012 and must be removed from the marketplace.

Gluten-free food in a catering setting

Labelling laws help coeliacs to make an informed choice about pre-packaged foods, but they do not apply to food such as ready-to-eat sandwiches, cakes, snacks, and meals served at restaurants. Sale of these foods is governed by the Sale of Food and Drugs Act (1875) and the Food Hygiene Regulations (1950 to 1989), which state that all food sold to the public must be of the 'nature, substance and quality demanded' by the customer and not be 'unfit'.

Food products that do not comply with the new labelling requirements will become illegal on 1 January 2012 and must be removed from the marketplace.



How can restaurants and eating establishments safely and effectively cater for coeliacs?

Advice about the suitability of particular foods can be offered by staff, and gluten-free options can be provided on request. The level of risk depends on how effectively the restaurant (or other food establishment) (a) controls the hazard and (b) provides accurate information to the customer. Analytical investigations at this level are not practical. Instead, the establishment should make sure that their staff are aware of the gluten-containing ingredients used in their foods, as well as the risk of cross-contamination. In a catering setting, cross-contamination can happen at any stage in food preparation - from storage to the use of contaminated ingredients. Despite these limitations the restaurant must still, by law, provide food that will not be injurious to their customer's health.

So the fundamental question is: can a coeliac customer obtain a gluten-free meal in a catering establishment on request?

Determining awareness of coeliac condition and the impact of gluten among catering staff

The Public Analyst's Laboratories (in Dublin, Cork and Galway) test foods that are labelled as gluten-free. However, the awareness and knowledge about gluten-free foods among food service staff has not been determined. Research has shown

that coeliacs are actually at greatest risk of exposure when they eat out.

The Hazard Analysis Critical Control Point principles help to prevent cross-contamination in the kitchens, but catering staff play a vital role. Their lack of knowledge can significantly increase the risk for coeliac customers. Exactly what level of awareness exists among catering staff has not been determined. Without testing, it is difficult to plan appropriate programmes to address knowledge gaps. This survey was designed to help us to do just that.

To effectively determine catering staff's level of awareness, we needed to assess them during real-life situations. The following survey consisted of two assessments. Catering staff were asked for information on the gluten content of a meal, and this information was recorded by our samplers in a questionnaire. We also carried out quantitative analysis of the gluten content of the meal.

The questions used in this survey (Appendix 2) were designed to capture the interaction that takes place between the customer and the catering staff. The establishments that were sampled were those where a coeliac might expect to find a gluten-free option, so we excluded certain kinds of premises, such as sandwich bars. This automatically includes the entire spectrum of restaurants and takeaways. We specified to our samplers that the meal should have been prepared on the premises. To maximize the number of restaurants surveyed, only one meal was purchased from any given place. We advised our samplers on the most appropriate approach to take when ordering food (Appendix 1). The sampler was free to choose their meal, bearing in mind the fact that certain meal ingredients (such as gravy) would lead them to discussions with the server that would help our investigations. Samplers were asked to purchase a meal only when they had been assured that it did not, or

probably did not, contain gluten (as it was, or with ingredients removed). For example, buying a meal containing a sauce made from wheat-based flour was pointless but, if the server offered to change or remove the sauce, it provided a suitable 'test' for the restaurant's reliability.

We advised our samplers on sample handling and questionnaire completion. The survey was designed to assess levels of knowledge, and it was made clear to all participating restaurants that there would be no enforcement action(s) taken as a result.

It was agreed that establishments serving unsatisfactory samples would be notified of the result by their local Environmental Health Service.

Project objectives

1. To determine the likelihood of purchasing a gluten-free meal in a catering establishment, using relevant methodology.
2. To determine the level of knowledge about the dietary needs of coeliacs among catering staff, and their ability to respond to a request for a gluten-free meal.
3. To quantitatively analyse all restaurant meal samples for gluten, using approved laboratory methodology.
4. To find out if knowledge and service levels differ between listed 'coeliac-friendly' restaurants and those which are not listed.
5. To identify measures that will improve the awareness of coeliac condition in the catering industry.

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Results

Sampling location

The survey was carried out in the Republic of Ireland (ROI) and Northern Ireland (NI). We sampled 248 premises in HSE Dublin/Mid Leinster (61), HSE Dublin/North East (51), HSE Southern (34), HSE Western (54) and the Local Councils of Northern Ireland (48). Samples were taken in 23 counties including Cavan, Cork, Donegal, Dublin, Galway, Kildare, Kilkenny, Laois, Limerick, Mayo, Meath, Roscommon, Sligo, Waterford, Westmeath, Wexford, and Wicklow in the ROI and counties Antrim, Armagh, Derry, Down, Fermanagh, and Tyrone in NI.

In the ROI, samples were taken by 29 student EHOs, one EHO and one Assistant PEHO located in 23 EH local offices. In NI, 26 EHOs participated in the sampling, representing 18 District Councils.

Samples

260 samples were analysed for gluten content (one purchase included three separate samples). Sampling took place between 10am and 7:50pm, with most samples being taken during the lunchtime period, 12pm to 2pm. Sampling took place between 5 June and 3 August 2009.

Of the 257 samples for which a description was given, most were main courses but starters, desserts, and snack foods were also sampled.

Eighty per cent (204) of samples were described as the sampler's 'first choice'. Where the first choice was unsuitable, the sample was usually the second or third choice.

The types of premises sampled were: bakery (3), cafe / deli (49), domestic fast food restaurant (2), international fast food restaurant (1), hotel (49), pub (36), restaurant (117), and one unknown. These were further categorised as staff canteen (1), training college restaurant (1), bakery (1), chain restaurant (17), ethnic restaurant (24), hotel (53), and independent restaurant (159). The ethnic restaurants were Chinese (11), Asian (1), Indian (4), Italian (3), Greek (2), French (1), Japanese (1), and Thai (1).

Most samplings involved one or two staff members (occasionally three and rarely four) with approximately half of all samplings involving the manager or chef. In approximately 40 per cent of samples, a member of staff consulted with the manager or chef. Of the 251 responses which included this information, 32 per cent of samplers interacted with servers, 25 per cent interacted with the manager and/or chef, and 43 per cent interacted with server and manager and/or chef.

The 260 samples were analysed for gluten content at the Western Region Public Analyst's Laboratory. This laboratory is accredited by INAB

for gluten analysis by the R5 ELISA. Each sample was accompanied by a questionnaire that had been completed by the sampler immediately after purchase.

Analytical results

We graded our samples against the gluten limits currently used by the Western Region Public Analyst's Laboratory. These limits are: a maximum of 20 mg/kg gluten in products that would be naturally gluten-free (e.g. products containing no wheat or other triticum species such as spelt, durum, rye, barley, or their crossbred varieties), and a maximum of 200 mg/kg in products containing ingredients from wheat, rye, barley, oats, spelt, or their crossbred varieties, which have been rendered gluten-free through processing. These limits are based on Codex Alimentarius Alinorm 97/26⁵. The revised Codex gluten-free standard (July 2008) doesn't apply until 1 January 2012, which precludes the application of the new limits in this case.

Of the 260 samples analysed, 219 (84 per cent) did not contain quantifiable levels of gluten (see Table 1). Of the 41 (16 per cent) samples that did, 14 were within the range of 10 -20 mg/kg, 7 samples had levels in the range of 21-100 mg/kg, and three samples had levels in the range of 100 - 200 mg/kg. This means that 243 samples had a gluten content of less than 200 mg/kg. Three samples had gluten levels in the range of 200 - 1000 mg/kg, while 14 samples had gluten levels in excess of 1000 mg/kg, with one sample at 6,740 mg/kg. All but one of these samples were prepared meals (one sample was a 'fruit & nut bar' with a detected gluten level of 1820 mg/kg: this sample was subsequently followed up by the EHS).

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Table 1 Gluten content of the samples

Gluten content (mg/kg)	Number of samples	Percentage of total
< 10	219	84.2%
10 – 20	14	5.4%
21 – 100	7	2.7%
101 – 200	3	1.2%
201 – 1000	3	1.2%
> 1000	14	5.4%
Total	260	

Gluten-free samples (< 10 mg/kg)

The 219 (84 per cent) samples that did not contain detectable levels of gluten were purchased in 218 establishments. Ninety-two per cent of staff at these premises were knowledgeable about coeliac condition, and practically all of them were knowledgeable about gluten-containing foods (Table 2). Approximately half of those who had no knowledge of the coeliac condition showed an awareness of the foods that contained gluten and most consulted with the manager or chef. Around 11 per cent of those who sold gluten-free samples were hesitant in interacting with the researcher, and most staff (more than 70 per cent) sought assistance from the chef or manager. This was probably because they were being cautious about the gluten-free food recommendation, but it might also reflect trepidation about being involved in this research.

Most of the samples (80 per cent) were the sampler's first choice. Most samples involved interaction with one or two members of staff, and 51 per cent involved interaction with the manager and/or the chef.

Table 2 Qualitative evaluation of staff interaction during the purchase of samples

	Gluten-free samples (<10mg/kg)	Gluten-positive samples (satisfactory & unsatisfactory) (11 – 6,740 mg/kg)	Unsatisfactory Gluten-positive samples (> 200 mg/kg)
Prevalence of samples	84%	16%	6.5%
Staff knowledgeable about coeliac condition	92%	85%	82%
Staff knowledgeable about gluten-containing foods	92%	85%	76%
Staff consulted with the manager or chef where a lack of knowledge was evident	Majority	Majority	Majority
Staff hesitant during interaction with sampler	11%	27%	24%
The sample was the first choice of sampler	80%	73%	71%
The sampler interacted with 1 or 2 members of staff	Majority	Majority	Majority
The manager and/or the chef were involved in the sampling	51%	60%	65%
The premises had notices/signs relating to the coeliac condition or gluten-free meals	30%	32%	18%
The staff had been given advice/training on this issue	37%	34%	41%
Difficulty in sampling due to language problems	No	No	No

Thirty per cent of the establishments that we tested had notices about coeliac requirements and their gluten-free options. These were mostly menu notices or gluten-free menu choices. In establishments without notices, some indicated that they would make food to order and others had a note on the menu asking customers to alert staff about specific dietary requirements. Approximately 37 per cent of premises that sold gluten-free samples indicated they had received some form of advice or training on dealing with requests from coeliac or allergic customers. This advice/training was varied and included: (1) in-house training from the chef; (2) instruction to consult with the chef; and (3) training of the head chef or manager. Generally there was no formal training for staff. Communication problems due to language difficulties were not an issue.

Gluten-positive samples (11 – 6,740 mg/kg)

In total, 41 gluten-positive samples (16 per cent) were recorded (Table 3). These were purchased in restaurants (including ethnic), hotels, a staff canteen, and a college canteen. They were mostly main course samples, but included starter and dessert samples as well. Most of the gluten-positive samples (73 per cent) had been the first choice of our samplers. They had involved interaction with at least one member of staff – over 60 per cent involved interaction with the manager and/or the chef (see Table 2). In 85 per cent of cases, our samplers said that staff had awareness of the issues of gluten-free and coeliac condition. In 27 per cent of establishments, staff were hesitant or doubtful about recommending a suitable meal and most consulted with the chef or manager. The majority (68 per cent) of these establishments did not have any notices or signs about their gluten-free choices. In 66 per cent of premises, the staff had not received training or advice on this issue. Language difficulty was a problem in just four (10 per cent) of these establishments.

Approximately 37 per cent of premises that sold gluten-free samples indicated they had received some form of advice or training on dealing with requests from coeliac or allergic customers.

‘Unsatisfactory’, gluten-positive samples (> 200 mg/kg)

While 41 samples were gluten-positive, 17 (6.5 per cent) had ‘unsatisfactory’ levels of gluten. Sixteen of these were purchased in ROI (see Table 3). Six were purchased in hotels, ten in restaurants, and one in a staff canteen. They were mostly main course samples, but included starter and dessert samples as well. Most of the samples (71 per cent) were our samplers’ first choice (see Table 2). The majority involved interaction with one or two members of staff and over 65 per cent of samplings involved the manager and/or the chef. Samplers reported that staff had knowledge or awareness of the issues of gluten-free (76 per cent) and coeliac condition (82 per cent). Only in 24 per cent of establishments did they show any hesitation or doubt about recommending a suitable meal. Interestingly, the chef or manager was consulted in just 35 per cent of establishments where the staff were hesitant. The majority (82 per cent) of establishments did not have any notices or signs concerning gluten-free-food. In 59 per cent of cases the staff had not received training or advice on this issue. Twelve per cent indicated that the chefs had received training. Language difficulty was a problem in just one case.

Table 3 Food samples with detectable levels of gluten

Food sampled	[Gluten] mg/kg	Satisfactory?
Cajun salad	10	Yes
Greek minced lamb steak	11	Yes
Chicken stir-fry with mixed vegetables	12	Yes
Coeliac pizza	13	Yes
Fried squid	13	Yes
Gluten-free toastie	15	Yes
Apple pie	16	Yes
Penne pasta with pesto and parmesan	16	Yes
Seafood chowder	18	Yes
Chicken stir fry + rice	18	Yes
Prawn pil-dil, prawn platter served in sizzling garlic and chilli oil baked bread	18	Yes
Marinated chilli chicken with wok fried vegetables	18	Yes
Queen cake	18	Yes
Vegan special gluten-free sandwich	20	Yes
Mince pie, mixed salad, wheaten bread	38	Yes
Chocolate meringue finished with various nuts	46	Yes
Spicy pumpkin soup	48	Yes
Beef stroganoff	57	Yes
Subji pakoora – ground flour, spices, vegetables, deep fried	67	Yes
Steak ribs with smoked hickory + maple honey dressing	87	Yes
Vegetable soup – gluten-free	95	Yes
Orange + almond cake	190	Yes
Chicken kebab skewers on curried rice	200	Yes
Terrain of paté	200	Yes
Cajun spiced chicken with a honey dressing with onions	300	No
Chicken curry	750	No
Chicken curry & rice	820	No

Cajun chicken baguette	1050	No
Chowder (seafood)	1290	No
Chicken curry and boiled rice	1520	No
Bolognaise	1620	No
Chocolate cranberry fruit & nut bar	1820	No
Doner kebab without the wrap	3180	No
Gluten-free gravy	3830	No
Chicken curry	5220	No
Cous cous	6120	No
Atlantic seafood chowder	6390	No
Ham burger	>6646*	No
Mini breakfast	>6681*	No
Lemon cheesecake with biscuit base	>6708*	No
Chicken stir fry with noodles	6740	No

* These samples yielded extracts with gluten levels above the normal calibration range.

Gluten-free sample choices

Twenty-two samples (8.5 per cent of the total) were described as 'gluten-free' on the menus or boards that were presented to the samplers (see Table 4). Four of these (18 per cent) contained gluten at satisfactory levels. The sample of 'gluten-free' gravy contained a very high level of gluten, potentially enough to cause acute illness in a coeliac customer (depending on the quantity consumed).

Table 4 Gluten content of identifiably 'gluten-free' sample choices

Menu choice	Gluten [mg/kg]
Gluten-free chocolate brownie	<10
Gluten-free pea + ham soup	<10
Shepherd's pie – gluten-free gravy used, mince, vegetables and potatoes.	<10
Arabiatta gluten-free pasta, tomato sauce with gluten-free paste	<10
Beef, potatoes + gluten-free gravy.	<10
Gluten-free, wheat free chocolate macaroon	<10
Gluten-free bread	<10
Gluten-free bagel with cream cheese	<10
Potatoes & gluten-free gravy	<10
Salmon with gluten-free white sauce	<10
Roast beef dinner – gluten-free	<10
Salmon gluten-free dinner	<10
Tuna salad gluten-free	<10
Steak with gluten-free pepper sauce	<10
Coeliac muffin	<10
Gluten-free vegetable soup	<10
Gluten-free soup	<10
Coeliac pizza	13
Gluten-free toastie	15
Vegan special gluten-free sandwich	20
Vegetable soup – gluten-free	95
Gluten-free gravy	3830

Coeliac-friendly restaurants

The Coeliac Society of Ireland supplied us with a list of 360 coeliac-friendly restaurants, as recommended by its members. The Society follows up each recommendation, sending the restaurant information including the Society's catering booklet and a reference sheet of gluten-free products. A list of 14 restaurants was obtained from Coeliac UK for Northern Ireland. Consulting these lists, we surveyed 41 coeliac-friendly restaurants in the ROI and four in NI (see Table 5): that is 20.5 per cent and 9.8 per cent, respectively, of all of the restaurants that we sampled. Four 'coeliac-friendly' restaurants provided samples that contained detectable levels of gluten, and two of these samples (4.4 per cent) contained more than 200 mg/kg. Compare this to the 7.0 per cent rate (15/215) for non-listed restaurants and 6.5 per cent (17/260) overall. The samples – a chicken curry with boiled rice, and a chocolate cranberry fruit & nut bar – had a gluten content of 1520 and 1820 mg/kg, respectively.

Four 'coeliac-friendly' restaurants provided samples that contained detectable levels of gluten.



Table 5 Gluten content of samples purchased in coeliac-friendly restaurants

Sample	[Gluten] mg/kg
Tomato and basil soup	<10
Cream of wild mushroom soup	<10
Shepherd's pie	<10
Chicken curry and rice	<10
Passion fruit pavlova	<10
Panfried scallops	<10
Macaroon biscuit	<10
Stuffed aubergine with garnish and cheese	<10
Pasta with tomato and basil sauce	<10
Deep fried chicken in Peking sauce with fried rice	<10
Pizza	<10
Mussels a la marinere	<10
Shepherds pie	<10
Creme brulée with almond bread	<10
Traditional Irish stew, beef with carrots. Potatoes and juice	<10
Salmon	<10
Gluten-free, wheat free chocolate macaroon	<10
Potatoes & gluten-free gravy	<10
Chicken curry	<10
Almond and orange cake/ brownie – flourless	<10
Tapas Spanish cheese with slices of beef, tomato and extra virgin olive oil	<10
Leak & potato soup	<10
Vegetable soup	<10
Chocolate roulade	<10
Roast beef dinner – gluten-free	<10
Fish in a white onion sauce	<10
Yellow, Thai curry	<10
Chocolate sponge cake	<10
Seafood- fillet of salmon with a lime & basil glaze & citrus herb cream	<10

Warm chicken & bacon salad	<10
Vegetable soup	<10
Soup and bread	<10
Humus	<10
Chicken with Pekinese sauce	<10
Stuffed peppers + cheese with rice and salad	<10
Pollo diavola chargrilled chicken with pepper spicy tomato and garlic sauce in salad	<10
Seafood pie – taste a memory foods	<10
Bacon and cabbage with parsley sauce and creamed potato	<10
Vanilla panna cotta	<10
Sandwich-salad, chicken, cucumber & tomato	<10
Chicken skewers	<10
Marinated chilli chicken with wok fried vegetables	18
Chocolate meringue finished with various nuts	46
Chicken curry and boiled rice	1520
Chocolate cranberry fruit & nut bar	1820



2

Discussion

Most of the restaurants that we surveyed demonstrated the competence and capacity to provide a gluten-free meal on request. Ninety-three and a half per cent of samples contained less than 200 mg/kg of gluten, which meets the current legal 'gluten-free' standard. We do not know whether the gluten-containing samples were the result of an attempt to produce a gluten-free version of an existing menu option, or a pre-existing in-house gluten-free menu option. Either way, the gluten-containing options present a risk to coeliac customers.

The three samples (1.2 per cent) that contained a moderate to high level of gluten (300 -820 mg/kg) carry a greatly increased risk for coeliacs. Given the nature of these foods – cajun spiced chicken, chicken curry, rice – it seems that the gluten content was probably caused by cross-contamination with (most likely) wheat flour during preparation.

This could have been caused by the use of wheat flour in the food preparation area, or by the inadequate cleaning of bowls or utensils. However, foods with higher contamination levels are more likely to include a gluten-containing ingredient, of which the preparer is unaware.

Fourteen samples had gluten levels in excess of 1000 mg/kg, with one sample at 6,740 mg/

kg. The risk of illness, and in particular acute gastrointestinal distress, increases significantly in those foods with a gluten content of more than 1000 mg/kg. It is unlikely that cross-contamination alone would produce such high gluten levels in these samples. We can speculate on the gluten-containing ingredients from the meal descriptions: wheat-based baguette, burger-buns, cheesecake biscuit base, noodles, pasta and cous-cous, wheat flour used to thicken chowder, sauces or gravy (despite the assertion that this was gluten-free). As for the 24 positive but satisfactory samples which contained gluten in the range of 10- 198 mg/kg, some cross-contamination is evident.

We can associate the unsatisfactory samples with a low level of general knowledge concerning coeliac condition and gluten in foods. It may not be significant, but the servers' hesitancy doubled in places where our samplers purchased gluten-positive meals (both satisfactory and unsatisfactory), and the meal was more likely to have been a second or third choice. Interestingly, the staff who served the unsatisfactory gluten-positive samples were more likely to consult their manager and/or chef, and less likely to have notices on display about gluten-free options.

Levels of staff training were higher at the restaurants where we obtained unsatisfactory

samples, but the difference is too small for us to draw any conclusions. Worryingly, two unsatisfactory samples came from listed 'coeliac-friendly' restaurants. However, the failure rate in these restaurants was half of that in non-listed restaurants, and this is probably indicative of efforts to meet the dietary needs of coeliac customers. Language problems did not present a barrier in the overwhelming majority of samplings.

Conclusions

- In general, there is a good chance that a request for a gluten-free meal can be accommodated in restaurants. This is especially true in listed 'coeliac-friendly' restaurants.
- Staff rely on advice and input from their chef or manager to accommodate these requests.
- The likelihood of being served unsafe food does not seem to be linked to the level of staff training. However, it can be linked to the general knowledge and confidence shown by serving staff. Staff training can address this knowledge gap.
- Staff confidence should not be taken as a guarantee of gluten-free food. Even establishments that cater for coeliac customers can make mistakes.
- Notices/signs relating to coeliac condition or gluten-free meals were uncommon and, even if present, were no guarantee against an adverse outcome.
- The presence of gluten-free menu choices did not guarantee risk-free dining.

How can we address these issues?

We recommend:

- (a) improving the knowledge and awareness of chefs and managers,
- (b) supporting in-house staff training, and
- (c) making better use of signage.

Such measures should enable coeliacs to eat out more safely and enjoyably, reducing the risk of ill health and underpinning confidence in the ability of a restaurant to cater for coeliac needs. This is especially important for restaurants that wish to cater for coeliac customers.

3

Project Recommendations

1. All service staff should receive proper training. This should address food allergies and intolerance (including coeliac condition) and the control of food allergens in a catering setting. **safefood**, who promote best practice in food safety, should address this as part of its food allergy training programme*.
 2. Resource materials for the trade should be developed. These will contain information about hazard control practices for gluten and other allergens**.
 3. Establishments serving coeliac customers should ensure that their staff understand which foods contain gluten. Help is available, in the form of the (annually-updated) Gluten-free Catering Booklet, available from the Coeliac Society of Ireland.
 4. Openness of communication is vital. Caterers must communicate any doubts to their coeliac customer, who can then make an informed decision about their purchase.
 5. It is important for those with the coeliac condition to initiate communication and be clear about their requirements, even in establishments which are 'coeliac-friendly', or have gluten-free menu choices.
- * The **safefood** training programme in food allergen control 2007 – 2009 was implemented to address this need. Over 550 Environmental Health Officers and third level catering lecturers have been trained. EHOs have also been empowered to cascade best practice food allergen management to the catering industry during routine inspections and lectures to catering course students who will subsequently work in the industry.
- ** Safe Catering and other FSAI publications and **safefood**'s 'Food Allergy & Intolerance: Guidance for the catering industry' will contribute substantially to this requirement.

Appendix 1

Methodology for sampling meals for gluten content in a restaurant setting

The instructions given to our samplers

The purpose of this survey of catering staff is to determine (a) the extent of awareness and knowledge about the coeliac condition and (b) their ability to accurately respond to a request for a gluten-free meal.

Sampling will be conducted in catering establishments throughout Ireland. Wherever possible, 'coeliac-friendly' establishments should also be sampled.

A sample consists of one individual dish – starter, main course, or dessert, chosen from a menu.

Choice of establishment

- The establishment must be a restaurant.
- The restaurant can be non-ethnic or ethnic. Please choose an establishment where you would expect to purchase one of the dishes listed on the inventory of 'Dishes that may contain gluten either as a direct or hidden ingredient' (Appendix 3).
- The restaurant should not be contacted in advance of the visit.
- The restaurant should be visited as early as possible during the period in which it is open

for business for lunch or dinner and preferably before the clientele builds up.

- A single meal (dish) should be purchased in a given restaurant.

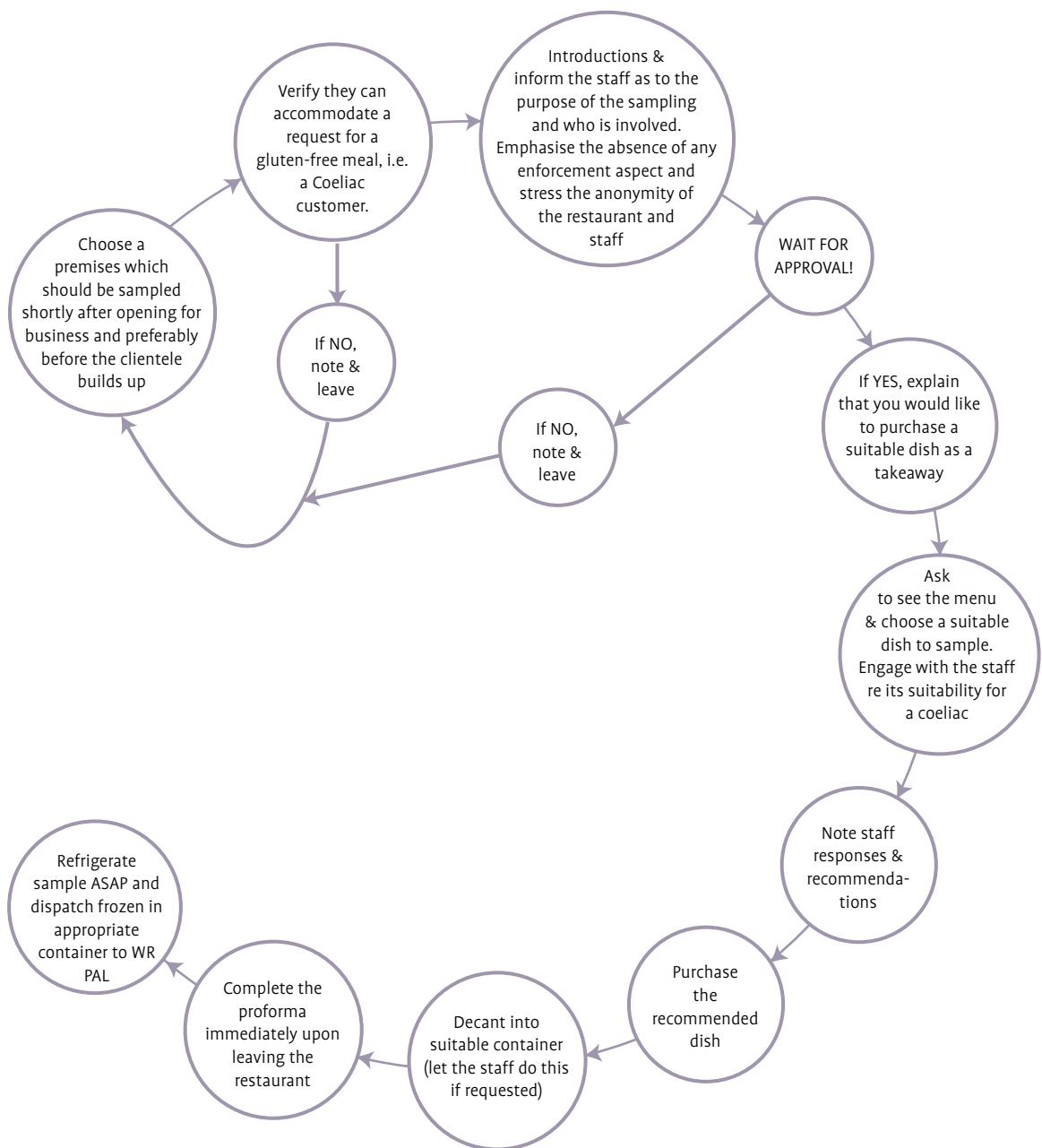
Sampling procedure

- On entering the premises, go directly to the counter (if there is one) or engage with the personnel. Note – do not ask to see the manager (this must be the prerogative of the staff member).
- At the outset, ask if they can cater for someone who has coeliac condition. If the response is 'No', discontinue the process. If the response is 'Yes', then proceed with the sampling. Whatever the response, it is vitally important to note the information you receive and the type of reaction you get.
- At this point, please introduce yourself and inform the staff that the sampling is part of a survey, being conducted by **safefood**, in conjunction with the HSE and the Coeliac Society of Ireland, to record the risk of exposure to gluten in a catering setting. The restaurant should be informed that the survey has no enforcement aspect and is purely to generate knowledge and find out if there is a need for education and training in the area of food sensitivity awareness. It must be stressed that the anonymity of the restaurant is guaranteed. Note – it is quite acceptable

for staff to contact the manager at this or any stage, if they wish to do so.

- Please explain that you would like to purchase a dish as a takeaway.
 - If the staff are willing to participate, ask to see the menu. If they are unwilling to participate, leave the premises and note the response on the questionnaire.
 - Choose a dish to sample. In order to test the restaurant's capabilities, choose a dish that is likely to contain gluten. Some dishes, such as plain meat and vegetable dishes that are not dressed, will definitely be gluten-free and therefore of no fundamental value to our survey. Staff may recommend a meal but judgement should be exercised before accepting their recommendation. Priority should be given to dishes that are not described as gluten-free on the menu, but which may be prepared as gluten-free by the restaurant. Appendix 3 can be used as a guideline for choosing a suitable dish which can be chosen from any section of the menu.
 - Ask if it is suitable in its current form for a coeliac or if it can be made suitable (the attached list of potential dishes to sample provides a guideline).
 - Advice on ingredients should not be given by you to the staff, except for the obvious – the need to avoid gluten-containing food such as bread (do not list the possible gluten-containing foods. This would defeat the purpose of the exercise).
- Note the response of the staff member(s).
 - Provide a suitable container in which to decant the dish. The staff may wish to do this behind the counter or in the kitchen and not in view of their other customers.
 - The dish should be paid for and the receipt kept. If the restaurant does not wish to receive payment, that is acceptable.
 - Ask the staff member(s) if they have received advice/training on how to deal with requests from coeliac and/or allergic customers.
 - Note any notices or signs relating to coeliac condition/gluten-free meals on display in the premises (including on the menu).
 - The accompanying questionnaire should be completed immediately upon leaving the restaurant to avoid recall bias.
 - Label the container appropriately.
 - The container should be immediately transferred to a cold storage unit and frozen prior to dispatch to Western Region Public Analyst's Laboratory together with the questionnaire (it is acceptable to store samples frozen until a batch of samples is obtained for transport to the laboratory).

Gluten sampling: proposed methodology



Appendix 2

Questionnaire

N.B. Please complete this form immediately after leaving the premises.

HSE, CSI, safefood: 2009

Sample reference number:.....

Cost (€ or £: please specify):

Samples collected by:
(name of sampling officer)

Reporting to:

Office address:

Office phone number:.....

Signature of sampler:.....

Samples collected at: (time) on (date)

Premises details

Sampling area:

Premises name:.....

Premises address:

Type of Restaurant (please tick one):

Non-Ethnic Ethnic (please specify)

Independent Hotel Chain Institutional

Other (please specify)

Sample details

Sample type: (as per menu).....

Sample description:.....
.....
.....

Was this the first choice asked for: Yes No

If No, then how many choices were made prior to this one?:
.....

How many staff members were involved in making the final recommendation?

Did you interact with the manager or the chef? Were these consulted or called for by other staff members?.....

Comments:.....
.....
.....

Did the staff show an awareness/knowledge of coeliac condition and the need for coeliacs to avoid gluten? Yes No

Comments:.....
.....
.....

Did the staff show an awareness/knowledge of the kind of foods that could contain gluten?

Yes

No

Comments:.....
.....
.....

Were the staff hesitant in any way? Did they express any doubt about the sample prior to the recommendation? Yes No

Comments:.....
.....
.....

Were there any notices or signs relating to coeliac condition/gluten-free meals on display in the premises (including on the menu)? Yes No

Comments:.....
.....
.....

Did the staff member(s) say if they had ever been given advice/training on how to deal with requests from coeliac and/or allergic customers? Yes No

Comments:.....
.....
.....

Was there any problem due to language difficulty? Yes No

Comments:.....
.....
.....

Any other comments:.....
.....
.....



Appendix 3

Dishes that may contain gluten, either as a direct or hidden ingredient. Note: Bland dishes, such as meat, two vegetables and potato (without gravy), should be avoided as it is highly unlikely that these will contain gluten and therefore pointless to sample. In order to choose a dish to be sampled, consider the possibility of gluten being present. Dishes marked with * will most certainly contain gluten (gluten-free versions may be available in coeliac friendly restaurants).

Starters:

- * All soups can contain gluten if thickened with flour.
- * Broths usually contain pearl barley which contains gluten.
- * Soups thickened with potato or cornflour can have croutons included.
- * Minestrone contains spaghetti. This can be of the gluten-free variety.
- * Dumplings in soups.
- * Anything with croutons (including salads)
- * Egg croquette
- * Tempura (Japanese dish of deep fried battered vegetables or seafood)

Salads with salami and assorted cooked or smoked sausages

Chicken wings

Fish mousse

Hash browns

Paté

Poppadums

Potato salad

Prawn cocktail

Spring rolls (made with rice paper). Gluten-free version)

Stuffed mushrooms

Sweetcorn fritters

Cheese fondue

Coeliac friendly restaurants may have gluten-free breads / pizzas / cakes / buns / biscuits / quiches / pasta dishes / pancakes / wraps on offer.

Despite assurances, these can be contaminated with gluten!

Vegetarian dishes include:

Bean & nut burgers
Bean goulash
Dishes with stir fried rice/fried rice
Gnocchi dishes – potato based
Lentil stews
Nut cutlets
Polenta/cornmeal – dishes – although gluten-free, dish can be contaminated
Potato cakes
Potato wedges
Potato/mushroom/vegetable gratins
Risotto
Stir fry vegetables
Tortillas – corn variety
Vegetable pies
Vegetables in sauce mixes
Rice noodles with sauces
Vegetable bakes
Mash – some restaurants can use instant mash
Spanish omelette

Also, dressings / sauces / mayonnaise / tartare sauce / horseradish sauce / mustard / soya sauce / stuffing /nut stuffing /rice stuffing / potato fillings/toppings/crumbles/dipping sauces / gravy can contain gluten.

Desserts:

* Tiramisu
Commercially made lemon curd and mincemeat usually contain gluten
Meringues
Soufflés
Fritters
Tortes (nut based cakes)
Roulades
Mousse
Cheese cakes – gluten-free variety
Macaroons
Fondues – fruit variety
Crepes – gluten-free variety
Pancakes – gluten-free variety
Ice cream cakes
Trifle – gluten-free variety
Flans – gluten-free variety
Fudge
Syllabub – liqueur/cream/macaroon type dessert

Main meals:

* Burgers – lamb/beef/vegetable

* Crumbed meat / fish

* Fried scampi

* Calamari (deep fried squid)

Barbecued pork

Barbecued spare ribs

Bean goulash

Beef madras

Beef olives

Beef stroganoff

Beef/lamb/chicken skewers with dipping sauce

Braised steaks

Cajun chicken

Carbonate of beef

Chicken á la king

Chicken sauté

Chicken tikka

Chilli con carne

Coq au Riesling – type of chicken stew

Cornish pasties

Cottage pie/shepherd's pie

Curried chicken

Curries

Deep fried chicken

Duckling in orange sauce

Fish cakes

Fish kedgeriee

Frankfurters

Fricassée of chicken

Fried liver

Fried potatoes/chips

Goujons gluten-free version

Goulash

Gram/buckwheat – dishes – although gluten-free, dish can be contaminated

Kebabs

Lasagne dishes – gluten-free lasagne uses lasagne sheets made with buckwheat

Meat / vegetable pies – potato toppings

Meat balls

Meat loaf

Moussaka

Nasi goreng (rice dish with chicken, bacon and soya sauce)

Paella

Pakora – chick pea deep fried snack

Pan fried fish can be dusted in flour

Pasta dishes – gluten-free variety

Ragu – type of stew

Rendang – beef curry

Stews

Stir fries – meats/fish/chicken etc

Stuffed peppers

Sweet & sour dishes

Tagine – type of stew

Tandoori chicken/prawns

Terrines

Tonkatsu – deep fried pork cutlet

Analytical methodology used in the analysis of food samples for gluten content

The analytical method chosen for this project is the R5 Elisa, which is based on a monoclonal antibody to the prolamins gliadin, secalin and hordein (from wheat, rye & barley respectively). The R5 antibody shows no cross-reactivity to the prolamins from oats (avenin), corn (zein) and rice. The R5 antibody detects the coeliac-toxic pentapeptide QQQFP, which occurs repetitively in the toxic prolamins. The R5 Elisa (which is now available commercially from a number of laboratory supply companies) is the analytical method accepted by both Codex Alimentarius (118-1979:2008) and the European Union (Regulation (EC) 41/2009) as the method for the control of gluten in gluten-free foods. Allied to the use of the R5 Elisa was the use of the Mendez cocktail extraction solution and method, which is the recommended method for the analysis of heat-treated foods for gliadin/gluten, because it is the best method for 'unravelling' the complex 3-dimensional structures that heat-treated proteins can assume. This analysis involved the homogenisation of the complete meal as received (e.g. all meat, vegetables and any sauces were homogenised together). This was done to mimic the consumption of the whole 'gluten-free' meal as it would have been served in the restaurant. (A slight disadvantage of this approach was that we could not, in the event of a high gluten result for a meal, ascertain which component contained gluten). Once the homogenised sample had been extracted using the Mendez cocktail method, it was first screened for the presence of gluten using the R5 Elisa. Any samples that gave a positive result were then subjected to quantitative confirmatory analysis, again using the R5 Elisa with the Mendez cocktail extraction method. All results were reported back to the submitter (via their Principal EHO in the case of samples submitted in the Republic of Ireland).

Appendix 4

General precautions which a person with coeliac condition must take in a restaurant setting

- Self service salad bars: The serving spoon will probably be moved from dish to dish with the potential for cross contamination, e.g. from a couscous or pasta salad.
- Removing batter from fish does not make it safe to eat.
- Fried rice often has soya sauce in it which is a source of gluten.
- Avoid deep fried foods (if anything breaded was cooked in the same oil your food will be contaminated).
- Carvery meats – ensure there is no stuffing in the joint or that the coating on the baked ham is gluten-free.
- Chips – check that the oil is not used to cook anything other than the chips. Some restaurants cook chips in clean oil but then refresh them before serving in oil that is used to cook breaded products.
- Ask if the meat has been pre-marinated (if so avoid it).
- Check if stock cubes are used in the soup, sauce or to boil the rice.
- Check if salad dressings are homemade or from a bottle.
- If your salad arrives with croutons ensure a fresh crouton-free salad is prepared, it is not safe to just remove the croutons from the plate.
- Remind the waiter not to put croutons on your salad, biscuit on your crème brulee or wafers on your ice-cream.
- Ask if the food has been coated in flour before frying.
- Find out if the mashed potatoes are from a mix.
- Check if a sauce is used to decorate the plate.
- Check that no soya sauce is used unless it is certified gluten-free.

Materials

The materials and methods used in the analysis for gluten used in this project were based on the requirements of the commercial Elisa kit used

1. r-biopharm RIDAScreen gliadin kit (A 96 well 'sandwich' ELISA, part no. R7001).
2. ELx 808 Universal microplate reader.
3. Round bottom 13 ml centrifuge tubes and standard 50 ml centrifuge tubes.
4. Centrifuges: Heraeus Sepatech Labofuge 200, or equivalent for 15 ml tubes.
IEC Centra CL2, or equivalent for 50 ml tubes.
5. Food homogenisation equipment: Foss Cyclotec mill.
Industrial blender – Foss Tecator.
Food Processor/Jug blender.
Coffee Blender – IKA A11 basic.
6. Graduated Class A pipettes.

7. Fixed & variable volume micropipettes.
8. 50-300 µl multi-channel pipette.
9. Biotek Elx 50 AutoStripwasher.
10. Stop watch
11. Sample mixer – Stuart Rotator SB3 or equivalent.
12. Weighing balance accurate to four decimal places.
13. Incubator – Grant OLS200 water bath set to 50 °C.
14. Vortex mixer– IKA MS2 Minishaker.
15. ‘Mendez’ cocktail solution (R-BioPharm Part Number R7006)
16. 80 per cent absolute ethanol
17. Distilled water
18. For food samples containing chocolate, coffee, cocoa or tannin: use fish gelatin solution:

To 60 per cent (v/v) ethanol p.a. in water add 10 per cent (w/v) fish gelatin (Sigma, product No. G-7765) and four per cent (w/v) polyvinylpyrrolidone (Sigma, product No. PVP 10). Dissolve fish gelatin and polyvinylpyrrolidone in ethanol (60 per cent) and store at room temperature. Mix well before use. & discard after three weeks.

Sample preparation

Frozen samples should be thawed-out at room-temperature before homogenisation.

Refrigerated samples can be homogenised directly:-

1. All samples must be homogenised to give an even and fully homogeneous preparation, the appropriate milling system must be used and every precaution taken to ensure that the mill used is fully cleaned to be absolutely certain that there is no carry-over from one sample to the next.
2. Approx. 0.2 (± 0.02) g (for meat products/ mueslis/other samples with a possible uneven gluten distribution one (± 0.02) g of sample was used) of sample was weighed accurately into a round bottom 13 ml centrifuge tube. The weight of sample taken was recorded to four decimal places. Clean Spatula with 80 per cent ethanol after each use.
3. 2.0 ml of the cocktail solution was added (10 ml where 1g sample taken).
4. The cocktail solution/sample mix was mixed thoroughly using a vortex mixer to ensure even sample dispersed in the liquid.
5. The cocktail solution/sample mix was incubated at 50 (± 2.0) °C for 40.0 (± 4.0) mins in a water bath and then allowed to cool to room temperature.
 - a. 6.0 ml (30 ml where 1g sample was taken) of 80 per cent ethanol was added and mix thoroughly using a vortex mixer to ensure sample is dispersed in the liquid.
 - b. Where tannin containing samples were being analysed (Materials 18, above) 3.0 ml of the fish gelatin solution and 3.0 ml 100 per cent ethanol were added to the sample solution

6. The solutions were placed on the sample mixer (25 – 30 rpm) for 60 (\pm 2.0) mins at room temperature.
7. The solutions were centrifuged for 10 (\pm 1.0) mins at 2,500 rpm in a suitable centrifuge and the supernatant decanted into a glass container.

(Note: The supernatant obtained after the centrifugation can be stored in the dark at room temperature for up to four weeks. If stored in the fridge, ensure it is brought up to room temperature before use).

Sample Analysis

All reagents were brought to room temperature before use and returned to 2 – 8°C immediately after use. Microwells were not allowed to dry between working steps and direct sunlight was avoided during all incubations, i.e. the microtiter plate was kept covered at all times.

1. The antibody enzyme conjugate was reconstituted (only the amount which actually was needed was diluted), before pipetting, the concentrate was shaken carefully. For reconstitution, the concentrate was diluted one in 11 with distilled water.
2. Washing buffer is provided as a 10X concentrate. Before use, the concentrate was allowed to equilibrate at room temperature to dissolve any crystals that may have formed in the solution (it can be heated to speed up this process). The buffer was reconstituted with distilled water.
3. The sample dilution buffer was provided as a concentrate (5 X). It was reconstituted with distilled water.
4. The supernatant was diluted (sample preparation VIII above) 1 in 21, mixed well and 100 μ l of this solution was used per well in the test. (For some samples further dilution may have been necessary to bring the samples within the range of the standards. In these cases the sample was diluted as appropriate with the sample dilution buffer).
5. A sufficient number of wells were inserted into the microwell holder for all standards and samples to be run in duplicate.
6. All the sample and standard solutions were mixed before starting the assay. 100 μ l of each standard solution or prepared sample was added to separate duplicate wells.
7. The microtitre plate was covered and incubated for 30 min at room temperature.
8. Unbound components were washed off the plate using the Elx50 Auto Strip Washer.
9. 100 μ l of the diluted enzyme conjugate was added to each well.
10. The well contents were mixed thoroughly by rotating the plate while it was held flat on a flat surface.
11. The microtitre plate was covered and incubated for 30 minutes at room temperature.
12. Unbound conjugate was washed off the plate using the Elx50 Auto Strip Washer.
13. Equal volumes of the substrate and chromogen were mixed together and 100 μ l of the mixture was added to each well.
14. The well contents were mixed thoroughly by rotating the plate while it was held flat on a flat surface.

15. The microtitre plate was covered and incubated for 30 minutes at room temperature.
16. 100 µl of the stop reagent was added to each well.
17. The plate was mixed well and the absorbance was measured at 450 nm against an air blank on the automated microplate reader. Each plate was read within 60 minutes following the addition of stop solution.
18. A standard curve of absorbance (the dependent variable – plotted on the y-axis) versus the concentration of the standard solutions analysed (the independent variable – plotted on the x-axis) was drawn and the concentration (in ppb) of each unknown sample was read from this graph.
19. Using information recorded for each sample (weight taken, dilutions used) the concentration of gliadin in each sample was calculated in mg/kg.
20. The gluten content of each sample was calculated by multiplying the gliadin result X2, this assumption is common practice in gluten analysis.
21. All internal quality control criteria were examined and it was ensured that all criteria were all within specification before a result from any analytical batch was accepted. Internal QC included the use of reference materials (negative for gluten and with a known concentration of gluten). The laboratory has also successfully participated in the FAPAS inter-laboratory proficiency testing scheme for many years.

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