CODEX ALIMENTARIUS COMMISSION

Food and Agriculture Organization of the United Nations

World Health Organization

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Agenda Item 4.1 **MAS44/CRD19**

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ORIGINAL LANGUAGE ONLY

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON METHODS OF ANALYSIS AND SAMPLING

44th Session

Virtual

5 – 8 May and 14 May 2025

INFORMATION ON ISO 1871 (DETERMINATION OF PROTEIN IN QUINOA)

(submitted by Chile and supported by Argentina, Colombia, Peru, Uruguay)

The seven (7) countries have provided the following information to support the CCMAS's reclassification of the ISO 1871 method for determining protein in quinoa from Type IV to Type I. In this regard, the method's validation is supported by metrological traceability to the SI (International System of Units) and has the required reproducibility and method robustness necessary to be classified as Type I.

The strength of this validation lies in the fact that the method was validated through an intercomparison between laboratories in quinoa-producing countries with the greatest experience with this food originating in the Andes mountain range in South America, and whose indigenous peoples continue to produce it. The method is being used by these countries, thus strengthening trade and ensuring the nutritional quality of quinoa, which supports the SDGs of ending poverty (1) by promoting peasant agriculture, Hunger (0) by strengthening access, and Health and Well-being (3) due to its nutritional properties.

This method, unlike other methods developed or validated by a single organization or testing laboratory, was validated by an intercomparison between seven national metrology institutes (NMIs) that are members of the International Bureau of Weights and Measures (BIPM).

- IBMETRO, Instituto de Metrología de Bolivia, Bolivia.
- INACAL, Instituto Nacional de Calidad, Perú.
- INEN, Servicio Ecuatoriano de Normalización, Ecuador.
- INM, Instituto Nacional de Metrología, Colombia.
- ISP, Instituto de Salud Pública de Chile, Chile.
- LATU, Laboratorio Tecnológico del Uruguay, Uruguay.
- INTI, Instituto Nacional de Tecnología Industrial, Argentina.

Therefore, it is expected that, in accordance with the technical evidence underlying the Codex and this committee, the method will be reclassified as Type I.

PROTEIN INTERCOMPARISON RESULTS FOR VALIDATION OF METHODS OF NUTRITIONAL CONSTITUENTS IN QUINOA

The laboratories of the National Metrology Institutes INM (or designated ID institutes) of the Inter-American Metrology System (SIM), which participated in the project:

The agreed criteria for the validation of the proximal parameter methods, in cases where they were not carried out, included at least the following points:

- Repeatability uncertainty component (or individual repeatability).
- Intermediate precision (or intermediate repeatability) uncertainty component.
- Bias uncertainty component for those parameters for which certified reference materials (Nitrogen for Protein).

The general established scheme, modifiable according to the useful space of the equipment, was as follows;

Week 1 day 1, 10 replicas of analyst 1.

Week 1 day 2, 10 responses from analyst 2.

Week 2 day 1, 10 replicas of analyst 1.

Week 2 day 2, 10 responses from analyst 2.

Each analyst performs, together with his or her series of samples, a duplicate humidity test for correction on a dry-mass basis according to ISO 712 Standard.

The respective INMs carried out the validations using commercial quinoa samples acquired in the respective local markets.

The factor conversion (Nx) It was assumed according to the Bolivian standard NB 312029:2006 of the Bolivian Institute of Standardization and Quality (IBNORCA), and according to Jones 1941 for foods in general.

Table I Criteria of validation

Attribute	**Protein**
	ISO 1871
Repeatability, *r,* g/100g	$r = (0.0063.\ wP) \times 2.8$; where wP is the protein content on a dry-mass basis
Intermediate precision, *ri*, g/100g	*2r*
Average value, g/100g, *U* *k*=2.	Nitrogen 1.562 + 0.014 (dry-mass basis) (CRM ERM BD 381-rye flour) Nitrogen 1.851 $\pm$ 0.017 (dry-mass basis) (ERM BD 382 wheat flour)
Bias	$\Delta \leq U_{\Delta}$ Δ is the absolute difference $U\Delta$ is the bias uncertainty
Factor conversion (Nx)	6.25

Table II

the specific chemicals used for the catalysts; • the different reagents and their concentrations that were used; and • what conditions for the method were used corresponding to the validation data provided.

Conditions/ Chemicals Reagent Used for:	**LAB 1**	**LAB 2**
Catalysts	3,5 g K_2SO_4 +0,62 g $CuSO_4x5H_2O$	2 unid (3,5 g K_2SO_4; 0,105 g Se; 0,105 g $CuSO_4x5H_2O$)
Digestion	Sulfuric acid 96 %	Sulfuric acid 96 %, 20mL
Control to Digestion	Glycine $\geq$ 99,5 % electrophoresis purity reagent	saccharose p.a. (0% N)

Time & temperature Digestion	410 °C x 2 h	Maximum temperature: 430 °C, heating ramp: 20 min up to 150 °C, 20 min hold, 20 min up to 300 °C, 20 min hold, 20 min up to 430 °C, 20 min hold.
Distillation	NaOH 32 %	NaOH 40%
Control to distillation	Ammonium sulfate 99,9999 % purity	Ammonium sulfate p.a. (0% N)
CRM	ERM BC381	ERM BC382
Volumetric titration	HCl 0,1 N Bromocresol Green + methyl red	HCL 0,1 N Bromocresol Green + methyl red
Conditions/ Chemicals Reagent Used for:	**LAB 3**	**LAB 4**
Catalysts	3,5 g K_2SO_4 +0,105 g $CuSO_4x5H_2O$ + 0,105 g TiO_2	5 g (47,7% Na_2SO_4; 47.7% g K_2SO_4;2.8% TiO_2; 1.8% $CuSO_4$)
Digestion	Sulfuric acid 96 %, 20 mL	Sulfuric acid 97 %, 20mL-25 mL
Control to Digestion	Glycine 100 % purity	Glycine 99 % purity
Time & temperature Digestion	Time: Increase 50 ºC every 30 min Until the 420 ºC. (Digest until clear, continue heating for another 30 min)	420 ºC x 2 h
Distillation	NaOH 33 %	NaOH 30%
Control to distillation	Ammonium sulfate p.a.	Paper free Nitrogen
CRM	ERM BC 382	ERM BC 382, ERM BC 381
Volumetric titration	HCl 0,1 N Bromocresol Green + methyl red	H_2SO_4 0,05 N Bromocresol Green + methyl red
Conditions/ Chemicals Reagent Used for:	**LAB 5**	**LAB 6**
Catalysts	15 g K_2SO_4 +0,5 g $CuSO_4$	7-10 g K_2SO_4 + 0,5 g $CuS0_4$
Digestion	Sulfuric acid 97,9 %	Sulfuric acid 95-97 %, 20-25 mL
Control to Digestion	Saccharose 100% Glycine 99,9 % purity	Saccharose 99.5%
Time & temperature Digestion	420 ºC x 2 h	420 °C ± 10 °C x 2 h ± 5 min
Distillation	NaOH 400 g/L	NaOH 40%
Control to distillation	Ammonium sulfate 99,8 %.	Ammonium dihydrogen phosphate (Nitrogen 12,130 ±0,047
CRM	ERM BC381	ERM BC381
Volumetric titration	HCl 0,1 M Potentiometric titration	HCl 0,1 N Thasiro Indicator: Methyl red-methylene blue.
Conditions/ Chemicals Reagent Used for:	**LAB 7**	
Catalysts	10,6 g (94,4 % K_2SO_4; 2,8% $CuSO_4$;2.8% TiO_2)	
Digestion	Sulfuric acid 98 % x 20 mL	
Control to Digestion	D(+) Saccharose USP-NF Glycine 99,9 % purity	
Time & temperature Digestion	420 °C x 2,5 h	
Distillation	NaOH 40%	
Control to distillation	Ammonium sulfate 99,0 %.	

CRM	ERM BC382
Volumetric titration	HCL 0,1M

Results of Validation Protein, ISO 1871

The results of the individual validation of the protein and humidity methods are presented below:

Attribute	Criteria	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7
RSDr, %	--	0.62	0,2	0.64	0.44	0.07	4.32	0.94
Repeatability, *r,* g/100g	*r* = (0.0063. wP) × 2.8	0.24	0,028	0.27	0.18	0.42	0.202	0.27
RSDri,%	--	0.65	0,4	0.85	0.38	0.08	3.68	0.98
Intermediate precision, *ri*, g/100g	*2r*	0.38	0,056	0.36	0.18	0,56	0.210	0.50
Reference value, N, CRM, g/100 g, *U*k=2.	--	1.562 ± 0.014	1.851 ± 0.017	1.851 ± 0.017	1.851 ± 0.017	1.562 ± 0.014	1.562 ± 0.014	1.851 ± 0.017
Value Media, N, g/100g	--	1.554	1,857	1,837	1,840	1.568	n.i	1,838
|Bias| (Δ)	--	0.008	0,006	0.014	0.011	0.006	n.i	0.016
Uncertainty Bias, U_{Δ} *k=2*	--	0.030	0,25	0,029	0,039	0.019	n.i	0,092
$\Delta \leq U_{\Delta}$	Yes	Yes	Yes	Yes	Yes	Yes	n.i	Yes

Intercomparison Laboratories for method validation

The validation was concluded with an intercomparison. The objective of this stage was to establish the degree of agreement between the measurements of the laboratories of the National Metrology Institutes INM (or designated ID institutes) and was carried out by all the laboratories in the respective parameters in which they contributed to the characterization value, according to the CIPM guideline MRA-D-05 Measurement comparisons Version 1.6. It was carried out over 8 months.

The NMI/ID laboratories carried out the tests on two samples according to the random assignment of use carried out at the conclusion of the batch preparation, to which they had to carry out at least two replicates per sample, reporting the value of each of the replicates obtained. And average of said values and their associated uncertainty.

The NMI/ID laboratories had to carry out a correct homogenization of the bottles prior to taking the aliquots and follow the previously validated methods.

In those parameters in which the number of results reported was greater than four, the value assigned by consensus through the median (KCRV) was calculated.

The standard uncertainty of the consensus value (*uME*) was calculated as:

$$uME = \frac{1{,}25 \times MADe}{\sqrt{n}}$$

$$MADe = MAD \times 1{,}483$$

$$MAD = median|xi - X| \; i = 1, \ldots, n$$

n: $number\ of\ \ NMI\ laboratory$

xi: $result\ report\ of\ NMI\ laboratory$

X: $median\ dof\ results\ report$

To calculate the degrees of equivalence, the difference between the reported value and the consensus value *(di)* and its associated uncertainty *U(di)* were determined according to the following equations.

$$di = xi - X$$

$$U(di) = 2 \text{ x } \sqrt{u(xi)^2 + u(ME)^2}$$

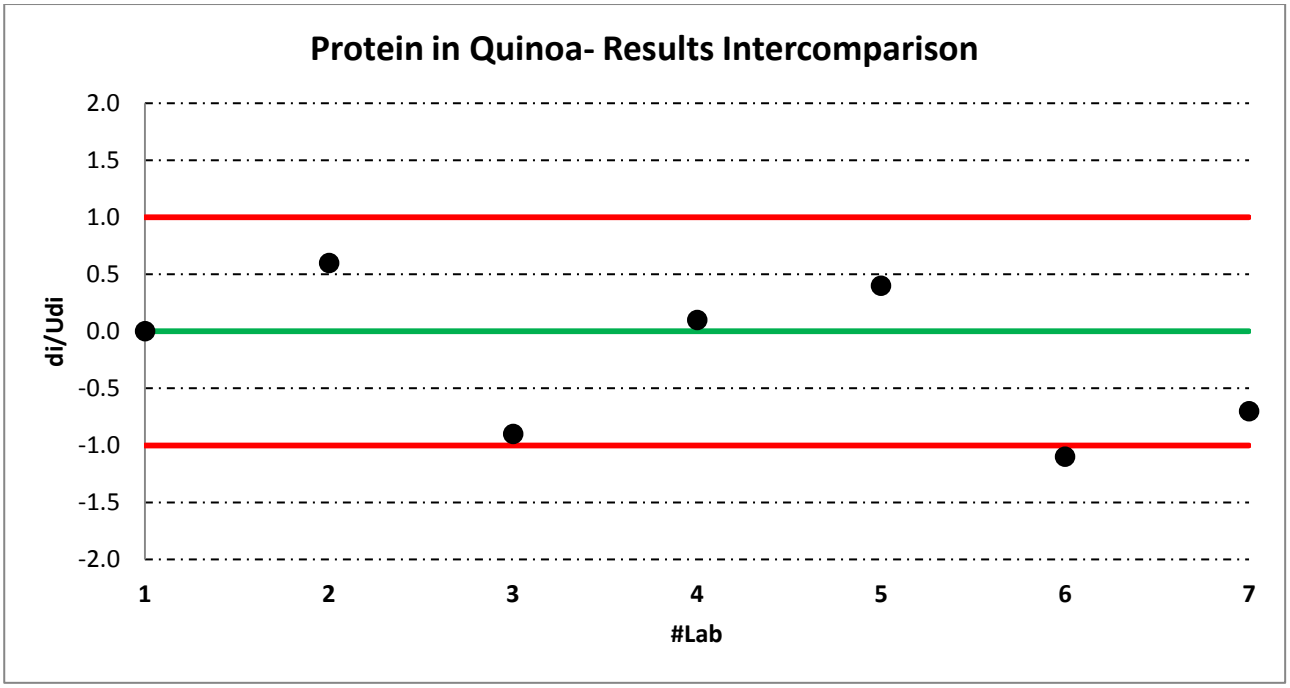
Throughout this stage, the necessary measurements were made to achieve degrees of equivalence (*DoE*) values within or close to the -1 range; 1, and the uncertainty calculations were revised.

In the parameters in which the number of data provided by the laboratories of the respective INMs was 4 or less, the normalized error was used, with the value to be achieved being E_n, ≤ 1.

In the event that a single NMI contributed value, successive measurements were compared using the normalized error.

Interlaboratory comparison results

Laboratory	Protein
	di/Udi
Lab 1	0.0
Lab 2	0.6
Lab 3	-0.9
Lab 4	0.1
Lab 5	0.4
Lab 6	-1.1
Lab 7	-0.7



Conclusion

The results of the validation carried out by the National Metrology Institutes, the method has adequate precision and bias for its intended purpose. The analytical procedure is not affected by small variations in reagents or method parameters, meaning the method has the required robustness. Based on its titrimetric principle it is a primary method and should be classified as a Type I method.