codex alimentarius commission

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

WORLD HEALTH ORGANIZATION

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Agenda Item 4 **CX/NFSDU 03/4**
October 2003

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON NUTRITION AND FOODS FOR SPECIAL DIETARY USES
Twenty-fifth Session

Report of the Working Group on Prolamin Analysis and Toxicity (WGPAT)

1. BACKGROUND

Coeliac disease is an autoimmune disease triggered by the cereal protein gluten. The causal relation between gluten and its "toxicity" in individuals genetically predisposed to develop coeliac disease is firmly established and forms the basis for inclusion of gluten in food regulations and declarations in order to prevent harmful effects of gluten-containing food or food components in coeliac patients. A network of genetic predisposition, of biochemical processes modifying the triggering protein and of a complex T-cell-mediated immune reaction at the small intestinal level characterizes the disease, which shows many clinical forms. Prevalence of coeliac disease including all these forms is now known to be as high as 1:200 in Europe. Therapy consists of a gluten-free diet.

The Working Group on Prolamin Analysis and Toxicity (WGPAT) exists since 1985. Working aims are co-ordination of research on laboratory gluten analysis in food and on clinical evaluation of patients' sensitivity to prolamins. Analytical work has made considerable progress in the last few years: a European gliadin reference is now available and a sensitive and reliable enzyme-linked immunoassay (ELISA) has been published. New clinical data on potential thresholds of gluten tolerance in coeliac patients have also been collected, definitive clinical results can be expected within the next two years (Stern *et al.*, 2001).

2. THE EUROPEAN GLIADIN REFERENCE IRMM-480

A European Gliadin Reference (IRMM-480) is now available by the EC Institute for Reference Materials and Measurements (IRMM). Final certification is expected for mid 2004. Gliadin is the

gluten fraction soluble in ethanol and contains epitopes active in coeliac disease. The reference material was shown to be soluble, homogenous and stable. All gliadin components from 28 wheat varieties commonly grown in Europe are present in the preparation. IRMM-480 can be used as a reference for immunochemical gliadin/gluten determinations (van Eckert, 2002). The gliadin reference is available on direct request to IRMM (European Commission, Directorate-General Joint Research Centre [JRC], Institute for Reference Materials and Measurements, Reference Materials Unit, Retieseweg, 2440 Geel, Belgium).

3. THE R5 SANDWICH ELISA FOR GLIADIN/GLUTEN DETERMINATION

A reliable ELISA system has been developed based on recognition of the potential coeliac-toxic epitope QQPFP contained in gliadin and related prolamins (Valdès *et al.*, 2003). Briefly, the assay consists of a food extraction step and a double monoclonal antibody sandwich ELISA using horse radish peroxidase conjugate. The system is specific for prolamins from wheat, rye and barley. It does not cross-react with oats. Limit of detection is 1.5 mg/kg gliadin. The test is robust and simple. Specificity and sensitivity are high. The intraassay variation was 7.5%, the interassay variation was 8.7% (Valdés *et al.*, 2003). The system was shown to work with unprocessed and heat-processes samples.

A large international collaborative trial was carried out by WGPAT (Immer *et al.*, 2003). Twelve samples (gliadin-spiked maize and rice, commercial samples of gluten-free flour) were analyzed by 20 laboratories. Independent statistical evaluation by IRMM showed comparable results for two kit systems based on the same method (see above). Standard deviation of relative repeatability was 19%, standard deviation of relative reproducibility was 30%.

Technical problems originating from heat treatment and matrix effects could be solved by the assay system. For hydrolyzed gluten products an alternative competitive ELISA system has been described recently based on the same R5 monoclonal antibody by the same group.

The R5 ELISA system is superior to earlier tests (Denery-Papini *et al.*, 1999). This method is now proposed by WPGAT for further evaluation by the Codex Committee on Methods of Analysis and Sampling (CCMAS). Further progress (development of new methods), however, should not be inhibited by any regulation.

4. CLINICAL DATA ON THRESHOLD ON GLUTEN SENSITIVITY

A gluten-free diet is the essential treatment of coeliac disease. Nevertheless in coeliac patients the relationship between the quantity of gluten ingested (trace amounts) and the severity of clinical symptoms and histological abnormalities is still undefined. Individual variation and clinical

heterogeneity of coeliac patients pose difficult problems for an attempt to find acceptable threshold values for trace amounts of gluten to be possibly allowed in gluten-free foods (Stern *et al.,* 2001). Only a very few controlled studies have been undertaken to solve this question. Very recently it was concluded from a controlled study (Peräaho *et al.,* 2003) that wheat starch containing gluten-free flour products were acceptable in the gluten-free diet. However, the daily intake of gluten by the patients investigated was not given in this study. At present previous *in vivo* challenge studies (Catassi *et al.,* 1993) which originally showed a "toxic" effect of 100 mg of gliadin daily intake are being extended to a prospective study including proper controls and appropriate inclusion and exclusion criteria. From both groups cited definitive data are expected in the next two years. Until these data are available a renewed level discussion is not justified. The current limit for gluten-free foods of [200] mg/kg gluten may require reconsideration in the near future.

5. CONCLUSION

Based on recent progress on the gliadin reference IRMM-480 and on the R5 sandwich ELISA method as published recently (Valdés *et al.,* 2003) WGPAT proposes to the Codex Committee on Nutrition and Foods for Special Dietary Use to recommend the R5 ELISA method for further evaluation by the Codex Committee on Methods on Analysis and Sampling and to take the necessary measures for this purpose.

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