Gluten analysis of fermented or hydrolyzed food products

Coeliac disease is a permanent intolerance to gluten that results in damage to the small intestine and is reversible when gluten is avoided by diet. Gluten is a mixture of prolamin and glutelin proteins present in wheat, rye and barley.

Currently, the FDA "is issuing a final rule to establish requirements concerning "gluten-free" labeling for foods that are fermented or hydrolyzed or that contain fermented or hydrolyzed ingredients". For this kind of food (e.g., beer, yogurt), R-Biopharm has developed a special method to detect gliadin/gluten or fragments of it (gliadin/gluten peptides) that are formed during fermentation and hydrolyzation process by proteolysis. The R5 competitive ELISA method (RIDASCREEN® Gliadin competitive (2nd generation)) is in contrast to sandwich ELISA methods able to react with small peptides too. Hence, competitive ELISA is the method of choice for proof of antigens that underwent proteolysis.

A new standard material was developed for assay calibration to respect the presence of gliadin fragments for quantification. The working group of Prof. Dr. Köhler (German Research Centre for Food Chemistry) produced the hydrolyzed standard. For this, wheat, rye and barley were digested by pepsin and trypsin. The assay can be related to the prolamin concentration and therefore to the limit values stipulated by the Codex Alimentarius.

The FDA states that there is not any suitable method for the detection and quantification of gluten in fermented or hydrolyzed food. The R5 Competitive ELISA is hence not suitable too. The main critical issue is that hydrolytic conditions (time, temperature, or composition under which the hydrolysis is occurring) during preparation of kit calibrators are different to those during food manufacturing process. FDA states that the peptide profile is hence likely to be different, and the assay is unlikely to generate accurate results. This is scientifically correct. However, this is the same with all diagnostic methods using calibrators for food analysis (e.g., sandwich ELISA, LC-MS/MS). Calibrators can never cover and mirror all conditions of food processing.

Method calibration is a compromise therefore and users need to know where limitations are given and how to interpret results correctly.

In consequence, the rationale of FDA means that each user has to establish own calibration standards according to the specific food process conditions. This needs to be done for each food product since each manufacturing step influences the immunochemical reactivity. Consequently, it will not be possible to standardize methods and to compare results between different labs. This would be a step back into the last century.

The long experience with immunochemical methods shows that users can trust results even when a method's calibration is a compromise only. Of course, it must be proven that the compromise is well chosen. Hence, R-Biopharm initiated an independent international study for the R5 Competitive ELISA. The outcome of the study was that the method is appropriate for its intended use. Consequently, the AOAC granted the assay an Official Method of Analysis (OMA) status (final action) in 2019.

Within hydrolyzed food, beer samples are of special interest. Therefore, beer samples were part of the samples that have been tested with the above mentioned international study.

The same study data set was accepted by AACC to grant the method the status of an AACC Approved Method of Analysis in 2013. An additional study was initiated with internationally known experts to show reliability of the R5 Competitive ELISA method for investigation of beer samples. A subcommittee of the American Society of Brewing Chemists recommended that the method for gluten determination by R5 Competitive ELISA be included in the Methods of Analysis.

This shows that the R5 competitive ELISA method is an intensively proven and widely accepted method of analysis for fermented or hydrolyzed food products. In the hands of professional technicians who know how to interpret results it is a reliable method that offers comparability between results from different labs.

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