Association des Amidonneries
de Céréales de l'Union Européenne

43, av. Des Arts – B 1040 Brussels
Tel: +32-2-289 67 60 Fax: +32-2-513 55 92
e-mail: aac@aac-eu.org
Website : www.aac-eu.org

AAC COMMENTS TO THE FDA on "GLUTEN-FREE" Public Meeting – August 19

As suppliers of "gluten free" food ingredients and encouraged by the ongoing revision of the Codex Standard for Gluten-Free Foods and by the provisions on allergen labeling in the E.U., the European Cereal Starch Industry Association (AAC) has made a great effort during the last years to improve the knowledge regarding gluten content and nitrogen compounds of wheat starch and wheat starch derivatives.
Therefore, the AAC would like to submit information and comments to FDA regarding Gluten content and analyses procedure for wheat starch and wheat starch derivatives in Europe.

Gluten content and Nitrogen compounds of Wheat starch:

In 1981 Codex Alimentarius defined "Gluten-Free" Foods as products containing less than 0.05 g nitrogen per 100 g on dry matter. However many products, including wheat starch, contain considerable amounts of non-gluten endogenous proteins and of non-protein nitrogen compounds which are also measured by total nitrogen determination.
The Codex Standard currently under revision aims to define a specific gluten threshold for gluten free foods based on gluten tolerance in celiac patients. The revision of the Codex Standard for Gluten-Free Foods was put on hold in 2001 until a reliable method for the determination of gluten and scientific data on the tolerable intake of gluten by celiac people become available. In the current proposal, two levels of gluten are considered for gluten-free foods: maximum 200 ppm/ds for products containing derivatives of gluten-containing cereals, and maximum 20 ppm/ds for naturally gluten-free foods.

Does wheat starch comply with those levels?
During 4 years, AAC collected data from the major European Producers on N content of wheat starch. The N content is one of the quality parameter in the production of wheat starch. The average content is 0.036 % N and all the values are below 0.056 % N. From those data, it can be concluded that 80-85% of the industrial wheat starch complies with the current Codex specification (0.05 g N /100 g D.S). The remaining 15-20% is only slightly higher.

Facing the revision of Codex Standard and the provisions on allergen labeling in the E.U., AAC members have conducted extensive analytical studies in order to clarify what is the contribution of different compounds containing nitrogen in total nitrogen content of wheat starch and wheat starch hydrolysates (see study in annex as part of the file submitted by the AAC in the context of the previsions for exemption of certain products from allergen labeling in the E.U.).

For wheat starch, it has been shown that 60% of total N originates from lipid compounds and only 30% from proteins. The major part of granule starch proteins is an enzyme involved in biosynthesis of starch, namely Granule Bound Starch Synthase.
Gluten in wheat starch was measured using the R5 Enzyme-Linked ImmunoSorbent Assay (ELISA) method.The gluten contents were principally between 50 and 150 ppm with a maximum value of 242 ppm.

Therefore, taking into account these results, the European Cereal Starch Industry Association is confident that a substantial part of wheat starch produced in Europe complies with the limit of 200 ppm gluten as proposed in the revised draft Codex Standard for Gluten-free foods.

Wheat starch derived products: glucose syrups and maltodextrins

Wheat starch derivatives are obtained through partial or total hydrolysis of wheat starch. During the manufacturing process wheat starch derivatives undergo efficient purification steps including protein removal by active carbon treatment and ion exchange.

The nitrogen content of wheat starch derivatives is ranging from about 15 ppm (detection limit of the method) to a maximum of 150 ppm which gives maximum 1000 ppm expressed as apparent protein content (Nx6,25).

A study conducted using amino acid analyses and mass spectrometry demonstrated that only trace amounts of protein (1-40 mg/kg) are detectable in wheat starch glucose syrups and maltodextrins. Part of these proteins is peptides that arise from degradation of gluten. The difference between apparent protein and true protein in wheat starch derivatives is explained by N-containing lipid residues.

By applying the sandwich and competitive R5 ELISA method, the response was below the detection limit of 3.1 and 2.4 ppm respectively.

Method of determination of gluten:

Most of the available methods for quantitative determination of gluten in foods are immunological.

Two types of commercial ELISA tests kits were used for several years: 1) a monoclonal antibody raised against an omega-gliadin peptide from the Australian wheat variety Timgalen, originally developed by Skerrit. The method has been approved as standard by AOAC (AOAC Official Method 991.19). 2) a polyclonal antibody raised against a mixture of alpha, beta, gamma and omega gliadin fraction purified from a mixture of fifteen different German wheat varieties originally developed by Riedel de Haen.
As it could be expected the reactivity of these two antibodies is different. Furthermore, the protein preparations included in each kit as standards being also slightly different in composition as indicated above, any direct quantitative comparison between the two methods is impossible.

A more recent second-generation approach, followed by several academic laboratories consisted in raising monoclonal antibodies directed against chemically synthesized peptides, the sequence of which are deduced from amino acids sequence analysis of cloned gliadin genes. The principle

is to choose a short non-repetitive sequence, which will allow the recognition of a single type of molecule. Although quite appealing in theory, this approach has led to low sensitivity tests which could not meet the requirements of an industrially applicable method.

Méndez et al. (Unidad de Gluten, Centro National de Biotecnologia, Madrid, Spain) have studied a monoclonal antibody, identified as R5. This method has been developed within the Prolamin Working Group. It uses a new monoclonal antibody, identified as R5 and originally isolated by Mendez. R5 recognizes several and small repetitive epitopes as QQPFP, LQPFP,QLPYP,QLPTP, QQTFP,PQPFP, QQPYP and PQPFP in gliadins.

R5 has been recently used as the base for two new kits developed by Ingenasa, a Spanish company, and R-Biopharm. The R5 antibody has a sensitivity of 1.5 ppm for gliadins (3 ppm for gluten) and it allows equivalent recognition of wheat, barley and rye glutens. Valdés et al. also showed by Western blotting the reactivity of R5 towards enzymatically or heat-modified wheat prolamines. Immer et al. (2002) performed a ring test with 20 laboratories on the R5 ELISA and they concluded that the method is able to detect gliadin in the range of 2-5 ppm using the Prolamin Working Group gliadin standard.

The classical sandwich ELISA R5 has some limitations that still have to be solved, and this method is hardly applicable to wheat starch hydrolysates and therefore should be considered with particular caution when applied to these ingredients or to food products containing them. The competitive R5 ELISA method, which is under development, aims to solve these problems. This should be a significant improvement, enabling us to get a practical and reliable method for determination of gluten in such products, but the method still needs to be validated against a standard reference method. Several commercial kits are now available based on the R5 antibody such as the Ridascreen gluten kit (R-Biopharm, Germany) and the Ingezim gluten kit (Ingenasa, Spain).

The Mendez's method has been proposed and submitted to Codex Alimentarius for approval. In April 2005, the Codex Committee on Methods of Analysis and Sampling has agreed to endorse temporarily the R5 ELISA method for the determination of gluten as a Type I method.

The European starch industry's position on Gluten-free

The revision of the Codex Standard for Gluten-Free Foods was postponed in 2001 until a reliable method for the determination of gluten, and scientific data relating to the tolerable levels of gluten for coeliac patients became available. Based on the current proposal, two levels of gluten are being considered for gluten-free foods: maximum 200 ppm/ds for products containing derivatives of gluten-containing cereals, and maximum 20 ppm/ds for naturally gluten-free foods. Both categories would be labelled as "gluten-free".

The maximum level of 200 ppm gluten is already being commonly used on the market for wheat-based gluten-free foods, and the same limit is also applicable to the wheat starch ingredient used in these foods. Recent data on the gluten content of wheat-based gluten-free foods show that these products generally contain about 100 ppm gluten or less. This is in line with the 200 ppm limit value used by products on the market for the following reasons:

- In order to be able to guarantee maximum 200 ppm gluten for wheat starch, a sampling and analytical error needs to be taken into account by the starch producer.
- Wheat-based gluten-free foods contain a maximum of 70-80% wheat starch.

- As indicated above, the gluten content of industrial wheat starch is generally between 50 and 150 ppm.

The upper limit for gluten-free foods will need to take into account data on tolerable levels for daily intake of gluten by coeliac patients, and data on exposure to gluten through the use of wheat-based gluten-free foods. Based on the data available today, a gluten content of wheat based gluten-free foods of about 100 ppm or less will result in daily exposure levels to gluten that are acceptable for a large majority of coeliac patients. A second category of gluten-free foods, guaranteeing lower gluten content, will be suitable for the most sensitive coeliac patients.

The cereal starch industry therefore believes that the maximum level of gluten in wheat starch can be maintained at 200 ppm, as proposed in the revised draft of the Codex Standard for Gluten-Free Foods. This will reinforce the present situation of the market, which offers wheat-based gluten-free foods containing about 100 ppm gluten or less.
One category of gluten-free foods with a low level of gluten acceptable for all coeliac patients would exclude the use of wheat starch in gluten-free foods.

Finally, it is important that a reliable and generally accepted reference method for the determination of gluten becomes available as soon as possible. Until then, the starch industry has no other choice but to continue using N as a parameter within the context of gluten-free product quality, even if it is not directly related to the protein and gluten content in starch and starch derivatives.

References:

AAC (2004) Gluten-free: the starch industry's view. Proceedings of the 19th meeting of the Working Group on Prolamin Analysis and Toxicity, Prague, 30 september-3 October 2004. Editor: Martin Stern, p. 165-169.

AOAC Official Method 991.19 Gliadin as a measure of Gluten in Foods – Colorimetric Monoclonal Antibody Enzyme Immunoassay Method First Action 1991, Final Action 2001

Ferre S., Garcia E, Méndez E. Measurement of hydrolysed gliadins by a competitive ELISA based on monoclonal antibody R5: analysis of syrups and beers. Proceedings of the 18th meeting of the Working Group on Prolamin Analysis and Toxicity, 2-5 October, Stockolm, 2003

Immer U, Vela C, Méndez E, Jenssen F. PWG collaborative trial of gluten in gluten-free food through "Cocktail ELISA". Proceedings of the 17th meeting of the Working Group on Prolamin Analysis and Toxicity, 3-6 October 2002, Verlag Wissenschaftliche Scripten, Zwickau, Germany 2003.

Valdés I., García E., Llorente M., Méndez E. Innovative approach to low-level gluten determination in foods using a novel sandwich enzyme-linked immunosorbent assay protocol. European Journal of Gastroenterology & Hepatology 2003;15:465-474