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RESIDUAL GLUTEN CONTAMINATION IN SOME FOODSTUFFS USED IN GLUTEN-FREE DIET BY COELIAC PATIENTS IN POLAND*

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The aim of the study was to evaluate gliadin contamination in gluten-free raw materials and processed products consumed by coeliac patients in Poland, by an immunoenzymatic assay (ELISA). Over a 4-year period (2000–2004), a total of 724 food samples collected from 300 different products were analysed. Thirty five samples were from imported food products and 689 samples were from the home industry. One hundred twenty products were analysed once, while 180 were analysed 2 to even 4 times on different occasions.

Gliadin – a marker of gluten contamination – was detected in 27 of 724 (3.7%) samples, and its content ranged of 5 mg/kg to 35 mg/kg (10–70 mg of gluten/kg). In 11 of these samples (40.7%), the quantity of gliadin exceeded 10 mg/kg, currently accepted in Poland as the upper limit for gluten-free food. Most of those positive samples were wheat starches or wheat-starch-based processed foods.

INTRODUCTION

Coeliac disease (CD; gluten-sensitive enteropathy) is an autoimmune disease of the small intestinal mucosa triggered by gluten proteins of wheat and similar prolamin proteins of related cereals. In individuals genetically predisposed to develop CD, the disease is caused by abnormal T-cell response to gluten proteins bound to the disease-associated HLA-DQ2 or HLA-DQ8 molecules [Sollid, 2002]. In consequence, activated T-cells produce IFN-γ with strong inflammatory properties, which is probably responsive for induction of tissue damage.

Upon digestion of gluten, susceptible patients suffer from self-perpetuating mucosal inflammation characterised by progressive villi atrophy and hyperplasia of the crypts. Although the disease often starts shortly after the introduction of wheat into the diet the individual severity of the symptoms is quite variable, ranging from very severe in some patients to nearly unnoticeable in others [Papadopoulos, 2001]. Removal of gluten from the diet of coeliac patients is the only effective way to stop the disease process.

A gluten-free diet excluding wheat (gliadins), rye (secalins) and barley (hordeins) is thus the treatment of choice in this disorder. However, despite the effort and care demonstrated by most patients and their relatives, the strict adherence to the "gluten-free" diet is complicated by several difficulties. Unintentional consumption of gluten may take place when gluten-free foods became contaminated with gliadin. This may occur in the fields, during harvesting, milling, manufacturing, packaging or transporting [Hekkens, 1995]. Unintentional transgressions of gluten may also take place when producers do not list all ingredients of food on the labels. It is common knowledge that gluten-containing grains, especially wheat and its derivatives, are frequently used in a variety of food products. The residual gliadins are found in many unexpected for patients foodstuffs such as processed meats, soups, blue cheeses or mould-covered cheeses, sauces, juices and beers, as well as in food additives like flavorings, emulsifiers, colorings and preservatives that are derived from gluten-containing grains. Because until now the relationship between the trace amounts of gluten ingested and the severity of clinical symptoms and histological abnormalities in coeliac patients as well as in patients with cereal allergy is not defined, the regular control of gluten-free foods intended for dietary use is of great importance [Rujner *et al*., 2002; Czaja-Bulsa *et al*., 2005].

The study addressed the quantitative analysis of residual gliadin content in various commercial gluten-free products available on the Polish market.

MATERIAL AND METHODS

Between January 2000 and December of 2004, a total of 724 food samples obtained from 300 raw materials or processed products, including commercially available gluten-free foods, maize, soya, starches, rice, meat, sausages, syrups, chocolate-based products, breads, biscuits, breakfast cereals, soups, *etc*., were analysed. Among them, 35 food samples were imported and 689 samples of raw or processed foods were from the home industry. One hundred and twenty products were analysed once, whereas 180 products from independent productive series were analysed 2 to 4 times.

Gliadin concentration, a marker of gluten contamination, in extracted food samples was determined by an enzyme immunoassay based on the double antibody sandwich ELISA technique. The method was standardised according to the

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Working Group on Prolamin Analysis and Toxicity (WGPAT) requirements [Gregorek, 1994]. Detection limit of the method was 5 mg of gliadin/kg of food (10 mg of gluten/kg) that was under requirements of the Codex Alimentarius Commission [Food and Agriculture Organization of the United Nations, 1997].

At the moment, according to the current proposition of the Codex Alimentarius Commission [Food and Agriculture Organization of the United Nations, 1997], the maximum level of gluten for "gluten-free foods" is set at 20 mg/kg, *i.e.* 10 mg of gliadin/kg.

RESULTS

The description of the gluten-free foodstuffs examined is presented in Table 1. Twenty seven (3.7 %) out of 724 food samples contained detectable amounts of gliadin ranging from 5 mg/kg to 35 mg/kg. In 11 (40.7%) out of 27 positive samples gliadin contamination exceeded currently accepted upper limit for gluten-free foods, *i.e.* 10 mg/kg (20 mg/kg of gluten). Six (54.5%) of these 11 samples were wheat-starches or wheat-starch-based bread-flour mixtures obtained from different manufactures. Gliadin content of these samples was between 14 and 35 mg/kg (28 to 70 mg/kg of gluten). The other 5 samples with gliadin concentration exceeding 10 mg/kg were as follows: 2 soups with gluten-free noodles containing wheat starch (16 and 19 mg of gliadin/kg), 2 ready-to-eat dishes with meat and vegetables (12 and 17 mg of gliadin/kg) and 1 starch syrup (26 mg of gliadin/kg).

Among 180 samples of raw materials or processed foods evaluated minimum twice, in 88.8% (160/180) gliadin was not detected (<5 mg/kg), while another 11.2% (20/180) contained 7 to 27 mg of gliadin/kg. Repeated examinations of the same samples collected from different productive series showed a similar or variable level of gliadin contamination, but were never negative. Again, these products mostly included wheat starch or starch-based flours.

DISCUSSION

In this study gliadin was detected in 27 (3.7%) controlled food samples and in 11 of them its amount exceeded the quantity allowed for a food to be considered gluten-free according to the Codex Alimentarius Commission of FAO/WHO.

It is important to emphasize that 6 out of 11 positive samples with the gliadin quantity over 10 mg/kg contained wheat starch. For years, wheat starch has been recommended for coeliac patients as it was believed to contain a very small amount of gluten or none at all [Skerrit *et al*., 1987]. However, when a more sensitive ELISA method was developed, it has been demonstrated that some wheat starches and some food prepared with their addition may contain too high amounts of gluten [Sdepanian *et al*., 2001]. Probably, the presence of residual gliadin in wheat starch is related to the inaccurate technology of wheat flour preparation. The consensus of opinion about the level of gliadin considered to be safe in the coeliac patient's diet is still open for discussion [Stern *et al*., 2001]. According to the Codex Alimentarius Commission [Food and Agriculture Organization of the United Nations, 1997], the intake of 10 mg/day should not be exceeded by susceptible patients. However, the tolerance to trace amounts of gliadin is wide-ranging among individuals, and in some of them the mucosal deterioration may start immediately after gluten challenge [Rujner *et al*., 2002; Kaukinen & Collin, 2003]. Because toxicity of gluten depends on individual sensitivity and the dose of antigen ingested, the safe limit of daily gluten intake should be evaluated individually and especially susceptible patients with coeliac disease should not consume food with any detectable gliadin, regardless of the quantity.

In this study, in 2 ready-to-eat dishes for children containing only meat, vegetables and naturally gluten-free ingredients the quantity of gluten exceeded 20 mg/kg. Considering the amount of gliadin in these foods, gluten contamina-

TABLE 1. The analysed gluten-free foodstuffs and their basic raw material composition.

Groups of products	Ingredients	Number	Percent of samples above 10 mg/kg
Ready-to-eat jar food for infants and young children			
– juices and syrups	Extracts of juices, water, sugar, starch syrups	147	0.7
– desserts	Fruits, corn starch, modified flour, corn flakes	114	-
– vegetable dishes	Vegetables, modified starch, thickening agents	12	-
– vegetables with meat dishes	Vegetables, meats, corn starch	120	1.7
– soups	Vegetables, corn starch, wheat starch, gluten-free noodles	72	2.7
Flour	Rice flour, buckwheat flour, corn flour, corn starch, wheat starch	21	-
Macaroni and noodles	Rice flour, corn flour, corn starch	12	-
Gluten-free breads, wheat starch	Rice flour, corn flour, corn starch, wheat starch, potato starch, eggs	11	55
Milk paps for infants	Powdered milk, corn starch, rice flour, fruits, sugar	83	-
Milk formula for infants	Powdered milk, rice gruel, corn gruel	45	-
Sauces	Modified starch, taste and flavour agents	7	-
Meats and sausages	Meats, modified starch, spices	10	-
Other	Creams, sweets, yoghurts	70	-
Total		724	1.5

tion possibly occurred during processing, for example, from equipment coated with residue of wheat flour. Thus, it is a very important warning for food manufacturers that contamination of naturally gluten-free products with gluten may occur at any stage between harvest and processing. On the other hand, our results clearly show that raw materials which have been rendered "gluten-free", like wheat starch, must be controlled for gluten contamination before use for food preparation.

Thus, the printing of information "gluten-free" on the labels of processed foods exclusively on the basis of ingredients without any laboratory analyses should not be allowed.

SUMMARY

As shown in this study, the vast majority of the gluten-free foods analysed in our laboratory contained <20 mg of gluten /kg. However, it is worth stressing that a contamination with gluten was found in some naturally gluten-free and wheat-based de-glutenised products available on the Polish market. Due to our finding of possible contamination with gluten of the dietary foods claimed to be gluten-free, especially wheat starch and wheat-starch-based products, systematic control of gluten-free foods for dietary use should be obligatory.

CONCLUSIONS

1. We concluded that the greater part of food samples analysed did not contain gluten.
2. Special attention should be paid to preparations of wheat starch intended to be used in gluten-free processed food. It is recommended to analyse gluten contamination in each batch of wheat starch as well as in ready for consumption products with its addition.
3. The contamination of dietetic products with gluten may be responsible for poor clinical and histological recovery, both in wheat-starch based as well as in naturally gluten-free foods. Thus, gliadin content must be measured in all gluten--free products to protect patients with coeliac disease.
4. It is very important to emphasize that the gluten-free products and foods containing gluten should be properly labeled.

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OBECNOŚĆ GLUTENU W NIEKTÓRYCH ARTYKUŁACH ŻYWNOŚCIOWYCH STOSOWANYCH PRZEZ PACJENTÓW CHORYCH NA CELIAKIĘ W POLSCE

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Od stycznia 2000 roku do grudnia 2004 roku zbadano ogółem 724 próbki żywności bezglutenowej uzyskane z 300 różnych surowców i gotowych wyrobów spożywczych, zarówno naturalnie nie zawierających glutenu lub z których gluten usunięto (tab. 1). Sto dwadzieścia produktów zbadano jednorazowo, podczas gdy 180, pobranych z różnych serii produkcyjnych, badano 2–4 krotnie. Zawartość gliadyny – markera obecności glutenu – badano metodą immunoenzymatyczną (ELISA) wystandaryzowaną zgodnie z obowiązującymi zaleceniami grupy ekspertów przy FAO/WHO. Obecność gliadyny wykazano w 27 próbkach, w tym w 11 z nich jej zawartość przekraczała dopuszczalny limit 10 mg/kg (20 mg/kg glutenu). W większości przypadków dotyczyło to preparatów skrobi pszennej i gotowych wyrobów ją zawierających.

Wyniki badań wskazują, że systematyczna kontrola żywności bezglutenowej stosowanej w diecie eliminacyjnej u chorych na celiakię jest niezbędna dla zapewnienia bezpieczeństwa i właściwej ochrony zdrowia pacjentów.