Measurement of gluten using a monoclonal antibody to a coeliac toxic peptide of A gliadin

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Abstract
Background—Future European Community regulations will require a sensitive and specific assay for measurement of coeliac toxic gluten proteins in foods marketed as gluten-free. To avoid spurious cross reactions with non-toxic proteins, specific antibodies and target antigens are required. A synthetic 19 amino acid peptide of A gliadin has been shown to cause deterioration in the morphology of small intestinal biopsy specimens of coeliac patients in remission.
Aims—To develop an assay for detection of gluten in foods, based on measurement of a known toxic peptide.
Methods—A monoclonal antibody raised against the toxic A gliadin peptide, with a polyclonal anti-unfractionated gliadin capture antibody, was used to develop a double sandwich enzyme linked immunosorbent assay (ELISA) for the measurement of gluten in foods.
Results—Standard curves for gliadin and for rye, barley, and oat prolamins were produced. The sensitivity of the assay was 4 ng/ml of gliadin, 500 ng/ml for rye prolamins, and 1000 ng/ml for oat and barley prolamins. The assay could detect gluten in cooked foods, although at reduced sensitivity. Prolamins from coeliac non-toxic rice, maize, millet, and sorghum did not cross react in the assay. A variety of commercially available gluten-free foods were analysed; small quantities of gluten were detected in some products.
Conclusion—The assay may form the basis of a sensitive method for measurement of gluten in foods for consumption by patients with coeliac disease.
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Keywords: gluten; gliadin; prolamin; coeliac disease; monoclonal antibodies

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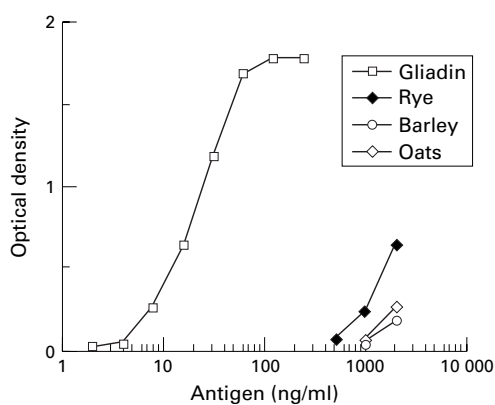Accepted for publication 18 February 1998

Coeliac disease is a permanent intolerance to the ethanol soluble storage proteins or prolamins in wheat, rye, barley, and possibly oats. The condition is treated by a gluten-free diet, with the exclusion of these cereals. Commercially available foods for patients with coeliac disease are frequently based on wheat starch, which contains residual gluten.[1 2]

A strict gluten-free diet significantly reduces the risk of small bowel lymphoma which may complicate coeliac disease.[3] UK regulations state that foods labelled as gluten-free must contain less than 0.3% of protein derived from wheat, rye, barley, and oats, determined by Kjeldahl nitrogen.[4] The European Community will require measurement of the wheat prolamin, gliadin, rather than all wheat derived proteins, which include albumins, globulins, and starch granule proteins.[2] The permissible level is likely to be set at 10 mg gliadin/100 g product. However, progress has been hampered by lack of a suitable assay. The assay should be accurate, robust, simple, and capable of detecting all prolamins toxic to coeliac patients, but should not cross react with coeliac non-toxic cereals, such as maize. Polyclonal antibody based assays suffer a lack of specificity[5 6]; monoclonal based systems allow quantitation of coeliac toxic epitopes.

A 19 amino acid peptide of A gliadin (sequence: LGQQQPFPPQQPYPQPQPF) caused morphometric and cellular changes in the small intestinal mucosa of coeliac patients in remission.[7] In this study we raised monoclonal antibodies against this 19-mer peptide to establish an enzyme linked immunosorbent assay (ELISA) for measurement of those cereal prolamins which exacerbate coeliac disease.

Prolamins in cooked foods are difficult to measure due to the low extractability of all but the minor low sulphur (ω gliadin) component.[8] Pretreatment of cooked foods with a reducing agent to break interchain disulphide bonds formed during cooking would render soluble the major α and γ (sulphur containing) gliadins. We therefore assessed the effect of reducing agents in our assay.

Methods

PEPTIDE SYNTHESIS, AND POLYCLONAL AND MONOCLONAL ANTIBODY PRODUCTION
Peptide synthesis was performed as described previously.[7] In addition to native peptide, we produced alanine point substituted analogues of the coeliac activating 19-mer, which comprises amino acids 31–49 of A gliadin. Amino acids 31, 33, 36, 38, 39, 41, 42, 44, 46, and 48 were substituted. These peptides were biotinylated. Polyclonal rabbit antibodies to gliadin and the murine antipeptide monoclonals were produced as previously described.[9]

CONCENTRATION AND PURIFICATION OF ANTIBODIES
The IgG fraction of polyclonal rabbit antigliadin and monoclonal antipeptide antibody PN3, which was of the IgG1 subclass, were purified by 45% ammonium sulphate precipitation and made up to 0.3 and 0.1 of their original volumes respectively.

Figure 1 Standard curve for wheat gliadin and curves for prolamins of rye, barley, and oats.

PREPARATION OF ANTIGENS
Gliadins
Whole gliadin and its α, β, γ, and ω subfractions were prepared as described previously from Kolibri wheat flour.[10] The subfractions did not however conform to the classification proposed by Autran *et al*,[11] based on N-terminal amino acid sequences. Rector strain gliadin was provided by Dr H Wieser, and was prepared as described, except that 60% ethanol was used for extraction instead of 70%.[12] The standard gliadin preparation was also obtained from a commercial kit for measurement of gluten (Transia Ltd, Lyon, France). No information is given by the manufacturers as to the strain of wheat used. Forty per cent ethanolic solutions of each gliadin standard were prepared at 1 mg/ml. These were then further diluted in water to 100 µg/ml. Solutions were centrifuged at 6500 *g* for 10 minutes to precipitate any gliadin which might have come out of solution following lowering of the ethanol concentration. Solutions were then analysed by the method of Lowry *et al*,[13] against bovine serum albumin (BSA) as a standard, and by absorbance at 280 nm under ultraviolet light. Standard solutions of gliadin 2000 ng/ml, according to the Lowry reaction, were made up in 5% BSA with 0.1% sodium azide and stored in aliquots at −20°C.

Other prolamins
The flours of rye (variety Rheidol), barley (variety Porter), and oats (variety Peniarth) were obtained from Plant Breeding International, Trumpington, Cambridge. Prolamins were extracted at 50 mg/ml in 50% ethanol by mixing for two hours at room temperature. Additionally the pure prolamins from oats (variety Alfred) were kindly supplied by Dr H Wieser.[14]

Table 1 Sensitivity of the assay to coeliac toxic prolamins

Protein	Sensitivity	Equivalent in flour
Gliadin	4 ng/ml	0.008 mg/100 g
α gliadin	4 ng/ml	0.008 mg/100 g
β gliadin	16 ng/ml	0.032 mg/100 g
γ gliadin	16 ng/ml	0.032 mg/100 g
ω gliadin	1000 ng/ml	2 mg/100 g
Rye	500 ng/ml	1 mg/100 g
Barley	1000 ng/ml	2 mg/100 g
Oats	1000 ng/ml	2 mg/100 g

Polymeric prolamins
Low molecular weight glutenins were obtained from Dr P Payne at Plant Breeding International.[15] These were similarly made into solutions of 2000 ng/ml Lowry positive protein and stored as above.

PREPARATION OF FOODSTUFFS
Wheat starches—Eight wheat starches were analysed, of which four were commercial brands (Nutregen, Wheatex, Glutafin, and Kinderm). Four were experimental starches obtained from the manufacturer of the Juvela brand (AB Semper, Stockholm) who provided Kjeldahl nitrogen analysis, from which we calculated total protein by using the factor 5.7.

Non-gluten foods—The following foods frequently used as additives and substitutes in gluten-free foods were tested in the assay: dried skimmed milk powder (Premier Brands, Staffs); sugar beet bran (Nutricia Dietary Products), soya flour (Community Foods, Brent Cross, London), chick pea flour (Ethos Health Foods, Nottingham), and gluten-free maize flour (Cantassium Food Company, Putney). Whole grains of rice, millet, and sorghum were kindly donated by Dr P Janssen of the regional food inspection service, Zuppen, The Netherlands. Pure grains of 15 varieties of wheat were obtained from AB Semper (Oslo). These were milled using a pestle and mortar.

Gluten-free products—At the request of Nutricia Dietary Products (Trowbridge, Wiltshire), one of the leading UK manufactures of gluten-free products, we analysed a variety of cooked and uncooked gluten-free products. A number of foods produced by a smaller company, Dr Schar (Postal, Italy) were also tested.

Cooked flour products—A test flour was spiked with 1 g/100 g of Rector strain gliadin standard, formed into a dough, and baked at 230°C for 10 minutes. The loaf was separated into the crust and crumb fractions. These fractions were provided by Dr H Wieser.

All the above samples were extracted by stirring at 50 mg/ml in 40% ethanol for one hour at room temperature, after twice defatting the whole wheat products with four volumes of *n*-butanol. Additionally, a reducing buffer was prepared containing 50% aqueous propan-1-ol, 1% β mercaptoethanol, Tris-HCl 0.08 mol/l (pH 7.5), and 2 mol/l urea. Cooked crust and crumb fractions were extracted in this buffer for one hour at 60°C under nitrogen. A set of gliadin standards was made up in a reducing buffer diluted 100-fold in phosphate buffered saline (PBS)/Tween.

DOUBLE SANDWICH ELISA
A double sandwich ELISA was performed using a modification of our previous method.[16] Briefly, polyclonal rabbit anti-unfractionated gliadin (50 µg/ml) acted as a capture antibody. Standard curves were constructed for wheat gliadin, range 1–250 ng/ml, and for rye secalin, barley hordein, and oat avenin over a range 1–2000 ng/ml. Extracts of food samples were diluted depending on the expected gliadin content as follows: neat and diluted 1/10 in

PBS/Tween, for samples of expected gliadin content zero; diluted 1/5 to 1/160 for wheat starches and 1/2000 to 1/60 000 for whole wheats and breads. The samples and standards were incubated for one hour at room temperature, followed by murine PN3 monoclonal antibody, diluted 1/3000, for one hour and rabbit antimouse IgG conjugated to alkaline phosphatase diluted 1/300 (Sigma Chemicals, Poole, Dorset), for one hour. The substrate was *p*-nitrophenyl phosphate 1 mg/ml in diethanolamine buffer, pH 9.8, incubated for 30 minutes at room temperature. In a later set of experiments, incubation steps at all stages were reduced to 30 minutes.

Sensitivity—This was calculated as the lowest concentration of antigen which gave an optical density (OD) greater than 1.5 times the mean of six assay blanks (PBS/Tween in place of antigen). The amount of each prolamin detectable in a flour product was calculated with respect to the quantities of product extracted for use in this assay (50 mg/ml). If the sensitivity to a particular antigen was—for example, 15 ng/ml, then that quantity of antigen could be detected in 50 mg of product. The sensitivity is expressed as mg of antigen per 100 g of product.

Repeatability—This was assessed by measuring the same two wheat starch extracts on three separate ELISA plates, on the same day, in parallel experiments. The coefficient of variation was calculated as follows: standard deviation/mean × 100%.

Reproducibility—This was calculated by comparing three extracts of the two wheat starches, and measuring these on three consecutive days. The coefficient of variation was calculated as above. Both of these assays were performed using the shortened form of the assay—that is, 30 minute incubations at each stage.

EPITOPE IDENTIFICATION

ELISA plates were coated overnight with avidin 10 μg/ml (Sigma Chemicals). Dilution curves of biotinylated 19-mer peptide and biotinylated point substitutions were incubated, followed by monoclonal antibody PN3 (1/2500) The assay was developed as described above.

Results

MEASUREMENT OF GLIADIN BY DOUBLE SANDWICH ELISA

Sensitivity

The sensitivity for unfractionated gliadin was 4 ng/ml—that is, an absolute sensitivity of 200 pg or 0.008 mg/100 g flour (fig 1). Table 1 shows the sensitivity of the assay for various prolamin fractions. Both varieties of oat prolamins were detected in the assay.

Non-gluten foodstuffs

Extracts of rice, maize, millet, sorghum flours, and miscellaneous ingredients used in gluten-free foods all tested negative (see table 2).

Whole wheats

Fifteen varieties of wheat had total gliadin contents in the range 1.2 to 9 g/100 g of whole grains (table 3).

Wheat starches

All had detectable gliadin, ranging from 0.50 to 7.75 mg/100 g (tables 4 and 5).

Commercially available gluten free foods

Table 6 shows the gliadin content of gluten-free products. Values obtained range from 0 to 6.7 mg/100 g.

Cooked products

The gliadin content of whole flour was 4.16 g/100 g; that of the resultant crumb and crust, extracted with 40% ethanol, was 1.68 and 0.7 g/100 g, respectively—that is, 17% of the value for uncooked flour. Using reducing conditions, the gliadin content of the crumb was 2 g/100 g, and of the crust 1.2 g/100 g. Gliadin standards made up in a 100-fold dilution of reducing buffer produced an attenuated standard curve, such that a standard of gliadin content 62 ng/ml, appeared to contain only 16 ng/ml, a 74% reduction.

Standards

Figure 2 shows the standard curves obtained for gliadins of different varieties. Rector and commercial standard gliadins produced similar parallel curves to that obtained for Kolibri gliadin. A sample with optical density

Table 3 Gliadin content of various wheat varieties

Name of strain	Type of wheat	Gliadin content
Sverno	Soft spring	6.0 g/100 g
Ring	Soft spring	1.2 g/100 g
Dragon	Strong spring	6.0 g/100 g
Kadett	Strong spring	4.5 g/100 g
Rang	Soft spring	6.0 g/100 g
Draband	Soft spring	5.0 g/100 g
Pompe	Soft spring	4.0 g/100 g
Holme	Autumn strong	5.0 g/100 g
Starke	Autumn strong	5.0 g/100 g
Folke	Autumn soft	5.3 g/100 g
Hilder	Autumn soft	8.4 g/100 g
Portal	Autumn soft	9.0 g/100 g
Solid	Autumn soft	6.0 g/100 g
Kosack	Autumn soft	4.5 g/100 g
Dinkle	Spelt (*Triticum spelta*)	5.9 g/100 g

All wheat varieties have been used for food purposes. Strong wheats have been used for bread making, soft varieties for other bakery products. All except Dinkle are varieties of *Triticum aestivum*.

Table 4 Protein and gliadin content of experimental wheat starches (provided by AB Semper)

Starch	Protein Kjeldahl N×5.7	Gliadin mg/100 g (3 analyses)			Mean
A	0.1995%	0.10	0	1.60	0.57
B	0.1995%	1.12	0.76	2.70	1.52
C	0.2132%	2.40	2.80	6.00	3.73
D	0.2286%	7.70	7.70	7.00	7.40

Table 5 Gliadin content of commercial wheat starches

Trade name	Gliadin mg/100 g (2 analyses)		Mean
Kinderm	6.50	7.80	7.20
Glutafin	0.50	0.50	0.50
Wheatex	1.00	1.00	1.00
Nutregen	8.00	7.50	7.75

Table 6   The gliadin content of gluten-free food products

Product	Gliadin mg/100 g
Wheat starch based products	
Cooked foods*	
Glutafin GF fibre loaf	0.128
Glutafin GF white bread with soya	0.200
Loprofin white bread (with salt, canned)	0.16
Loprofin white bread (no added salt, canned)	0.12
Low protein dp chocolate chip cookies	0.24
Uncooked products	
Glutafin GF white mix	0.9
Glutafin GF fibre mix	0.9
Rite Diet low protein flour mix	0
Loprofin low protein mix	1.4
Wheat-free products	
Cooked products†	
Rite Diet GF chocolate digestive biscuits	0.184
Rite Diet GF custard creams	1.18
Glutafin GF tea biscuits	0.21
Glutafin GF crackers	0.144
Glutafin GF biscuits	0.35
Glutafin GF digestive biscuits	0.152
Loprofin orange flavour cream wafers	0.144
Rite Diet GF mince pies	1.28
Uncooked products‡	
Glutafin pasta spirals	6.7
Glutafin pasta spirals	0.56
Glutafin vermicelle	3.7
Glutafin vermicelle	0.07
Glutafin spaghetti	2.2
Glutafin spaghetti	5.44
Glutafin macaroni penne	3.6
Glutafin macaroni penne	0.3
Glutafin macaroni	0.5
Products made by Dr Schar (Italy): all wheat-free	
Uncooked products	
Penne	0
Pasta Alfabeto	0.028
Capelli d'Angello	0.014
Spaghetti	0
Pipette	0
Cooked products	
GF biscuits	1.6
Snack GF chocolate wafers with hazelnut	0
GF biscuits with chocolate	0.3
GF hazelnut wafers	0
GF cocoa wafers	0
GF vanilla wafers	0.6

* Other wheat starch containing cooked products listed below contained less than 0.1 mg/100 g: Loprofin chocolate chip cookies, Glutafin GF white loaf, Rite Diet GF white bread, Rite Diet GF fibre rolls, Rite Diet low protein white bread with added fibre, Loprofin LP white rolls, Loprofin LP loaf.

† Other wheat-free cooked products listed below contained less than 0.1 mg/100 g: Glutafin GF WF white rolls, Glutafin GF WF white loaf, Nutricia Erma WF white bread, Rite Diet GF ginger-nut cookies, Rite Diet GF chocolate chip cookies, Rite Diet GF bourbon, Loprofin crackers, Loprofin chocolate flavour cream biscuit, Loprofin vanilla flavour cream wafers, dp butterscotch cookies, Loprofin chocolate flavour cream wafers, GF dietary GF thin wafers, Glutafin magdalens, Glutafin chocolate snack, Zonnatura snack wafels, Zonnatura rijstwafels, Zonnatura carobean, Rijstwafels, Zonnatura puffed rice cereal, Glutafin chocolate cake, Rite Diet banana cake, Rite Diet date and walnut cake, Rite Diet lemon cake, Rite Diet GF Christmas pudding, Rite Diet GF rich fruit cake.

‡ Other wheat-free uncooked products listed below contained less than 0.1 mg/100 g: Glutafin tagliatelle, Glutafin GF multigrain fibre mix, Glutafin GF multigrain white mix, Rite Diet GF white bread mix, Rite Diet GF brown bread mix, Rite Diet GF flour mix, Rite Diet GF/WF baking powder, Loprofin egg replacer, Loprofin PKU milk, Sesame crunch, Loprofin tagliatelle, Loprofin spirals pasta, Zilversliesrijst, Quinoa, Gierst millet, Zonnatura linzen lentils.

1.0 would appear to have a gliadin content of 60 ng/ml against Rector gliadin, 70 ng/ml against Kolibri gliadin (used in our experiments), and 100 ng/ml against commercial standard.

Storage and stability
Storage of the gliadin standard solution for five years at −20°C led to a decrease in immunogenicity so that a sample with OD 1.0 would have an apparent gliadin content of 22 ng/ml when measured against the new standard, but 50 ng/ml against the old standard. Exposing the concentrated purified antibody solution to nil, one, and two freeze/thaw cycles did not lead to any decrease in activity.
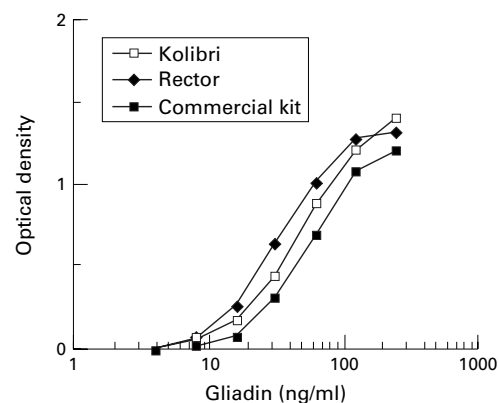

Figure 2   Standard curves for gliadin from three varieties of wheat.

Shorter incubation times
These did not decrease the sensitivity of the assay, which remained at 4 ng gliadin/ml.

Repeatability
Assays employed the shorter incubation times. Values for the first starch extract were 1.44, 1.20, and 1.28 mg/100 g (coefficient of variation 9.3%). Values for the second starch extract were 3.20, 3.04, and 3.60 mg/100 g (coefficient of variation 8.7%).

Reproducibility
Values for the first starch extract were 1.20, 1.66, and 1.73 mg/100 g (coefficient of variation 18.8%). Values for the second starch extract were 3.04, 4.80, and 4.30 mg/100 g (coefficient of variation 22.3%).

IDENTIFICATION OF THE EPITOPE RECOGNISED BY THE ANTIPEPTIDE MONOCLONAL ANTIBODY
Substitution of amino acids Q (33), P (36), and P (38) greatly reduced the binding of PN3 to native peptide, suggesting that the epitope of importance may be in the region of the sequence QQQPFP. Other substitutions had no effect.

Discussion
Our assay is 1000-fold more sensitive to gliadin than will be required for the proposed new WHO-Codex regulations. The similarity of the standard curves produced by the assay for three standard gliadin preparations, and the cross reaction of 15 strains of wheat flour suggest that the epitope measured is widely distributed among wheat gliadin varieties. The epitope in the region of QQQPFP, recognised by our monoclonal antibody, is found in α and ω type gliadins. Avenins contain QQQPF, while the lesser motifs QQQP and QQPFP are found in γ type gliadins, hordeins, and secalins.[8] The low sensitivity of the assay to ω gliadins is probably due to the low reactivity of polyclonal antigliadin antiserum to ω gliadins.[17]

Whether measurement of this epitope allows a true assessment of gliadin concentration is a more difficult question. Wheat flour, depending on its strength, contains 3.0 to 7.5 g/100 g gliadin—that is, 40% of the total protein. The majority of our measurements for whole flours were within this range.

Wheat starch samples obtained from the manufacturers of the Juvela brand (AB Semper Stockholm) were available, with Kjeldahl nitrogen analysis. All fell within the current limit of 0.3% total protein, and within the proposed limit of 10 mg gliadin/100 g. The relation of total protein to gliadin content varies for different wheat starches. Up to 0.25%, most protein consists of starch granule protein; over 0.25%, any increase in protein content is entirely gliadin.[2] In a series of 16 wheat starches, five samples of 0.2–0.3% protein had a gliadin content of approximately 5 mg/100 g. Our experimental wheat starches had protein contents of 0.2–0.23%; thus gliadin measurements of 0.57, 1.52, 3.73, and 7.4 appear reasonable estimates in the light of the data of Skerritt and Hill.[2] However, individual starches may vary considerably from mean values of protein to gliadin content.

We also assessed four commercially available starches. Kinderm, Glutafin, and Wheatex starches are currently available, comply with the current regulations, contain less than 0.3% protein, and do not, to our knowledge, induce symptoms in most patients. Using our assay these starches would also comply with the proposed new regulations. Nutregen wheat starch was withdrawn from the market some years ago because it contained more than 0.3% protein. Our assay found only an acceptable level, 8 mg/100 g gliadin in this product; thus we may underestimate the gliadin content of some wheat starches.

In order to measure accurately gliadin in wheat starches, it is essential that the standard is of the same composition as the sample. However, since our standard is whole gliadin and wheat starches comprise those proteins which remain after washing, differing solubilities of the subfractions may profoundly influence the measurement of gliadin. Repeated washing with water of wheat starches under laboratory conditions leads to a stripping of the α and ω gliadins from the surface of the starch granules, whereas the γ gliadins are retained longer (Donald D Kasarda, personal communication). It is possible that γ gliadins may be more highly represented in a washed wheat starch than the other gliadins. This alteration of composition might account for some results being lower than expected. However this problem would emerge in any assay of this type where unfractionated gliadin is used as the standard.

Our assay detects rye and barley prolamins, but at much lower sensitivity than wheat; however, they could be estimated quantitatively if the appropriate antigen was used as a standard curve. Rye or barley flours analysed against gliadin standards would register as not gluten-free, having 54 mg/100 g and 16.5 mg/100 g of "gliadin".

Careful feeding studies have suggested that oat prolamins are not coeliac toxic.[18] However, in vitro studies have shown activation of intestinal cell mediated immunity by oat prolamins[19]; the subject therefore remains to be clarified. Two samples of pure strain oat prolamins cross reacted at low levels in our assay, depending on the source. This suggests genuine cross reactivity of oat prolamins with gliadin, as opposed to wheat contamination during cultivation, milling, or storage. Whole oat flour gave an apparent gliadin content of 34.6 mg/100 g and would register as unacceptable. Our monoclonal, PN3, raised against a peptide of known coeliac enterotoxicity, recognises an epitope in the region of the motif QQQPFP. The motif QQQPF occurs within oat prolamins,[8] and this may confer the cross reactivity with PN3. However, T cell antigenicity may involve a different or larger motif within the gliadin peptide. Studies of T cell antigenicity and HLA class II binding of gliadin peptides[20 21] may help to elucidate minimal epitopes for coeliac toxicity and should establish the role of oat prolamins in coeliac disease.

The coeliac toxicity of glutenin subunits is unknown. Low molecular weight subunits have a similar amino acid composition to gliadin and cross reacted in our assay. These proteins contain the motif QQQP, hence their reactivity with PN3; whether this sequence confers toxicity is unknown.

Our assay does not give quantitative results for cooked foods. Use of reducing agents prior to extraction proved unsuccessful. The sensitivity of the standard curve was attenuated, presumably due to the denaturation of the capture antibody. Steps could be taken to remove reducing agents; however the methodology is not suitable for use as a fast routine test in a small scale quality control laboratory. A kit, measuring ω gliadin,[22 23] the heat stable fraction, attempted to circumvent these problems, but has received mixed reviews.[24] ω Gliadins may comprise 6–20% of the total gliadins, depending on strain, leading to potential errors of −44% to +80% when extrapolating total gliadin from ω gliadin content.[25] We suggest that there is no ideal method for measurement of gliadin in cooked foods.

We employed our assay to screen some of the gluten-free products made by Nutricia Dietary Products. While traces of gliadin could be detected in the majority of wheat starch based products, the highest was for Loprofin low protein mix, at 1.4 mg/100 g; this is not however available on presciption for gluten sensitive enteropathy.

The cooked products are more difficult; only 17% of the gliadin in flour was measurable in the outer crust of a loaf of bread. Assuming our assay measures only 15% of the actual gluten content, all products would fall well within permissible levels. The majority of the wheat-free products contain no or only a trace of gliadin, even allowing for the possibility that only 15% of gliadin was detected. The assay however highlighted some issues. For example, Rite Diet mince pies with 1.28 mg/100 g and Rite Diet custard creams with 1.18 mg/100 g, are both cooked and therefore might contain up to 8.53 and 7.87 mg gliadin/100 g respectively. Similarly some of the uncooked pasta samples contained up to 6.7 mg/100 g gliadin. All of these products however contained than less 10 mg gliadin/100 g, and therefore comply with current guidelines for

gluten-free foods. Examination of different batches of pasta revealed variation, with, in one case, no gliadin detected, suggesting the potential for contamination during processing. Subsequent changes in working practice by the company have lead to production of sequential batches of pasta products in which negligible quantities of gliadin were detected.

Analysis of products from a smaller European manufacturer showed that—for example, gluten-free biscuits made from flours of maize, tapioca, and soya flour contained 1.6 mg gliadin/100 g. This might represent only 15% of the gliadin content in the uncooked product, which could potentially be 10.7 mg/100 g.

These data suggest that our assay is effective in detecting gliadin in gluten-free foods. In the case of cooked products extrapolation can give an approximate idea of gliadin content.

The assay described here is robust and repeatable with a coefficient of variation of less than 10%. The reproducibility was less good: a coefficient of variation of 20% is usually the maximum tolerated for this type of assay; our measurements were just above and just below this. Further standardisation will be required.

In conclusion, we feel that this assay may form the basis of a sensitive method for measurement of gluten in foods based on wheat, rye, barley, and oats.

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