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Assessment of Gliadin in Supposedly Gluten-Free Foods Prepared and Purchased by Celiac Patients

Vera Lucia Sdepanian, *Isabel Cristina Affonso Scaletsky, Ulysses Fagundes-Neto, and Mauro Batista de Morais

*Pediatric Gastroenterology Division, and *Department of Microbiology, Universidade Federal de São Paulo-Escola Paulista de Medicina, São Paulo, Brazil*

ABSTRACT

Background: The present study was designed to evaluate the presence of gliadin in homemade foods prepared by patients with celiac disease and/or their relatives, as well as in processed products consumed by such patients in São Paulo, Brazil, by enzyme immunoassay (EIA) and Western blot (WB) analysis.

Methods: One hundred ninety samples were analyzed: 108 homemade foods prepared in homes of patients with celiac disease, 81 processed products, and 1 positive control of homemade food. All samples were analyzed by EIA based on monoclonal antibodies to heat stable ω-gliadins and related prolamins from wheat, rye, and barley. Samples were also analyzed using the WB technique.

Results: Only one (0.9%) of 108 homemade foods contained detectable amounts of gliadin, as determined by EIA. Twelve of 81 processed products contained gliadin by EIA, as follows: 5 of 61 without gluten listed in the ingredients, 2 of 11 malt extracts, 1 of 2 wheat starches, 1 of 2 types of beer, and all 3 positive control products. Gliadin content of these products was between 4 and 10 mg of gliadin/100 g of product, except for the wheat starch sample (28 mg of gliadin/100 g) and all 3 samples with gluten (>4000 mg of gliadin/100 g). The positive control of homemade food contained 152 mg of gliadin/100 g. One hundred three of 190 samples were analyzed by WB, and 21 of these were gliadin positive. A comparison of results obtained by EIA and WB showed no statistical differences between the methods.

Conclusions: The greater part of the foods prepared in homes of patients with celiac disease and most processed products supposed to be gluten-free did not contain gliadin. Therefore, celiac patients adequately prepare gluten-free homemade food and have the expertise to purchase processed gluten-free food in São Paulo, Brazil. ***JPGN 32:65–70, 2001.*** **Key Words:** Celiac disease—Enzyme immunoassay—Food analysis—Gliadin—Gluten—Western blot analysis.

The treatment of celiac disease is essentially based on the elimination of wheat, rye, barley, oats, and their by-products from the diet (1,2). In daily practice, the adherence and control needed to assure the intake of a gluten-free diet are threatened by several difficulties, despite the effort and care demonstrated by most patients and their relatives. Unintentional transgressions may occur when processed foods do not list their true composition on their labels (3). Unintentional transgressions may also take place when gluten-free foods became unknowingly contaminated with gliadin. This contamination may occur in the field or during crop handling, storage, transport, milling, manufacturing, or packaging (4). Nevertheless, voluntary transgressions are known to occur despite information available to patients on the deleterious effects of gluten in their diet (5–8). However, it is well recognized that many patients with celiac disease, after a variable period of time consuming a gluten-free diet, may tolerate certain amounts of gluten and remain asymptomatic (6) or exhibit only minor symptoms of the disease (9). However, the noxious effects gluten exerts on the small intestinal mucosa are still present (6) and will become apparent in the long run. These visible effects include short stature (10) or even intestinal malignancies (11).

Prolamins are the toxic protein fractions soluble in ethanol that are part of gluten composition. Each of the offending cereals for celiac patients has a specific prolamin: gliadin-wheat, hordein-barley, secalin-rye, and avenin-oat (12). According to the Codex Alimentarius Commission of the Food and Agriculture Organization and the World Health Organization (FAO/WHO), a food is considered gluten-free when the level of prolamin detected in its composition is lower than 10 mg/100 g (13,14). This level of sensitivity can be achieved by us-

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Address correspondence and reprint requests to Vera Lucia Sdepanian, Rua dos Otonis 880 apt 102, CEP: 04025-002, São Paulo, SP, Brazil (e-mail sdepanian@nw.com.br).

ing monoclonal or polyclonal antibody–based techniques (15). The enzyme immunoassay (EIA), using the monoclonal ω-gliadin antibody, is the elected technique to identify gliadin and related prolamins in raw, cooked, or processed foods (13–18). The Western blot (WB) technique allows identification of protein fractions that react with the antigliadin antibody through molecular weight detection (19). Thus, the combination of both techniques is highly appropriate for identification and quantification of prolamins present in foods.

The present study was designed to evaluate the presence of gliadin in homemade foods prepared by patients with celiac disease and/or their relatives, as well as in processed products consumed by such patients in the city of São Paulo, Brazil, using both the EIA and the WB technique.

MATERIALS AND METHODS

One hundred eight homemade foods were prepared by patients with celiac disease and/or their relatives and collected at the annual party of the Brazilian Celiac Association. The members at this party had no prior knowledge that the homemade food samples would be collected and affirmed that none of the 108 samples contained gluten. As for the ingredients present in these homemade foods, 85 of them contained some type of the following cereal or tuber byproducts: corn starch (28 samples), corn flour (18 samples), corn meal (9 samples), rice flour (17 samples), manioc meal (4 samples), manioc flour (13 samples), potato starch (5 samples), and a baking mix labeled gluten-free containing wheat starch (1 sample). Ten of these 85 homemade foods contained two types of cereal, whereas 75 of them contained only one cereal. We also decided to analyze a carrot cake baked at home by one of the authors of the present study (VLS) as a positive control of a wheat-containing homemade food.

The 81 processed products were divided into six categories. The first category comprised 61 products that did not contain wheat, rye, barley, oats, wheat starch, or malt extract, according to the ingredient specification listed on the label. They are widely consumed according to information provided by patients and members of the Brazilian Celiac Association and are commonly purchased in regular food outlets in São Paulo, Brazil. Within this category we analyzed naturally gluten-free flours: corn starch (6 samples), manioc meal (3 samples), manioc flour (3 samples), corn flour (2 samples), buckwheat flour (2 samples), cookies made of manioc flour (2 samples) and corn starch (1 sample), cheese bread made of manioc flour (6 samples), dessert made of gelatin (1 sample), yeast (2 samples), coffee (5 samples), sauces and seasonings (5 samples), soups (2 samples), pasta made of rice (1 sample), dairy products (10 samples), sausage-type products (3 samples), and soft drinks (7 samples). We point out that the only products purchased at a bakery were two samples of cheese bread (cheese bread A, cheese bread B) and the dessert made of gelatin.

The second category analyzed included two different brands of toothpaste, because they are the most commonly used toothpaste by children with celiac disease in Brazil, according to the patients' self-reports. Toothpaste was included in the list of processed products, because children mention that they very frequently swallow toothpaste while brushing their teeth. The other four categories included: 11 samples of malt extract (7 breakfast cereals, 2 chocolate powders, 1 chocolate milk, and 1 chocolate bar), wheat starch (2 samples), and beer (2 samples) and as positive controls, wheat flour (2 samples) and gluten powder (1 sample). These last four items analyzed are the most representative samples of the processed products categories and are not included in the regular diet of patients with celiac disease. Therefore, 190 samples were analyzed: 109 homemade foods and 81 processed products.

Extraction of Gliadin from Foods

For extraction of gliadin from foods, samples of 1 g were extracted with 10 mL of 40% (vol/vol) ethanol solution by high-speed (20,000 rpm for 30 seconds) dispersion using Ultraturrax probe (Janke & Kunkel, Dontingen, Germany) at room temperature (16). Before use, extracts were centrifuged at 2,500 rpm for 10 minutes and recentrifuged at 14,000 rpm for 10 minutes (16). A 100-μL aliquot of the supernatant was prepared by analysis for EIA and 200 μL by WB.

Enzyme Immunoassay

An enzyme immunoassay (EIA) test kit (16) (Gluten Lab-Test-Transia; Diffchamb S.A., Lyon, France) was used to analyze gluten in samples according to the manufacturers' instructions. An aliquot of 100 μL of supernatant of each sample was diluted in diluent buffer, depending on the expected gliadin content: 1:50 for homemade and processed foods, 1:500 for carrot cake, and 1:5,000 for wheat flour and gluten powder. An amount of 100 μL of the diluted samples were incubated with mouse anti-gliadin monoclonal antibody for 30 minutes at room temperature (16). Microwells were washed five times with wash buffer and horseradish peroxidase–conjugated monoclonal antibody to gliadin was incubated for 30 minutes. After five washes, substrate-chromogen solution was incubated for 10 minutes. Color development was terminated by acidification and absorbances read at 450 nm. The samples containing polyphenol such as cocoa, coffee, tea, beer, and hops were extracted using polyphenol-binding additives to the 40% ethanol solution (16). All samples were analyzed in duplicate. According to the manufacturers' specification, the sensitivity of the test is 4 mg of gliadin/100 g of food.

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis

A 200-μL aliquot of the supernatant was diluted in equal volumes of sample buffer (0.015 M Tris-HCl buffer [pH 6.5], containing 25% glycerol, 1% sodium dodecyl sulfate [SDS], 2.5 % β-mercaptoethanol, and 0.05% bromophenol blue dye). Before being loaded onto the gels, samples were boiled for 6 minutes. Ten microliters of each sample were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) with a linear 10% gel and a 3% stacking gel by the method of Laemmli (20). On each gel, a set of six standard marker proteins (14,300–200,000 molecular weight, Gibco-BRL, Life Technologies, Grand Island, NY, U.S.A.) was included.

Western Blot

After electrophoresis, proteins were transferred to nitrocellulose membranes by the method of Towbin et al. (21). Electrophoretic transfer was performed overnight at room temperature at 12 V. The unabsorbed sites on the membranes were blocked by incubation for 6 hours at room temperature with phosphate-buffered saline (PBS) containing Tween 20 0.25% (PBST) and 3% bovine serum albumin (BSA). The membranes were then incubated with 10 mL of polyclonal anti-gliadin biotin conjugate (diluted 1:500 in PBST and 3% BSA; Sigma Chemical Co., St. Louis, MO, U.S.A.) and with anti-IgG peroxidase conjugate (diluted 1:200 in PBST and 3% BSA; Pierce, Rockford, IL, U.S.A.) for 2 hours at room temperature and washed in PBS containing 0.05% Tween 20 for 45 minutes with three changes of buffer. After washing, the membranes were incubated for 30 minutes in streptavidin-peroxidase conjugate (diluted 1:100,000 in PBST and 3% BSA; Boehringer–Mannheim, Mannheim, Germany) and washed again for 45 minutes. Immunoblots were visualized using enhanced chemiluminescence detection (Amersham Life Science, Ltd., Little Chalfont, UK).

Statistical Analysis

Results of gliadin detection obtained by EIA and WB were compared using the McNemar test. Fisher's exact test was used to compare the results of homemade foods with and without corn flour and to compare gliadin presence detected by the WB technique with different concentrations found by EIA. Statistical analysis was performed using Sigma-Stat (Jandel Scientific, Corte Madera, CA, U.S.A.) (22).

RESULTS

Table 1 shows the results of the 190 samples analyzed by EIA. Only 1 (0.9%) of 108 homemade foods prepared in homes of patients with celiac disease contained detectable amounts of gliadin (6.7 mg of gliadin/100 g) as determined by EIA. This sample was a corn flour cake made of eggs, milk, sugar, corn flour, cheese, and yeast. The positive control of homemade food (carrot cake made of the wheat flour) contained 152 mg of gliadin/100 g of product. According to EIA, 12 of 81 processed products contained gliadin, as follows: 5 of 61 without gluten in the ingredients (cheese bread A and a dessert made of gelatin bought in a bakery, manioc meal, buckwheat flour A, and buckwheat flour B), 2 of 11 malt extracts (chocolate powder A and chocolate bar), 1 of 2 wheat starches (potato with wheat starch), 1 of 2 types of beer, and all three positive control samples (2 wheat flour

TABLE 1. *Enzyme immunoassay results of 190 samples and Western blot results of 103 samples*

	Enzyme immunoassay			Western blot		
	Negative	Positive		Negative	Positive	
Homemade food						
Prepared by celiac patients	107	1	corn flour cake A*	33	5	corn flour cake A* corn flour cake B corn flour cake C corn flour cake D sweet made of corn starch
Positive control	0	1	carrot cake with wheat flour‡	0	1	carrot cake with wheat flour‡
Processed product						
Without gluten	56	5	cheese bread A* sweet made out of gelatine* manioc meal* buckwheat flour A* buckwheat flour B*	37	7	cheese bread A* cheese bread B cheese bread C sweet made out of gelatine* buckwheat flour A* soft drink seasoning
Malt extract	9	2	chocolate powder A* chocolate bar*	9	2	chocolate powder A chocolate milk
Wheat starch	1	1	potato with wheat starch†	1	1	potato with wheat starch†
Beer	1	1	beer A*	0	2	beer A* beer B
Toothpaste	2	0		2	0	
Positive control	0	3	wheat flour A§ wheat flour B§ gluten powder§	0	3	wheat flour A§ wheat flour B§ gluten powder§
Sub-total	176	14		82	21	
Total	190			103		

EIA, enzyme immunoassay, lower limit = 4 mg gliadin/100 g.
* 4 mg/100 g < gliadin content <10 mg gliadin/100 g.
† Gliadin content = 28 mg/100 g.
‡ Gliadin content = 152 mg/100 g.
§ Gliadin content > 4000 mg/100 g.

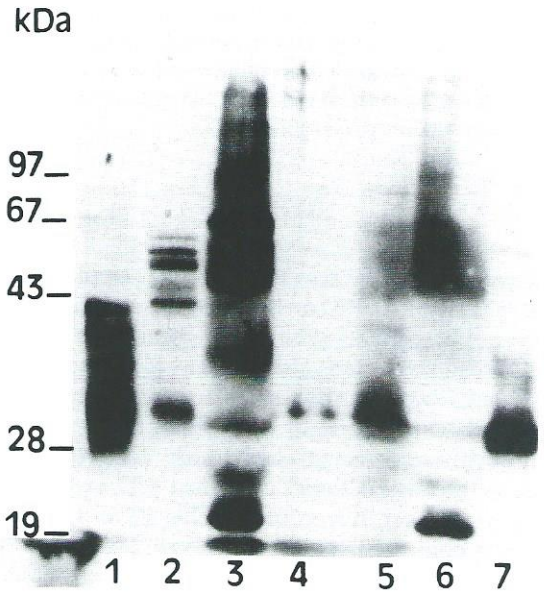

FIG. 1. Western blot of sodium dodecyl sulfate—polyacrylamide gel electrophoresis (SDS-PAGE) gel developed with anti-gliadin biotin conjugated. Lane 1: Gliadin; lane 2: positive control of homemade food; lane 3: corn flour cake A; lane 4: buckwheat flour B; lane 5: buckwheat flour A; lane 6: corn flour cake B; and lane 7: potato with wheat starch. Positions of prestained molecular weight markers are indicated: phosphorylase B, 97 kd; bovine serum albumin, 67 kd; ovalbumin, 43 kd; carbonic anhydrase, 28 kd; and β-lactoglobulin, 19 kd.

samples and 1 gluten powder). The gliadin content in these products was between 4 and 10 mg of gliadin/100 g of product, except for the wheat starch sample, which contained 28 mg of gliadin/100 g and all three positive control products, which contained more than 4000 mg of gliadin/100 g.

All 14 positive samples by EIA (gliadin content higher than 4 mg/100 g) were analyzed through WB assay. Although the sensitivity of the test was 4 mg/100 g by EIA, all 16 samples containing 2 to 4 mg of gliadin/100 g were also analyzed by WB. Seventy-three samples selected that randomly contained less than 2 mg of gliadin/100 g were analyzed as well. One hundred three of 190 samples were analyzed using this technique: 39 homemade foods and 64 processed products (Table 1). Five (13%) of 38 homemade foods prepared by patients were WB positive, but only one of these samples was EIA positive. The homemade positive control was also positive by WB assay. Fifteen of 64 processed products were positive by WB: 7 of 44 samples without gluten (3 samples of cheese bread, one of those bought at a bakery; dessert made of gelatin; buckwheat flour A; soft drink; and seasoning), 2 of 11 samples of malt extract (chocolate powder A and chocolate milk), 1 of 2 samples of wheat starch (potato with wheat starch), the 2 types of beer, and all 3 positive control products (2 samples of wheat flour and 1 gluten powder). Thus, 21 samples were positive by WB.

Figure 1 shows the WB of gliadin (Sigma), three homemade foods, and three processed products. The homemade foods analyzed were the positive control and two corn flour cakes prepared by patients (corn flour cake A and corn flour cake B). The corn flour cake A (Fig. 1, sample 3), was the only homemade food prepared by patients with celiac disease that was positive by EIA. The processed products were samples of two types of buckwheat flour (buckwheat flour A and buckwheat flour B) and the potato with wheat starch. All these samples were positive by WB, except the buckwheat flour B (Fig. 1, sample 4).

In observing the positive results using the WB technique, it is worthwhile to mention that there was a significant difference ($P = 0.019$, Fisher test) of positivity using the WB technique for homemade foods containing corn flour (4/11; 36.4%) in comparison with those without corn flour (1/27; 3.7%).

Three different levels of gliadin concentration were compared as follows: more than 4 mg/100 g, 2 to 4 mg/100 g, and less than 2 mg/100 g as detected by EIA and WB technique. There was 80.0% of positivity with WB when EIA was higher than 4 mg/100 g, 43.8% when EIA was 2 to 4 mg/100 g, and 4.1% when EIA was lower than 2 mg/100 g.

A comparison of the results obtained by EIA and WB revealed no statistical difference between these methods (Table 2).

DISCUSSION

Patients with celiac disease should know how to prepare at home, as well as to commercially purchase, gluten-free products, especially in countries where a large variety of gluten-free processed food is not available.

Because a large portion of food consumed by patients is prepared at home, we analyzed homemade foods. Published data on this subject are sparse. Two points are important in preparing homemade gluten-free foods: adequate selection of raw and processed products and preparing food without gluten contamination. As a strategy for collection, sample food was gathered at a Brazilian Celiac Association party to which each patient brought food previously prepared at home, as they are used to doing at every Celiac Association party. They were not

TABLE 2. *Comparison of results obtained by Enzyme immunoassay and Western blot*

	Western blot		
	Positive	Negative	Total
EIA			
Positive	11	3	14
Negative	10	79	89
Total	**21**	**82**	**103**

EIA, enzyme immunoassay.
McNemar test, $P = 0.096$.

aware that samples would be analyzed. We believe that this strategy for collection might provide samples more representative of food consumed on a day-to-day basis than those collected at previously scheduled visits to health clinics or from homes. This second approach would necessitate prior notification of the patient, and extra care might be taken for such an event.

Gliadin was detected in five homemade foods prepared in homes of patients with celiac disease by the WB method, although only one sample was EIA positive. The amount of gliadin was just above the limit of detection and of doubtful clinical significance. It remains unclear whether the presence of gliadin characterizes contamination during processing or during preparation. It is possible to postulate that the elevated percentage of positivity of WB in foods prepared with corn flour may be attributable to a greater frequency of cross reaction with the polyclonal antibody used in WB, than the monoclonal antibody used in the EIA. It is important to emphasize that this issue has never been approached in the published literature. Comparing the results of the 103 samples analyzed by the two assays, we observed a 12.6% (13/103) disagreement between the methods; nevertheless, no statistical difference was observed. The selection of processed products analyzed in this study was based on information provided by the patients. Since 1992, a federal law in Brazil has required food manufacturers to print the warning "contains gluten" on the labels of processed foods containing gluten. Food manufacturers have been printing this warning exclusively based on product ingredients, without any laboratory analyses to confirm the presence of gliadin. Thus, there is no consideration of a possible contamination of naturally gluten-free foods with gluten. Gluten contamination may occur at any stage between harvest and processing.

In this study, of only three processed foods purchased at a bakery, two were positive by EIA as well as WB, and the remaining one was positive by WB only. Considering the low amount of gliadin in these foods, gluten contamination possibly occurred during processing, from utensils and equipment coated with wheat flour residue. A further study must be conducted to evaluate a greater number of gluten-free bakery samples to study the security of purchasing gluten-free products prepared at the bakery. Both buckwheat samples analyzed contained gliadin. However, this cereal should not contain gliadin, because it bears no relation to true monocotyledonous wheat (23). There are two explanations to account for this positive result: either buckwheat was contaminated with wheat flour or there was a cross reaction between the antibodies used in EIA and WB with buckwheat, as previously related by Ellis and Ciclitira (15). The results obtained affirm that the majority of processed food with no gluten listed in the ingredients indeed contain no gliadin.

For years, wheat starch has been an alternative for patients with celiac disease, because it was believed to contain very small amounts of gluten or none at all (24). However, with improvements in gluten detection techniques, it has been demonstrated that some foods prepared with wheat starch may contain more than the quantity allowed for a food to be considered gluten-free (25). We analyzed only two products containing wheat starch sold in food stores, and in one gliadin was detected using both techniques. Therefore, it is prudent for patients to avoid wheat starch foods.

There is also controversy about whether malt extract should be included in a gluten-free diet. Malt extract is produced from malt grain derived from barley. Depending on the extraction technique used in its production, malt extract may contain gluten. In this study, no breakfast cereal sample contained gliadin. On the contrary, samples of chocolate powder, chocolate milk, and chocolate bar containing malt extract presented discordant results not only among the samples, but also in gluten detection assays.

Ellis et al. (26) demonstrated that the hordein content of foods and beverages was underestimated and considered barley products unsuitable for patients with celiac disease. We detected gliadin in the samples of the two types of beer using WB and in one sample by EIA, confirming the presence of the prolamin, which is toxic to patients, in this beverage.

The maximum quantity of gliadin permitted for food to be considered gluten-free, according to the Codex Alimentarius Commission of FAO/WHO is 200 ppm of gluten, equivalent to 10 mg gliadin/100 g (13,14). Although there is no consensus in the literature or solid scientific agreement, some researchers consider this level too high to protect susceptible populations (15). According to the Codex Commission, the intake of 10 mg gliadin/day should not be exceeded by susceptible groups (13,14). Nevertheless, it seems unlikely that the patient can properly monitor intake to avoid exceeding this recommended maximum daily intake. Therefore, we believe patients with celiac disease should not consume food with any detectable gliadin, regardless of the quantity.

It is important to emphasize that the gliadin content of all positive samples detected by EIA was lower than the FAO/WHO limit of 10 mg/100 g, except for the processed foods containing wheat starch and samples known to contain gluten, such as wheat flour, gluten powder, and the carrot cake made with wheat flour.

We concluded that the greater part of the foods prepared in homes of patients with celiac disease, as well as most processed products supposed to be gluten-free, did not contain gliadin. Therefore, patients adequately prepare gluten-free homemade food and have the expertise to purchase processed gluten-free food in São Paulo, Brazil.

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REFERENCES

1. Berge-Henegouwen GP, Mulder CJJ. Pioneer in the gluten free diet: Wille-Karel Dicke 1905–1962, over 50 years of gluten free diet. *Gut* 1993;34:1473–5.
2. Walker-Smith JA. Celiac disease. In: Walker WA, Durie PR, Hamilton JR, et al. eds. *Pediatric Gastrointestinal Disease.* 2nd ed. St. Louis, MO: Mosby; 1996:840–61.
3. Skerritt JH, Devery JM, Hill AS. Chemistry, coeliac-toxicity and detection of gluten and related prolamins in foods. *Panminerva Med* 1991;33:65–74.
4. Hekkens WThJM. Background information regarding the standard for gluten-free foodstuffs for patients with coeliac disease. In: *Proceedings of the 10th European Working Group of Prolamin Analysis and Toxicity.* Brussels, Belgium. November 1–3 1995. Brussels, Belgium: Association des Aminodenneries de Cereales de L.U.E., 1995:63–72.
5. Kumar PJ, Walker-Smith J, Milla P, et al. The teenage coeliac: follow-up study of 102 patients. *Arch Dis Child* 1988;63:916–20.
6. Auricchio S, Troncone R. Effects of small amounts of gluten in the diet of coeliac patients. *Panminerva Med* 1991;33:83–5.
7. Mayer M, Greco L, Troncone R, et al. Compliance of adolescents with coeliac disease with a gluten-free diet. *Gut* 1991;32:881–5.
8. Fabiani E, Catassi C, Villari A, et al. Dietary compliance in screening-detected coeliac disease adolescents. *Acta Paediatr* 1996;85: 65–7.
9. Walker-Smith J, Murch S. Coeliac disease. In: Walker-Smith J, Murch S. *Diseases of the Small Intestine in Childhood.* 4th ed. Oxford, UK: Isis Medical Media; 1999:235–77.
10. Groll A, Candy DCA, Preece MA, et al. Short stature as the primary manifestation of coeliac disease. *Lancet* 1980;2(8204): 1097–9.
11. Holmes GKT, Prior P, Lane MR, et al. Malignancy in coeliac disease: effect of a gluten-free diet. *Gut* 1989;30:333–8.
12. Ciclitira PJ, Ellis HJ. Determination of gluten content of foods. *Panminerva Med* 1991;33:75–82.
13. Food and Agriculture Organization of the United Nations. Codex Alimentarium Commission. Proposed draft revised standard for gluten-free foods (step 3 of the procedure). In: *Codex Alimentarius. Report of the Nineteenth Session of the Codex Committee on Nutrition and Foods for Special Dietary Uses.* Bonn-Bad Godesberg, Germany. March 27–31, 1995. Rome: FAO; 1995;7:21–5. (ALINORM 95/26).
14. Food and Agriculture Organization of the United Nations. Codex Alimentarium Commission. Proposed draft revised standard for gluten-free foods (step 5 of the procedure) In: *Codex Alimentarius. Report of the Twentieth Session of the Codex Committee on Nutrition and Foods for Special Dietary Uses.* Bonn-Bad Godesberg, Germany. October 7–11, 1997. Roma, FAO, 1997:5–6, 33–41. (ALINORM 97/26).
15. Ellis HJ, Ciclitira PJ. Measurement of gluten and gliadin in food. In: Mäki M, Collin P, Visakorpi JK, eds. Coeliac disease. *Proceedings of the 7th International Symposium on Coeliac Disease.* Tampere, Finland, Coeliac Disease Study Group 1996. 1997: 213–9.
16. Skerritt JH, Hill AS. Monoclonal antibody sandwich enzyme immunoassays for determination of gluten in foods. *J Agric Food Chem* 1990;38:1771–8.
17. Skerritt JH, Hill AS. Enzyme immunoassay for determination of gluten in foods: collaborative study. *J Assoc Anal Chem* 1991;74: 257–64.
18. Hekkens WThJM. The determination of prolamins in gluten-free food: introductory remarks. *Panminerva Med* 1991;33:61–4.
19. Freedman AR, Galfre G, Gal E, et al. Western immunoblotting of cereal proteins with monoclonal antibodies to wheat gliadin to investigate coeliac disease. *Int Arch Allergy Appl Immunol* 1988; 85:346–50.
20. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970;227:680–5.
21. Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci USA* 1979;76:4350–54.
22. Fox E. User's Manual. Sigma Stat: Statistical Software for Windows [on CD-ROM] Berlin: Germany, Jandel Scientific, 1994.
23. Pomeranz Y, Marshall HG, Robbins GS, et al. Protein content and amino acid composition of maturing buckwheat (Fagopyrum esculetum Moench). *Cereal Chem (St. Paul)* 1975;52:479–85.
24. Skerritt JH, Wrigley CW, Wilkinson W, et al. Wheat starch and the gluten-free diet [letter]. *Med J Aust* 1987;147:262–3.
25. Wieser H. Collaborative studies on the quantitative determination of gliadin in wheat starch. *Proceedings of the 11th Working Group Of Prolamin Analysis And Toxicity.* Tecklenburg, Germany, November 6–8, 1996. Tubigen, Germany: Kroner Starke; 1997:43–60.
26. Ellis HJ, Freedman AR, Ciclitira PJ. Detection and estimation of the barley prolamin content of beer and malt to assess their suitability for patients with coeliac disease. *Clin Chim Acta* 1990;189: 123–30.