CODEX ALIMENTARIUS COMMISSION

Food and Agriculture Organization of the United Nations

World Health Organization

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Agenda Item 3 **CX/MAS 23/42/3 Add.1**

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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

1. This document contains the methods of analysis for provisions proposed by the following Committee and Member Countries (Appendix I and Appendix II) and the updated information submitted by following Codex Observers (Appendix III):

- Codex Committee on Contaminants in Foods
    - Sampling plans for total aflatoxins in certain cereals and cereal based products including foods for infants and young children in the *General Standard for Contaminants and Toxins in Food and Feed* (CXS 193-1995).
- Uruguay, Argentina, and Brazil
    - Determination of moisture in powdered dairy products in the *Recommended Methods of Analysis and Sampling* (CXS 234-1999)
- AOAC International (AOAC) and International Association for Cereal Science and Technology (IACST)
    - Dietary fibre provisions in the *Recommended Methods of Analysis and Sampling* (CXS 234-1999).
- International Dairy Federation (IDF) and the International Organization for Standardization (ISO)
    - Methods for determination of lactose in dairy permeate powders and determination of fat contents in various dairy matrices in the *Recommended Methods of Analysis and Sampling* (CXS 234-1999).
    - Determination of moisture content in dried milk products in the in the *Recommended Methods of Analysis and Sampling* (CXS 234-1999).

CODEX COMMITTEE ON CONTAMINANTS IN FOODS (CCCF16, 2023)[1]

2. The Committee is invited to endorse the sampling plans for total aflatoxins in certain cereals and cereal-based products including foods for infants and young children (Appendix I).

URUGUAY, ARGENTINA, AND BRAZIL

3. The Group responsible for preparing this document (Uruguay, Argentina, and Brazil) supported by other CCLAC countries recommends to CCMAS to:

- Endorse the Method described in Appendix II, Annex II as Type I for the determination of moisture in the following commodities: Blend of skimmed milk and vegetable fat in powdered form, Reduced fat blend of skimmed milk powder and vegetable fat in powdered form, Dairy permeate powders, Milk powders and cream powders, Whey powders.
- Describe the method in CXS 234-1999 as Appendix VII.
- Amend CXS 234-1999, according to the item “Summary of proposed changes in CXS 234, including retyping of existing methods and recommendations to CCMAS” of this document (Appendix II, Annex I).

[1] CX/CF 23/16/7, CX/CF 23/16/7 Add.1

CODEX OBSERVERS

4. IACST recommend CCMAS to take the following actions on dietary fibre provisions (Appendix III):

   - Endorse AOAC 2022.01/ICC Standard 191 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.
   - Remove AOAC 2011.25/AACCI 32-50.01 from CXS 234-1999.

5. IDF and ISO recommend CCMAS to take the following actions on determination of lactose and fat content (Appendix III):

   - Endorse ISO 22662 | IDF 198 as Type II method for the determination of lactose in dairy permeate powders.
   - Endorse ISO 23319 | IDF 250: 2022 - Cheese and processed cheese products, caseins and caseinates — Determination of fat content — Gravimetric method – Schmid-Bondzynski-Raztlaff principle, to replace the following two standards in CXS 234:
     a. ISO 1735 | IDF 5: 2004 - Cheese and processed cheese products - Determination of fat content - Gravimetric method (Reference method).
     b. ISO 5543 | IDF 127 - Caseins and caseinates – Determination of fat content – Gravimetric method (Reference method).
   - Endorse ISO 23318 | IDF 249: 2022 - Milk, dried milk products and cream — Determination of fat content — Gravimetric method - Röse-Gottlieb principle, to replace the following six standards:
     a. ISO 1211 | IDF 1: 2010 - Milk - Determination of fat content - Gravimetric method (Reference method).
     b. ISO 1736 | IDF 9: 2008 - Dried milk and dried milk products - Determination of fat content - Gravimetric method (Reference method).
     c. ISO 2450 | IDF 16: 2008 - Cream - Determination of fat content - Gravimetric method (Reference method).
     d. ISO 8381 | IDF 123: 2008 - Milk-based infant foods - Determination of fat content (Reference method).
     e. ISO 1737 | IDF 13: 2008 - Evaporated milk and sweetened condensed milk - Determination of fat content - Gravimetric method (Reference method).
     f. ISO 1854 | IDF 59: 2008 - Whey cheese - Determination of fat content - Gravimetric method (Reference method).
   - Consider updating the reference of ISO 1211|IDF 1 to ISO 23319|IDF 250 for aqueous coconut products.

6. IDF and ISO recommend CCMAS to maintain the current method listed for determination of moisture content in dried milk products as Codex Type I method for checking compliance with existing Codex provisions on the moisture content in dried milk products in Codex standards 251, 207, 289 and 331, as listed in CXS 234.

APPENDIX I

CODEX COMMITTEE ON CONTAMINANTS IN FOODS (CCCF16)

Sampling Plans Provisions in the General Standard for Contaminants and Toxins in Food and Feed (CXS 193-1995)

SAMPLING PLANS FOR TOTAL AFLATOXINS IN CERTAIN CEREALS AND CEREAL-BASED PRODUCTS INCLUDING FOODS FOR INFANTS AND YOUNG CHILDREN

Sampling plans and performance criteria for aflatoxin (AFB1+AFB2+AFG1+AFG2) in maize grain, destined for further processing.

Maximum level	15 µg/kg AFB1+AFB2+AFG1+AFG2
Increments	Increments of 100g, depending on the lot weight (≥0.5 tons)
Sample preparation	dry grind with a suitable mill (particles smaller than 0.85 mm – 20 mesh)
Laboratory sample weight	≥5 kg
Number of laboratory samples	1
Test portion	25 g
Method	Selected according to the established performance criteria in Table 3
Decision rule	If the sum of test results of AFB1, AFB2, AFG1 and AFG2 for the laboratory sample is equal to or less than 15 µg/kg, accept the lot. Otherwise, reject the lot.

Sampling plans and performance criteria for aflatoxin (AFB1+AFB2+AFG1+AFG2) in flour meal, semolina and flakes derived from maize

Maximum level	10 µg/kg AFB1+AFB2+AFG1+AFG2
Increments	10 x 100g
Sample preparation	dry grind with a suitable mill (particles smaller than 0.85 mm – 20 mesh), if necessary for coarse samples
Laboratory sample weight	1 kg
Number of laboratory samples	1
Test portion	25g
Method	Selected according to the established performance criteria in Table 3
Decision rule	If the sum of test results of AFB1, AFB2, AFG1 and AFG2 for the laboratory sample is equal to or less than 10 µg/kg, accept the lot. Otherwise, reject the lot

Sampling plans and performance criteria for aflatoxin (AFB1+AFB2+AFG1+AFG2) in husked rice

Maximum level	20 µg/kg AFB1+AFB2+AFG1+AFG2
Increments	Increments of 100g, depending on the lot weight (≥0.5 tons)
Sample preparation	dry grind with a suitable mill (particles smaller than 0.85 mm – 20 mesh)
Laboratory sample weight	≥5 kg
Number of laboratory samples	1
Test portion	25g
Method	Selected according to the established performance criteria in Table 3
Decision rule	If the sum of test results of AFB1, AFB2, AFG1 and AFG2 for the laboratory sample is equal to or less than 20 µg/kg, accept the lot. Otherwise, reject the lot

Sampling plans and performance criteria for aflatoxin (AFB1+AFB2+AFG1+AFG2) in polished rice

Maximum level	5 µg/Kg AFB1+AFB2+AFG1+AFG2
Increments	Increments of 100g, depending on the lot weight (≥0.5 tons)
Sample preparation	dry grind with a suitable mill (particles smaller than 0.85 mm – 20 mesh)
Laboratory sample weight	≥5 kg
Number of laboratory samples	1

Test portion	25g
Method	Selected according to the established performance criteria in Table 3
Decision rule	If the sum of test results of AFB1, AFB2, AFG1 and AFG2 for the laboratory sample is equal to or less than 5 µg/kg, accept the lot. Otherwise, reject the lot

Sampling plans and performance criteria for aflatoxin (AFB1+AFB2+AFG1+AFG2) in sorghum

Maximum level	10 µg/kg AFB1+AFB2+AFG1+AFG2
Increments	Increments of 100g, depending on the lot weight (≥0.5 tons)
Sample preparation	dry grind with a suitable mill (particles smaller than 0.85 mm – 20 mesh)
Laboratory sample size	≥5 kg
Number of laboratory weight	1
Test portion	25g
Method	Selected according to the established performance criteria in Table 3
Decision rule	If the sum of test results of AFB1, AFB2, AFG1 and AFG2 for the laboratory sample is equal to or less than 10 µg/kg, accept the lot. Otherwise, reject the lot

Sampling plans and performance criteria for aflatoxin (AFB1+AFB2+AFG1+AFG2) in cereal-based food for infants and young children

Maximum level	5 µg/kg AFB1+AFB2+AFG1+AFG2
Increments	10 x 100g
Sample preparation	dry grind with a suitable mill (particles smaller than 0.85 mm – 20 mesh), if necessary for coarse samples
Laboratory sample weight	1 kg
Number of laboratory samples	1
Test portion	25g
Method	Selected according to the established performance criteria in Table 3
Decision rule	If the sum of test results of AFB1, AFB2, AFG1 and AFG2 for the laboratory sample is equal to or less than 5 µg/kg, accept the lot. Otherwise, reject the lot

Sampling plans and performance criteria for aflatoxin (AFB1+AFB2+AFG1+AFG2) in cereal-based food for infants and young children destined for food aid programs

Maximum level	10 µg/kg AFB1+AFB2+AFG1+AFG2
Increments	10 x 100g
Sample preparation	dry grind with a suitable mill (particles smaller than 0.85 mm – 20 mesh), if necessary for coarse samples
Laboratory sample size	1 kg
Number of laboratory weight	1
Test portion	25g
Method	Selected according to the established performance criteria in Table 3
Decision rule	If the sum of test results of AFB1, AFB2, AFG1 and AFG2 for the laboratory sample is equal to or less than 10 µg/kg, accept the lot. Otherwise, reject the lot

Definitions:

Lot	An identifiable quantity of a food commodity delivered at one time and determined by the official to have common characteristics, such as origin, variety, type of packing, packer, consignor, or markings.
Sublot	Designated part of a larger lot in order to apply the sampling method on that designated part. Each sublot must be physically separate and identifiable.
Sampling plan	It is defined by an aflatoxin test procedure and an accept/reject level. An

	aflatoxin test procedure consists of three steps: sample selection, sample preparation and analysis or aflatoxin quantification. The accept/reject level is a tolerance usually equal to the Codex maximum level (ML).
Incremental sample	The quantity of material taken from a single random place in the lot or sublot.
Aggregate sample	The combined total of all the incremental samples that is taken from the lot or sublot. The aggregate sample has to be at least as large as the laboratory sample or samples combined.
Laboratory sample	The smallest quantity of cereal grains, shelled cereal grains and cereal-based products comminuted in a mill. The laboratory sample may be a portion of or the entire aggregate sample. If the aggregate sample is larger than the laboratory sample (s), the laboratory sample (s) should be removed in a random manner from the aggregate sample in such a way to ensure that the laboratory sample is still representative of the sublot sampled.
Test portion	A portion of the comminuted laboratory sample. The entire laboratory sample should be comminuted in a mill. A portion of the comminuted laboratory sample is randomly removed for the extraction of the aflatoxin for chemical analysis.

SAMPLING PLAN DESIGN CONSIDERATIONS

MATERIAL TO BE SAMPLED

1. Each lot of cereal grains and cereal-based products, which is to be examined for AFs, must be sampled separately. Lots larger than 50 tons should be subdivided into sublots to be sampled separately. If a lot is greater than 50 tons, the lot should be subdivided into sublots according to Table 1.

Table 1. Subdivision of cereal grains sublots according to lot weight – Maize grain, sorghum, polished rice and husked rice

Lot weight (t)	Maximum weight or minimum number of sublots	Number of incremental samples	Minimum laboratory sample weight (kg)
≥ 1500	500 tons	100	5
> 300 and < 1500	3 sublots	100	5
≥ 100 and ≤ 300	100 tons	100	5
≥ 50 and < 100	2 sublots	100	5
< 50	-	3-100*	5

*see Table 2

2. Considering that the weight of the lot is not always an exact multiple of the weight of sublots, the weight of the sublot may exceed the mentioned size by a maximum of 20%.

INCREMENTAL SAMPLE

3. The suggested minimum size of the incremental sample of cereal grains and cereal-based products should be 100 g for lots ≥ 0.5 tons.

4. For lots less than 50 tons of cereal grains and cereal-based products, the sampling plan must be used with 3 to 100 incremental samples, depending on the lot weight. For very small lots (< 0.5 tons) a lower number of incremental samples may be taken, but the aggregate sample uniting all incremental samples shall be also in that case at least 5 kg. Table 2 may be used to determine the number of incremental samples to be taken.

Table 2. Number of incremental samples of cereal grains to be taken depending on the weight of the lot- Maize grain, sorghum, polished rice and husked rice

Lot weight (t)	Number of incremental samples	Minimum laboratory sample weight (kg)
≤ 0.05	3	5
> 0.05 - ≤ 0.5	5	5
> 0.5 - ≤ 1	10	5
> 1 - ≤ 3	20	5
> 3 - ≤ 10	40	5
> 10 - ≤ 20	60	5
> 20 - < 50	100	5

STATIC LOTS

5. A static lot can be defined as a large mass cereal grains and cereal-based products contained either in a large single container such as a wagon, truck or railcar or in many small containers such as sacks or boxes and the cereal grains and cereal-based products is stationary at the time a sample is selected. Selecting a truly random sample from a static lot can be difficult because all containers in the lot or sublot may not be accessible.

6. Taking incremental samples from a static lot usually requires the use of probing devices to select product from the lot. The probing devices should be specifically designed for the commodity and type of container. The probe should (1) be long enough to reach all products, (2) not restrict any item in the lot from being selected, and (3) not alter the items in the lot. As mentioned above, the aggregate sample should be a composite from many small incremental samples of product taken from many different locations throughout the lot.

7. For lots traded in individual packages, the sampling frequency (SF), or number of packages that incremental samples are taken from, is a function of the lot size (LT), incremental sample size (IS), aggregate sample size (AS) and the individual packing size (IP), as follows:

SF = (LT x IS)/ (AS x IP).

8. The sampling frequency (SF) is the number of packages sampled. All sizes should be in the same mass units such as kg.

DYNAMIC LOTS

9. Representative aggregate samples can be more easily produced when selecting incremental samples from a moving stream of cereal grains and cereal-based products as the lot is transferred from one location to another. When sampling from a moving stream, take small incremental samples of product from the entire length of the moving stream; composite the incremental samples to obtain an aggregate sample; if the aggregate sample is larger than the required laboratory sample(s), then blend and subdivide the aggregate sample to obtain the desired size laboratory sample(s).

10. Automatic sampling equipment such as a cross-cut sampler is commercially available with timers that automatically pass a diverter cup through the moving stream at predetermined and uniform intervals. When automatic sampling equipment is not available, a person can be assigned to manually pass a cup through the stream at periodic intervals to collect incremental samples. Whether using automatic or manual methods, incremental samples should be collected and composited at frequent and uniform intervals throughout the entire time the cereal flow past the sampling point.

11. Cross-cut samplers should be installed in the following manner: (1) the plane of the opening of the diverter cup should be perpendicular to the direction of the flow; (2) the diverter cup should pass through the entire cross-sectional area of the stream; and (3) the opening of the diverter cup should be wide enough to accept all items of interest in the lot. As a general rule, the width of the diverter cup opening should be about two to three times the largest dimensions of items in the lot.

12. The size of the aggregate sample (S) in kg, taken from a lot by a cross cut sampler is:

S= (D x LT) / (T x V),

where, D is the width of the diverter cup opening (cm), LT is the lot size (kg), T is interval or time between cup movement through the stream (seconds), and V is cup velocity (cm/sec).

13. If the mass flow rate of the moving stream, MR (kg/sec), is known, then the sampling frequency (SF), or number of cuts made by the automatic sampler cup can be computed as a function of S, V, D, and MR. SF = (S x V) / (D x MR).

PACKAGING AND TRANSPORTATION OF SAMPLES

14. Each laboratory sample shall be placed in a clean, inert container offering adequate protection from

contamination, sunlight, and against damage in transit. All necessary precautions shall be taken to avoid any change in composition of the laboratory sample, which might arise during transportation or storage. Samples should be stored in a cool dark place.

SEALING AND LABELLING OF SAMPLES

15. Each laboratory sample taken for official use shall be sealed at the place of sampling and identified. A record must be kept of each sampling, permitting each lot to be identified unambiguously and giving the date and place of sampling together with any additional information likely to be of assistance to the analyst.

SAMPLE PREPARATION PRECAUTIONS

16. Sunlight should be excluded as much as possible during sample preparation, since aflatoxin gradually breaks down under the influence of ultra-violet light. Also, environmental temperature and relative humidity should be controlled and not favour mould growth and aflatoxin formation.

HOMOGENIZATION - GRINDING

17. As the distribution of aflatoxin is extremely non-homogeneous, laboratory samples should be homogenized by grinding the entire laboratory sample received by the laboratory. Homogenization is a procedure that reduces particle size and disperses the contaminated particles evenly throughout the comminuted laboratory sample.

18. The laboratory sample should be finely ground and mixed thoroughly using a process that approaches as complete homogenization as possible. Complete homogenization implies that particle size is extremely small, and the variability associated with sample preparation is minimized. After grinding, the grinder should be cleaned to prevent aflatoxin cross-contamination.

TEST PORTION

19. The suggested weight of the test portion taken from the comminuted laboratory sample should be approximately 25 g. If the laboratory sample is prepared using a liquid slurry, the slurry should contain 25 g.

20. Procedures for selecting the 25 g test portion from the comminuted laboratory sample should be a random process. If mixing occurred during or after the comminution process, the 25 g test portion can be selected from any location throughout the comminuted laboratory sample. Otherwise, the 25 g test portion should be the accumulation of several small portions selected throughout the laboratory sample.

ANALYTICAL METHODS

21. A criteria-based approach, whereby a set of performance criteria is established with which the analytical method used should comply, is appropriate. The criteria-based approach has the advantage that, by avoiding setting down specific details of the method used, developments in methodology can be exploited without having to reconsider or modify the specific method. A list of possible criteria and performance levels is shown in Table 3. Utilizing this approach, laboratories would be free to use the analytical method most appropriate for their facilities.

Table 3. Method criteria for total aflatoxins in cereals, considering AFB1: AFB2+AFG1+AFG2 of 50:50.

Commodity	Analyte	ML (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)	Precision (%)	Minimal applicable range (µg/kg)	Recovery (%)
Maize grain	AF B1+B2+G1+G2	15	≤ 3	≤ 6	≤44	8.4 - 21.6	60-115
	AFB1	-	≤1.5	≤ 3.0	≤44	4.2 - 10.8	60-115
	AFB2	-	≤ 0.5*	≤ 1*	≤44	1.4 - 3.6	40-120
	AFG1	-	≤ 0.5*	≤ 1*	≤44	1.4 - 3.6	40-120
	AFG2	-	≤ 0.5*	≤ 1*	≤44	1.4 - 3.6	40-120
Maize flour, meal, semolina and flakes derived from maize; Sorghum grain; cereal-based foods for infants and young children for food aid programs	AF B1+B2+G1+G2	10	≤2	≤4	≤44	5.6 - 14.4	60-115
	AFB1	-	≤1.0	≤2.0	≤44	2.8 - 7.2	60-115

	AFB2	-	≤0.33*	≤0.67*	≤44	0.9 - 2.4	40-120
	AFG1	-	≤0.33*	≤0.67*	≤44	0.9 - 2.4	40-120
	AFG2	-	≤0.33*	≤0.67*	≤44	0.9 - 2.4	40-120
Husked Rice	AF B1+B2+G1+G2	20	≤4	≤8	≤44	11.2 - 28.8	60-115
	AFB1	-	≤2.0	≤4.0	≤44	5.6 - 14.4	60-115
	AFB2	-	≤0.67*	≤1.33*	≤44	1.9 - 4.8	60-115
	AFG1	-	≤0.67*	≤1.33*	≤44	1.9 - 4.8	60-115
	AFG2	-	≤0.67*	≤1.33*	≤44	1.9 - 4.8	60-115
Polished Rice; Cereal-based food for infants and young children	AF B1+B2+G1+G2	5	≤1	≤2	≤44	2.8 - 7.2	40-120
	AFB1	-	≤0.5	≤1	≤44	1.4 - 3.6	40-120
	AFB2	-	≤0.17*	≤0.33*	≤44	0.5 - 1.2	40-120
	AFG1	-	≤0.17*	≤0.33*	≤44	0.5 - 1.2	40-120
	AFG2	-	≤0.17*	≤0.33*	≤44	0.5 - 1.2	40-120

*If those values could not be validated, LOD and LOQ for AFB2, AFG1 and AFG2 could be up to parameters for AFB1.

APPENDIX II

URUGUAY, ARGENTINA, AND BRAZIL

Determination of Moisture in Powdered Dairy Products in the Recommended Methods of Analysis and Sampling (CXS 234-1999)

Executive Summary

At CCMAS41 several Members presented CDR25 regarding the endorsement of the method for moisture in dairy products ISO 5537 I IDF 26. The Members highlighted that this method could cause limitations in trade (REP21/MAS paras 36 – 41 and para 42 ii).

CCMAS agreed to request the PWG on endorsement to assess the data to support the endorsement of AOAC 927.05 and consideration should also be given to the accessibility and cost of the methods recommended for endorsement.

A collaborative study was performed considering more accessible and less costly methods (AOAC 927.05 and Moisture at normal pressure Method at 102 ºC described in Annex II below) in Milk Protein Concentrate, Skimmed milk in powder (SMP), Whey powder, Whey protein concentrate, Whole milk powder and Infant formula and Powdered milk mix with vegetable fat.

Results of the collaborative study showed that the moisture at normal pressure Method (Annex I) is fit for purpose and aligned with the general selection criteria of methods of analysis of The Codex Procedure Manual and with the Codex general methods applicable to several products. Some advantages of using moisture at normal pressure Method should be noted by the Committee, such as its practicality and common use by laboratories, the use of equipment with greater ease of access and calibration under usual conditions in the laboratory routine, as well as the fact that it does not require specific inputs, nor does it generate waste that affects the environment.

In order to help the PWG to make a decision, it is important to consider the comments and data presented below, which supports the recommendation of the method described in Annex I.

Recommendations to CCMAS

The Group responsible for preparing this document (Uruguay, Argentina and Brazil) supported by others CCLAC countries recommends to CCMAS to:

- Endorse the Method described in Annex II as Type I for the determination of moisture in the following commodities: Blend of skimmed milk and vegetable fat in powdered form, Reduced fat blend of skimmed milk powder and vegetable fat in powdered form, Dairy permeate powders, Milk powders and cream powders, Whey powders
- Describe the method in CXS 234-1999 as Appendix VII
- Amend CXS 234-1999, according to the item “Summary of proposed changes in CXS 234, including retyping of existing methods and recommendations to CCMAS” of this document (see Annex I below).

Proposal for the determination of moisture in powdered dairy products

- **Scope**: This Standard specifies a method for the determination of moisture content for all types of powdered milk, powdered creamer, powdered permeated dairy products, infant formulas, and mixtures of powdered skimmed milk with vegetable fat.

- **Validated matrices:** Milk Protein Concentrate (MPP), Skimmed milk in powder (SMP), Whey powder (AWP), Whey protein concentrate (RWP), Whole milk powder (WMP), Infant formula (PIF) and Powdered milk mix with vegetable fat (PMV). Additional validation data for whole milk powder and skimmed milk powder are published in CRD06 of the CCMAS41.

- **Description of the principle**: A portion of the sample is dried in an oven under normal pressure set at (102 ± 2) °C until constant weight and weighed to determine the loss of mass.

- **Other methods already listed in CXS 234**: ISO 5537 I IDF 26 is the current method for moisture in milk powder products.

The ISO 5537 I IDF 26 method principle of measurement is determining the moisture content by drying in a specifically designed oven at 87 °C for 5 hours while an air current of specified composition passes through the sample at a fixed flow of 33 mL/min. This method requires equipment and other specific inputs, including an oven of exclusive design with only two manufacturers in the world as described in the standard itself. Moreover,

the oven is very expensive and exclusively used for dairy products. Flow and temperature calibration capabilities and air composition are not readily available in some regions and generates costs that do not allow its applicability in all countries, a requirement for the officialization of a Codex method.

This method is exclusive, in terms of equipment and application for powdered milk products, therefore it deviates from the method selection criteria available in the Codex Procedural Manual, especially considering its practicability and applicability under normal conditions of the laboratory and the preference for methods for several groups of matrices.

The method proposed (Annex I) is a method aligned with the criteria required to be proposed as a Codex method with the following characteristics:

- Proven safety based on performance data appropriate to the related product standard.
- Practicable and commonly used in laboratories.
- Principle of measurement of similar characteristics to the one used when establishing the technical specifications of the product standard.
- Codex general method applicable to several products.
- Equipment with greater ease of access and calibration. The temperature calibration is in a range usually used in the laboratory routine,
- It does not require specific inputs, nor does it generate waste that affects the environment.

- Summary table of the validation data and other relevant information from the collaborative study.

During 2022 a collaborative study was performed by several laboratories in Latin American Countries to evaluate alternative methods of ISO 5537 I IDF 26, for the purpose of evaluating their performance data and compliance with the General Criteria for the selection of methods of analysis, established in the Codex Procedural Manual. The collaborative study was designed considering a moisture method with a vacuum oven (AOAC 927.05) and a moisture method in a normal oven at 102 ºC (Annex I) in the following matrices: Milk Protein Concentrate (MPP), Skimmed milk in powder (SMP), Whey powder (AWP), Whey protein concentrate (RWP), Whole milk powder (WMP), Infant formula (PIF) and Powdered milk mix with vegetable fat (PMV). Additional complementary data related with the ISO 5537 I IDF 26 are obtained.

These studies were coordinated by the National Metrology Institute of Uruguay (LATU, ISO/IEC 17043 accredited under the ISO 5725 series) within the framework of ISO/IEC 17043, with the participation of 12 laboratories from Brazil, Uruguay, Panama, Argentina and Costa Rica. The samples were obtained from Global Proficiency (New Zealand) except for Powdered milk mix with vegetable fat that was prepared by LATU (Table 3).

Results of this collaborative study are shown in Tables 1, 2 and 3 for the AOAC 920, Annex I and ISO 5537 I IDF 26 methods.

Table 1-Results of a collaborative study of several laboratories. Method AOAC 927.05 (moisture method using vacuum oven)

Attribute	RWP	AWP	MPP	PIF	SMP	WMP	PMV
Number of participants after removing outliers	6	5	6	4	6	6	5
Average value, g/100 g	4,78	2,21	6,21	2,32	4,05	2,85	2,20
Repeatability standard deviation, s_r g/100 g	0,074	0,070	0,083	0,041	0,068	0,052	0,059
Repeatability coefficient of variation, %	1,54	3,16	1,33	1,77	1,67	1,82	2,69
Repeatability limit, r , (2.8 s_r) , g/100 g	0,207	0,195	0,232	0,115	0,190	0,146	0,166

Reproducibility standard deviation, s_R, g/100 g	0,354	0,440	0,443	0,528	0,367	0,109	0,279
Reproducibility Coefficient of Variation, %	7,41	19,97	7,14	22,76	9,08	3,83	12,68
Reproducibility limit, R, $(2.8\ s_R)$, g/100 g	0,992	1,233	1,242	1,478	1,028	0,306	0,782

Table 2-Results of a collaborative study of several laboratories carried out with a moisture method in an oven at normal pressure (102 ± 2) ° C

Attribute	RWP	AWP	MPP	PIF	SMP	WMP	PMV
Number of participants after removing outliers	9	8	9	8	10	9	8
Average value, g/100 g	4,67	1,63	5,95	1,98	3,93	2,79	1,99
Repeatability standard deviation, s_r g/100 g	0,063	0,072	0,072	0,052	0,037	0,045	0,039
Repeatability coefficient of variation, %	1,35	4,45	1,21	2,65	0,95	1,61	1,93
Repeatability limit, r, $(2.8\ s_r)$, g/100 g	0,177	0,203	0,202	0,147	0,105	0,126	0,108
Reproducibility standard deviation, s_R, g/100 g	0,119	0,141	0,185	0,060	0,072	0,092	0,072
Reproducibility Coefficient of Variation, %	2,55	8,68	3,12	3,06	1,84	3,30	3,61
Reproducibility limit, R, $(2.8\ s_R)$, g/100 g	0,334	0,395	0,519	0,169	0,203	0,258	0,202

Table 3-Results of a collaborative study of several laboratories carried out with ISO 5537 I IDF 26: 2004 (Informative data)

Attribute	RWP	AWP	MPP	PIF	SMP	WMP	PMV
Number of participants after removing outliers	2	2	2	2	2	2	1
Average value, g/100 g	4,803	1,862	5,990	2,173	4,183	2,934	2,338
Repeatability standard deviation, s_r g/100 g	0,038	0,034	0,070	0,033	0,045	0,048	0,057
Repeatability coefficient of variation, %	0,80%	1,81	1,18	1,53	1,07	1,62	2,46
Repeatability limit, r , (2.8 s_r) , g/100 g	0,107	0,094	0,197	0,093	0,126	0,133	0,161
Reproducibility standard deviation, s_R , g/100 g	0,031	0,127	0,005	0,014	0,103	0,059	---
Reproducibility Coefficient of Variation, %	0,64	6,80	0,08	0,63	2,46	2,00	---
Reproducibility limit, R , (2.8 s_R) , g/100 g	0,087	0,354	0,013	0,038	0,288	0,164	---

A comparison of reference material is shown in Table 4.

Table 4 – Reference material description

Reference Material				**Average value g/100 g**		**% with respect to the assigned value[1]**	
Matrix	**Assigned Value g/100 g**	**Acceptance Range g/100 g**	**Sample Provider**	**AOAC 927.05**	**Moisture 102 ºC (normal pressure)**	**AOAC 927.05**	**Moisture 102 ºC (normal pressure)**
RWP	4,70	4,49 – 4,91	Global Proficiency	4,78	4,67	102	99,4
AWP	1,64	1,60 – 1,68	Global Proficiency	2,21	1,63	135	99,4
MPP	6,00	5,88 – 6,12	Global Proficiency	6,21	5,95	103	99,2
PIF	2,00	1,78 – 2,21	Global Proficiency	2,32	1,98	116	99,0
SMP	3,88	3,77 – 4,00	Global Proficiency	4,05	3,93	104	101
WMP	2,73	2,63 – 2,84	Global Proficiency	2,85	2,79	104	102
PMV	1,93	1,58 – 2,29	LATU	2,20	1,99	114	103

Note[1]: Recovery acceptance criteria (Codex Procedure Manual): unit g/100 g, 97 to 103 %

Considering the results obtained and the codex selection criteria for methods of analysis available in the Procedures Manual, the milk products experts committee involved in this study recommend the method of moisture normal pressure at 102 ºC (Annex I) to CCMAS.

Validation data for ISO 5537 I IDF 26: 2004 published on CCMAS 41 Meeting MAS/CDR06

Table 1. Results of a multi-lab comparison study with ISO 5537 | IDF 26:2004 and IDF 93A:1993 on 3 skimmed milk powders (SMP) and 3 whole milk powders (WMP) as conducted by Grobecker et al., 1999.

	ISO 5537 | IDF 26:2004						IDF 26A:1993					
	SMP 1	SMP 2	SMP 3	WMP 1	WMP 2	WMP 3	SMP 1	SMP 2	SMP 3	WMP 1	WMP 2	WMP 3
Number of participating labs after eliminating outliers	8	8	8	8	8	8	8	8	8	8	8	8
Mean value, % m/m	3.62	3.57	3.93	3.16	2.52	2.38	3.72	3.74	4.02	3.21	2.57	2.44
Repeatability standard deviation s_r% m/m	0.052	0.085	0.053	0.035	0.045	0.049	0.081	0.092	0.082	0.057	0.069	0.080
Coefficient of variation of repeatability, %	1.44	2.38	1.34	1.11	1.80	2.06	2.18	2.46	2.04	1.78	2.68	3.28
Repeatability limit, r (2,8*s_r), % m/m	0.146	0.238	0.148	0.098	0.126	0.137	0.227	0.258	0.230	0.160	0.193	0.224
Reproducibility standard deviation s_R% m/m	0.058	0.097	0.074	0.060	0.055	0.098	0.177	0.175	0.167	0.157	0.155	0.150
Coefficient of variation of reproducibility, %	1.61	2.69	1.89	1.89	2.19	4.11	4.76	4.68	4.15	4.89	6.03	6.15
Reproducibility limit, R (2,8*s_R), % m/m	0.162	0.272	0.207	0.168	0.154	0.274	0.496	0.490	0.468	0.440	0.434	0.420

Table 2. Results of a multi-lab validation study with ISO 5537 | IDF 26:2004 on rennet whey powder (RWP), acid whey powder (AWP) whey permeate powder (WPP),), milk permeate powder (MPP), cream powder (CP) and powdered infant formula (PIF) as conducted by IDF/ISO in 2020. To be published.

	ISO 5537 | IDF 26:2004					
	RWP	AWP	WPP	MPP	CP	PIF
Number of participating labs after eliminating outliers	11	12	12	12	11	12
Mean value, % m/m	2.06	2.57	1.52	1.52	2.57	1,87
Repeatability standard deviation s_r% m/m	0.026	0.030	0.037	0.046	0.024	0.039
Coefficient of variation of repeatability, %	1.27	1.16	2.46	3.01	0.93	2.07
Repeatability limit, r (2,8*s_r), % m/m	0.073	0.083	0.104	0.128	0.067	0.109
Reproducibility standard deviation s_R% m/m	0.064	0.098	0.083	0.102	0.068	0.072
Coefficient of variation of reproducibility, %	3.10	3.82	5.44	6.71	2.66	3.85
Reproducibility limit, R (2,8*s_R), % m/m	0.179	0.274	0.231	0.285	0.191	0.202

Annex I: Summary of proposed changes in CXS 234, including retyping of existing methods and recommendations to CCMAS

Recommended Methods of Analysis and Sampling (CXS 234-1999)

Commodity	Provision	Method	Principle	CXS	Proposed Type
Blend of skimmed milk and vegetable fat in powdered form	Water (moisture)[2]	Described in Appendix VII	Gravimetry, drying at 102°C	CXS 251	I
Blend of skimmed milk and vegetable fat in powdered form	Milk protein in MSNF	Described in Appendix VIIand ISO 1736 | IDF 9 and ISO 8968-1 | IDF 20-1	Calculation from total solids content, fat content and protein content Gravimetry, drying at 102ºC and Gravimetry (Röse-Gottlieb) and Titrimetry (Kjeldahl)	CXS 251	IV
Blend of skimmed milk and vegetable fat in powdered form	Milk protein in MSNF	Described in Appendix VII and ISO 1736 | IDF 9 and ISO 8968-1 | IDF 20-1	Calculation from total solids content, fat content and protein content Gravimetry, drying at 102ºC and Gravimetry (Röse-Gottlieb) and	CXS 251	IV

			Titrimetry (Kjeldahl)		
Reduced fat blend of skimmed milk powder and vegetable fat in powdered form	Water (moisture)[2]	Described in Appendix VIIAnnex I	Gravimetry, drying at 102°C	CXS 192	I
Reduced fat blend of skimmed milk powder and vegetable fat in powdered form	Milk protein in MSNF	Described in Appendix VII Annex I and ISO 1736 | IDF 9 and ISO 8968-1 | IDF 20-1	Calculation from total solids content, fat content and protein content Gravimetry, drying at 102ºC and Gravimetry (Röse -Gottlieb) and Titrimetry (Kjeldahl)	CXS 192	IV
Reduced fat blend of skimmed milk powder and vegetable fat in powdered form	Milk protein in MSNF	Described in Appendix VII and ISO 1736 | IDF 9 and AOAC 991.20	Calculation from total solids content, fat content and protein content Gravimetry, drying at 102ºC and Gravimetry (Röse -Gottlieb) and Titrimetry (Kjeldahl)	CXS 192	IV
Dairy permeate powders	Water (moisture)[2]	Described in Appendix VII	Gravimetry, drying at 102°C	CXS 331	I
Milk powders and cream powders	Water (moisture)[2]	Described in Appendix VII	Gravimetry, drying at 102°C	CXS 207	I
Whey powders	Water (moisture)[2]	Described in Appendix VII	Gravimetry, drying at 102°C	CX 289	I

Note[2] Water content excluding the crystallized water bound to lactose (generally known as “moisture content”)

ANNEX II – Test moisture method at normal pressure (102 ± 2) °C

Products	Parameter
Powdered milk, Powdered creamer, Powdered permeated milk, Infant formulas and Blend of skimmed milk powder with vegetable fat	Moisture

DESCRIPTION OF THE METHOD: DETERMINATION OF MOISTURE

1. SCOPE

This Standard specifies a method for the determination of moisture content for all types of powdered milk, powdered creamer, powdered permeated dairy products, infant formulas, and mixtures of powdered skimmed milk with vegetable fat.

2. DEFINITION

The content is the mass loss determined by the procedure specified in this Standard. It is expressed in percentage by mass g/100 g.

3. PRINCIPLE

A portion of the sample is dried in an oven set at (102 ± 2) °C until constant weight and weighed to determine the loss of mass.

4. EQUIPMENT

Common laboratory equipment and, in particular, the following.

4.1. Analytical balance, capable of weighing with a precision of 1 mg, with a minimum resolution of 0.1 mg.

4.2. Drying oven, with good ventilation, as far as possible with forced ventilation, capable of being thermostatically maintained at (102 ± 2) °C throughout the workspace, with a temperature controller.

4.3. Desiccator, with freshly dried silica gel with hygrometric indicator or another effective desiccant.

4.4. Flat-bottomed dishes, approximately 25 mm deep, approximately 50 mm in diameter, and made of an appropriate material (for example, glass, stainless steel, nickel, or aluminium), fitted with tight-fitting, removable lids easily.

5. SAMPLING

It is important that the laboratory receive a truly representative sample and that it has not been damaged or changed during transport or storage.

Sampling is not part of the method specified in this Standard. A recommended sampling method is provided in ISO 707.

6. TEST SAMPLE PREPARATION

Transfer the entire sample to a dry, tightly closed container with a capacity of approximately twice the volume of the sample. Mix thoroughly by turning and shaking the container.

7. PROCEDURE

7.1 Preparation of the dish.

7.1.1. Heat the uncovered capsule and its lid (4.4) in the oven (4.2) controlled at (102 ± 2) °C, for 1 h.

7.1.2. Transfer the capped dish to the desiccator (4.3), allow it to cool to room temperature in the balance room, and weigh (4.1) to the nearest 0.1 mg.

7.2. Test sample

7.2.1. Place 1 - 1.5 g of the prepared test sample (6) in the dish, cover with the lid and weigh to the nearest 0.1 mg.

7.3. Determination

7.3.1. Uncover the capsule and place it together with the lid in the oven (4.2), controlled at (102 ± 2) °C for 2 hrs.

7.3.2. Replace the cap, transfer the capped dish to the desiccator, allow to cool to balance room temperature, and weigh to the nearest 0.1 mg.

7.3.3. Uncover the capsule and heat again, along with its lid, on the oven for 1 h. Then repeat operation 7.3.2.

7.3.4. Repeat this process until the difference in mass between two successive weighings does not exceed 0.5 mg. Record the lowest mass.

8. CALCULATION AND EXPRESSION OF RESULTS

8.1. Calculation

The moisture content in the sample, expressed in g/100 g, is equal to:

$$\text{moisture} = \frac{(m_1 - m_2) \times 100}{(m_1 - m_0)}$$

where,

m_0 is the mass, in grams, of the dish and lid (7.1.2)

m_1 is the mass, in grams, of the dish, lid and test sample before drying (7.2.1)

m_2 is the mass, in grams, of the dish, lid and test sample after drying (7.3.4)

8.2. Expression of test results

Express the sample results to two decimal places.

APPENDIX III

CODEX OBSERVERS

Dietary Fibre Provisions in the Recommended Methods of Analysis and Sampling (CXS 234-1999)

Executive summary:

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.

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 fibre 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 fibre (TDF) in 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 fibre (SDF) has been completed and method AOAC 2022.01/ICC Standard 191 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 fibre methodology within Codex where the recommended Type 1 methods for a) Total Dietary Fibre and b) Soluble and Soluble Dietary Fibre, 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, AOAC 2022.01/ICC Standard 191, that corrects all issues identified with AOAC 2011.25/AACCI 32-50.01 as outlined in detail in Annex A below.

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

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 AOAC 2022.01/ICC Standard 191 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 Fibre 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 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

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-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.

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 AOAC 2022.01/ICC Standard 191, 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 fibre component IDF and SDFP and the soluble fibre fraction: SDFS.

- Comparison with existing methods: Differences between AOAC 2022.01/ ICC Standard 191 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**
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
sr, g/100g	0.47-1.41	0.59-1.35
sR, g/100g	0.95-3.14	1.11-3.05
RSDr, %	2.43-8.60	1.58-3.57

RSDR, %	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

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

IACST recommends CCMAS to:

1. Endorse AOAC 2022.01/ICC Standard 191 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.

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

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 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 20142 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, 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 RS4 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 2022.01/ICC Standard 191, the measured TDF content of RS4 and RS2 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 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 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 AOAC 2022.01/ICC Standard 191 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 AOAC 2022.01/ICC Standard 191.

b. A simplified procedure for desalting samples prior to HPLC analysis was introduced in AOAC 2022.01/ICC Standard 191. 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 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 3250.01 and AOAC 2022.01/ICC Standard 191 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 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.

Determination of lactose and fat content in the Recommended Methods of Analysis and Sampling (CXS 234-1999)

Executive Summary

IDF and ISO/TC34/SC5 would like to inform the CCMAS that several IDF/ISO standards have been revised.

- ISO 22662 | IDF 198 has been revised to broaden the scope of matrices to include dairy permeate liquid and dairy permeate powder in light of the recently published Codex standard on Dairy Permeates (CXS 331). A new entry line for CXS 234 is therefore proposed.
- Standards for the determination of fat in various dairy matrices have been reorganized and revised. The revision of 10 IDF/ISO standards for various dairy matrices led to the combination into 2 standards. The first of them is focused on matrices requiring the use of the Schmid-Bondzynski-Raztlaff principle, while the second one is based on the Röse-Gottlieb principle. The merger of these standards into 2 aims at a full editorial alignment in the description for the concerned matrices and facilitating their utilization by the users of the standards.

  Consequently, the references to the standards listed in CXS 234 need to be updated.

IDF and ISO recommend the CCMAS to:

1. Endorse ISO 22662 | IDF 198 as Type II method for the determination of lactose in dairy permeate powders
2. Endorse ISO 23319 | IDF 250: 2022 - Cheese and processed cheese products, caseins and caseinates — Determination of fat content — Gravimetric method – Schmid-Bondzynski-Raztlaff principle, to replace the following two standards in CXS 234:
   a. ISO 1735 | IDF 5: 2004 - Cheese and processed cheese products - Determination of fat content - Gravimetric method (Reference method)
   b. ISO 5543 | IDF 127 - Caseins and caseinates – Determination of fat content – Gravimetric method (Reference method)
3. Endorse ISO 23318 | IDF 249: 2022 - Milk, dried milk products and cream — Determination of fat content — Gravimetric method - Röse -Gottlieb principle, to replace the following six standards:
   a. ISO 1211 | IDF 1: 2010 - Milk - Determination of fat content - Gravimetric method (Reference method)
   b. ISO 1736 | IDF 9: 2008 - Dried milk and dried milk products - Determination of fat content - Gravimetric method (Reference method)
   c. ISO 2450 | IDF 16: 2008 - Cream - Determination of fat content - Gravimetric method (Reference method)
   d. ISO 8381 | IDF 123: 2008 - Milk-based infant foods - Determination of fat content (Reference method)
   e. ISO 1737 | IDF 13: 2008 - Evaporated milk and sweetened condensed milk - Determination of fat content - Gravimetric method (Reference method)
   f. ISO 1854 | IDF 59: 2008 - Whey cheese - Determination of fat content - Gravimetric method (Reference method)
4. Consider updating the reference of ISO 1211|IDF 1 to ISO 23319|IDF 250 for aqueous coconut products.

Method(s) for determination of lactose content

- ISO 22662 | IDF 198 (Ed 2) Milk and milk products — Determination of lactose content by high-performance liquid chromatography (Reference method)

ISO 22262 | IDF 198 standard proposes a reference method to quantify lactose content in fluid milk (raw and heat-treated), dried milk and cream (raw and pasteurized). The method was earlier multi-lab validated on 18 samples (six samples of fluid milk, six samples of cream and six samples of milk powder).

With the increase of exchange of Dairy Permeate Powders (DPP - from milk and whey), a need of a method to quantify lactose content in these products has arisen.

The main difference between the dried milk in the former method and DPP is a difference in lactose concentration. Considering this, the reflection was directed towards a reduction of the test portion of the same order as the difference in lactose concentration (to keep an equivalent final amount of lactose in the sample injected in the HPLC device). Performed tests have confirmed the validity of such an approach, whereby compliance was shown with the with the repeatability limit earlier established for dried milks. The results of these tests are described in Annex 1 of this document.

The standard is available from ISO as Draft International Standard here:
https://www.iso.org/standard/84827.html

The full International Standard is expected to be published later in 2023.

Description of the principle:

An internal standard [D(+)-melezitose] is added to a weighed volume of sample and to lactose standards. A chemical reagent (Biggs-Szijarto solution) is added to precipitate out the fat and the protein component fractions of milk. The sample is filtered twice prior to injection, first through paper filter and then through a 0.45 µm nylon filter. The lactose and the internal standard are separated by a cation exchange column in the lead form and detected by a differential refractometer detector or other suitable detector. As mobile phase, HPLC grade water is used.

Attribute – Lactose	ISO 22662 | IDF 198
Matrices, samples used in collaborative study	18 samples (six of fluid milk, six of cream and six of milk powder) + complementary comparison tests on dairy permeate powders
Concentration range of matrices validated	N/A
Repeatability (RSD_r or s_r)	0.37 %
Reproducibility (RSD_R or s_R)	2.94 %
CXS 331 – Minimum lactose, anhydrous(a) (m/m)	76%

Method(s) for determination of fat content - matrices requiring the use of the principle of Schmid-Bondzynski-Raztlaff

- **ISO 23319 | IDF 250 (**Gravimetric method – Schmid-Bondzynski-Raztlaff principle).

Scope

This document specifies a method for the determination of the fat content of all types of cheese and processed cheese products containing lactose of below 5 % (mass fraction) of non-fat solids, and all types of caseins and caseinates. The method is not applicable to fresh cheese types containing, for example, fruits, syrup or muesli. For such products, the Schmid-Bondzynski-Ratzlaff (SBR) principle is not applicable due to high concentrations of sugars. For these products, the method using the Weibull-Berntrop principle (see ISO 8262-3 | IDF 124-3[4]) is appropriate.

This first edition cancels and replaces ISO 1735 | IDF 5:2004 and ISO 5543 | IDF 127:2004, which have been merged and technically revised.

The Bulletin of the IDF No. 235/1988 - Interlaboratory collaborative studies (second series) – FIL-IDF (page 55) contains the reports of the collaborative studies on cheese, caseins and caseinates referred to in annexes C and D with the method.

The method is published by IDF and ISO, and includes a summary of the validation data (which have not changed).

Description of the principle (including reagents, standards, temperatures, equipment)

A test portion is digested with hydrochloric acid, then ethanol is added. The acid-ethanolic solution is subsequently extracted with diethyl ether and light petroleum. The solvents are removed by distillation or evaporation. The mass of the substances extracted, which are soluble in light petroleum, is determined.

NOTE This is usually known as the Schmid-Bondzynski-Ratzlaff principle.

New standard	**ISO 23319 | IDF 250** (Gravimetric method – Schmid-Bondzynski-Raztlaff principle)	
Previous versions	**ISO 1735 | IDF 5:2004**	**ISO 5543 | IDF 127:2004**
Matrices, samples used in collaborative study	Cheese and processed cheese products containing lactose of below 5 % (mass fraction) of non-fat solids. Tested in Emmentaler, Gouda, Brie and processed cheese.	Caseins, caseinates and rennet caseins

The data with repeatability and reproducibility is not provided since there is no change to the data contained in the revised standard. The main change is to update the new reference relevant for endorsed provisions.

Method(s) for determination of fat content- matrices requiring the use of the Rose-Gottlieb principle

- **ISO 23318 | IDF 249 (**Gravimetric method – Rose-Gottlieb principle).

Scope

This document specifies the method for the determination of fat content in:

a) raw milk (cow, sheep, goat), reduced fat milk, skimmed milk, chemically preserved milk and processed liquid milk;

b) dried milk products (e.g. whole, partially skimmed, skimmed milk powder; dairy permeate powder; whey powder; blend skimmed milk powder and vegetable fat; milk based infant formula powder);

c) raw, processed and sour cream;

d) evaporated milk and sweetened condensed milk (e.g. liquid sweetened and unsweetened concentrated milk);

e) whey cheese as defined in CXS 284-1999;

f) liquid whey and buttermilk;

g) milk-based edible ices and ice mixes;

h) liquid concentrated infant foods.

The method does not apply in the following cases:

- For b), when the powder contains hard lumps which do not dissolve in ammonia solution. This is noticeable by a distinct smell and the result of the determination will be too low. In such cases, a method using the Weibull-Berntrop principle is suitable, e.g. ISO 8262-3|IDF 124-3.
- For c), to sour creams with starch or other thickening agents. When separation or breakdown of fat occurs, a method using the Weibull-Berntrop principle is suitable, e.g. ISO 8262-3|IDF 124-3.
- For e), to products which do not dissolve completely in ammonia solution, as the result of the determination will be too low. With such products, a method using the Weibull-Berntrop principle is suitable, e.g. ISO 8262-3|IDF 124-3.
- For g), to milk-based edible ices and ice mixes in which the level of emulsifier, stabilizer or thickening agent or of egg yolk or of fruits, or of combinations of these constituents, makes the Röse-Gottlieb method unsuitable. With such products, a method using the Weibull-Berntrop principle is suitable, e.g. ISO 8262-2|IDF 124-2.

For the products under a), b) and c) the precision figures are given in Annexes C to G with the method. These precision figures are derived from interlaboratory studies fully conforming to the requirements from ISO 5725-2.

For the products under d) to h), the precision figures are given in Annex H with the method. These precision figures are derived from interlaboratory studies not fully conforming to the requirements from ISO 5725-2 in terms of number of samples (< 6) and number of participating laboratories (< 8).

This standard replaces the following standards: ISO 1211 | IDF 1, ISO 1736 | IDF 9, ISO 2450 | IDF 16, ISO 8381 | IDF 123, ISO 1737 | IDF 13, ISO 1854 | IDF 59, ISO 7208 | IDF 22 and ISO 7328 | IDF 116.

The method is published by IDF and ISO, and includes a summary of the validation data.

Description of the principle (including reagents, standards, temperatures, equipment)

- An ammoniacal ethanolic solution of a test portion is extracted with diethyl ether and light petroleum. The solvents are removed by distillation or evaporation. The mass of the substances extracted is determined. NOTE This principle is usually described as the “Rose Gottlieb” principle.

Attribute – Fat content	**ISO 23318 | IDF 249 (**Gravimetric method – Rose Gottlieb principle)					
Previous versions	ISO 1211 | IDF 1;	ISO 1736 | IDF 9,	ISO 2450 | IDF 16,	ISO 8381 | IDF 123,	ISO 1737 | IDF 13;	ISO 1854 | IDF 59,
Matrices, samples used in collaborative study	Milk: raw cow milk, raw sheep milk, raw goat milk, reduced fat milk, skimmed milk, chemically preserved milk, and processed liquid milk Milk from sheep and goats.	Dried whole, dried partially skimmed and dried skimmed milk, dried whey, dried buttermilk and dried butter serum	Raw, processed and sour creams	Milk based infant foods	Evaporated milk and sweetened condensed milk (liquid sweetened and unsweetened concentrated milk)	Whey cheeses

The data with repeatability and reproducibility is not provided since there is no change to the data contained in the revised standard. The main change is to update the new reference relevant for endorsed provisions.

Summary of proposed changes in CXS 234, including update to references of existing methods and recommendations to CCMAS

Table 1. Recommended Methods of Analysis and Sampling (CXS 234-1999)

Commodity	**Provision**	**Method**	**Principle**	**Codex STAN**	**Proposed Type**
Blend of evaporated skimmed milk and vegetable fat	Total fat	~~ISO 1737 | IDF 13~~ **ISO 23318 | IDF 249**	Gravimetry (Röse-Gottlieb)	250	I
Blend of evaporated skimmed milk and vegetable fat	Milk solids-not-fat[13] (MSNF)	ISO 6731 | IDF 21 and ~~ISO 1737 | IDF 13~~ **ISO 23318 | IDF 249**	Calculation from total solids content and fat content Gravimetry, drying at 102°C and Gravimetry (Röse-Gottlieb)	250	I
Blend of evaporated skimmed milk and vegetable fat	Milk protein in MSNF[13]	ISO 6731 | IDF 21 and ~~ISO 1737 | IDF 13~~ **ISO 23318 | IDF 249** and ISO 8968-1 | IDF 20-1	Calculation from total solids content, fat content and protein content Gravimetry, drying at 102°C and Gravimetry (Röse-Gottlieb) and Titrimetry (Kjeldahl)	250	IV
Blend of evaporated skimmed milk and vegetable fat	Milk protein in MSNF[13]	ISO 6731 | IDF 21 and ~~ISO 1737 | IDF 13~~ **ISO 23318 | IDF 249** and AOAC 991.20	Calculation from total solids content, fat content and protein content Gravimetry, drying at 102°C and Gravimetry (Röse-Gottlieb) and Titrimetry (Kjeldahl)	250	IV
Reduced fat blend of evaporated skimmed milk and vegetable fat	Total fat	~~ISO 1737| IDF 13~~ **ISO 23318 | IDF 249**	Gravimetry (Röse-Gottlieb)	250	I
Reduced fat blend of evaporated skimmed milk and vegetable fat	Milk solids-not-fat (MSNF) [13]	ISO 6731 | IDF 21 and ~~ISO 1737 | IDF 13~~ **ISO 23318 | IDF 249**	Calculation from total solids content and fat content Gravimetry, drying at 102°C and Gravimetry (Röse-Gottlieb)	250	I

Commodity	Provision	Method	Principle	Codex STAN	Proposed Type
Reduced fat blend of evaporated skimmed milk and vegetable fat	Milk protein in MSNF[13]	ISO 6731 | IDF 21 and ~~ISO 1737 | IDF 13~~ **ISO 23318 | IDF 249** and ISO 8968-1 | IDF 20-1	Calculation from total solids content, fat content and protein content Gravimetry, drying at 102°C and Gravimetry (Röse-Gottlieb) and Titrimetry (Kjeldahl)	250	IV
Reduced fat blend of evaporated skimmed milk and vegetable fat	Milk protein in MSNF[13]	ISO 6731 | IDF 21 and ~~ISO 1737 | IDF 13~~ **ISO 23318 | IDF 249** and AOAC 991.20	Calculation from total solids content, fat content and protein content Gravimetry, drying at 102°C and Gravimetry (Röse-Gottlieb) and Titrimetry (Kjeldahl)	250	IV
Blend of skimmed milk and vegetable fat in powdered form	Total fat	~~ISO 1736 | IDF 9~~ **ISO 23318 | IDF 249**	Gravimetry (Röse-Gottlieb)	251	I
Blend of skimmed milk and vegetable fat in powdered form	Milk protein in MSNF[13]	ISO 5537 | IDF 26 and ~~ISO 1736 | IDF 9~~ **ISO 23318 | IDF 249** and ISO 8968-1 | IDF 20-1	Calculation from total solids content, fat content and protein content Gravimetry, drying at 87°C and Gravimetry (Röse-Gottlieb) and Titrimetry (Kjeldahl)	251	IV
Blend of skimmed milk and vegetable fat in powdered form	Milk protein in MSNF[2]	ISO 5537 | IDF 26 and ~~ISO 1736 | IDF 9~~ **ISO 23318 | IDF 249**and AOAC 991.20	Calculation from total solids content, fat content and protein content Gravimetry, drying at 87°C and Gravimetry (Röse-Gottlieb) and Titrimetry (Kjeldahl)	251	IV
Reduced fat blend of skimmed milk powder and vegetable fat in powdered form	Total fat	~~ISO 1736 | IDF 9~~ **ISO 23318 | IDF 249**	Gravimetry (Röse -Gottlieb) I	251	I

[2] Milk total solids and Milk solids-not-fat (MSNF) content include water of crystallization of lactose

Commodity	Provision	Method	Principle	Codex STAN	Proposed Type
Reduced fat blend of skimmed milk powder and vegetable fat in powdered form	Milk protein in MSNF[13]	ISO 5537 | IDF 26 and ~~ISO 1736 | IDF 9~~ **ISO 23318 | IDF 249** and ISO 8968 - 1 | IDF 20 - 1	Calculation from total solids content, fat content and protein content Gravimetry, drying at 87 °C and Gravimetry (Röse -Gottlieb) and Titrimetry (Kjeldahl)		IV
Reduced fat blend of skimmed milk powder and vegetable fat in powdered form	Milk protein in MSNF[13]	ISO 5537 | IDF 26 and ~~ISO 1736 | IDF 9~~ **ISO 23318 | IDF 249** and AOAC 991.20	Calculation from total solids content, fat content and protein content Gravimetry, drying at 87 °C and Gravimetry (Röse -Gottlieb) and Titrimetry (Kjeldahl)	251	IV
Blend of sweetened condensed skimmed milk and vegetable fat	Total fat	~~ISO 1737 | IDF 13~~ **ISO 23318 | IDF 249**	Gravimetry (Röse -Gottlieb)	252	I
Blend of sweetened condensed skimmed milk and vegetable fat (for products sweetened with sucrose only)	Milk solids -not -fat[13] (MSNF)	ISO 6734 | IDF 15 and ~~ISO 1737 | IDF 13~~ **ISO 23318 | IDF 249** and ISO 2911 | IDF 35 ~~and~~	Calculation from total solids content, fat content and sucrose content Gravimetry, drying at 102 °C and Gravimetry (Röse -Gottlieb) and Polarimetry	252	IV
Blend of sweetened condensed skimmed milk and vegetable fat (for products sweetened with sucrose only)	Milk protein in MSNF[13]	ISO 6734 | IDF 15 and ~~ISO 1737 | IDF 13~~ **ISO 23318 | IDF 249** and ISO 2911 | IDF 35 and ISO 8968 -1|IDF 20 - 1	Calculation from total solids content, fat content, sucrose content and protein content Gravimetry, drying at 102 °C and Gravimetry (Röse -Gottlieb) and Polarimetry and Titrimetry (Kjeldahl)	252	IV

Commodity	Provision	Method	Principle	Codex STAN	Proposed Type
Blend of sweetened condensed skimmed milk and vegetable fat (for products sweetened with sucrose only)	Milk protein in MSNF[13]	ISO 6734 | IDF 15 and ~~ISO 1737 | IDF 13~~ **ISO 23318 | IDF 249** and ISO 2911 | IDF 35 and AOAC 991.20	Calculation from total solids content, fat content, sucrose content and protein content Gravimetry, drying at 102 °C and Gravimetry (Röse-Gottlieb) and Polarimetry and Titrimetry (Kjeldahl)	252	IV
Reduced fat blend of sweetened condensed skimmed milk and vegetable fat	Total fat	~~ISO 1737 | IDF 13~~ **ISO 23318 | IDF 249**	Gravimetry (Röse -Gottlieb)	252	I
Reduced fat blend of sweetened condensed skimmed milk and vegetable fat (for products sweetened with sucrose only)	Milk solids -not -fat[13] (MSNF)	ISO 6734 | IDF 15 and ~~ISO 1737 | IDF 13~~ **ISO 23318 | IDF 249** and ISO 2911 | IDF 35	Calculation from total solids content, fat content and sucrose content Gravimetry, drying at 102 °C and Gravimetry (Röse -Gottlieb) and Polarimetry	252	IV
Reduced fat blend of sweetened condensed skimmed milk and vegetable fat (for products sweetened with sucrose only)	Milk protein in MSNF[13]	ISO 6734 | IDF 15 and ~~ISO 1737 | IDF 13~~ **ISO 23318 | IDF 249** and ISO 2911 | IDF 35 and ISO 8968 -1|IDF 20 - 1	Calculation from total solids content, fat content, sucrose content and protein content Gravimetry, drying at 102 °C and Gravimetry (Röse -Gottlieb) and Polarimetry and Titrimetry (Kjeldahl)	252	IV

Commodity	Provision	Method	Principle	Codex STAN	Proposed Type
Reduced fat blend of sweetened condensed skimmed milk and vegetable fat (for products sweetened with sucrose only)	Milk protein in MSNF[13]	ISO 6734 | IDF 15 and ~~ISO 1737 | IDF 13~~ **ISO 23318 | IDF 249** and ISO 2911 | IDF 35 and AOAC 991.20	Calculation from total solids content, fat content, sucrose content and protein content Gravimetry, drying at 102 °C and Gravimetry (Röse-Gottlieb) and Polarimetry and Titrimetry (Kjeldahl)	252	IV
Cheese	Milkfat	~~ISO 1735 | IDF 5~~ **ISO 23319 | IDF 250**	Gravimetry (Schmid-Bondzynski - Ratzlaff)	283	I
Cheeses, individual	Milkfat in dry matter	ISO 5534 | IDF 4 ~~ISO 1735 | IDF 5~~ **ISO 23319 | IDF 250**	Calculation from dry matter content and fat content Gravimetry, drying at 102°C and Gravimetry	263 to 278	I
Cheeses in brine	Milkfat in dry matter (FDM)	ISO 5534 | IDF 4 ~~ISO 1735 | IDF 5~~ **ISO 23319 | IDF 250**	Calculation from dry matter content and fat content Gravimetry, drying at 102°C and Gravimetry (Schmid- BondzynskiRatzlaff)	208	I
Cottage cheese	Fat-free dry matter	ISO 5534 | IDF 4 and ~~ISO 1735 | IDF 5~~ **ISO 23319 | IDF 250**	Calculation from dry matter content and fat content Gravimetry, drying at 102 °C Gravimetry (Schmid-Bondzynski-Ratzlaff)	273	I
Cottage cheese (for samples containing lactose up to 5%)	Milkfat in dry matter	ISO 5534 | IDF 4 and ~~ISO 1735 | IDF 5~~ **ISO 23319 | IDF 250**	Calculation from dry matter content and fat content Gravimetry, drying at 102 °C and Gravimetry (Schmid-Bondzynski-Ratzlaff)	273	I

Commodity	Provision	Method	Principle	Codex STAN	Proposed Type
Cottage cheese (for samples containing lactose up to 5%)	Milkfat	~~ISO 1735 | IDF 5~~ **ISO 23319 | IDF 250**	Gravimetry (Schmid-Bondzynski-Ratzlaff)	273	I
Cream cheese	Moisture on fat-free basis	ISO 5534 | IDF 4 ~~ISO 1735 | IDF 5~~ **ISO 23319 | IDF 250**	Calculation from fat content and moisture content Gravimetry drying at 102°C (forced air oven) Gravimetry (Schmid-Bondzynski-Ratzlaff)	275	I
Dairy permeate powders	Milkfat	~~ISO 1736 | IDF 9~~ **ISO 23318 | IDF 249**	Gravimetry (Röse-Gottlieb)	331	I
Dairy permeate powders	**Lactose**	**ISO 22662 | IDF 198**	**High performance liquid chromatography**	**331**	**II**
Edible casein products	Milkfat (Total fat)	~~ISO 5543 | IDF 127~~ **ISO 23318 | IDF 249**	Gravimetry (Schmid-Bondzynski-Ratzlaff)	290	I
Mik powders and cream powders	Milkfat	~~ISO 1736 | IDF 9~~ **ISO 23318 | IDF 249**	Gravimetry (Röse-Gottlieb)	207	I
Mozzarella	Milkfat in dry matter – with high moisture	ISO 5534 | IDF 4 and ~~ISO 1735 | IDF 5~~ **ISO 23319 | IDF 250**	Calculation from dry matter content and fat content Gravimetry, drying at 102oC and Gravimetry (Schmid-Bondzynski-Ratzlaff)	262	I

Commodity	Provision	Method	Principle	Codex STAN	Proposed Type
Mozzarella	Milkfat in dry matter – with low moisture	ISO 5534 | IDF 4 and ~~ISO 1735 | IDF 5~~ **ISO 23319 | IDF 250**	Calculation from dry matter content and fat content Gravimetry, drying at 102°C and Gravimetry (Schmid-Bondzynski-Ratzlaff)	262	I
Whey cheeses by coagulation	Milkfat	~~ISO 1735 | IDF 5~~ **ISO 23319 | IDF 250**	Gravimetry (Schmid-Bondzynski-Ratzlaff)	284	I
Whey cheeses by coagulation	Milkfat in dry matter	~~ISO 1735 | IDF 5~~ **ISO 23319 | IDF 250** and ISO 5534 | IDF 4	Calculation from fat content and dry matter content Gravimetry (Schmid-Bondzynski-Ratzlaff) Gravimetry, drying at 102°C	284	I
Fermented milks	Milkfat	~~ISO 1211 | IDF 1~~ **ISO 23318 | IDF 249**	Gravimetry (Röse-Gottlieb)	243	I
Cream	Milkfat	~~ISO 2450 | IDF 16~~ **ISO 23318 | IDF 249**	Gravimetry (Röse-Gottlieb)	288	I
Creams lowered in milkfat content	Milkfat	~~ISO 2450 | IDF 16~~ **ISO 23318 | IDF 249** / AOAC 995.19	Gravimetry (Röse-Gottlieb)	288	I
Evaporated milks	Milkfat	~~ISO 1737 | IDF 13~~ **ISO 23318 | IDF 249**	Gravimetry (Röse-Gottlieb)	281	I
Evaporated milks	Milk Protein in MSNF[13]	~~ISO 1737 | IDF 13~~ **ISO 23318 | IDF 249**	Gravimetry (Röse-Gottlieb)	281	I
Sweetened condensed milk	Milkfat	~~ISO 1737 | IDF 13~~ **ISO 23318 | IDF 249**	Gravimetry (Röse-Gottlieb)	282	I
Sweetened condensed milks (for products sweetened with sucrose only)	Milk Protein in MNSF[13]	ISO 6734 | IDF 15 and ~~ISO 1737 | IDF 13~~ **ISO 23318 | IDF 249** and ISO 2911 | IDF 35 and ISO 8968-1 | IDF 20-1	Calculation from total solids content, fat content, sucrose and protein content Gravimetry, drying at 102 °C and Polarimetry Gravimetry (Röse-Gottlieb) Titrimetry (Kjeldahl)	282	I

Commodity	Provision	Method	Principle	Codex STAN	Proposed Type
Whey cheeses by concentration (carbohydrate contents below 5%)	Milkfat (Total fat)	~~ISO 1854 | IDF 59~~ **ISO 23318 | IDF 249**	Gravimetry (Röse Gottlieb)	284	I
Whey cheeses by concentration (for carbohydrate content under 5%)	Milkfat in dry matter (total fat in dry matter)	~~ISO 1854 | IDF 59~~ **ISO 23318 | IDF 249** and ISO 2920 | IDF 58	Calculation from fat content and dry matter content Gravimetry (Röse Gottlieb) Gravimetry, drying at 88 °C	284	I
Whey powders	Milkfat	~~ISO 1736 | IDF 9~~ **ISO 23318 | IDF 249**	Gravimetry (Röse-Gottlieb)	289	I
Infant formula	Total fat	AOAC 989.05 ~~ISO 8381|IDF 123~~ **ISO 23318 | IDF 249**	Gravimetry (Röse-Gottlieb)	72	I

Other updates necessary in CXS 234 to be considered

- 2 lines for aqueous coconut products refer to ISO 1211 | IDF 1, and shall need to be updated to ISO 23318|IDF 249
- 2 lines on page 56 of the 2021 version of CXS 234 - * DETERMINATION OF WATER CAPACITY OF CONTAINERS (CAC/RM 46)

Annex 1 – REPORT on analysis of lactose in milk permeate powder samples (ISO 22662|IDF 198)

At the last meeting of the IDF Standing Committee on Analytical Methods for Composition held during the IDF/ISO analytical week in Prague in 2019, it was requested to investigate if the standard ISO 22662|IDF 198 can be applied to analyze lactose in samples of milk permeate powder with the objective to include this matrix in the scope. Milk powder which is a similar matrix, to some extent, to the milk permeate powder, was already included in the scope of this standard.

Lactanet (Canada), who performed the initial collaborative study for ISO 22662|IDF 198, performed 12 analysis of milk permeate powder by following the procedure described in the standard ISO 22662|IDF 198 with one modification by lowering the test sample weight to 0,2 g in order to get peak areas within the range of the concentration of the calibration samples. We also analyzed skim milk powder in order to verify that the repeatability of the results for both products are comparable.

The table below shows that the results obtained with milk permeate powder are much better than those obtained with skim milk powder. This observation is not a surprise since the permeate powder contains a low content of protein and almost no fat as compared to the regular milk powder. Also to mention, the results of skim milk powder are still robust as compared to the repeatability data reported in ISO 22662|IDF 198. In conclusion, the inclusion of milk permeate powder in the scope of this standard is recommended.

Table-1: Results for lactose (g/100 g)

Sample	Skim milk powder	Milk permeate powder
1	49,09	82,02
2	49,10	82,15
3	48,67	82,19
4	48,93	82,09
5	49,01	82,03
6	49,08	82,22
7	49,16	82,07
8	49,14	81,85
9	49,19	82,07
10	49,17	81,95
11	49,15	81,95
12	49,16	81,93
Mean	**49,07**	**82,04**
STD	**0,15**	**0,11**
RSD	**0,30**	**0,13**

Date: 2020-04-08

Determination of Moisture Content in Dried Milk Products in the Recommended Methods of Analysis and Sampling (CXS 234-1999)

Summary

CCMAS41 deferred decision on the methods for moisture determination in dried milk products to CCMAS42, and the working group on Endorsement.

IDF and ISO wish to bring to CCMAS the following information relevant for the discussion:

- History and explanations on changes to the principle needed to improve performance of the method,
- Additional validation data obtained on 6 matrices, using the template for consideration of methods, conducting to an update of ISO 5537 | IDF 26:2004.
- Guidance on the control of drying ovens used in the application of ISO 5537 | IDF 26 is now included in the revised version of the standard to be published mid-2023.

An evaluation of the development and endorsement process with ISO 5537 | IDF 26:2004 confirms that the favorable performance is well underpinned with results from comparisons against other methods, with multi-lab validation data on skimmed milk powder and whole milk powder some 20 years ago. Moreover, its favorable performance was confirmed with more recent results in proficiency testing and with the results of a complementary conducted multi-lab validation study with other dried milk products.

With the information provided in more details hereafter, IDF and ISO recommend maintaining the current method listed for determination in dried milk products as Codex Type I method for checking compliance with existing Codex provisions on the moisture content in dried milk products in Codex standards 251, 207, 289 and 331, as listed in CXS 234.

IDF and ISO also encourage users of alternative methods for moisture determination in dried milk products to secure traceability of their results to ISO 5537 | IDF 26:2004, whereby ISO 5537 | IDF 26:2004 is to remain as the method of choice in case of measurements for official purposes or when dispute resolution is required.

Alternative methods can still be used for other intended purposes.

Commodity	Provision	Method	Principle	Type	Codex STAN
Blend of skimmed milk and vegetable fat in powdered form	Water (Moisture)	ISO 5537 | IDF 26	Gravimetry, drying at 87 °C	I	251
Reduced fat blend of skimmed milk powder and vegetable fat in powdered form	Water (Moisture)	ISO 5537 | IDF 26	Gravimetry, drying at 87 °C	I	251
Dairy permeate powders	Moisture	ISO 5537 | IDF 26	Gravimetry, drying at 87 °C	I	331
Milk powders and cream powders	Water (Moisture)	ISO 5537 | IDF 26	Gravimetry, drying at 87 °C	I	207
Whey powders	Water (Moisture)	ISO 5537 | IDF 26	Gravimetry, drying at 87 °C	I	289

Introduction

During CCMAS40, Uruguay raised concerns on the inclusion of ISO 5537 | IDF 26 in CXS 234-1999 as this Type I method required for the determination of moisture in dried milk was sophisticated, was limited to analysis in powders, and that other methods were available for such determinations for which validation data were available.

CCMAS41 could not reach consensus on confirming method ISO 5537 | IDF 26 for determination of moisture content in dried milk.

Proposals were made for AOAC 927.05 as the preferred Type I method, noting that this was a standard method widely used for determination of moisture in dried milk in many countries around the world; and the IDF/ISO method had limitations for use especially since the equipment and utensils were not widely available, were costly and led to environmental waste, and was therefore not accessible to many countries. The EWG Chair explained, that it was necessary to consider performance data to evaluate replacement of a Type I method which was already listed in CXS 234 for many years, and reminded CCMAS that according to its own rules in the Comprehensive guidance for the process of submission, consideration and endorsement of methods for inclusion in CXS 234, performance / validation data should be submitted in the template provided 60 days prior to a Session of CCMAS.

CCMAS42 therefore deferred decision on the methods for moisture content to CCMAS42; and agreed to request the PWG on endorsement to consider this matter. Data supporting other method(s) should be submitted according to the template in Comprehensive guidance for the process of submission, consideration and endorsement of methods for inclusion in CXS 234; and consideration should also be given to accessibility and cost implications.

Development, validation and adoption of ISO 5537|IDF 26:2004

Moisture in dried milk is a method-defined analytical parameter. Any method-defined parameter requires meticulously standardized analytical methodology in order to secure precise and accurate reference test results. Possible interferences are to be identified and counteracted through the use of well-defined and optimized equipment and proper materials, thereby strictly following a prescribed analytical protocol.

Reference method determination of the moisture content of food matrices relies upon the principle of weight loss upon drying under controlled conditions. Moisture is removed by heating a test portion in a drying oven until constant mass of the test portion is obtained. During the eighties of the last century, it was observed that the then reference method for the determination of moisture in dried milk products, IDF 26A:1993, in practice exhibited unacceptable high values for repeatability and reproducibility. This initiated the quest for a more robust and precise method.

Critical points in the proper operation of drying oven methods are:

- Intensive air ventilation is essential to obtain an accurate and uniform temperature inside the drying oven. However, the force of ventilation that can be applied is limited because the test portion is dried in an open dish.
- The relative air humidity in the testing environment. For instance, tests carried out showed a systematic difference of 0.3% (m/m) between the moisture content of a whole milk powder determined in winter (lower relative humidity of the air in the testing environment) and summer (higher relative humidity of the air in the testing environment.

With one of the initiatives at the time, these critical points were addressed with the development of a drying oven with controlled air flow, wherewith test portions of dried milk could be dried under much better standardized conditions, i.e. uniform temperature and constant relative humidity. Testing conditions were optimized to arrive at on average equivalent results with IDF 26A:1994 (de Knegt & van den Brink, 1998). This resulted in a revised method description including the use of the new developed test apparatus under optimized test conditions.

The global implementation of the method was promoted by involving two manufacturers of laboratory equipment who did bring compact, easy-to-operate and well affordable versions of the required drying oven to the market, equipment which is still globally purchasable today.

The performance of this revised method was verified in a multi-lab validation study with a comparison of the performance against three other methods, amongst them IDF 26A:1993, on three whole milk powder samples and three skimmed milk powder samples. The study was conducted under the aegis of the EU Joint Research Centre with participation of 8 laboratories. The results of this study are summarized in Table 1.

Table 1. Results of a multi-lab comparison study in 8 laboratories on 3 whole milk powders and 3 skimmed milk powders with 4 methods for the determination of moisture/water in dried milk as conducted by Grobecker et al., 1999. All values in % m/m.

Method	Mean	Standard deviation of repeatability (s_r)	Standard deviation of reproducibility (s_R)
IDF 26A:1993	3.28	0.067	0.142
ISO 5537 | IDF 26:2004	3.20	0.048	0.066

Klein Vacuum Oven Method	3.53	0.071	0.109
Karl Fisher Titration	3.69	0.054	0.084

From these data it was concluded that best precision was achieved with the method described in ISO 5537 | IDF 26:2004 and the Karl Fisher titration. Especially with reproducibility ISO 5537 | IDF 26:2004 showed much more favorable performance than IDF 26A:1993. The calculated mean value with ISO 5537 | IDF 26:2004 was close to the calculated mean value from the results with IDF 26A:1993, which was in accordance with the aim during method development. Understandably, the calculated mean value for the Karl Fisher titration was higher as this method measures not only the “free water”, but all water present in the sample.

ISO and IDF therefore concluded that ISO 5537 | IDF 26:2004 was the proper method to replace IDF 26A:1994 as the reference method for the determination of moisture content in dried milk. The method was therefore adopted as an IDF/ISO standard. It was subsequently also adopted by CEN as an EN Standard. CCMAS endorsed the method as a Type I method for the determination of water[3] content in:

- blends of skimmed milk and vegetable fat in powdered form, in reduced fat blends of skimmed milk powder and vegetable fat in powdered form, and in milk powders and cream powders in 2006 (ALINORM 06/29/23);
- whey powders in 2008 (ALINORM 08/31/23);
- dairy permeate powders in 2018 (REP 18/MAS).

Additional validation for other dried dairy matrices and revised ISO 5537 | IDF 26: 2023

The adoption of the method in Codex Alimentarius for application in the additional dried dairy matrices relied on single lab validation data and expert opinion. In light of the review of Codex-adopted methods of analysis and sampling in recent years, it was therefore considered appropriate to underpin the applicability of the method for the wider scope of dried milk products through a complementary international multilab validation study. This study was conducted in 2021 according to ISO 5725 part 1 and part 2, involving 14 laboratories in eight countries. Test samples were rennet whey powder, acid whey powder, whey permeate powder, milk permeate powder, cream powder and powdered infant formula.

The results of the initial collaborative study with skim milk powders and whole milk powders (Grobecker et al., 1999) and the results of this complementary study (link to be inserted) prompt the inclusion of values of 0.15% and 0.25%, respectively for the repeatability limit and the reproducibility limit, in a revised version of ISO 5537|IDF 26 for the determination of the moisture content in dried milk and dried milk products. This revised version also contains additional guidance on how to control the functioning of the drying oven.

Some considerations on ISO 5537 | IDF 26 and alternative methods

ISO 5537 | IDF 26:2004 is applied as reference method in several laboratories around the world. However, also alternative methods are widely applied. Laboratory performance with the different methods can be compared through proficiency testing.

On request, we were given insight in results collected by two proficiency test organizers. Test results from recent years on the determination of the moisture content in skimmed milk powder, whole milk powder and cream powder[4] and test results on the determination of dry matter content in whey powder[5] underline the more favorable precision of ISO 5537 | IDF 26:2004 as compared to methods with traditional oven drying methods at 102 °C.

It is acknowledged that in routine also other methods are in use, both in laboratories and in production environments. Feedback from the evaluated proficiency studies indicates application in practice of:

- IDF Provisional Standard 26A:1993 – Dried Milk and Dried Cream – Determination of Water Content

[2] Water content excluding the crystallized water bound to lactose (in fact to read moisture content).
[4] Dairychek (Powders), operated by Global Proficiency (NZ), www.global-proficiency.com.
[5] EPQS studies on dry matter in whey powder, operated by MUVA Kempten GmbH (DE), www.muva.de.

- AOAC Official Method 927.05. Loss on Drying (Moisture) in Dried Milk. 1927
- Undefined drying methods with drying at 102 °C
- Near infrared spectroscopy as guided by ISO 21543 | IDF 201:2006 – Guidelines for the application of near infrared spectroscopy

Although these alternative methods have no formal status in Codex Alimentarius, in routine their use may be preferred over ISO 5537 | IDF 26:2004 for reasons such as still achieving an acceptable degree of accuracy and precision, available equipment and facilities, robustness, ease of use in operation and cost.

Recommendations

An evaluation of the development and endorsement process with ISO 5537 | IDF 26:2004 learns that the favorable performance is well underpinned with results from comparisons against other methods, with multi-lab validation data on skimmed milk powder and whole milk powder some 20 years ago. Moreover, its favorable performance was confirmed with more recent results in proficiency testing and with the results of a complementary conducted multi-lab validation study with other dried milk products.

IDF and ISO recommend maintaining the current method listed for determination in dried milk products as Codex Type I method for checking compliance with existing Codex provisions on the moisture content in dried milk products in Codex standards 251, 207, 289 and 331, as listed in CXS 234.

References

Association of Official Agricultural Chemists. 1927. AOAC Official Method 927.05. Loss on Drying (Moisture) in Dried Milk**.**

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Annex 1 - submission of validation data

Method for determination of moisture

- ISO 5537 | IDF 26 Milk and milk products — Determination of moisture content in dried milk
- The method was validated on 6 samples: rennet whey powder (RWP), acid whey powder (AWP), whey permeate powder (WPP), milk permeate powder (MPP), cream powder (CP) and powdered infant formula (PIF).
- The full report of the validated data of collaborative study is published in (link to be inserted)
- Description of the principle:

  A test portion is dried in a drying oven set at 87 °C for 5 h while dry air is passed through the test portion. The loss of mass of the test portion (which is related to the content of “non-chemically bound” water) is determined.

Attribute – Moisture	ISO 5537 | IDF 26:2023
Matrices, samples used in multilab validation study	six samples: rennet whey powder (RWP), acid whey powder (AWP), whey permeate powder (WPP), milk permeate powder (MPP), cream powder (CP) and powdered infant formula (PIF).
Level range of moisture in samples tested	1.52 % to 2.57 %
Repeatability (RSD_r or s_r)	
rennet whey powder (RWP)	0.026 %
acid whey powder (AWP)	0.030 %
whey permeate powder (WPP)	0.037 %
milk permeate powder (MPP)	0.046 %
cream powder (CP)	0.024 %
powdered infant formula (PIF)	0.039 %
Reproducibility (RSD_R or s_R)	
rennet whey powder (RWP)	0.064 %
acid whey powder (AWP)	0.098 %
whey permeate powder (WPP)	0.083 %
milk permeate powder (MPP)	0.102 %
cream powder (CP)	0.068 %
powdered infant formula (PIF)	0.072 %
Limit of Quantitation	NA
CXS 331 – Dairy permeate powders	Max 5% (m/m)
CXS 207 - Milk powders and cream powders	Max 5 % (m/m)
CXS 289 - Whey powders	Max 5 % (m/m)

Attribute – Moisture	ISO 5537 | IDF 26 2004
Matrices, samples used in collaborative study	
Level range of moisture in samples tested	2,38 % to 3,93 %.
Repeatability (RSD_r or s_r)	
Whole milk powder 1	0.052 %
Whole milk powder 2	0.085 %
Whole milk powder 3	0.053 %
Skimmed milk powder 1	0.045 %
Skimmed milk powder 2	0.035 %
Skimmed milk powder 3	0.049 %
Reproducibility (RSD_R or s_R)	
Whole milk powder 1	0.058 %
Whole milk powder 2	0.096 %
Whole milk powder 3	0.074 %
Skimmed milk powder 1	0.055 %
Skimmed milk powder 2	0.060 %
Skimmed milk powder 3	0.098 %
Limit of Quantitation	NA
CXS 251 – Blend of skimmed milk and vegetable fat in powdered form	Max 5 % (m/m)
CXS 207 - Milk powders and cream powders	Max 5 % (m/m)