CODEX ALIMENTARIUS COMMISSION

Food and Agriculture Organization of the United Nations

World Health Organization

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Agenda Item 3 **CRD19**

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JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON METHODS OF ANALYSIS AND SAMPLING

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ENDORSEMENT OF METHODS OF ANALYSIS AND SAMPLING PLANS FOR PROVISIONS IN CODEX STANDARDS

(Submitted by International Association for Cereal Science and Technology)

CRD from IACST supporting replacement of AOAC 2011.25/AACCI 32-50.01 with ICC Standard 191/AOAC 2022.01 in CXS 234-1999 as a Type I method for the measurement of Total Dietary Fibre.

Executive summary:

In 2009, a definition for dietary fibre that included resistant starch (RS) and non-digestible oligosaccharides (NDOs) was adopted by CODEX Alimentarius Commission. Analytical methodology to measure total dietary fibre (TDF) as defined by CODEX, namely AOAC 2009.01/AACCI 32-45.01, was also adopted at this time and included in CXS 234-1999. Method AOAC 2009.01/AACCI 32-45.01 was followed by method AOAC 2011.25/AACCI32-50.01 which, due to a modification of the method workflow, allows for the measurement of insoluble and soluble fiber separately. In evaluating these two methods since their initial publication, a number of limitations have been identified. These limitations were addressed by method AOAC 2017.16/ICC Standard 185 which was recognised as an improved method and in 2021, was accepted as a Type 1 method for the measurement of total dietary fiber (TDF) in CODEX CXS 234-1999 and replaced AOAC 2009.01/ AACCI 32-45.01. The equivalent update of method AOAC 2011.25/AACCI 32-50.01 for the measurement of insoluble (IDF) and soluble dietary fiber (SDF) has been completed and ICC Standard 191 / AOAC 2022.01 was developed. Following on from the acceptance of AOAC 2017.16/ICC Standard 185 as Type I method, we now have an anomaly in dietary fiber methodology within CODEX where the recommended Type 1 methods for a) Total Dietary Fiber and b) Soluble and Soluble Dietary Fiber, are no longer harmonised. In keeping with the best principles of CODEX, it is recommended that the standard should now be updated by replacing AOAC 2011.25/AACCI 32-50.01 with an improved, fully validated method, ICC Standard 191 / AOAC 2022.01, that corrects all issues identified with AOAC 2011.25/AACCI 32-50.01 as outlined in detail in Appendix A.

Agenda Item #3: Endorsement of Methods of Analysis Provisions and Sampling Plans in Codex Standards

Methods of analysis for provisions in the Standard for determining the content of dietary fibres of higher and lower molecular weight in food that may or may not contain resistant starches (CXS 234-1999).

ICC Standard 191 / AOAC 2022.01

Codex Committee decision: CCNFSDU41 (Dusseldorf, November 2019) recommended replacement of AOAC 2009.01/AACCI 32-45.01 with AOAC 2017.16/ICC Standard 185. The equivalent replacement of AOAC 2011.25/ AACCI 32-50.01 with ICC Standard 191 / AOAC 2022.01 is also required now that a harmonised, validated method is available for this provision.

- **Title and method description:** Determination of Insoluble, Soluble, and Total Dietary Fiber in Foods Using a Rapid Integrated Procedure of Enzymatic-Gravimetric-Liquid Chromatography. Briefly, a defatted, lyophilised, homogenous food sample is incubated with pancreatic α-amylase (PAA) plus amyloglucosidase (AMG) at 37°C for 4 hours to simulate human intestinal digestion followed by protease. Insoluble dietary fiber (IDF) is recovered through filtration and measured gravimetrically. An ethanol solution is added to the filtrate

to recover fibre which precipitates in the presence of 78% aqueous ethanol (SDFP) which is measured gravimetrically. Allowance is made for residual ash and protein content. Dietary fibre that is soluble in 78% aqueous ethanol (SDFS) is recovered and measured by high-performance liquid chromatography (HPLC). Soluble dietary fiber is the sum of SDFP and SDFS. Insoluble dietary fiber is IDF. Total dietary fibre (TDF) is the sum of the insoluble fiber fraction (IDF) and the soluble dietary fiber (SDFP + SDFS).[1]

1. McCleary, B. V., Sloane, N. and Draga, A. (**2015**). Determination of total dietary fibre and available carbohydrates: A rapid integrated procedure that simulates in vivo digestion. *Starch-Stärke*, **67**, 860-883. https://onlinelibrary.wiley.com/doi/full/10.1002/star.201500017

- **Scope and validated matrices:** An interlaboratory validation study involving 17 laboratories around the world was conducted in conjunction with AOAC and ICC. Eight blind duplicate samples were selected to cover a range of relevant food samples comprising canned kidney beans, carrots (steamed), dark rye crispbread, high-fiber barley flour, oat bran, miso soup powder containing resistant maltodextrins, chocolate containing resistant maltodextrins and a health food nutrition bar containing fructo-oligosaccharides. The performance of the method in terms of repeatability and reproducibility was marginally better than that reported for AOAC 2011.25/AACCI 32-50.01.

McCleary, B. V. and McLoughlin C. Determination of Insoluble, Soluble, and Total Dietary Fiber in Foods Using a Rapid Integrated Procedure of Enzymatic-Gravimetric-Liquid Chromatography: First Action 2022.01 *Journal of AOAC International*, 2023, **106**, 127-145. **Note that this method is now First Action.**

https://academic.oup.com/jaoac/article/106/1/127/6668272

- **Description of the method principle:** The full method protocol is available for download from AOAC or ICC and a summary is outlined below.

AOAC 2022.01/ICC Standard 191 is based on a similar principle to AOAC 2011.25/AACCI 32-50.01 but significant changes have greatly improved the method performance, particularly for some important sample types. In ICC Standard 191 / AOAC 2022.01, duplicate test portions are incubated for 4 hours at 37°C and pH 6 with 4 KU pancreatic □-amylase (PAA) and 1.7 KU amyloglucosidase (AMG) while stirring or shaking in 250 mL bottles. This incubation mimics *in-vivo* digestion, solubilising and hydrolysing non-resistant starch. The reaction is terminated by adjustment of the pH to 8.2 and increasing the temperature to ~95°C to inactivate both PAA and AMG. This is followed by a protease incubation for 30 minutes at 60°C at pH 8.2 to hydrolyse protein in the sample.

After the enzymatic hydrolysis is completed, the pH is adjusted to 4.3 to inactivate protease and the sample is filtered through a crucible containing Celite, washed, dried and weighed to measure the IDF fraction. This residue weight is corrected for protein, ash and the blank value for the final calculation.

Four volumes of 95% aqueous ethanol are then added to the incubation mixture and stirred to precipitate SDFP which is recovered on a crucible, washed, dried and weighed. This residue weight is corrected for protein, ash and the blank value for the final calculation.

The aqueous ethanol filtrate is concentrated, deionised and analysed by HPLC using TSKgel® PWXL analytical and guard columns to allow for accurate measurement of SDFS versus an internal standard, diethylglycerol (or glycerol). Total dietary fibre is calculated as the sum of the insoluble fiber component IDF and SDFP and the soluble fiber fraction: SDFS.

- **Comparison with existing methods:** Differences between ICC Standard 191 / AOAC 2022.01 and AOAC 2011.25/AACCI 32-50.01 are outlined in detail in Appendix A.

Validation Summary

Interlaboratory study attribute	AOAC 2011.25/AACCI 32-50.01	ICC Standard 191 / AOAC 2022.01
Matrices, samples used	Cabbage, mixed grains with apple flakes, chocolate with fructooligosaccharides, biscuits containing fructooligosaccharides, defatted cookies with oat graham and polydextrose and RS2 starch, peanuts, oat bran, whole wheat bread with 2% α-cyclodextrin;	canned kidney beans, carrots (steamed), dark rye crispbread, high-fiber barley flour, oat bran, miso soup powder containing resistant maltodextrins, chocolate containing resistant maltodextrins and a health food nutrition bar containing fructo-oligosaccharides
No. of laboratories	19	17
TDF concentration, g/100g	11.8-29.9	22.87-41.19
s_r, g/100g	0.47-1.41	0.59-1.35
s_R, g/100g	0.95-3.14	1.11-3.05
RSD_r, %	2.43-8.60	1.58-3.57
RSD_R, %	6.85-14.48	4.55-9.26
CXS 234-1999 Provision	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.	

Summary of proposed changes in CXS 234-1999, Methods of analysis for dietary fibre: Guidelines for Use of Nutrition and Health Claims: Table of Conditions for Claims (see p28)

General methods that measure both the higher (monomeric units > 9) and the lower molecular weight fraction (monomeric units <=9)				
Standard	**Provisions**	**Method**	**Principle**	**Proposed Type**
All foods	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.	**AOAC 2022.01/ ICC Standard 191**	**Enzymatic-Gravimetry High Pressure Liquid Chromatography**	**I**
		~~AOAC 2011.25~~ ~~AACC Intl 32-50.01~~	~~Enzymatic-Gravimetry High Pressure Liquid Chromatography~~	~~I~~

Recommendations to CCMAS

AOACI and IACST recommends CCMAS to take the following actions:

1. Endorse ICC Standard 191 / AOAC 2022.01 as Type I for the determination of insoluble and soluble dietary fibres of higher and lower molecular weight in food that may or may not contain resistant starches.

2. Remove AOAC 2011.25/AACCI 32-50.01 from CXS 234-1999.

Appendix A. Technical issues with AOAC 2011.25/AACCI 32-50.01 now rectified with ICC Standard 191 / AOAC 2022.01:

1) Resistant maltodextrin artefacts: It was discovered that during the analysis of starchy foods such as bread and pasta, highly resistant maltodextrin compounds were produced as an artefact of the enzymatic incubation conditions employed in AOAC 2011.25/AACCI 32-50.01.[1] These compounds were then incorrectly included in the SDFS fraction resulting in an overestimation of TDF. The absolute value of the overestimation was typically 1-2 g/100g but given that the foods most affected typically exhibited very low TDF content, this can have significant implications for nutrient content claim labelling. In a specific example, the TDF value for Kellogg's Corn Flakes was erroneously increased from 3.8 to 6.0 g/100g2 which according to CAC/GL 23-1997 would allow for the manufacturer to make a "high" fibre claim while the correct TDF value of 3.8 g/100g qualifies only for a "source" fibre claim. An equivalent case was also observed for certain breads.[1]

A modification to AOAC 2011.25 was introduced in 2014[2] to address this limitation but this was not adopted by CODEX at CCNFSDU36 as the modified method was not fully validated through a multi-laboratory study. In response, the method author completely redeveloped AOAC 2011.25/AACCI 32-50.01 to arrive at ICC Standard 191 / AOAC 2022.01, moving from a 16-hour enzymatic incubation time to a more physiologically relevant period of 4 hours that avoided the undesired formation of the resistant maltodextrin compounds referenced above.

2) Resistant starch underestimation: It had also been suggested that AOAC 2011.25/AACCI 32-50.01 failed to accurately measure certain forms of resistant starch, most notably RS_4 a synthetic phosphate cross-linked starch.[3] This issue was also resolved by the new, shorter, enzymatic incubation conditions that match closely with those found in the human digestive system where the residence time for food is approximately 4 hours. In moving from AOAC 2011.25/AACCI 32-50.01 to ICC Standard 191 / AOAC 2022.01, the measured TDF content of RS_4 and RS_2 increased from ~30 g/100g to ~60 g/100g, and ~50 g/100g to ~59% g/100g, respectively. Given the adoption of physiologically relevant enzyme incubation conditions, the new results obtained are deemed to be more accurate.

3) Fructo-oligosaccharides (FOS) underestimation: Fructotriose, a significant component in FOS mixtures, was incorrectly not included as part of the SDFS fraction when the AOAC 2011.25/AACCI 32-50.01 was performed with the recommended Waters SugarPak HPLC column. ICC Standard 191 / AOAC 2022.01 removes the option to use this column and specifies that only a TSK-Gel HPLC column can be employed for the quantification of SDFS. This procedure ensures that fructotriose elutes before DP2 oligosaccharides and thereby eliminates the FOS underestimation issue. The chromatography conditions for ICC Standard 191 / AOAC 2022.01 match those that are described in AOAC 2001.03.

4) Isomaltooligosaccharides overestimation: AOAC 2011.01/AACCI 32-50.01 quantified the TDF content of typical IMO food ingredients at ~30 g/100g which has been shown to be a significant overestimation.[4,5] ICC Standard 191 / AOAC 2022.01 reduces this value to ~10 g/100g and once again, given the adoption of physiologically relevant enzyme incubation conditions, the new result obtained is deemed to be more accurate.

5) Further improvements: In addition to the errors that have been corrected as outlined above, practical method improvements have also been implemented following feedback from laboratory analysts using AOAC 2011.25/AACCI 32-50.01.

a. Sodium azide, an acute toxic chemical, was included in the enzymatic incubation conditions in AOAC 2011.25/AACCI 32-50.01 to prevent microbial growth contamination during the assay. Reducing the incubation period from 16 hours to 4 hours removed the requirement for sodium azide in ICC Standard 191 / AOAC 2022.01.

b. A simplified procedure for desalting samples prior to HPLC analysis was introduced in ICC Standard 191 / AOAC 2022.01. This improvement, in addition to the shortened enzyme incubation period, reduces resource requirement for analysts resulting in lower analytical laboratory costs.

c. The use of diethylene glycol (DEG) in ICC Standard 191 / AOAC 2022.01 as the recommended internal standard for the measurement of SDFS to replace sorbitol, the internal standard in AOAC 2011.25/AACCI 32-50.01, makes the method more universally applicable given that DEG is not a typical food ingredient, while sorbitol can be present in some food matrices.

Lastly but most importantly, it must be stressed that the major difference between AOAC 2011.25/AACCI 32 50.01 and ICC Standard 191 / AOAC 2022.01 is the reduction in the enzyme incubation period to match that of the average residence time for food in the small intestine. This change will “future-proof” ICC Standard 191 / AOAC 2022.01 to ensure that the analysis of functional food ingredients that will continue to be developed in the future will result in TDF values that closely reflect their behaviour in the human digestive system.

1. Brunt K, Sanders P. Improvement of the AOAC 2009.01 total dietary fibre method for bread and other high starch containing matrices. *Food Chem*. 2013;140(3):574-580. doi:10.1016/j.foodchem.2012.10.109.

2. McCleary B V. Modification to AOAC Official Methods 2009.01 and 2011.25 to Allow for Minor Overestimation of Low Molecular Weight Soluble Dietary Fiber in Samples Containing Starch. *J AOAC Int*. 97(3):896-901. https://www.ingentaconnect.com/content/aoac/jaoac/2014/00000097/00000003/art00032.

3. Shukri R, Zhu L, Seib PA, Maningat C, Shi Y-C. Direct in-vitro assay of resistant starch in phosphorylated cross-linked starch. *Bioact Carbohydrates Diet Fibre*. 2015;5(1):1-9. doi:https://doi.org/10.1016/j.bcdf.2014.11.001.

4. Lowery RP, Wilson JM, Barninger A, et al. The effects of soluble corn fibre and isomaltooligosacharides on blood glucose, insulin, digestion and fermentation in healthy young males and females. *J Insul Resist*. 2018;3(1):1-6. doi:http://dx.doi.org/10.4102/jir.v3i1.32.

5. Gourineni V, Stewart LM, Icoz D, Zimmer PJ. Gastrointestinal Tolerance and Glycemic Response of Isomaltooligosaccharides in Healthy Adults. *Nutr* . 2018;10(3). doi:10.3390/nu10030301.