



# ISO 24167| IDF 261 Standard for *in vitro* protein digestibility and DIAAS



DRAFT  
International  
Standard



ISO/DIS 24167  
IDF 261

Milk and milk products — In  
vitro digestion protocol for  
the determination of protein  
digestibility and in vitro digestible  
indispensable amino acid score  
(DIAAS)

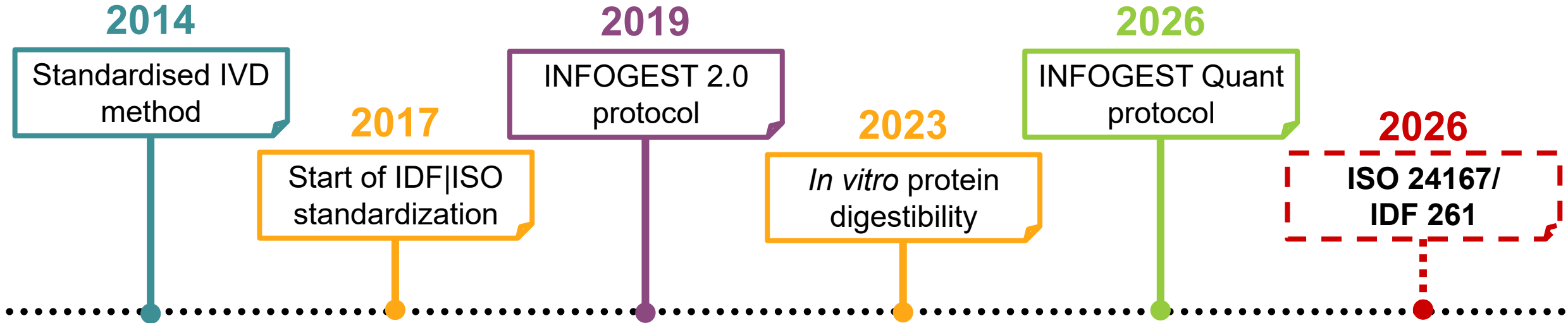
ICS: 07.100.30; 67.100.01

ISO/TC 34/SC 5  
Secretariat: NEN

**9<sup>th</sup> International Conference on FOOD DIGESTION, 19 - 21 May 2026 | Gdańsk Poland**

Lotti Egger, Ana Blanco, Reto Portmann, and collaborators of the INFOGEST network

# The journey of the ISO|IDF protein digestibility standard



Fo  
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P

nature protocols



Protocol extension

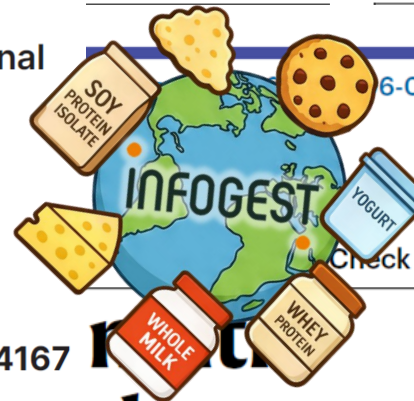
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## INFOGES<sup>®</sup> determin DIAAS of INFOGES<sup>®</sup>

Milk and milk products — In vitro digestion protocol for the determination of protein digestibility and in vitro digestible indispensable amino acid score (DIAAS)

ICS: 07.100.30; 67.100.01

DRAFT  
International  
Standard



6-025-01307-9

Check for updates

ISO/DIS 24167  
IDF 261

ISO/TC 34/SC 5  
Secretariat: NEN

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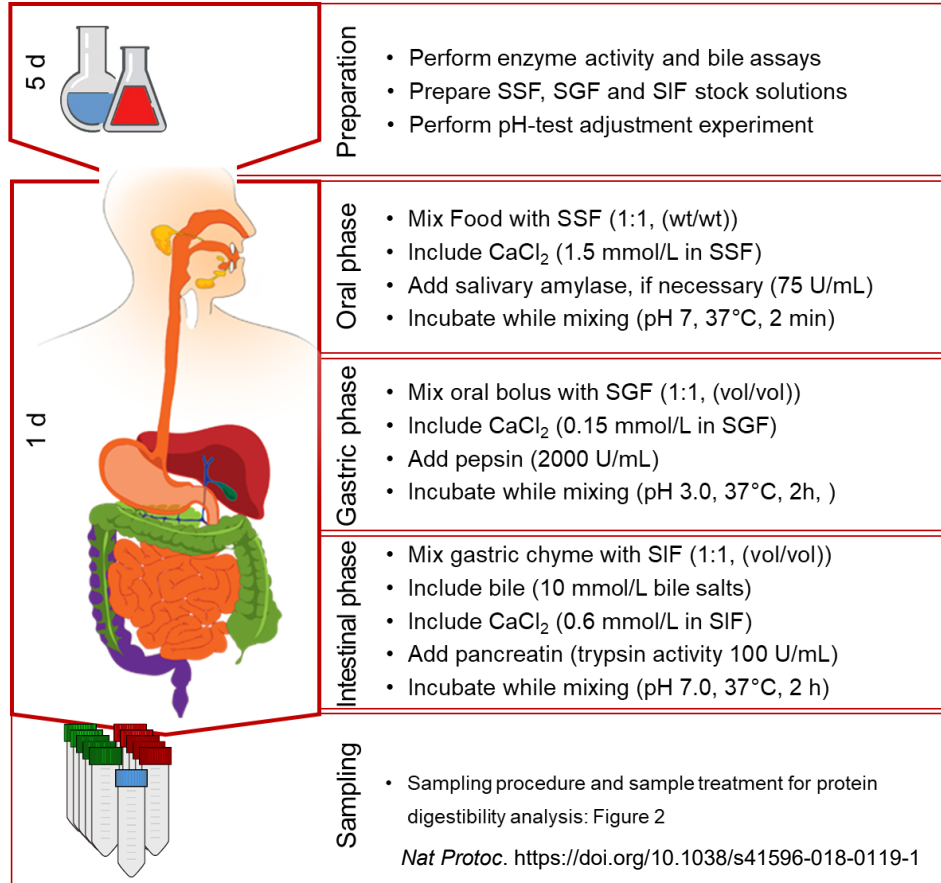
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u<sup>8</sup>, Fréi  
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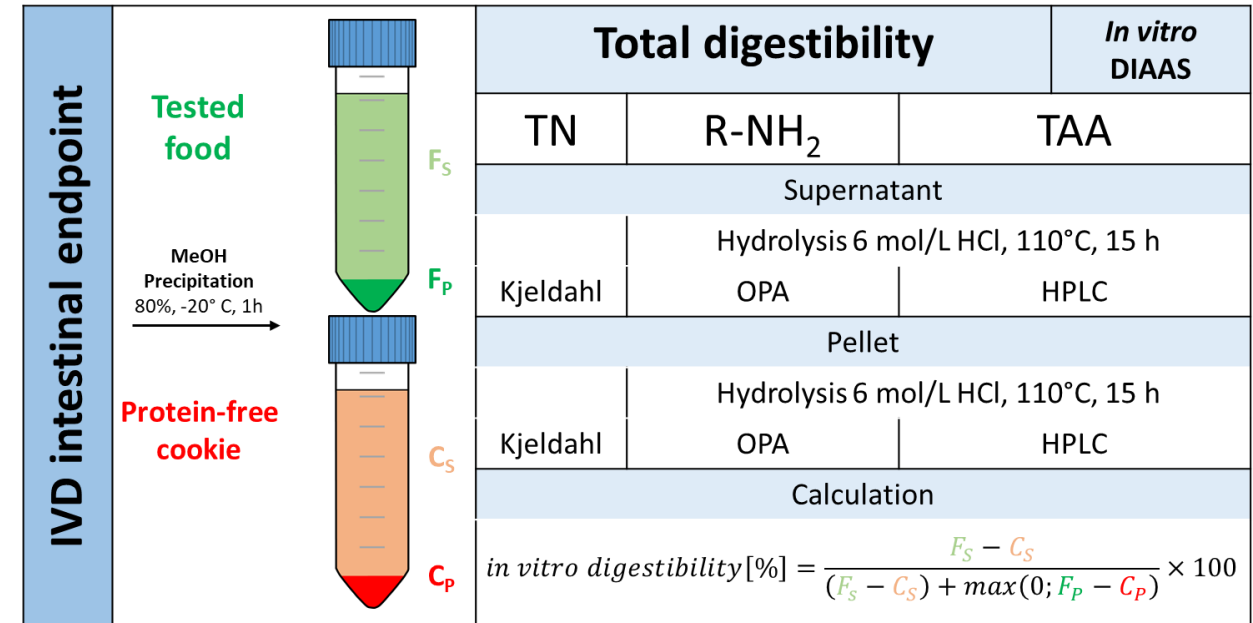


# *In vitro* protein digestibility and DIAAS workflow based on the static INFOGEST protocol

## INFOGEST static IVD



## Workflow for protein digestibility

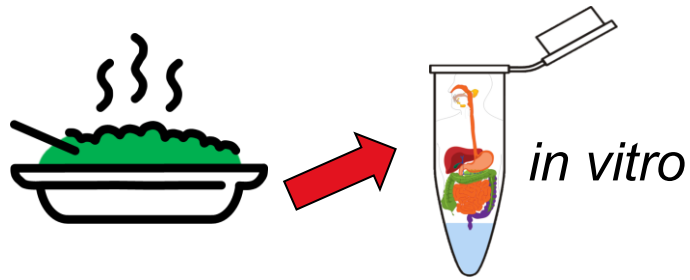


## Main adaptations:

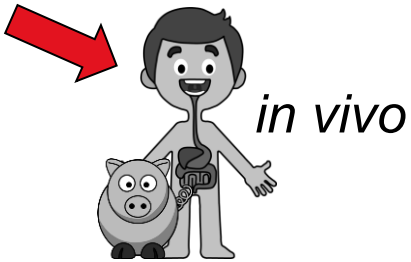
- Solubilization of pancreatin (supernatant after ultrasound and centrifugation)
- Normalization to the amount of protein input (40 mg/ 1 g of Food)

# *In vitro* digestibility and DIAAS workflow: validation

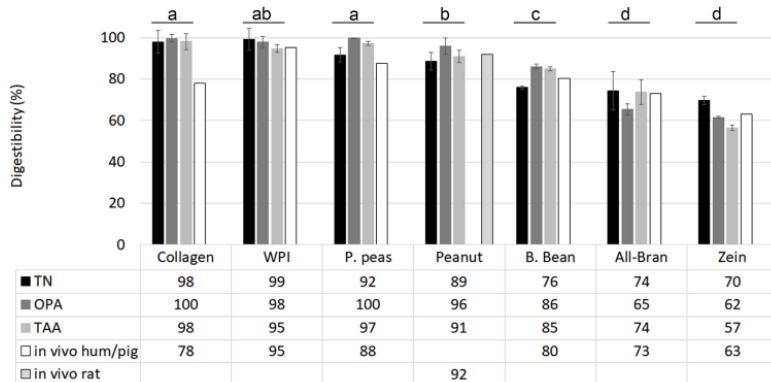
Validation *in vivo* / *in vitro*



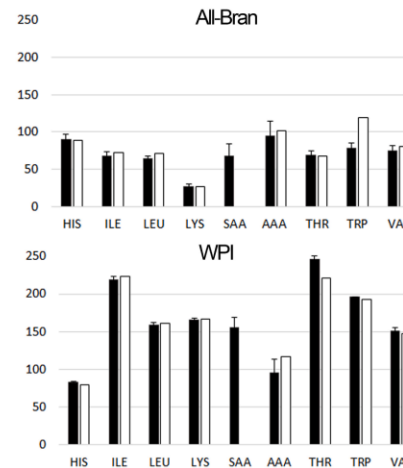
7 food sources  
and isolated  
proteins of  
different  
digestibility



digestibility



DIAAS



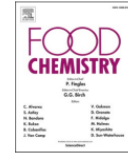
Food Chemistry 404 (2023) 134720



Contents lists available at ScienceDirect

Food Chemistry

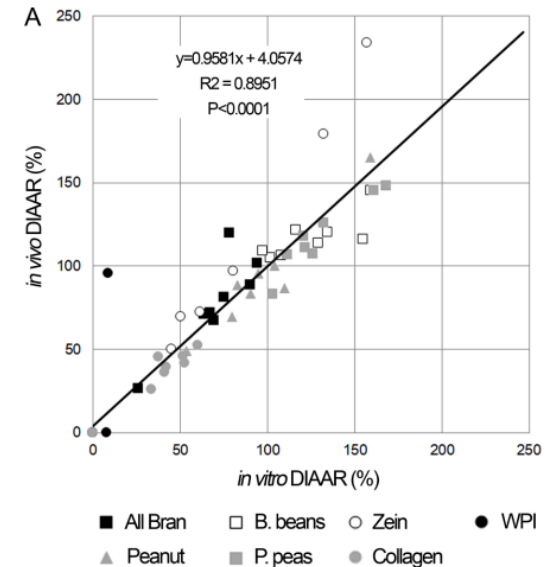
journal homepage: [www.elsevier.com/locate/foodchem](http://www.elsevier.com/locate/foodchem)



*In vitro* digestibility of dietary proteins and *in vitro* DIAAS analytical workflow based on the INFOGEST static protocol and its validation with *in vivo* data

Raquel Sousa<sup>a,b</sup>, Isidra Recio<sup>b</sup>, Dominique Heimo<sup>c</sup>, Sébastien Dubois<sup>c</sup>, Paul J. Moughan<sup>d</sup>, Suzanne M. Hodgkinson<sup>d</sup>, Reto Portmann<sup>a,1</sup>, Lotti Egger<sup>a,\*,1</sup>

Correlation: 0.96

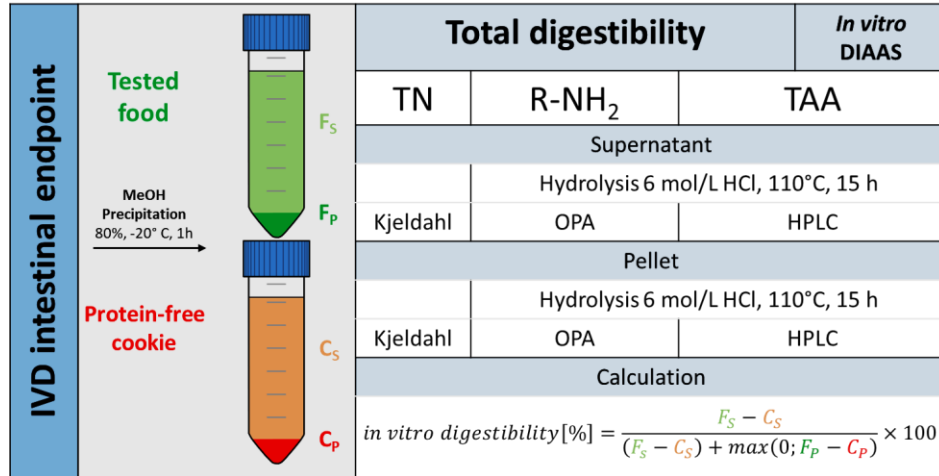


Main results:

- *In vitro* protein digestibility correlates well with *in vivo* results for the exact same substrates
- Total digestibility obtained with three analytical endpoints TN, OPA, and TAA are not different



# Ringtrial: ISO/WD 24167:2024 | IDF 261:2024



Ringtrial to obtain repeatability and reproducibility of the method

RT 1: 5 dairy products: 26 participants

skim milk powder, whole milk powder, whey protein isolate, yoghurt (freeze-dried), Cheese (freeze-dried)

RT 2: 5 dairy and 2 plant-based products: 23 participants

Additional: soy protein isolate, chick pea

→ Participants selected between the three analytical methods: TN, OPA, TAA

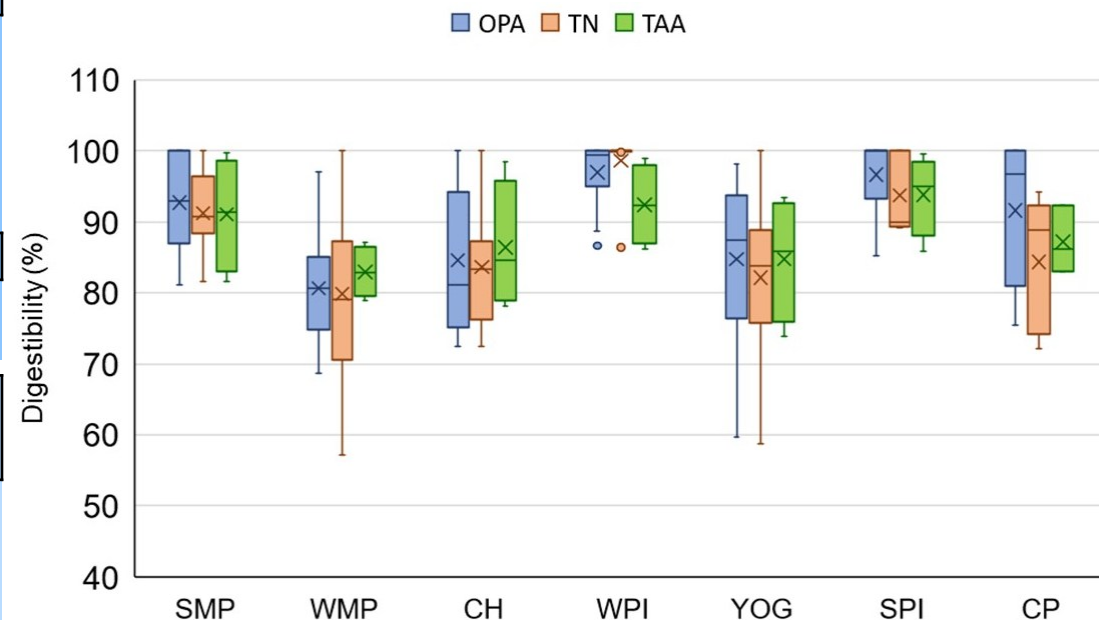


# Ringtrial results: Total digestibility

|                                 | SMP        | WMP         | Cheese      | WPI        | Yoghurt     | SPI        | Chick<br>Pea |
|---------------------------------|------------|-------------|-------------|------------|-------------|------------|--------------|
| <b>OPA</b>                      |            |             |             |            |             |            |              |
| N                               | 16         | 14          | 15          | 18         | 14          | 15         | 13           |
| <b>Digestibility (%)</b>        | 92.93      | 80.71       | 81.06       | 99.40      | 87.43       | 100        | 96.67        |
| SD (standard deviation of mean) | 6.35       | 10.01       | 7.16        | 3.21       | 8.82        | 2.91       | 6.06         |
| SEM (standard error of mean)    | 1.6        | 2.7         | 1.8         | 0.8        | 2.4         | 0.8        | 1.7          |
| <b>S<sub>R</sub></b>            | <b>7.1</b> | <b>10.9</b> | <b>12.5</b> | <b>4.1</b> | <b>11.7</b> | <b>4.2</b> | <b>10.8</b>  |
| r (repeatability)               | 13.4       | 25.4        | 23.0        | 11.7       | 26.5        | 8.0        | 22.3         |
| R (reproducibility)             | 20.2       | 30.8        | 35.5        | 11.7       | 33.7        | 11.7       | 30.5         |

|                                 | SMP        | WMP         | Cheese     | WPI        | Yoghurt     | SPI        | Chick<br>Pea |
|---------------------------------|------------|-------------|------------|------------|-------------|------------|--------------|
| <b>TN</b>                       |            |             |            |            |             |            |              |
| N                               | 14         | 15          | 15         | 15         | 15          | 6          | 6            |
| <b>Digestibility (%)</b>        | 90.7       | 78.1        | 83.3       | 100.0      | 82.3        | 90.0       | 88.8         |
| SD (standard deviation of mean) | 4.0        | 4.1         | 8.2        | 0.2        | 8.6         | 6.2        | 6.9          |
| SEM (standard error of mean)    | 1.3        | 1.3         | 2.5        | 0.1        | 2.7         | 2.8        | 3.1          |
| <b>S<sub>R</sub></b>            | <b>7.0</b> | <b>13.3</b> | <b>9.6</b> | <b>5.0</b> | <b>12.9</b> | <b>6.6</b> | <b>16.6</b>  |
| r (repeatability)               | 10.8       | 33.7        | 22.3       | 2.6        | 36.0        | 14.2       | 20.9         |
| R (reproducibility)             | 19.5       | 37.2        | 26.8       | 14.1       | 36.0        | 18.4       | 46.5         |

|                                 | SMP  | WMP   | Cheese | WPI  | Yoghurt | SPI | Chick<br>Pea |
|---------------------------------|------|-------|--------|------|---------|-----|--------------|
| <b>TAA</b>                      |      |       |        |      |         |     |              |
| N                               | 4    | 4     | 4      | 4    | 4       | 3   | 2            |
| <b>Digestibility (%)</b>        | 91.4 | 82.81 | 84.54  | 92.3 | 85.81   | 95  | 86.2         |
| SD (standard deviation of mean) | 2.2  | 4.3   | 3.6    | 2.4  | 4.5     | 2.7 | 4.2          |



- Different analytical methods: TN, OPA, and TAA give equal total digestibility results
- Not enough datasets for statistical evaluation of TAA and individual AA digestibility



# ISO 24167|IDF 261 repeatability and reproducibility

## 14.4 Repeatability

The absolute difference between three independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, will not exceed 10 % of the mean in more than 5 % of cases.

## 14.5 Reproducibility

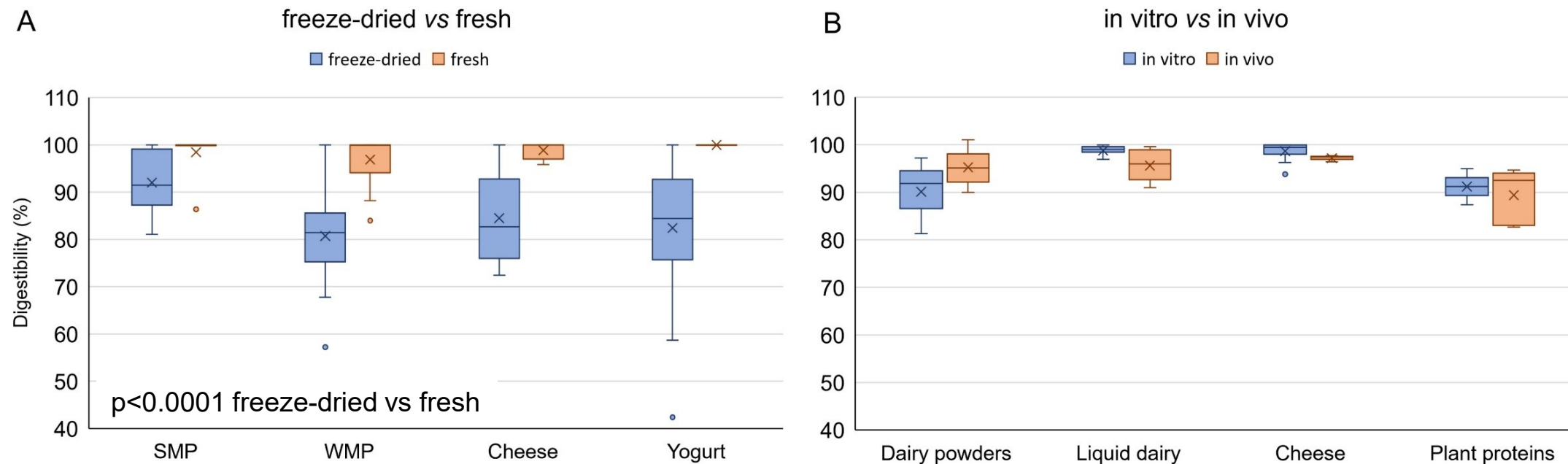
The absolute difference between three independent single test results, obtained using the same method on identical test material in different laboratories by different operators using different equipment, will not be more than 5 % of the cases greater than 15 % of the mean of the three determinations.

Acceptance criteria between replicates:

→ Intralab: 10 %

→ Interlab: 15 %

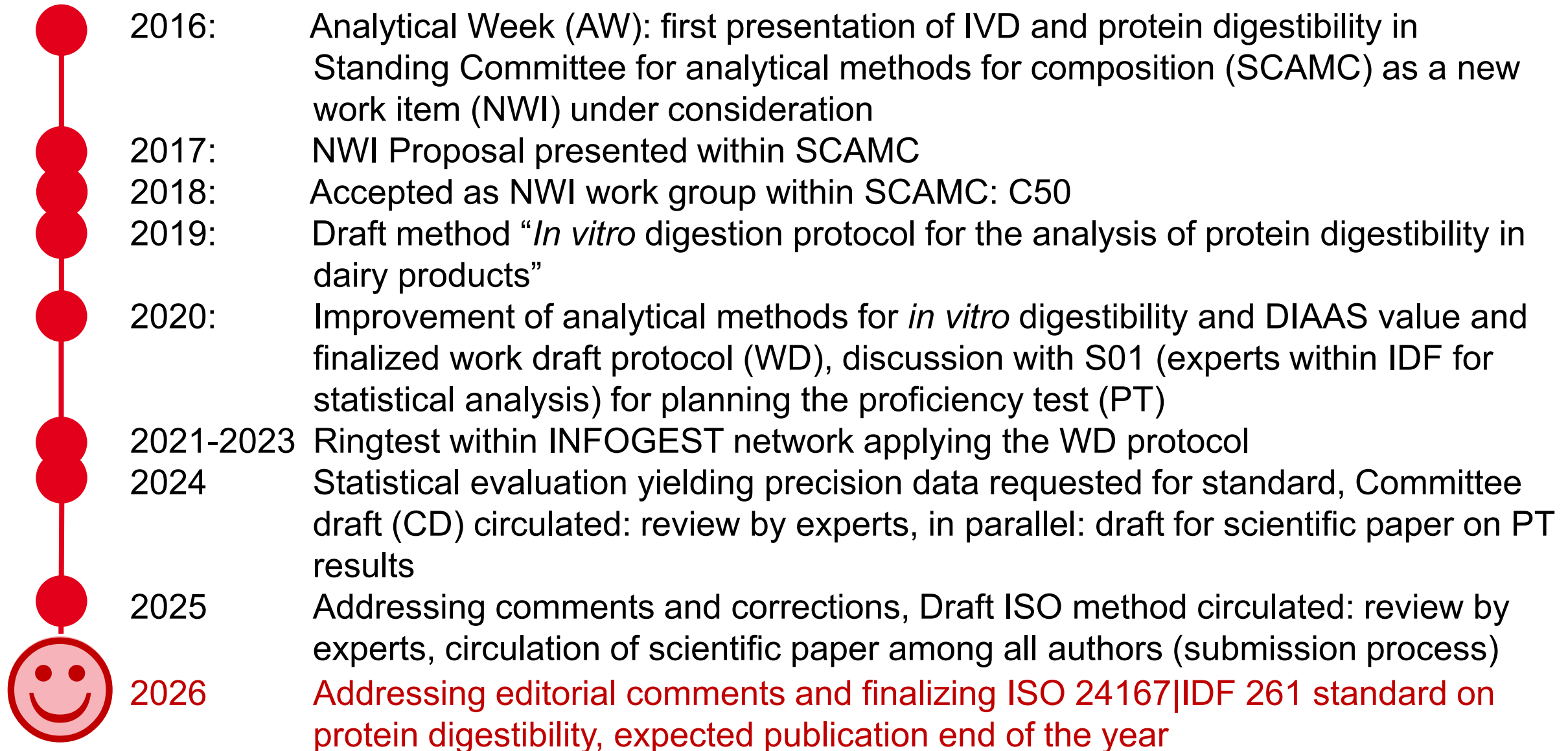
# Joint Publication of Ringtrial results



→ Digestibility of freeze-dried products < fresh products

→ *In vitro* results comparable to *in vivo* digestibility

# Standardization process within IDF and ISO: 10 years in...





## Summary

### **Drive the publication of the standard ISO 24167| IDF 261 forward**

We aim to reach the next step by the end of this year.

### **Joint scientific publication → paper submitted**

All results and findings are to be published in a peer-reviewed paper together with all participating RT collaborators.

### **TAA method described in the standard ISO 24167|IDF 261 but not fully validated for digesta as requested for DIAAS**

Generation of precision data in a collaborative study: ongoing



# All for One and One for All

University of Parma

INTA Universidad de Chile

Polskiej Akademii Nauk w. Olsztynie

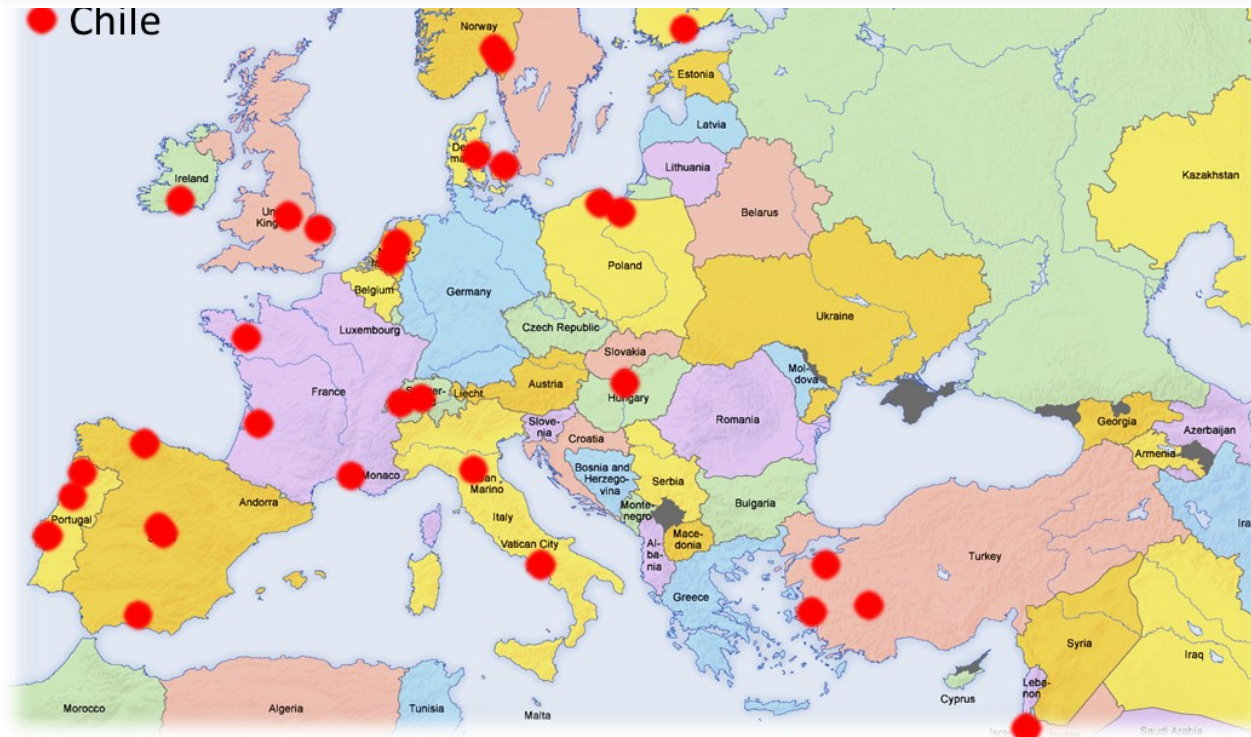
UMR STLO INRAE

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