CODEX ALIMENTARIUS COMMISSION

Food and Agriculture Organization of the United Nations

World Health Organization

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Agenda Item 2 **MAS/36 CRD/15**

Original Language Only

JOINT FAO/WHO FOOD STANDARDS PROGRAMME
CODEX COMMITTEE ON METHODS OF ANALYSIS AND SAMPLING

36th Session
Budapest, Hungary, 23 – 27 February 2015

MATTERS REFERRED BY THE CODEX ALIMENTARIUS COMMISSION AND OTHER SUBSIDIARY BODIES

(Comments of AACC International)

Methods for Dietary Fibre

18. CCNFSDU/36 agreed to recommend to CCMAS to:

- Retain AACCI 32-45.01 as the Type I method for dietary and adopt AACCI 32-50.01 as the Type 1 method for insoluble and soluble parts of dietary fibre (which can be summed up to total dietary fibre) as they have different scopes and are collaboratively studied and designed to match the Codex definition.
- Review if AOAC 2009.01 should now be considered as Type IV because it has been modified and not been collaboratively studied and is no longer considered equivalent to AACCI 32-45.01;
- Adopt AOAC 2011.25 as Type IV method because it has been modified and not been collaboratively studied and is no longer considered equivalent to AACCI 32-50.01.

Further to discussions at the IAM 20 February 2015, AACC International recommends the following changes to Standard 234.

STAN 234 with proposed changes

Commodity	Provision	Method	Principle	Type
All foods (1)	Method applicable for determining the content of dietary fibres of higher and lower molecular weight. The method is applicable in food that may, or may not, contain resistant starches.	**AACC Intl 32-45.01 (2009)** **AOAC 2009.01**	Enzymatic-Gravimetry High Pressure Liquid Chromatography	Type I
All foods (1)	**Method applicable for determining the content of insoluble and soluble dietary fibres of higher and lower molecular weight. The method is applicable in food that may, or may not, contain resistant starches.**	**AACC Intl 32-50.01 (2011)** **AOAC 2012.25**	**Enzymatic-Gravimetry** **High Pressure Liquid Chromatography**	**Type I**

Proposed changes are underlined

Method for detection of the toxic fraction in gluten harmful for individuals intolerant to gluten: ELISA G12 method

AACC International (AACC Intl) appreciates the opportunity to comment on this topic. AACC Intl recommends an update to the method currently in Standard 234 for detection of gluten in low-gluten products. As fully validated methods in specific matrices are now available to cover products based on maize and products based on rice.

Standard 234 with proposed changes
Foods for Special Dietary Uses

Commodity	Provision	Method	Principle	Type
~~Gluten-free foods~~	~~Gluten~~	~~Enzyme-Linked Immunoassay R5 Mendez (ELISA) Method *Eur J Gastroenterol Hepatol* 2003; 15: 465-474~~	~~Immunoassay~~	~~I~~
Gluten-free foods, maize matrices	Gluten	AACC Intl 38-50.01	Immunoassay	I
Gluten-free foods, rice matrices	Gluten	AACC Intl 38-52.01	Immunoassay	I

AACC Intl has recently adopted two methods for the quantitation of intact (low level) gluten in various food matrices: AACC 38-50.01 and AACC 38-52.01. Both methods have been through extensive collaborative studies and review by the AACC Intl approved methods technical committee chairs. The results have been reported in Cereal Foods World.

AACC Intl was careful in stipulating that 38-50.01 has been validated for corn-based matrices; 38-52.01 has been validated for rice-based matrices. Effect of matrices (e.g. recovery) should be taken into account at all times when using ELISA methods or expanding an ELISA method to matrices different from those that have been used for original testing in a collaborative study.

A current concern, in particular by celiac societies, is on the appropriateness of having more than one proprietary ELISA method for gluten quantitation validated and/or allowed. Here, the main features of the two methods approved by AACC Intl are discussed alongside the Codex 118 statements on Methods of Analysis and Sampling in chapter 5.

Codex 118: The quantitative determination of gluten in foods and ingredients shall be based on an immunologic method or other method providing at least equal sensitivity and specificity.

AACC Intl method 38-50.01 is an immunologic method based on the R5 antibody, the proprietary test-kit that has been evaluated by AACC Intl is the R-Biopharm test-kit. The antibody is raised against the ω-type of rye prolamins (ω-secalins); it is directed toward the epitope glutamine-glutamine-proline-phenylalanine-proline (QQPFP) in gliadins, hordeins, and secalins.

AACC Intl method 38-52.01 is an immunologic method based on the G12 antibody, the proprietary test-kit evaluated by AACC Intl is the Romer Labs test-kit. The antibody has been raised against a 33-mer fragment from α-gliadins with high immunogenicity that is highly resistant to degradation by digestive enzymes. The monoclonal antibody G12 targets the amino acid sequence QPQLPY within the 33-mer peptide.

Codex 118: The antibody used should react with the cereal protein fractions that are toxic for persons intolerant to gluten and should not cross-react with other cereal proteins or other constituents of the foods or ingredients.

Both antibodies fulfill this requirement. The R5 antibody targets a known epitope in gliadin related to celiac disease. The amino acid sequence QQPFP is part of numerous epitopes that have been found to be immunogenic or toxic. The G12 antibody targets a part of the 33-mer peptide. The amino acid sequence QPQLPY has also been found to be part of various peptides with celiac toxicity.

For both methods no cross-reactivity with non-toxic cereals has been noticed e.g. corn or rice. The G12 antibody has a cross-reactivity towards some oats cultivars.

Codex 118: Methods used for determination should be validated and calibrated against a certified reference material, if available.

To this date there is no certified reference material for calibration and validation. Therefore the only available option was followed. As calibrator PWG-gliadin (gliadin fraction of a mixture of 28 wheat varieties) was used (R5), or a commercial gluten preparation (G12) with known gliadin concentration (verified with PWG-gliadin).

Sample preparation.
On sample preparation it is important to note the following in both collaborative study reports:
A wheat flour was used for preparing gluten-containing test-samples with a known gluten target value. The gliadin and gluten contents of wheat flour from German cv. Genius were determined using an extraction/RP-HPLC method. Target concentrations were arrived at independently from the immunological methods that were tested to reveal the low levels of gluten contamination with reasonable recovery.

Codex 118: The detection limit has to be appropriate according to the state of the art and the technical standard. It should be 10 mg gluten/kg or below.

Both methods AACC Intl 38-50.01 (R5) and AACC Intl 38-52.01 (G12) are the current state-of-the-art of gluten testing with monoclonal antibodies. They have LODs well below the 10 mg gluten/kg, actually LODs are below 5 mg gluten/kg.

Codex 118: Method ELISA R5 (Mendez Method).

Standard 118 mentions specifically the R5 (Mendez Method). At the time of the latest revision in 2008 this was the method with the best performance. However, recent research has shown that there are various mono- and polyclonal antibodies being studied for use in new generation gluten-kits. In summary, the science on gluten ELISAs is improving fast. In the future, fixing Codex recommended gluten methodology on a single method could get in conflict with other statements in Codex 118 that “gluten (ELISA) methods should comply with current technical standards and the state-of-the-art of the science”.