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Agenda Item 4.2 **CX/MAS 25/44/5**

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JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON METHODS OF ANALYSIS AND SAMPLING

44th Session

Virtual

5 – 8 May and 14 May 2025

DETERMINATION OF MOISTURE COTENT IN WHEY POWDER

(Prepared by New Zealand with assistance from Australia, Brazil, European Union, Uruguay and the International Dairy Federation (IDF))

BACKGROUND

1. CCMAS43 (2024) agreed that Australia, Brazil, the European Union (EU), New Zealand, Uruguay and the International Dairy Federation (IDF) (hereafter referred to as the group), should develop a discussion paper on the application of the determination of moisture[1] to:

   i. gather the full data, including outliers, from studies on determination of moisture in whey powders according to the 102 °C method and the data related with ISO 5537 | IDF 26 validation and share within the group;

   ii. if it is necessary, gather additional comparison data on determination of moisture in whey powders according to both methods (102 °C method contained in the *Recommended methods of analysis and sampling* (CXS 234-1999) – Appendix III, ISO 5537 | IDF 26), to be provided, and share within the group;

   iii. evaluate, based on this data, if the 102 °C method could be exceptionally listed as Type IV for determination of moisture in whey powders, with the note "Due to accessibility to equipment and calibration of the method ISO 5537 | IDF 26, the method as described in Appendix III is listed as Type IV"; and

   iv. provide a recommendation for consideration by the working group on endorsement prior to CCMAS44 (2025).

SUMMARY

2. New Zealand led a process that involved representatives from Australia, Brazil, the EU, Uruguay and IDF to undertake the CCMAS43 actions.

3. New Zealand would like to thank all the representatives (and expert colleagues) for their focus and willingness to attend the virtual meetings, to provide technical information, and importantly to respond to all requests in a timely and informed way and actively and positively work together to ensure the process was robust.

4. Key points and further information for each proposal are presented in Appendix I Table 1 and Table 2.

DISCUSSION

5. In May 2024, New Zealand set out the intended process in the preparation of the discussion paper on determination of moisture in whey powders (*Standard for whey powders* (CXS 289-1995)) – evaluation of candidate Type IV method. New Zealand acted in a Chair role for this work – facilitating the process and collating information. In acting in a Chair role, New Zealand remained neutral through the process.

6. This process included the following:

   - Raw data was shared from testing with the 102 °C method and/or the ISO/IDF method on whey powder.
   - Cross review/analysis. Each party was free to make their own review/analysis involving other parties as needed and using their own way. The summary was already available for the 102 °C method from the Latin American and Caribbean (LAC) countries, and the IDF/ISO method from IDF/ISO. A reasonable

[1] REP24/MAS paragraph 17 and 20 (vi)

expectation was at least to have a review from LAC countries of the IDF/ISO data, and a review from the LAC data by IDF/ISO.

- The outcome from each participant would be shared, and the review would include: general remarks, statistical analysis from the data, referring to the principle/method used to assess the data (such as ISO 5725).
- Further steps were to be decided based on outcome of discussions. An introduction to the group setting out the intended process in the preparation of the discussion paper on determination of moisture in whey powders (CXS 289 - 1995) – evaluation of candidate type IV method.

7. In June 2024, New Zealand shared the raw data from testing with the 102 °C method and/or ISO 5537 | IDF 26 on whey powder(s) provided by Uruguay and IDF with the group. The group undertook a cross review/analysis of this data and the comments from each participant were shared with the group.

8. In December 2024, New Zealand convened a virtual meeting of the group to discuss the data provided and comments to date.

9. The comments raised a range of technical concerns as follows:

- There was no information on the set-up of the study. Was it conducted in conformity with ISO 5725?
- No information was available on sample stability and homogeneity.
- Was multiplicate analysis on the same sample performed or were multiplicate samples of the same whey powder provided to each lab?
- No details on the applied drying method were shared with each participant.
- No mention was made of possible comments from the participants.
- With lab 8 bringing a Grubbs outlier and lab 4 bringing a Cochran outlier, only data from 6 labs remained for the calculation of the precision parameters.
- The repeatability and reproducibility data obtained differed from the ones presented: r=0.25 and R=0.72 (versus r=0.195 and R= 1.233, as presented in CX/MAS 23/42/3 Add.1).
    - One of the data files “idf_wmp 102c method.xlsx” was replaced with ‘idf wmp 102 using AOAC template outliers removed.xlsx’. Both files were available to the group to provide traceability about which observations had been removed as outliers.
    - In addition, Uruguay requested ‘additional information related with ISO 5537 | IDF 26 validation study. They wanted to better understand discarding as part of their review of the the statistical treatment of moisture data by ISO 5537 | IDF 26 i.e. the cause analysis that led to the elimination of the outliers required by ISO 5725-2. This information was provided to Uruguay from IDF and included the AOAC template and the DIS version of ISO5725-2.
    - IDF also advised that ISO 5725-2 typically deals only with the special case of data in blind duplicates (n=2) but a later part of ISO 5725 does expand to cases with n>2, and that the outlier tests are intended to indicate if an observation might be an outlier and then it should be the statistician and the study director who decide if the removal provides better estimates of r and R than reflects reality of routine use.

10. In February 2025, New Zealand summarised the responses from the group following the December meeting. A background, options and questions based on these options were sent out to the group for review prior to the next meeting in March 2025, which can be found in Appendix II.

11. In March 2025, New Zealand convened a virtual meeting of the group to discuss the questions and comments to these, and consider the content of a discussion paper for consideration by CCMAS44. The main comments raised at this meeting can be found in Appendix III. The meeting participants agreed:

- that there was no consensus;
- that both positions should be presented to CCMAS; and
- to state the process that has been followed, report that no consensus has been reached, and prepare a discussion paper with the two options to present to the working group on endorsement that will convene prior to CCMAS44.

12. The sequence of activity of the group is presented in Appendix I, Table 3.

CONCLUSION

13. There was no consensus reached and two proposals are tabled for consideration by CCMAS44, depending on CCMAS44's decision on whether the data provided is adequate to support the endorsement. The proposals are as follows:

- **Proposal 1:** the data provided supports endorsement of the 102 °C method as Type IV for whey powders, and with explanatory notes on use conditions as footnotes in CXS 234-1999.
- **Proposal 2:** the data provided does not support endorsement of the 102°C method for whey powders, and that the multi-laboratory trial is to be repeated, along with a comparative study to estimate the bias relative to the ISO 5537 | IDF 26 method.

RECOMMENDATION

14. CCMAS44 is invited to:

i. consider the two proposals presented in paragraph 13 based on the information provided in Appendix I Table 1: Key points for each proposal; and

ii. decide whether the data provided is adequate to support the endorsement.

Appendix I

Table 1: Key points for each proposal

Proposal one: Supporting endorsement of the 102 °C method as Type IV for whey powders	**Proposal two:** Not supporting endorsement of the 102 °C method as Type IV for whey powders
That following review of the full data, including outliers, from studies on determination of moisture in whey powders according to the 102 °C method and the data related with ISO 5537 | IDF 26 validation, the 102 °C method is **supported** to be exceptionally listed as Type IV for determination of moisture in whey powders, with the note "Due to accessibility to equipment and calibration of the method ISO 5537 | IDF 26, the method as described in Appendix III is listed as Type IV" in 234 Key points: • The South American countries are not requesting equivalence, the Type I method would still be used for disputes with the Type IV method used in more routine applications. • The use of the 102 °C method as a traditional method is for national and control purposes. The ISO 5537 | IDF 26 Type I method will be used in the event of a dispute. • The concern about the potential precedent of the coexisting Type I and Type IV methods – endorsement as Type IV is requested only as an exceptional case noting that this has already been done for one of the fats and oils methods • There are differences in the experimental design used for the review of the methods • Both designs have similar uncertainties according to ISO 5725 so are fit for purpose • There are little differences in the statistical tests performed - Both data processing methods comply with ISO 5725 • There are differences in the outlier exclusion criteria; the exclusion of outliers is based on statistical and physical deviations, especially when it comes to method validation studies • There are no comparative studies of both methods in whey powder.	That following review of the full data, including outliers, from studies on determination of moisture in whey powders according to the 102 °C method and the data related with ISO 5537 | IDF 26 validation that the 102 °C method is **not supported** to be listed as Type IV for determination of moisture in whey powders, with the note "Due to accessibility to equipment and calibration of the method ISO 5537 | IDF 26, the method as described in Appendix III is listed as Type IV" Key points: • The current multi-laboratory trial data for the 102 °C method is inadequate for drawing definitive conclusions regarding its fitness-for-purpose: - Data has been reviewed following the AOAC protocol which is equivalent to the IUPAC and ISO5725. - Analysing the data without removing outliers, results in precision values/estimates poorer than for ISO5537 | IDF 26. Removal of outliers improves precision but raises questions about the number of laboratories with the conclusion that the study is inconclusive. • Comparison with the ISO5537 | IDF 26 method is required due to the potential bias of the 102°C method for whey powders; assessment of bias (accuracy) is required by the Codex Procedural Manual • The group found differences between the AWP summary data based on the Uruguay raw data provided to the group, and the 2022 LAC Collaborative study summary data reported to CCMAS previously. CCMAS44 should consider amending the 2022 LAC Collaborative study summary data presented in CX/MAS 23/42/3 Add.1 and MAS43/CRD19. • Controls on the 102 °C method are needed in Appendix III particularly regarding humidity control and temperature control. • The 102 °C method has issues when testing products containing lactose at levels greater than those found in whole milk powder and skim milk powder:

Proposal one: Supporting endorsement of the 102 °C method as Type IV for whey powders	**Proposal two:** Not supporting endorsement of the 102 °C method as Type IV for whey powders
	- Temperatures greater than 90 °C cause colour changes and the lactose starts to lose its water of crystallization. - This explains why the precision is poorer for whey powders than for ordinary milk powders (SMP and WMP). - The basis for the Type IV status of the 102 °C method for skim milk powder and whole milk powder is supported by data from the study by Grobecker er al. (1999). • Endorsement of the method as Type IV raises issues of coexistence of Type I and Type IV methods and the potential for multiple Type IV methods could create issues in the future. • There are no comparative studies of both methods as the 102 °C method was discontinued for not being fit for purpose and having developed a fit for purpose alternative method in ISO 5537 | IDF 26. The 102 °C method is considered unfit for moisture determination in whey powders because of the specific issues with these type of matrices. There was no driver to make such a comparison between the two methods.

In addition, and due to the complexity of the topic, information supporting the proposals, along with the sequence of activity including the technical information provided by representatives to the group for review and consideration is below in the Tables 2 and 3. Provision of this background information is important for the wider consideration by CCMAS44.

Table 2: More information on each proposal

Proposal one: Supporting endorsement of the 102 °C method as Type IV for whey powders	**Proposal two:** Not supporting endorsement of the 102 °C method as Type IV for whey powders
Q1: If it is necessary to have additional data validation for 102 °C oven' method considering that this method meets Type IV Codex method definition as it is a method which has been used traditionally, the technical evidence reviewed and the applicability aspects among other criteria for selection of methods defined in the Codex Procedural Manual. [based on Option 1.1]	Q1: whether the current data is adequate or whether a full validation study should be carried out and if so the form of that study in particular whether it should include estimation of bias relative to the ISO 5537 method? [based on: Options 2.1, 2.2 and 2.3] - Noting that as the current data either shows with all participant data included that the precision for whey powder is 'unsatisfactory' or if we remove a significant number of 'outliers' the study as 'inconclusive'. Therefore, this doesn't specifically support the '102 ^{o}C oven method' inclusion for CCMAS endorsement as 'adequate', and a further study is the only means we can see to overcome this. - If a further study is performed then it would be advisable to design the study to ensure a 'conclusive' outcome, but also suggest inclusion of some if not all the suggested additional 'controls' to see where participants can comply with these 'additional controls' (and have issues or not), and also guide any required updates to the method if the '102 ^{o}C oven method' proves to be 'fit-for-purpose' for whey powders. - Noting many of these 'controls' would align with ISO 17025 accreditation (or equivalent), e.g. temperature controlled chambers including ovens with initial manufacturers specification as providing '102 ± 2^{o}C temperature' control, then spatial uniformity calibration every 3 years using IEC 60068 over 3 points in the temperature range; verifying the temperature yearly; and on use monitor temperature at a minimum one point; plus routine inclusion of reference materials; control chart assessments of testing. - The independent review of the collaborative study data using the method described in Annex III of CXS 234-1999 (oven drying at 102 ^{o}C) showed that two of the participating laboratories were identified as outliers (one Cochran outlier, due to increased within-laboratory variation, one Grubbs outlier due to increased between-laboratory variation). Removing the outlying data from those two laboratories left only six data sets for further statistical evaluation. Widely accepted international standards for multi-laboratory validation of testing methods, e.g. ISO 5725, require at least eight data sets. Consequently, all reviewers agreed that the study

Proposal one: Supporting endorsement of the 102 °C method as Type IV for whey powders	**Proposal two:** Not supporting endorsement of the 102 °C method as Type IV for whey powders
	outcome is inconclusive. Therefore, the current data is not sufficient to endorse the method as a Type IV for the determination of moisture in whey powder and the collaborative study should be repeated, where the study design has to ensure that enough useable data are available for statistical evaluation.
Q2: whether the current data is adequate or whether additional data validation should be carried out ? [based on Option 1.2]	Q2: whether the additional information on controls necessary when using the old method should be included in Appendix III of CXS 234? [based on Option 2.4] - It may not be necessary to include all these controls in the method specifically, as some would be recommended for laboratories involved in the import and export control of foods with ISO 17025 (or equivalent) accreditations (see CXG 27 - 1997 *Guidelines for the assessment of the competence of testing laboratories* Involved in the Import and Export Control of Food) and potentially utilising the '102^{o}C oven method'. - The risk to accuracy and precision from potential degradation of the whey sample at the '102 ± 2 ^{o}C temperature', elevated temperature fluctuations(above the '102 ± 2 ^{o}C temperature' during testing without good temperature controls, rapid moisture re-adsorption if the laboratory environment is not controlled, plus differential in relative sensitivity of the '102 ^{o}C oven method' and 'ISO 5537 | IDF 26: 2004' which arises largely from the different sample masses, and which the '102 ^{o}C oven method' cannot increase due to the mechanics of the 'flat-bed' versus 'column' drying process. - It is generally accepted that the results obtained by the 102 ^{o}C drying method are especially affected by the relative humidity of the air in the laboratory where the test is carried out (de Knegt & van den Brink, International Dairy Journal 8 (1998) 733-738), thus leading to a relatively high standard deviation under reproducibility conditions. Therefore, the environmental laboratory conditions, in particular humidity, should be carefully controlled, while the other factors appear less important.

Background: What the group found	
• That in reviewing the ISO 5537 | IDF 26:2023 and '102 °C oven method' performance data it supports the retention of the ISO 5537 | IDF 26 as the Type I considering the limitation of applicability of the method, but there continues to be no consensus on whether the '102 °C oven method' should be endorsed as a Type IV method for Whey powders. Some participants understand that even though ISO 5537 | IDF 26 had little better precision figures than the '102°C oven method', 102 °C oven method' fully meets Type IV Codex method definition since it is a method which has been used traditionally and is fit fur purpose for food safety and trade market. Both methods complain with Codex performance criteria for method of analysis defined in the Codex Procedural Manual.	• That in reviewing the ISO 5537 | IDF 26: 2004 and '102 °C oven' method performance data it supports the retention of the ISO 5537 | IDF 26: 2004 as the Type I, but there continues to be no consensus on whether the '102 °C oven method' should be endorsed as a Type IV method for Whey powders.
• Considering the experimental design of the Collaborative Studies for AWP, after individual group member statistical assessment of '102 °C oven method' and ISO 5537 | IDF 26 opinions are as follows, - Some participants found during the statistical assessment of original eight individual participant data for AWP moisture by '102 °C oven method' from the 2022 LAC collaborative study, at least two and possibly three participants data was found to be 'outliers'. But this makes the revised dataset too small (5-6 of original 8 laboratories) thus the study 'inconclusive'. - Other participants found that Collaboratory studies of LAC and IDF have different designs of experiments (LAC 7-8 labs with 5 replicates each / total 35-45 individual results, IDF 12-11 labs by duplicate each / total 22-24 individual results) that provide comparable uncertainties considering ISO 5725-2 approach.	• After individual group member statistical assessment of the original eight individual participant data for Acid Whey Powder (AWP) moisture by '102 °C oven method' from the 2022 LAC collaborative study, at least two and possibly three participants data was found to be 'outliers'. But this makes the revised dataset too small (5-6 of original 8 laboratories) thus the study 'inconclusive'.
• Differences were found related with statistical data analysis of LAC and IDF studies. LAC Collaborative study data analysis includes as discarding outlier criteria Cochran and Grubbs test whilst IDF includes additional ones (h and k test). Both data analysis complains with the recommendations of ISO 5725-2 standard. Differences were found between the data analysis of the participants of the group.	• The 2022 LAC Collaborative summary table 4 data in Moisture Content of Whey Powder V01 241214LF emailed on 14 Dec 2024 by Uruguay now differs significantly to the summary data submitted to CCMAS provided for AWP in CX/MAS 23/42/3 Add.1 page 10 Table 2, and MAS43/CRD19 page 5 Table 2, but Uruguay's statistical summary provided on 14 Dec 24 is in better agreement with the statistical assessments of the other group members. This would also mean the MAS43/CRD19 page 5 Table 3, Moisture 102 °C (normal pressure) values would have to be amended also.

• Differences were found related with statistical data analysis of LAC and IDF studies between criteria regarding the data discarding that lead to slightly different results of precision between both methods. LAC Collaborative study statistical analysis are aligned with ASTM E178 – 21 that recommends discarding outliers only in cases of finding statistical or physical deviations, which is in line with the impartiality criteria established in most ISO standards, whilst IDF data analysis consider additional discarding criteria to lead with outliers as recommendation of technical experts.	• Outlier analysis has been conducted in strict conformance with ISO 5725. ISO 5725 demands to first get an indication on possible outliers by doing Mandel h and k statistics and then to confirm with Cochran and Grubbs test. Cochran and Grubbs are decisive, provided there is solid ground from additional information from the participant on deleting such data pairs. Additional criteria were not applied in the outlier evaluation process with the extension of ISO 5536|IDF 26 to whey powders.
Options:	
Option 1.1 Ask CCMAS44 to reassess whether '102 °C oven method' for AWP should be found fit-for purpose as a Type IV.	Option 2.1 The 2022 LAC PT summary data for AWP presented be amended based on the statistical summaries presented to the group and ask CCMAS44 to reassess whether '102 °C oven method' should be found fit-for purpose as a Type IV, taking into account the observations from the group.
Option 1.2 State the application for the '102 °C oven method' was declined by several group participants (but not a group consensus) as there was not clear evidence of method performance provided to the group from the 2022 LAC Collaborative study data for AWP to confirm it as 'fit-for-purpose'.	Option 2.2 State the application for the '102 °C oven method' was declined by the group majority (but not a group consensus) as there was not clear evidence of method performance provided to the group from the 2022 LAC PT data for AWP to confirm it as 'fit-for-purpose' for whey powders, based on precision but also trueness. While CXS 234 lists other methods without validation data as type IV, the particularity of the request here is to add a method as an alternative to a type I method. Therefore, the trueness is a key aspect in demonstrating fitness for purpose.
Option 1.3 Suggest the '102 °C oven method' for AWP is modified with further controls to hopefully provide better precision. These would include: - Stated in the method repeatability limit 'r' and reproducibility limit 'R' and sufficient replicates for within an analytical batch for the repeatability limit assessment. Inclusion of a duplicate 'control'	Option 2.3 On review of the individual participant data for AWP from the 2022 LAC Proficiency Test (PT) data set showed disparities with the statistical summary data provided in CX/MAS 23/42/3 Add.1 and MAS43/CRD19, all the data from the other milk powder matrices should be reviewed to identify any other anomalies and corrected. Noting precision and trueness have been demonstrated for whole and skimmed milk, see ref [1] and ref [2].

[1] Grobecker et al. 1999. Determination of the water content in milk powder: Report of a collaborative study performed in the period June-July 1999. European Commission Report, EU-DG JRC-IRMM & IHCP
[2] de Knegt, R.J. & van den Brink, H. 1998. Improvement of the Drying Oven Method for the Determination of the Moisture Content of Milk Powder. Int. Dairy J. 8, p 733-738.

sample within each analytical batch would allow compliance assessment. - inclusion of reference materials within each analytical batch; - routine inclusion of certified reference materials (if they become available)	
	Option 2.4 Suggest the '102 °C oven method' is modified with further controls to hopefully provide better precision; but which would necessitate a further collaborative study to validate but noting there is no guarantee that the outcome of validation will meet the criteria approach, if the 102°C drying temperature is fundamentally 'unfit-for-purpose' for Whey powders. These would include: a. Include in the method that the analyst records the 'Environmental conditions during measurement' i.e. laboratory temperature, and humidity. Where the relative humidity should be maintained between 40 % and 60 % which is the recommended limits provided by most balance manufacturers and by OIML R 111. Further, for high precision measurement, the temperature should not vary by more than ± 1 °C in a 24 h period throughout the year and should vary by less than 0.5 °C during any one measurement series (typically less than 1 h). Otherwise, a comfortable working environment is considered 20 to 25 °C. b. indicate in the method, examples of drying oven model/make which can achieve 102±2°C oven chamber spatial variation, as this is not easily achieved. c. As the ISO 5537:IDF 26: 2004 uses a 5.0 g±0.3 g test sample, and the '102°C oven method' uses a 1 g-1.5 g test sample, to achieve comparable sensitivity for the same weight loss by both methods, the '102°C oven method' may need to utilise an analytical balance with 3.3-5x greater sensitivity. Available analytical balance specifications can achieve for a range up to 30g, a readability of 0.01mg and repeatability of 0.03mg. d. Consider if a better endpoint assessment is available instead of the '0.5mg constant weight' or alternatively use a lower 'constant weight threshold' e.g. 0.2 mg, as we doubt the endpoint correlates with an inflection point on the drying curve. This should make it more reproducible but hopefully not shift the endpoint significantly and

	would require an analytical balance with greater sensitivity than currently specified. e. Stated in the method repeatability limit 'r' and reproducibility limit 'R' and sufficient replicates for within an analytical batch for the repeatability limit assessment. The Bulletin of IDF 2023 Collaborative study derives an amended repeatability and reproducibility specification for ISO 5537 | IDF 26: 2004 based on their collaborative study data. A similar assessment and specification could be derived for the '102°C oven method' based on a 'second larger collaborative study with additional method parameter controls' data and give a guide to competence and ongoing quality control. Inclusion of a duplicate 'control' sample within each analytical batch would allow compliance assessment. f. Inclusion of reference materials within each analytical batch; g. Routine inclusion of certified reference materials (if they become available); h. Control charts assessment between analytical batches has the potential to assure repeatability and reproducibility are within limits. Noting that introducing the mentioned controls, when already feasible in routine operation, will make the 102 °C method more complex and laborious in its execution than the ISO 5537 | IDF 26:2004 method.

Sequence of activity

Table 3: Sequence of activity within the group and related documents – NOTE ALL RELATED DOCUMENTS ARE AVAILABLE HERE

Date and title	Summary of activity
May 2024 Discussion paper on determination of moisture in whey powders (CXS 289 - 1995) – evaluation of candidate type IV method	An introduction to the group setting out the intended process in the preparation of the discussion paper on determination of moisture in whey powders (CXS 289) – evaluation of candidate type IV method. This included: • To share raw data from testing with the 102 °C method and/or the ISO/IDF method on whey powder • Cross review/analysis. Each party was free to make their own review/analysis involving other parties as needed and using their own way. The summary is already available for the 102°C method from the CCLAC countries, and the IDF/ISO method from IDF/ISO. A reasonable expectation is at least to have a review from CCLAC countries of the IDF/ISO data, and a review from the CCLAC data by IDF/ISO. • The outcome from each participant to be shared, and the review to include general remarks, statistical analysis from the data, referring to the principle/method used to assess the data (such as ISO 5725). • Further steps to be decided based on outcome of discussions
June 2024 Discussion paper on determination of moisture in whey powders (CXS 289) – evaluation of candidate type IV method - next steps	IDF and Uruguay provided raw data from testing with the 102 °C method and/or ISO 5537 | IDF 26 on whey powder(s). The raw data is attached as follows: Uruguay: Lab 1_WP.xlsx - Lab 8_WP.xlsx IDF: Whey powder results from IDF collaborative study.xlsx, Bulletin of the IDF B524 Moisture eCodex.pdf The Group was asked to undertake a cross review/analysis of this data
December 2024 Meeting	Information for the meeting (17/12/2025) provided to the Group: • Agenda • The review of the methods from Uruguay/Brazil and IDF and the responses from Australia and EU Noting: • IDF: Information on the Type I method compared to the Type IV method for determination of moisture in whey powder (attached) (and comment) - *IDF experts have conducted their review of the data obtained on whey powders and we came to the following comments and questions:* - *There is no information on the set-up of the study. Was it conducted in conformity with ISO 5725?*

Date and title	Summary of activity
	- *No information is available on sample stability and homogeneity,* - *Was multiplicate analysis on the same sample performed or were multiplicate samples of the same whey powder provided to each lab?* - *No details on the applied drying method with each participant,* - *No mention made of possible comments from the participants,* - *With lab 8 bringing a Grubbs outlier and lab 4 bringing a Cochran outlier, only data from 6 labs remain for the calculation of the precision parameters,* - *The repeatability and reproducibility data obtained differ from the ones presented: r=0.25 and R=0.72 (versus r=0.195 and R= 1.233, as presented in CX/MAS 23/42/3 Add.1). Attached is our calculation file (with and without outliers).* In addition: • IDF: - *The removal of "'idf_wmp 102c method.xlsx" and replacement with the 'idf wmp 102 using AOAC template outliers removed.xlsx'. Both files are included to provide traceability about which observations have been removed as outliers.* In addition: • Uruguay: - *requested 'additional information related with ISO 5537 I IDF 26 validation study. We are analysing the statistical treatment of moisture data by ISO 5537 | IDF 26, so we are trying to understand in a better way data discarding. That is why we need the cause analysis that led to the elimination of the outliers required by ISO 5725-2. Would it be possible to send us this information so that we can understand the criteria involved?'* • A response to Uruguay from IDF: - *The AOAC template is attached. The DIS version of ISO 5725-2 is attached.* - *ISO 5725-2 typically deals only with the special case of data in blind duplicates (n=2) but a later part of ISO5725 does expand to cases with n>2* - *The outlier tests are intended to indicate if an observation might be an outlier and then should be the statistician and the study director decide if the removal provides better estimates of r and R than reflects reality of routine use.*
February 2025 RE: Determination of moisture in whey powder	New Zealand summarised the information received, noting our role as neutral. We set out questions and background for review by the Group to consider prior to the meeting in March 2025: *Q1: whether the current data is adequate or whether a full validation study should be carried out and if so the form of that study in particular whether it should include estimation of bias relative to the 5537 method?*

Date and title	Summary of activity
	[based on options 1 & 2 below] *Q2: whether the additional information on controls necessary when using the old method should be included in Appendix III of CXS 234?* *[based on option 4 below]* ***Background:*** *What the group found:* • *That in reviewing the ISO 5537|IDF 26: 2004 and '102^{o}C oven' method performance data it supports the retention of the ISO 5537|IDF 26: 2004 as the Type I, but there continues to be no consensus on whether the '102^{o}C oven method' should be endorsed as a Type IV method for Whey powders.* • *After individual group member statistical assessment of the original eight individual participant data for Acid Whey Powder (AWP) moisture by '102^{o}C oven method' from the 2022 LAC collaborative study, at least two and possibly three participants data was found to be 'outliers'. But this makes the revised dataset too small (5-6 of original 8 laboratories) thus the study 'inconclusive'.* • *The 2022 LAC Collaborative summary table 4 data in Moisture Content of Whey Powder V01 241214LF emailed on 14 Dec 2024 by Uruguay now differs significantly to the summary data submitted to CCMAS provided for AWP in CX/MAS 23/42/3 Add.1 page 10 Table 2, and MAS43/CRD19 page 5 Table 2, but Uruguay's statistical summary provided on 14 Dec 24 is in better agreement with the statistical assessments of the other group members. This would also mean the MAS43/CRD19 page 5 Table 3, Moisture 102 ^{o}C (normal pressure) values would have to be amended also.* *Options available for the group:* 1. *Have the 2022 LAC Collaborative study summary data for AWP presented in CX/MAS 23/42/3 Add.1 and MAS43/CRD19 amended based on the statistical summaries presented to the group and ask CCMAS44 to reassess whether '102oC oven method' should be found fit-for purpose as a Type IV.* 2. *State the application for the '102oC oven method' was declined by the group majority (but not a group consensus) as there was not clear evidence of method performance provided to the group from the 2022 LAC Collaborative study data for AWP to confirm it as 'fit-for-purpose'.* 3. *On review of the individual participant data for AWP from the 2022 LAC Collaborative study showed disparities with the statistical summary data provided in CX/MAS 23/42/3 Add.1 and MAS43/CRD19, all the data from the other milk powder matrices should be reviewed to identify any other anomalies and whether the result of this review may impact the considerations and decision made by CCMAS42 for inclusion of the '102oC oven method' for the determination of moisture content for all types of powdered milk, powdered cream, and mixtures of powdered skimmed milk with vegetable fat.* 4. *Suggest the '102^{o}C oven method' is modified with further controls to hopefully provide better precision; but which would necessitate a further collaborative study to validate but noting there is no guarantee that the outcome of validation will meet*

Date and title	Summary of activity
	the criteria approach, if the 102°C drying temperature is fundamentally 'unfit-for-purpose' for Whey powders. These would include: *i. Include in the method that the analyst records the 'Environmental conditions during measurement' i.e. laboratory temperature, and humidity. Where the relative humidity should be maintained between 40 % and 60 % which is the recommended limits provided by most balance manufacturers and by OIML R 111. Further, for high precision measurement, the temperature should not vary by more than ± 1 °C in a 24 h period throughout the year and should vary by less than 0.5 °C during any one measurement series (typically less than 1 h). Otherwise, a comfortable working environment is considered 20 to 25 °C.* *j. indicate in the method, examples of drying oven model/make which can achieve 102±2°C oven chamber spatial variation, as this is not easily achieved.* *k. As the ISO 5537:IDF 26: 2004 uses a 5.0 g±0.3 g test sample, and the '102°C oven method' uses a 1 g-1.5 g test sample, to achieve comparable sensitivity for the same weight loss by both methods, the '102°C oven method' may need to utilise an analytical balance with 3.3-5x greater sensitivity. Available analytical balance specifications can achieve for a range up to 30g, a readability of 0.01mg and repeatability of 0.03mg.* *l. Consider if a better endpoint assessment is available instead of the '0.5mg constant weight' or alternatively use a lower 'constant weight threshold' e.g. 0.2 mg, as we doubt the endpoint correlates with an inflection point on the drying curve. This should make it more reproducible but hopefully not shift the endpoint significantly and would require an analytical balance with greater sensitivity than currently specified.* *m. Stated in the method repeatability limit 'r' and reproducibility limit 'R' and sufficient replicates for within an analytical batch for the repeatability limit assessment. The Bulletin of IDF 2023 Collaborative study derives an amended repeatability and reproducibility specification for ISO 5537 | IDF 26: 2004 based on their collaborative study data. A similar assessment and specification could be derived for the '102°C oven method' based on a 'second larger collaborative study with additional method parameter controls' data and give a guide to competence and ongoing quality control. Inclusion of a duplicate 'control' sample within each analytical batch would allow compliance assessment.* *n. inclusion of reference materials within each analytical batch;* *o. routine inclusion of certified reference materials (if they become available);* *p. Control charts assessment between analytical batches has the potential to assure repeatability and reproducibility are within limits.*

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March 2025	Information for the meeting 11/3/2025 provided to the group: • Agenda • The draft paper prepared by New Zealand and the responses from Australia, EU and IDF supporting the paper in principle • The response from Uruguay and Brazil not supporting the paper and providing alternative text
March 2025 Minutes from the meeting	Minutes were circulated. Responses were received from all participants. The version including all comments is attached.

Appendix II

Summary of background, questions and options for review prior to the March 2025 meeting

1. **Background: What the group found**:

- That in reviewing the ISO 5537 | IDF 26: 2004 and '102 °C oven' method performance data supported the retention of the ISO 5537 |IDF 26: 2004 as the Type I, but there continued to be no consensus on whether the '102 °C oven method' should be endorsed as a Type IV method for Whey powders.
- After individual group member statistical assessment of the original eight individual participant data for Acid Whey Powder (AWP) moisture by '102 °C oven method' from the 2022 LAC collaborative study, at least two and possibly three participants data were found to be 'outliers'. But this made the revised dataset too small (5-6 of original 8 laboratories) thus the study 'inconclusive'.
- The 2022 LAC Collaborative summary table 4 data in Moisture Content of Whey Powder V01 241214LF emailed on 14 Dec 2024 by Uruguay differed significantly to the summary data submitted to CCMAS provided for AWP in CX/MAS 23/42/3 Add.1 page 10 Table 2, and MAS43/CRD19 page 5 Table 2, but Uruguay's statistical summary provided on 14 Dec 24 was in better agreement with the statistical assessments of the other group members. This would also mean the MAS43/CRD19 page 5 Table 3, Moisture 102 °C (normal pressure) values would have to be amended also.

2. Questions for the group:

Q1: whether the current data is adequate or whether a full validation study should be carried out and if so the form of that study in particular whether it should include estimation of bias relative to the 5537 method?

[based on options 1 & 2 below]

Q2: whether the additional information on controls necessary when using the old method should be included in Appendix III of CXS 234?

[based on option 4 below]

3. **Options available for the group**:

Option 1: Have the 2022 LAC Collaborative study summary data for AWP presented in CX/MAS 23/42/3 Add.1 and MAS43/CRD19 amended based on the statistical summaries presented to the group and ask CCMAS44 to reassess whether '102 °C oven method' should be found fit-for purpose as a Type IV.

Option 2: State the application for the '102 °C oven method' was declined by the group majority (but not a group consensus) as there was not clear evidence of method performance provided to the group from the 2022 LAC Collaborative study data for AWP to confirm it as 'fit-for-purpose'.

Option 3: Review of the individual participant data for AWP from the 2022 LAC Collaborative study showed disparities with the statistical summary data provided in CX/MAS 23/42/3 Add.1 and MAS43/CRD19, therefore all the data from the other milk powder matrices should be reviewed to identify any other anomalies and whether the result of this review may impact the considerations and decision made by CCMAS42 for inclusion of the '102 °C oven method' for the determination of moisture content for all types of powdered milk, powdered cream, and mixtures of powdered skimmed milk with vegetable fat.

Option 4: Suggest the '102 °C oven method' is modified with further controls to hopefully provide better precision; but which would necessitate a further collaborative study to validate but noting there is no guarantee that the outcome of validation will meet the criteria approach, if the 102 °C drying temperature is fundamentally 'unfit-for-purpose' for whey powders. These would include:

a. Include in the method that the analyst records the 'Environmental conditions during measurement' i.e. laboratory temperature, and humidity. Where the relative humidity should be maintained between 40 % and 60 % which is the recommended limits provided by most balance manufacturers and by OIML R 111. Further, for high precision measurement, the temperature should not vary by more than ± 1 oC in a 24 h period throughout the year and should vary by less than 0.5 °C during any one measurement series (typically less than 1 h). Otherwise, a comfortable working environment is considered 20 to 25 °C.

a. Indicate in the method, examples of drying oven model/make which can achieve 102±2 °C oven chamber spatial variation, as this is not easily achieved.

b. As the ISO 5537:IDF 26: 2004 uses a 5.0 g±0.3 g test sample, and the '102 °C oven method' uses a 1 g-1.5 g test sample, to achieve comparable sensitivity for the same weight loss by both methods, the '102 °C oven method' may need to utilise an analytical balance with 3.3-5x greater sensitivity. Available

analytical balance specifications can achieve for a range up to 30g, a readability of 0.01mg and repeatability of 0.03mg.

c. Consider if a better endpoint assessment is available instead of the '0.5mg constant weight' or alternatively use a lower 'constant weight threshold' e.g. 0.2 mg, as we doubt the endpoint correlates with an inflection point on the drying curve. This should make it more reproducible but hopefully not shift the endpoint significantly and would require an analytical balance with greater sensitivity than currently specified.

d. Stated in the method repeatability limit 'r' and reproducibility limit 'R' and sufficient replicates for within an analytical batch for the repeatability limit assessment. The Bulletin of IDF 2023 Collaborative study derives an amended repeatability and reproducibility specification for ISO 5537 | IDF 26: 2004 based on their collaborative study data. A similar assessment and specification could be derived for the '102 °C oven method' based on a 'second larger collaborative study with additional method parameter controls' data and give a guide to competence and ongoing quality control. Inclusion of a duplicate 'control' sample within each analytical batch would allow compliance assessment.

e. inclusion of reference materials within each analytical batch;

f. routine inclusion of certified reference materials (if they become available);

g. control charts assessment between analytical batches has the potential to assure repeatability and reproducibility are within limits.

Appendix III

Summary of the main comments raised at the March 2025 meeting

Australia

- the current data is inadequate and comparison with the ISO5537 | IDF 26 method is required for a more conclusive answer.
- controls on the method are needed particularly regarding humidity control and temperature control.
- endorsement of the method as Type IV raises issues of coexistence of Type I and Type IV methods and the potential for multiple Type IV methods that could create issues in the future.
- comparative data are needed to assess bias/accuracy and equivalence; it is noted that the previous work on equivalence had been abandoned and there had been no suggestions for how this might be progressed.

Brazil

- the South American countries are not requesting equivalence, the Type I method would still be used for disputes with the Type IV method used in more routine applications.
- the concern about the potential precedent of the coexisting Type I and Type IV methods – endorsement as Type IV is requested only as an exceptional case noting that this has already been done for one of the fats and oils methods.
- there are two opposing points of view, Brazil would like to see both opinions included in the final document that is forwarded to the Physical Working Group (PWG) to decide on the matter.
- Brazil also mentioned that they did not consider the review of endorsements of the same method for other provisions to be within the current scope.

EU

- agree in principle with Australia.
- data has been reviewed following the AOAC protocol which is equivalent to the IUPAC and ISO5725.
- analysing the data without removing outliers, results in precisions poorer than ISO5537 | IDF 26.
- removal of outliers improves precision but raises questions about the number of laboratories with the conclusion that the study is inconclusive.
- need to have more robust validation data; need to repeat the trial with at least 10 laboratories to ensure there are sufficient laboratories after outlier screening.
- comparison to ISO5537 | IDF 26 could be an advantage but, by definition, empirical methods are unbiased that makes it difficult to talk about the equivalence.
- CCMAS considered equivalence of Type I methods but the proposals by USA were not pursued, it is debatable whether there is any benefit in conducting such a study.
- it could open a can of worms if a Type IV method was allowed to sit beside a Type 1 method in the context of the Codex typing system.

Uruguay

- we have not heard any new positions; we don't want to repeat it since ours was submitted in the Uruguay & Brazil feedback document.
- emphasize that: there are differences in the experimental design.
- understand that both designs have similar uncertainties according to ISO 5725 so are fit for purpose.
- there are little differences in the statistical tests performed; both data processing methods comply with ISO 5725
- there are differences in the outlier exclusion criteria; we understand that the exclusion of outliers is based on statistical and physical deviations, especially when it comes to method validation studies.
- emphasize that there are no comparative studies of both methods in whey powder, since the study conducted in the past was on powdered milk.

IDF

- agree with Australia and the EU.
- the study is inconclusive for the reasons mentioned and a new study should be carried out under controlled conditions.
- the 102 °C method has issues when testing products containing lactose at levels greater than those found in whole milk powder and skim milk powder. Temperatures greater than 90 °C cause colour changes and the lactose starts to lose its water of crystallization.
- this explains why the precision is poorer for whey powders than for ordinary milk powders (skimmed milk powder and whole milk powder.
- therefore, IDF believes that the multi-laboratory trial should be repeated following ISO 5725 or an equivalent procedure and including critical controls on temperature and humidity.
- comparisons should be made with the ISO5537 | IDF 26 method which was developed to be equivalent to the 102 °C method for whole milk and skim milk powders but with better precision.
- there are questions whether the 102 °C method is equivalent for whey powder.
- IDF also reminded their request to have incorrect data in the 2022 LAC Collaborative study summary data for AWP presented in CX/MAS 23/42/3 Add.1 and MAS43/CRD19 amended based on the statistical summaries presented to the group.