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Agenda Item 10 **CRD05 Rev**

JOINT FAO/WHO FOOD STANDARDS PROGRAMME
CODEX COMMITTEE ON NUTRITION AND FOODS FOR SPECIAL DIETARY USES

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(Proposals submitted by AOAC INTERNATIONAL, C&G[1], ICC, IDF, ISDI and ISO)

AOAC INTERNATIONAL, C&G and ICC

Replacement of AOAC 2011.25/AACC 32-50.01 with AOAC 2022.01/ICC Standard 191/AACC 32-61.01 in CXS 234-1999 as a Type I Method for the Measurement of Soluble, Insoluble and Total Dietary Fibre

EXECUTIVE SUMMARY

This document outlines a proposal for the 44th Session of the Codex Committee on Nutrition and Foods for Special Dietary Uses (CCNFSDU) to consider replacement of AOAC 2011.25/AACC 32-50.01 with AOAC 2022.01/ICC Standard 191/AACC 32-61.01 in CXS 234-1999 as a Type I method for the measurement of insoluble, soluble and total dietary fibre.

AOAC 2017.16/ICC Standard 185/AACC-23-60.01 was accepted as a Type I method for the measurement of total dietary fibre in CXS 234-1999 through CCNFSDU41 and CCMAS41 (2019-2021), replacing the AOAC 2009.01/AACC 32-45.01 method. The equivalent update of method AOAC 2011.25/AACC 32-50.01 for the measurement of insoluble, soluble and total dietary fibre has now been completed and approved as AOAC 2022.01/ICC Standard 191/AACC 32-61.01 and should also be reflected in CXS 234-1999.

Summary of proposed changes in CXS 234-1999, Table 4. Methods of analysis for dietary fibre: *Guidelines for use of nutrition and health claims* (CXG 23-1997): Table of conditions for claims

General methods that measure both the higher (monomeric units > 9) and the lower molecular weight fraction (monomeric units <=9)				
Standard	**Provisions**	**Method**	**Principle**	**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 2011.25~~ ~~AACC Intl 32-50.01~~ AOAC 2022.01/ AACC 32-61.01/ ICC Standard No. 191	Enzymatic-Gravimetry High Pressure Liquid Chromatography	I

BACKGROUND

In 2009, a definition for dietary fibre that included resistant starch (RS) and non-digestible oligosaccharides (NDOs) was adopted by Codex Alimentarius Commission (see CXG-2). Analytical methodology to measure total dietary fibre (TDF) as defined by Codex, namely AOAC 2009.01/AACC 32-45.01, was also adopted at this time and included in CXS 234-1999. Method AOAC 2009.01/AACC 32-45.01 was followed by method AOAC 2011.25/AACC32-50.01 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

[1] Formerly known as AACC International

AOAC 2017.16/ICC Standard 185/AACC 32-60.01 for total dietary fibre (TDF) and AOAC 2022.01/ICC Standard 191/AACC 32-61.01 for IDF, SDF and TDF – see Appendix A for details.

PROPOSAL AND RATIONALE

Following on from the acceptance of AOAC 2017.16/ICC Standard 185 as Type I method in 2021, an anomaly now exists in dietary fibre methodology within CXS 234-1999, where the recommended Type I methods for a) TDF and b) IDF, SDF and TDF, are no longer harmonized. Therefore, it is recommended that the CXS 234-1999 should now be updated by replacing AOAC 2011.25/AACC 32-50.01 with the improved, validated method, AOAC 2022.01/ICC Standard 191/AACC 32-61.01, that corrects issues identified with AOAC 2011.25/AACC 32-50.01.

AOAC 2022.01/ICC Standard 191/AACC 32-61.01 method and interlaboratory validation study summary are provided below.

AOAC 2022.01/ICC Standard 191/AACC 32-61.01 Method and Validation Study Summary

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

Method principle: A defatted, lyophilized, 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).[2]

Scope and validated matrices: An interlaboratory validation study involving 17 laboratories around the world was conducted.[3] 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 those reported for AOAC 2011.25/AACC 32-50.01.

Comparison with existing methods: Specific technical differences between AOAC 2022.01/ICC Standard 191/AACC 32-61.01 and AOAC 2011.25/AACC 32-50.01 are outlined in Appendix A.

Interlaboratory Study Attribute	**AOAC 2011.25/AACC 32-50.01**	**AOAC 2022.01/ICC Standard 191/AACC32-61.01**
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

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

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

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.	

RECOMMENDATION

That the Committee request CCMAS to:

1. 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.
2. Revoke AOAC 2011.25/AACC 32-50.01 for that provision.

Appendix A. Technical issues with AOAC 2011.25/AACC 32-50.01 now rectified with AOAC 2022.01/ICC Standard 191/AACC 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/AACC 32-50.01.[1,2] 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 a corn flakes product was erroneously increased from 3.8 to 6.0 g/100g[2] which according to CXG 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/AACC 32-50.01[3] to arrive at AOAC 2022.01/ICC Standard 191/AACC 32-61.01[4], moving from a 16-hour enzymatic incubation time to a more physiologically relevant period of 4 hours and employing higher concentrations of pancreatic a-amylase and amyloglucosidase, 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/AACC 32-50.01 failed to accurately measure certain forms of resistant starch, most notably RS_4 a synthetic phosphate cross-linked starch.[5] 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/AACC 32-50.01 to 2022.01/ICC Standard 191/AACC 32-61.01, the measured TDF content of specific samples 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/AACC 32-50.01 was performed with the recommended Waters SugarPak HPLC column. AOAC 2022.01/ICC Standard 191/AACC 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/AACC 32-61.01 match those that are described in AOAC 2001.03.

4) Isomaltooligosaccharides overestimation: AOAC 2011.01/AACC 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.[3,6,7] AOAC 2022.01/ICC Standard 191/AACC 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[7]: In addition to the limitations that have been addressed as outlined above, practical method improvements have also been implemented following feedback from laboratory analysts using AOAC 2011.25/AACC 32-50.01 and inhouse improvements.

a. Sodium azide, an acute toxic chemical, was included in the enzymatic incubation conditions in AOAC 2011.25/AACC 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/AACC 32-61.01.

b. A simplified procedure for desalting samples prior to HPLC analysis was introduced in AOAC 2022.01/ICC Standard 191/AACC 32-61.01. This improvement, extends the effective “life” of HPLC deionization cartridges by up to 10-times, resulting in lower analytical laboratory costs.

c. The use of diethylglycerol (DEG) in AOAC 2022.01/ICC Standard 191/AACC 32-61.01 as the recommended internal standard for the measurement of SDFS to replace sorbitol, the internal standard in AOAC 2011.25/AACC 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; also, sorbitol is not suitable as an internal standard when using the recommended TSK-Gel HPLC column as it elutes at the same point as glucose.

The major difference between AOAC 2011.25/AACC 32 50.01 and AOAC 2022.01/ICC Standard 191/AACC 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 and the increase of the levels of pancreatic a-amylase and amyloglucosidase to ensure that resistant starch values of reference samples are in-line with ileostomy values. This change will “future-proof” AOAC 2022.01/ICC Standard 191/AACC 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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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*. 2014, 97(3), 896-901. https://www.ingentaconnect.com/content/aoac/jaoac/2014/00000097/00000003/art00032.

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AOAC INTERNATIONAL, ISO and IDF

Methods of Analysis in the *Standard for infant formula and formulas for special medical purposes intended for infants* (CXS 72-1981) and *Follow-up formula for older infants and product for young children* (CXS 156-1987)

EXECUTIVE SUMMARY

This document outlines a proposal to replace or add new methods of analysis for the determination of nutrients in follow-up formula and infant formula, which are listed in CXS 234-1999 and referenced in CXS 72-1981, CXS 156-1987 and CXG 10-1979. It is proposed that these methods be considered during the 44th Session of the Codex Committee on Nutrition and Foods for Special Dietary Uses (CCNFSDU) in October 2024 and three primary actions be taken: (1) replace and retype current Type II/III/IV methods that may be outdated, and/or were not validated for all types of infant/follow-up formula; 2) add new Type II/III methods for a selection of nutrients for which such methods currently do not exist, and (3) request the Codex Committee on Methods of Analysis and Sampling (CCMAS) to consider them for endorsement.

Regarding the addition of new methods, they were developed jointly by AOAC INTERNATIONAL (AOAC), ISO and IDF through the AOAC Stakeholder Program on Infant Formula and Adult Nutritionals (SPIFAN) and are listed in CXS 234-1999 as Type II/III for infant formula.

INTRODUCTION

Since 2015, the Codex Alimentarius Commission has adopted multiple analytical methods for inclusion in CXS 234-1999 to verify compliance with provisions for nutrients in infant formula as referred to in CXS 72-1981, section A, on infant formula. As agreed by the AOAC SPIFAN Nutrient Expert Review Panel in June 2024, these methods are also fit for purpose to verify compliance of these nutrients in follow-up formula as referred to in CXS 156-1987 because of similarities between infant and follow-up formulas, specifically regarding definition and composition.

BACKGROUND

Since 2015, more than 20 methods of analysis for nutrients in infant formula were jointly developed and validated by AOAC INTERNATIONAL, ISO and IDF through the AOAC-SPIFAN program and approved as AOAC Official Methods and published by ISO and IDF as ISO or ISO/IDF standards. Most of these methods have been submitted to Codex for adoption. Methods are introduced by CCNFSDU, referred to CCMAS for technical review, typing and endorsement, and then submitted to the Codex Alimentarius Commission (CAC) for adoption. To date, methods for vitamin A, vitamin C, vitamin B_{12}, vitamin D, vitamin E, vitamin K, thiamine, riboflavin, niacin, pyridoxine, biotin, pantothenic acid, folic acid, myo-inositol, choline, carnitine, fatty acid profile, total nucleotides, iodine, chloride, calcium, magnesium, phosphorus, potassium, sodium, copper, iron, manganese, zinc, chromium, selenium, molybdenum, total amino acids and tryptophan have been brought through this process and all have been adopted by CAC as Type II methods (for infant formula) for the purpose of dispute resolution.

PROPOSAL AND RATIONALE

The Committee is requested to consider recommending the methods to assess compliance with provisions in CXS 156-1987 on follow-up formula for older infants (section A) as listed in Table 1 below and be referred to CCMAS for technical review and typing. There are currently only five provisions and seven test methods listed in CXS 234-1999 for follow-up formula and several of the methods are outdated and no longer fit for purpose. And so, addition of new state-of-the art methods would be an improvement for quality assurance and regulatory compliance.

It should be noted that regarding methods of analysis for provisions in the *Standard for follow-up formula for older infants and product for young children* (CXS 156-1987), CCNFSDU41 agreed to replace AOAC 999.15 / EN 14148 for vitamin K with AOAC 2015.09 / ISO 21446 as Type II. The latter methods, developed by the AOAC-SPIFAN program, are included in CXS 234-1999 as a Type II method for infant formula in CXS 72-1981. Thus, there is a precedent in Codex for extending methods for infant formula to follow-up formula in CXS 234-1999. It should be noted that members of the AOAC-SPIFAN Nutrient Expert Review Panel achieved consensus on the proposal in Table 1 during their meeting in June 2024. IDF experts also endorsed the proposal in Table 1 in August 2024.

Regarding methods of analysis for provisions in the *Standard for Infant Formula and Formulas for Special Medical Purposes Intended for Infants* (CXS 72-1981), it should be noted that the recommended method ISO 20631 for Folic acid is identical to AOAC 2011.06, which is already included in CXS 234-1999 as a Type II method for infant formula. The Committee is therefore requested to consider recommending ISO 20631 to assess compliance with the provision on Folic acid for infant formula according to CXS 72-1981, section A.

RECOMMENDATION

That the Committee refers to CCMAS the methods listed in Table 1 for review, (re)typing, revocation and endorsement as Type II/Type III methods for the determination of nutrients in infant formula (CXS 72-1981, Section A) and follow-up formula (CXS 156-1987, Section A).

It is recommended to request CCMAS to consider revoking/retyping of methods for follow-up formula currently listed in CXS 234-1999 as follows:

- Revoke AOAC 992.24 for iodine:
  CCMAS37 indicated that AOAC 992.24 is not fit for purpose to verify compliance with the provision on Iodine for infant formula according to CXS 72-1981.

- Revoke AOAC 974.29, AOAC 992.04, AOAC 992.06 for vitamin A:
  CAC39 in 2016 adopted AOAC 2012.10 / ISO 20633 as Type II for provisions on infant formula in CXS 72-1981 (Section A), as proposed by CCNFSDU37 and endorsed by CCMAS39. AOAC 992.04 and AOAC 992.06 (endorsed as Type II and Type III respectively for provisions in CXS 72-1981 at CCMAS30 in 2009) were revoked from CXS 234-1999 for infant formula. Together with AOAC 974.29, these methods are no longer in CXS 234-1999 for infant formula and consequently should be revoked for provisions on follow-up formula.
  It should be noted that as compared to AOAC 974.29, AOAC 992.04, and AOAC 992.06, AOAC 2012.10 / ISO 20633 has been collaboratively studied using infant formula matrices representative of the wide range and diversity of current formulations for these product categories.

- Retype AOAC 992.07 for pantothenic acid:
  CAC39 adopted AOAC 2012.16 / ISO 20639 as Type II method, as proposed by CCNFSDU and endorsed by CCMAS for verification of the provision on pantothenic acid in infant formula in CXS 72-1981.
  In 2019, CCNFSDU41 agreed to retain microbiological methods for the determination of vitamins as they are still in use. It is therefore proposed to retype AOAC 992.07 on pantothenic acid as a Type III method for follow-up formula. It should be noted that results generated by AOAC 992.07 are not different from results generated by AOAC 2012.16 / ISO 20639, as confirmed by Andrieux et al: JAOAC 95, 2, 2012:143.

Table 1: Methods proposed for review, (re)typing, and endorsement as Type II/Type III method for inclusion in CXS 234-1999 for follow-up formula and infant formula.

Commodity	Provision	Method	Principle	Type
Follow-up formula	Vitamin A	AOAC 2012.10 / ISO 20633	HPLC-UV	II
		~~AOAC 992.04~~	~~HPLC~~	~~II~~
		~~AOAC 992.06~~	~~HPLC~~	~~III~~
		~~AOAC 974.29~~	~~Colorimetry~~	~~IV~~
	Vitamin E	AOAC 2012.10 / ISO 20633	HPLC	II
	Vitamin D	AOAC 2016.05 / ISO 20636	LC-MS	II
	Thiamine	AOAC 2015.14 / ISO 21470	Enzymatic digestion and UHPLC-MS/MS	II
	Riboflavin	AOAC 2015.14 / ISO 21470	Enzymatic digestion and UHPLC-MS/MS	II
	Niacin	AOAC 2015.14 / ISO 21470	Enzymatic digestion and UHPLC-MS/MS	II
	Vitamin B_6	AOAC 2015.14 / ISO 21470	Enzymatic digestion and UHPLC-MS/MS	II
	Vitamin B_{12}	AOAC 2011.10 / ISO 20634	HPLC	II
		AOAC 2014.02	LC-UV	III
	Pantothenic acid	AOAC 2012.16 / ISO 20639	UHPLC-MS/MS	II
		AOAC 992.07	Microbioassay	~~II~~ III
	Folic Acid	AOAC 2011.06 / ISO 20631	LC-MS/MS	II
	Vitamin C	AOAC 2012.22 / ISO 20635	HPLC-UV	II
	Biotin	AOAC 2016.02 / ISO 23305	HPLC-UV	II
	Iron	AOAC 2015.06 / ISO 21424 | IDF 243	ICP-MS	II
		AOAC 2011.14 / ISO 15151 | IDF 229	ICP emission spectroscopy	III
	Calcium	AOAC 2015.06 / ISO 21424 | IDF 243	ICP-MS	II
		AOAC 2011.14 / ISO 15151 | IDF 229	ICP emission spectroscopy	III
	Phosphorus	AOAC 2015.06 / ISO 21424 | IDF 243	ICP-MS	II
		AOAC 2011.14 / ISO 15151 | IDF 229	ICP emission spectroscopy	III
	Magnesium	AOAC 2015.06 / ISO 21424 | IDF 243	ICP-MS	II
		AOAC 2011.14 / ISO 15151 | IDF 229	ICP emission spectroscopy	III
	Sodium	AOAC 2015.06 / ISO 21424 | IDF 243	ICP-MS	II
		AOAC 2011.14 / ISO 15151 | IDF 229	ICP emission spectroscopy	III
	Chloride	AOAC 2016.03 / ISO 21422 | IDF 242	Potentiometry	II
	Potassium	AOAC 2015.06 / ISO 21424 | IDF 243	ICP-MS	II
		AOAC 2011.14 / ISO 15151 | IDF 229	ICP emission spectroscopy	III
	Manganese	AOAC 2015.06 / ISO 21424 | IDF 243	ICP-MS	II
		AOAC 2011.14 / ISO 15151 | IDF 229	ICP emission spectroscopy	III
	Iodine	AOAC 2012.15 / ISO 20647 | IDF 234	ICP-MS	II
		~~AOAC 992.24~~	~~Ion selective potentiometry~~	~~II~~
	Selenium	AOAC 2011.19 / ISO 20649 | IDF 235	ICP-MS	II

Commodity	Provision	Method	Principle	Type
	Copper	AOAC 2015.06 / ISO 21424|IDF 243	ICP-MS	II
		AOAC 2011.14 / ISO 15151|IDF 229	ICP emission spectroscopy	III
	Zinc	AOAC 2015.06 / ISO 21424|IDF 243	ICP-MS	II
		AOAC 2011.14 / ISO 15151|IDF 229	ICP emission spectroscopy	III
	Total nucleotides	AOAC 2011.20 / ISO 20638	LC	II
	Choline	AOAC 2015.10 / ISO 21468	UHPLC-MS/MS	II
	Myo-inositol	AOAC 2011.18 / ISO 20637	LC-pulsed amperometry	II
	L-carnitine	AOAC 2015.10 / ISO 21468	UHPLC-MS/MS	II
	Total amino acids (excluding taurine and tryptophan) for use according to section 3.1.3 (a) notes 2) and 3) of CXS 156-1987	AOAC 2018.06 / ISO 4214|IDF 254 /AACC 07-50.01	UHPLC-UV	II
	Tryptophan	AOAC 2017.03	HPLC	II
	Total fatty acids	AOAC 2012.13 / ISO 16958 |IDF 231	Gas chromatography	II
Infant formula	Folic acid	AOAC 2011.06/ISO 20631	LC-MS/MS	II

IDF, ISDI and ISO

Methods of Analysis in the *Standard for follow-up formula for older infants and product for young children* (CXS 156-1987)

EXECUTIVE SUMMARY

This document outlines a joint proposal by IDF, ISDI & ISO to add a method of analysis for the determination of nitrogen content for determination of crude protein in Follow-up formula for older infants and product for young children to be listed in the Standard on *Recommended Methods of Analysis and Sampling* (CXS 234-1999).

It is proposed that this method be considered during the 44th Session of the Codex Committee on Nutrition and Foods for Special Dietary Uses (CCNFSDU) in October 2024 and a primary action taken: (1) add Type I method ISO 8968-1|IDF 20-1:2014 (Titrimetry; Kjeldahl) for crude protein in follow-up formula.

BACKGROUND

The following discrepancy can be noted for infant and follow-up formulas:

- The *Standard for infant formula and formulas for special medical purposes intended for infants* (CXS 72-1981) contains a provision for protein with reference to a nitrogen-to-protein conversion factor and the *Recommended Methods of Analysis and Sampling* (CXS 234-1999) stipulates a Type I method (ISO 8968-1|IDF 20-1:2014) for crude protein.

- On the other hand, the *Standard for follow-up formula for older infants and product for young children* (CXS 156-1987) contains a provision for protein with reference to a nitrogen-to-protein conversion factor but the *Recommended Methods of Analysis and Sampling* (CXS 234-1999) does not refer to any method for follow-up formula.

PROPOSAL AND RATIONALE

IDF, ISDI & ISO propose to endorse ISO 8968-1 | IDF 20-1 (Milk and milk products – Determination of nitrogen content - Part1: Kjeldahl principle and crude protein calculation) for determination of crude protein in follow-up formula listed in CXS 234-1999.

As mentioned above, there is currently no method for crude protein for follow-up formula in CXS 234-1999.

Although ISO 8968-1|IDF 20-1 was not specifically validated for follow-up formula, the method was validated for similar matrices, including liquid cow's, goat's and sheep's whole milk and dried milk and milk products (including infant formula). And, considering the similarities between infant and follow-up formulas, specifically regarding definition and composition, ISO 8968-1|IDF 20-1 is also considered fit for purpose for verifying compliance of crude protein in follow-up formula. Endorsement of ISO 8968-1|IDF 20-1 as a Type I method for crude protein in follow-up formula would align follow-up formula with infant formula, which already references ISO 8968-1|IDF 20-1 as a Type I method for crude protein in CXS 234-1999.

RECOMMENDATION

That the Committee refers to CCMAS the method listed in Table 1 for review and endorsement as Type I for determination of crude protein in follow-up formula.

Commodity	Provision	Method	Principle	Type
Follow-up formula	Crude protein	ISO 8968-1 | IDF 20-1	Titrimetry (Kjeldahl)	I

The note relevant to the nitrogen-to-protein conversion factor included in CXS 156-1987 may be inserted where appropriate to reflect the CAC/CCNFSDU decisions regarding the placement of these factors in the Codex texts.