Department of Food and Environmental Sciences
University of Helsinki
Finland

Immunochemical analysis of prolamins in gluten-free foods

Päivi Kanerva

ACADEMIC DISSERTATION

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ABSTRACT

People with coeliac disease have to maintain a gluten-free diet, which means excluding wheat, barley and rye prolamin proteins from their diet. Immunochemical methods are used to analyse the harmful proteins and to control the purity of gluten-free foods. In this thesis, the behaviour of prolamins in immunological gluten assays and with different prolamin-specific antibodies was examined. The immunoassays were also used to detect residual rye prolamins in sourdough systems after enzymatic hydrolysis and wheat prolamins after deamidation. The aim was to characterize the ability of the gluten analysis assays to quantify different prolamins in varying matrices in order to improve the accuracy of the assays.

Prolamin groups of cereals consist of a complex mixture of proteins that vary in their size and amino acid sequences. Two common characteristics distinguish prolamins from other cereal proteins. Firstly, they are soluble in aqueous alcohols, and secondly, most of the prolamins are mainly formed from repetitive amino acid sequences containing high amounts of proline and glutamine. The diversity among prolamin proteins sets high requirements for their quantification. In the present study, prolamin contents were evaluated using enzyme-linked immunosorbent assays based on ω- and R5 antibodies. In addition, assays based on A1 and G12 antibodies were used to examine the effect of deamidation on prolamin proteins. The prolamin compositions and the cross-reactivity of antibodies with prolamin groups were evaluated with electrophoretic separation and Western blotting.

The results of this thesis research demonstrate that the currently used gluten analysis methods are not able to accurately quantify barley prolamins, especially when hydrolysed or mixed in oats. However, more precise results can be obtained when the standard more closely matches the sample proteins, as demonstrated with barley prolamin standards. The study also revealed that all of the harmful prolamins, i.e. wheat, barley and rye prolamins, are most efficiently extracted with 40% 1-propanol containing 1% dithiothreitol at 50 °C. The extractability of barley and rye prolamins was considerably higher with 40% 1-propanol than with 60% ethanol, which is typically used for prolamin extraction.

The prolamin levels of rye were lowered by 99.5% from the original levels when an enzyme-active rye-malt sourdough system was used for prolamin degradation. Such extensive degradation of rye prolamins suggest the use of sourdough as a part of gluten-free baking. Deamidation increases the diversity of prolamins and improves their solubility and ability to form structures such as emulsions and foams. Deamidation changes the protein structure, which has consequences for antibody recognition in gluten analysis. According to the resuts of the present work, the analysis methods were not able to quantify wheat gluten after deamidation except at very high concentrations. Consequently, deamidated gluten peptides can exist in food products and remain undetected, and thus cause a risk for people with gluten intolerance.

The results of this thesis demonstrate that current gluten analysis methods cannot accurately quantify prolamins in all food matrices. New information on the prolamins of rye and barley in addition to wheat prolamins is also provided in this thesis, which is essential for improving gluten analysis methods so that they can more accurately quantify prolamins from harmful cereals.

Australia, under the name Gliadin assay, from ELISA Technologies, USA, under the name GlutenELISA, from Neogen, USA, under the name Tepnel Biokits Gluten Assay Kit and from Diagnostics Innovations Ltd., UK, under the name HAVen Gluten Kit.

R5 ELISA – the Méndez method

The R5 ELISA method was developed by Méndez and co-workers (Valdés et al. 2003). It quickly replaced ω-gliadin ELISA, since it was able to detect barley hordeins and was not as affected by the cultivars as the previous method. The international ring trial of the method was organized by the Prolamin Working Group (PWG) during 2002 (Méndez et al. 2005) and involved 20 laboratories and two test systems (Ingenasa and R-Biopharm). The samples studied in the trial contained heated and spiked maize bread samples, unheated spiked rice bread samples and contaminated gluten-free samples. The repeatability and reproducibility were found to be 20% and 32% for the Ingenasa kit and 18% and 30% for the R-Biopharm kit. These values were considered acceptable for immunological analysis.

The first step in developing the R5 ELISA was an ELISA method that was based on an antibody cocktail of three antibodies: Rye3, Rye5 and 13B4 (Sorell et al. 1998). Later on, the group developed the method further and limited it to only one single antibody, R5 (Valdés et al. 2003). The cocktail extraction procedure is recommended to be used with sandwich R5 ELISA. This means that prolamins are reduced before extraction by 2-mercaptoethanol. The extraction is further enhanced by guanidine hydrochloride (a disaggregating agent). The cocktail solution was introduced in 2000 at the 15th Meeting of the Prolamin Working Group and published in 2005 (García et al. 2005). Later, the group of Méndez compared extraction with the cocktail solution to that with aqueous ethanol (García et al. 2003). The recoveries obtained with the cocktail solution were from 70 to 98%, while the recoveries of gluten from the same samples with 60% ethanol were about 30 to 50%.

Competitive R5 ELISA was introduced at the 18th PWG meeting in 2003 (Ferre et al. 2004). It detected prolamins from beer and syrup samples more efficiently than the sandwich method. The standards used in the first competitive assay were PWG gliadin digested with pepsin, trypsin, pepsin+trypsin, chymotrypsin or with a pool of these enzymes. The results for the beer samples were from 1.9 to 17 times higher with competitive R5 than with sandwich R5. However, higher results were obtained for breakfast cereals with sandwich method than the competitive method (Hernando et al. 2005). This was suggested to occur because of heat treatment. Extraction with the cocktail solution is not compatible with competitive ELISA (Immer and Haas-Lauterbach 2005b), and heat-treated proteins may therefore remain unextracted when ethanol extraction without reduction is used.

The method is available from BioControl Systems, USA (Transia Plate Prolamins), Ingenasa, Madrid, Spain (Ingezim Gluten), Neogen, USA (Veratox for Gliadin R5) and R-Biopharm, Darmstadt, Germany (Ridascreen Gliadin and Ridascreen Gliadin Competitive). It should be noted, however, that the gliadin standard in the ELISA test of BioControl is from the wheat variety Timgalen, the intensity of which is about a half of that of PWG gliadin (Hasselberg et al. 2004). PWG gliadin is used in the ELISA by Ingenasa, and the standard of R-Biopharm is calibrated to PWG gliadin. The standards are described in more detail below.