ENZYME LINKED IMMUNO SORBENT ASSAY FOR THE QUANTITATIVE ANALYSIS OF PROLAMINS OF SORYZ (ELISA)

R. Siminiuc, PhD
Technical University of Moldova

INTRODUCTION

The detection of the content of prolamins plays an important role to enable the selection of food for patients who suffer from any form of gluten intolerance, especially for celiac and allergic individuals. Prolamins are protein fraction of wheat, rye, barley, and other cereals, that are not soluble in 0.15 M NaCl solution, but soluble in 40-70% ethanol. All the wheat prolamins (gliadins), rye prolamins (secalins), and barley prolamins (hordeins) are toxic for celiac patients. There is no agreement concerning toxicity of oat prolamins (avenins), while rice prolamins (oryzins) and corn prolamins (zeins) are not toxic at all. The percentage of prolamins in gluten is approximately 50 % for wheat. According to Codex alimentarius, "gluten free" foods are those containing less than 10 mg of gliadins (prolamins) in 100 g of dry substance (20 mg of gluten per 100 g of dry substance) [1].

1. GENERAL

Gluten is the characteristic term for the protein mixture of glutelins and prolamines (gliadins) found in cereals. The proportion of glutelin to gliadin in the protein mixture is approximately the same. One exception are starches: Their prolamine /gluten proportion is depending on the washing degree of the starches (factor between 1.6 and 2.6). There exist various groups of gliadins. Their contents vary from cereal to cereal. Gluten is found in many cereal products, however, due to its inherent physicochemical properties as binding and extending agent it is commonly used as an additive in foods.

Detection of gluten plays a role in the quality control and selection of foods for individuals with gluten intolerance. In cases of gluten intolerance enteropathy, celiac disease, sprue and related allergic reactions, a diet free from gluten contained in wheat, rye, barley and in some cases oat would be necessary.

In the Codex Standard for gluten free foods the term „gluten free“ is defined as follows: „In accordance with this standard "gluten free“ means, that the total amount of the used gluten of wheat, rye, barley and oat in the products or those crossed species in food or ingredients is not more than 200 ppm (mg/kg) on the dry substance basis“. A limit of Gluten to 20 ppm is in discussion.

Prolamines are those gluten fractions, which can be extracted with ethanol (40 - 70 %). The prolamine content of gluten generally is 50 %. Therefore, the limit for prolamines is 100 ppm (mg/kg) corresponding to an approx. gluten content of 0.02 %.

The common detection of gluten is based on microscopic, electrophoretic and chromatographic methods. These methods seldom yield acceptable quantitative results, particularly in the case of processed and cooked foods. In addition, these methods are time consuming and require expensive laboratory equipment. In accordance with the "Codex Standard for gluten free foods“ (temporary draft) the gluten detection in foods and ingredients has to be based on an immunological method and the detection limit should be at least 10 ppm in products based on dry substance. With the RIDASCREEN®FAST Gliadin test it is possible to detect gluten from wheat, rye and barley quantitatively with a detection limit of 10 ppm. It works in raw as well as in processed foods [2, 4].

2. TEST PRINCIPLE

The basis of the test is the antigen-antibody reaction. The wells of the microtiter strips are coated with specific antibodies to gliadins. By adding the standard or sample solution to the wells, present gliadin will bind to the specific capture antibodies. The result is an antibody-antigen-complex. Sample components not bound by the antibodies are then removed in a washing step. The bound gliadin is detected by an antibody conjugated to peroxidase (enzyme conjugate). Any unbound enzyme conjugate is then removed in a washing step. Enzyme substrate (urea peroxide) and

chromogen (tetramethylbenzidine) are added to the wells and incubated. Bound enzyme conjugate converts the colorless chromogen into a blue product. The addition of the stop reagent leads to a color change from blue to yellow. The measurement is made photometrically at 450 nm [2, 4].

3. MATERIALE ŞI METODE

As materials for making experiments were used native grains of soriz ”Alimentar 1”;

Method for determining is Enzyme Linked Immunoassay R5 Mendez (ELISA) :the official method proposed by the Codex Alimentarius;

Measurements were made this year in Laboratory of Applied Research and Analysis ”R&C Lab” S.r.l., (Uni En Iso 9001:2000), Vicenza, Italy.

4. RESULTS

The results are read off the calibration curve which can be used only to determine the gliadin concentration in samples assayed at the same time as the calibrators.

The mean values of the absorbance values obtained for the standards are entered in a system of coordinates on semi logarithmic graph paper against the gliadin concentration in µg/kg (ppb). The gliadin concentration corresponding to the absorbance of each sample can be read from the calibration curve

The results show that the soriz grains (Sorghum oryzoidum) the concentration of toxic prolamine is undetectable, or null, which includes soriz in the category of recommended cereals for celiac alimentation.

CONCLUSION

According to the results we can conclude that the soriz is a gluten-free cereal that can be recommended with certain for people with gluten intolerance or sensitivity. This allows the expansion of local gluten-free cereals for human food, increasing food security.

Biblyography

1. Immer, U., Haas-Lauterbach, S. *R-Biopharm AG, Darmstadt, Germany; Proceedings of the 17th meeting of Working Group on Prolamin analysis and toxicity, 3. - 6. Oktober 2002, London, UK, England: RIDASCREEN® / RIDA® gliadin test systems.*

2. Codex Alimentarius Commission. *Twenty-seventh Session Geneva, Switzerland, Report of the 25th session of the Codex committee on nutrition and foods for special dietary uses 28 June– 4 July 2004.*

3. Lopez, E., *et al. Proceedings of the 15th meeting of Working Group on Prolamin analysis and toxicity, 9. - 12. November 2000, Meran: A quantitative gluten „Cocktail extraction“ procedure for heat-processed foods.*

4. Wieser, H., *et al. Journal of Cereal Science. Quantitative Determination of Gliadin Subgroups from Different Wheat Cultivars. Pag. 19, 149 – 153, 1994.*

5. Hernando, A.*, et al. Proceedings of the 16th meeting of Working Group on Prolamin analysis and toxicity, 8. - 11. November 2001, Sitges, Spain: New strategy for the analysis of gliadins by MALDI-TOF/MS in maize or rice based foods: Fractionation of gliadins from maize or rice prolamines by acidic treatment.*

Recommended for publication: 18.05.2011