CODEX ALIMENTARIUS COMMISSION

Food and Agriculture Organization of the United Nations

World Health Organization

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Agenda Item 2 **MAS-CRD/03**

ORIGINAL LANGUAGE ONLY

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON METHODS OF ANALYSIS SAMPLING

MATTERS REFERRED TO THE COMMITTEE BY THE CODEX ALIMENTARIUS COMMISSION AND OTHER SUBSIDIARY BODIES

Information and comments submitted by AOAC and ICC supporting replacement of AOAC 2009.01/AACCI 32-45.01 with AOAC 2017.16/ ICC Standard 185 in CXS 234-1999 as a Type I method for the measurement of Total Dietary Fibre

Executive Summary

This document presents recommendations and supporting information from the Association of Official Analytical Collaboration International (AOAC) and the International Association of Cereal Science and Technology (ICC) regarding an update to dietary fibre methodology recommended in CXS 234-1999.

In 2009, a definition for dietary fibre that included resistant starch (RS) and non-digestible oligosaccharides (NDOs) was adopted by the 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. In evaluating this method since its initial publication in 2007, a number of limitations have been identified. It is therefore recommended that the standard be updated by replacing AOAC 2009.01/AACCI 32-45.01 with an improved, fully validated method, AOAC 2017.16/ICC Standard 185, that corrects all issues identified with AOAC 2009.01/AACCI 32-45.01, as outlined in detail in Appendix A.

Recommendations to CCMAS41

AOAC and ICC recommend CCMAS41 to take the following actions:

1. Endorse AOAC 2017.16/ICC Standard 185 as Type I for the determination of dietary fibres of higher and lower molecular weight in food that may or may not contain resistant starches.
2. Remove AOAC 2009.01/AACCI 32-45.01 from CXS 234-1999.

Agenda Item #2: Matters Referred to the Committee by the Codex Alimentarius Commission and Other Subsidiary Bodies

Codex Committee on Nutrition and Foods for Special Dietary Uses (CCNFSDU41)

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).

AOAC 2017.16/ICC Standard 185

- **Codex Committee decision:** CCNFSDU41 (Dusseldorf, November 2019) recommended replacement of AOAC 2009.01/AACCI 32-45.01 with AOAC 2017.16/ICC Standard 185.

- **Title and method description:** Total Dietary Fibre in Foods: Enzymatic-gravimetric-HPLC method. 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. Higher molecular weight dietary fibre (HMWDF) comprising insoluble dietary fibre (IDF) and fibre which precipitates in the presence of 78% ethanol (SDFP) 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). Total dietary fibre (TDF) is the sum of HMWDF (IDF + SDFP) and SDFS.[1]

- **Scope and validated matrices:** An interlaboratory validation study involving 13 laboratories around the world was conducted in conjunction with AOAC, ICC and AACCI. Eight blind duplicate samples were selected to cover a range of relevant food samples comprising RS_4, kidney beans, bran cereal, cookies containing FOS, oat bran, cookies containing RS_2 and polydextrose, dark rye crispbread, wholemeal bread. The performance of the method in terms of repeatability and reproducibility was marginally better than that reported for AOAC 2009.01/AACCI 32-45.01.[2][3][4]

- **Description of the method principle:** The full method protocol is available for download from AOACI (http://www.eoma.aoac.org/methods/info.asp?ID=51726) or ICC (https://icc.or.at/publications/icc-standards/standards-overview/185-standard-method) and a summary is outlined below.

AOAC 2017.16/ICC Standard 185 is based on a similar principle to AOAC 2009.01/AACCI 32-45.01 but significant changes have greatly improved the method performance, particularly for some important sample types. In AOAC 2017.16/ICC Standard 185, duplicate test portions are incubated for 4-hours at 37°C at pH 6 with 4 KU pancreatic a-amylase (PAA) and 1.7 KU amylglucosidase (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.

Four volumes of 95% aqueous ethanol are then added to the incubation mixture and stirred to precipitate SDFP. The HMWDF (comprising IDF and SDFP) is recovered on a crucible, washed, dried and

[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

[2] McCleary, B. V. Total Dietary fibre (CODEX Definition) in Foods and Food Ingredients by a Rapid Enzymatic-Gravimetric Method and Liquid Chromatography: Collaborative Study, First Action 2017.16. *Journal of AOAC International*, 2019, **101**, 196-207. **Note that this method is now Final Action.** https://academic.oup.com/jaoac/article/102/1/196/5658193

[3] McCleary, B. V., Cox, J. and McKie, V. A. AACC International Approved Methods Technical Committee Report: Collaborative Study on Determination of Total Dietary fibre (Digestion-Resistant Carbohydrates per Codex Definition) by a Rapid Enzymatic-Gravimetric Method and Liquid Chromatography. *Cereal Foods World*, 2018, **63**, 80-84. https://www.cerealsgrains.org/publications/plexus/cfw/pastissues/2018/mar-apr/Pages/CFW-63-2-0080.aspx

[4] Note that new terminology/nomenclature/language that was introduced by AACCI during the Final Action approval stage could not be accepted by the method author and as such, AACCI method 32-60.01 has not yet been advanced to Final Action Status by AACCI.

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® PW_{XL} analytical and guard columns to allow for accurate measurement of SDFS versus an internal standard, glycerol. Total dietary fibre is calculated as the sum of HMWDF and SDFS.

- **Comparison with existing methods:** Differences between AOAC 2017.16/ ICC Standard 185 and AOAC 2009.01/AACCI 32-45.01 are outlined in detail in Appendix A.

Validation Summary

Interlaboratory study attribute	**AOAC 2009.01/AACCI 32-45.01**	**AOAC 2017.16/ICC Standard 185**
Matrices, samples used	RS_2, kidney beans, bran cereal, broccoli, carrots, haricot beans, wholegrain bread, wholegrain pasta	RS_4, kidney beans, bran cereal, cookies containing FOS, oat bran, cookies containing RS2 and polydextrose, dark rye crispbread, wholemeal bread
No. of laboratories	16	13
TDF concentration, g/100g	11.6-47.8	6.8-60.6
s_r, g/100g	0.41-1.43	0.29-0.74
s_R, g/100g	1.18-5.44	0.57-4.67
RSD_r, %	1.65-12.34	1.22-6.34
RSD_R, %	4.7-17.97	2.64-13.38
CXS 234-1999 Provision	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.	

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 dietary fibres of higher and lower molecular weight. The method is applicable in food that may or may not contain resistant starches.	**AOAC 2017.16/ ICC Standard 185**	**Enzymatic-Gravimetry High Pressure Liquid Chromatography**	**I**
		~~AACC Intl 32-45.01 AOAC 2009.01~~	~~Enzymatic-Gravimetry High Pressure Liquid Chromatography~~	~~I~~

Appendix A. Technical issues with AOAC 2009.01/AACCI 32-45.01 now rectified with AOAC 2017.16/ICC Standard 185:

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 2009.01/AACCI 32-45.01.[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 Kellog's Corn Flakes was erroneously increased from 3.8 to 6.0 g/100g[2] 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 2009.01 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 2009.01/AACCI 32-45.01 to arrive at AOAC 2017.16/ICC Standard 185, 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 2009.01/AACCI 32-45.01 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 2009.01/AACCI 32-45.01 to 2017.16/ICC Standard 185, 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 2009.01/AACCI 32-45.01 was performed with the recommended Waters SugarPak HPLC column. ICC Standard 185/AOAC 2017.16 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.

4) Isomaltooligosaccharides overestimation: AOAC 2009.01/AACCI 32-45.01 quantified the TDF content of typical IMO food ingredients at ~30 g/100g which has been shown to be a significant overestimation.[4,5] AOAC 2017.16/ICC Standard 185 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 2009.01/AACCI 32-45.01 over the previous 8 years.

a. Sodium azide, an acute toxic chemical, was included in the enzymatic incubation conditions in AOAC 2009.01/AACCI 32-45.01 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 AOAC 2017.16/ICC Standard 185.

b. A simplified procedure for desalting samples prior to HPLC analysis was introduced in ICC Standard 185/AOAC 2017.16. This improvement, in addition to the shortened enzyme incubation period, reduces resource requirement for analysts resulting in lower analytical laboratory costs.

Lastly but most importantly, it must be stressed that the major difference between AOAC 2009.01/AACCI 32 45.01 and AOAC 2017.16/ICC Standard 185 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" AOAC

2017.16/ICC Standard 185 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.
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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.