CODEX ALIMENTARIUS COMMISSION

Food and Agriculture Organization of the United Nations

World Health Organization

Viale delle Terme di Caracalla, 00153 Rome, Italy - Tel: (+39) 06 57051 - E-mail: codex@fao.org - www.codexalimentarius.org

Agenda item 3

MAS44/CRD04
April 2025
ORIGINAL LANGUAGE ONLY

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON METHODS OF ANALYSIS AND SAMPLING

44th Session
Virtual
5-8 May and 14 May 2025

Replacement of AOAC 2011.25/AACCI 32-50.01 with AOAC 2022.01/ICC Standard 191/AACCI 32-61.01 in CXS 234-1999 as a Type I method for the measurement of soluble, insoluble and total dietary fibre

(Information provided by AACC, AOAC and ICC)

Executive summary:

The Codex Committee on Nutrition and Foods for Special Dietary Uses (CCNFSDU44) recommended the replacement of AOAC 2011.25/AACCI 32-50.01 with AOAC 2022.01/ICC Standard 191/AACCI 32-61.01 in CXS 234-1999 as a Type I method for the measurement of Soluble, Insoluble and Total Dietary Fibre.

The proposed replacement will ensure harmonization of a method for total dietary fibre (AOAC 2017.16/ICC Standard 185/ AACCI 32-60.01) and a method for insoluble, soluble and total dietary fibre (AOAC 2022.01/ICC Standard 191/AACC 32-61.01) in CXS 234-1999 as these methods are based on the same incubation conditions.

This document presents recommendations and supporting information from AOAC INTERNATIONAL (AOAC), International Association for Cereal Science and Technology (ICC) and Cereals & Grains Association (AACC) regarding this topic to be discussed during the 44th Session of the Codex Committee on Methods of Analysis and Sampling (CCMAS44).

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/ AACCI 32-61.01**	**Enzymatic-Gravimetry High Pressure Liquid Chromatography**	**I**
		~~AOAC 2011.25 AACC Intl 32-50.01~~	~~Enzymatic-Gravimetry High Pressure Liquid Chromatography~~	~~I~~

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 separate measurement of insoluble (IDF) and soluble dietary fibre (SDF). 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/AACCI 32-60.01, which was internationally recognised as an improved

method and in 2021, was accepted as a Type I method for the measurement of total dietary fibre (TDF) in CXS 234-1999, replacing AOAC 2009.01/AACCI 32-45.01. The equivalent update of method AOAC 2011.25/AACCI 32-50.01 for the measurement of IDF, SDF and TDF has been completed and approved as AOAC 2022.01/ICC Standard 191/AACCI 32-61.01. Following on from the acceptance of AOAC 2017.16/ICC Standard 185/AACCI 32-60.01as Type I method in 2021, an anomaly now exists in dietary fibre methodology within CODEX, where the recommended Type I methods for a) Total Dietary Fibre and b) Insoluble, Soluble and Total Dietary Fibre, are no longer harmonised. In keeping with the best principles of CODEX, and as recommended by CCNFSDU44, CXS 234-1999 should now be updated by replacing AOAC 2011.25/AACCI 32-50.01 with an improved, validated method, AOAC 2022.01/ICC Standard 191/AACCI 32-61.01, that corrects all issues identified with AOAC 2011.25/AACCI 32-50.01 as outlined in detail in Appendix A.

Specifically, CCNFSDU44 agreed to request CCMAS to:

- Endorse AOAC 2022.01/ICC Standard 191/AACC 32-61.01 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. The CCNFSDU44 suggested that a footnote as follows should be inserted:

  Isolated, purified, and/or synthetic fibres captured by AOAC 2022.01/ICC Standard 191/AACC 32-61.01 that do not meet the Codex definition of dietary fibre in the Guidelines on nutrition labelling (CXG 2-1985) should be subtracted from the final measurement, where deemed appropriate by competent authorities.

- Revoke AOAC 2011.25/AACC 32-50.01 for use with the same provision.

AOAC, ICC and AACC consider the proposed footnote inconsistent with existing practices in CXS 234-1999 because this footnote addresses a regulatory concern and not an analytical method issue. Furthermore, this statement, while true, already applies to all nine total dietary fibre methods that are included in CXS 234-1999, given that the definition of dietary fibre in CXG-2 provides additional restrictions on isolated and synthetic dietary fibres. The definition located in CXG-2 is transcribed below for clarity:

Dietary fibre means carbohydrate polymers[1] with ten or more monomeric units[2] , which are not hydrolysed by the endogenous enzymes in the small intestine of humans and belong to the following categories:

- Edible carbohydrate polymers naturally occurring in the food as consumed,
- carbohydrate polymers, which have been obtained from food raw material by physical, enzymatic or chemical means and which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities,
- synthetic carbohydrate polymers which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities.

It is the position of AOAC, ICC and C&G that it is already abundantly clear that if an isolated or synthetic dietary fibre does not meet the Codex definition, but is captured in any analytical method, then it "should be subtracted from the final measurement, where deemed appropriate by competent authorities." The proposed footnote seems therefore redundant. If CCMAS44 determines that such a footnote is required, then it should apply to all total dietary fibre methods in CXS 234-1999 and not only to AOAC 2022.01/ICC Standard 191/AACC 32-61.01.

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).

AOAC 2022.01/ICC Standard 191/AACCI 32-61.01

Codex Committee decision: CCNFSDU44 (Dresden, October 2024) recommended replacement of AOAC 2011.25/AACCI 32-50.01 with AOAC 2022.01/ICC Standard 191/AACCI 32-61.01.

- **Title and method description:** Determination of Insoluble, Soluble, and Total Dietary Fibre in Foods Using a Rapid Integrated Procedure of Enzymatic-Gravimetric-Liquid Chromatography. Briefly, a

1 When derived from a plant origin, dietary fibre may include fractions of lignin and/or other compounds associated with polysaccharides in the plant cell walls. These compounds also may be measured by certain analytical method(s) for dietary fibre. However, such compounds are not included in the definition of dietary fibre if extracted and re-introduced into a food.

2 Decision on whether to include carbohydrates from 3 to 9 monomeric units should be left to national authorities."

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 fibre (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 fibre is the sum of SDFP and SDFS. Insoluble dietary fibre is IDF. Total dietary fibre (TDF) is the sum of the insoluble fibre fraction (IDF) and the soluble dietary fibre (SDFP + SDFS).[1]

- **Scope and validated matrices:** An interlaboratory validation study involving 17 international laboratories was conducted.[2] Eight blind duplicate samples were selected to cover a range of relevant food samples comprising canned kidney beans, carrots (steamed), dark rye crispbread, high-fibre 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.

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

  AOAC 2022.01/ICC Standard 191/AACCI 32-61.01 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 certain important sample types. In AOAC 2022.01/ICC Standard 191/AACCI 32-61.01, duplicate test portions are incubated for 4 hours at 37°C and pH 6 with 4 KU pancreatic a-amylase (PAA) and 1.7 KU amyloglucosidase (AMG) while stirring or shaking in 250 mL bottles. This incubation mimics *in-vivo* digestion[3], 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® PW_{XL} 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 fibre component IDF and SDFP and the soluble fibre fraction: SDFS.

- **Comparison with existing methods:** Differences between AOAC 2022.01/ ICC Standard 191/AACCI 32-61.01 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**	**AOAC 2022.01/ICC Standard 191/AACCI 32-61.01**
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-fibre 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)

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Recommendations to CCMAS

AOAC, ICC and AACC recommends the following actions for CCMAS' consideration:

1. Endorse AOAC 2022.01/ICC Standard 191/AACCI 32-61.01 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. Revoke AOAC 2011.25/AACCI 32-50.01 from CXS 234-1999 for use with the same provision.
3. If the accompanying footnote proposed by CCNFSDU44 is needed in CXS 234-1999, apply it to all total dietary fibre methods in that standard.

Appendix A. Technical issues with AOAC 2011.25/AACCI 32-50.01 now rectified with AOAC 2022.01/ICC Standard 191/AACCI 32-61.01:

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.[4] 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/100g[5] 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.[4]

   A modification to AOAC 2011.25 was introduced in 2014[5] 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 AOAC 2022.01/ICC Standard 191/AACCI 32-61.01, 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.[6] 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 2022.01/ICC Standard 191/AACCI 32-61.01, 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. AOAC 2022.01/ICC Standard 191/AACCI 32-61.01 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 AOAC 2022.01/ICC Standard 191/AACCI 32-61.01 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.[7,8] AOAC 2022.01/ICC Standard 191/AACCI 32-61.01 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 as a result of continued research conducted by the method author, combined with 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 AOAC 2022.01/ICC Standard 191/AACCI 32-61.01.

   b. A simplified procedure for desalting samples prior to HPLC analysis was introduced in AOAC 2022.01/ICC Standard 191/AACCI 32-61.01. 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 diethylglycerol (DEG) in AOAC 2022.01/ICC Standard 191/AACCI 32-61.01 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 AOAC 2022.01/ICC Standard 191/AACCI 32-61.01 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" AOAC 2022.01/ICC Standard 191/AACCI 32-61.01 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.

References:

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