# Inter-laboratory comparison of in vitro protein digestibility based on the static INFOGEST method

Lead authors: Lotti Egger, Raquel Sousa and Reto Portmann; corresponding author: charlotte.egger@agroscope.admin.ch Agroscope, 3003 Bern, Switzerland; www.agroscope.ch

## Context

As recommended by the FAO, the digestible indispensable amino acid score (DIAAS) is the best measure for assessing protein quality. Recently, a workflow for protein digestibility quantification and DIAAS calculation was developed based on the static INFOGEST in vitro digestion method. A first interlaboratory study showed promising results and the standardisation process within the International Dairy Federation (IDF) and ISO was initiated to establish an international ISO/IDF standard method. Currently, a second inter-laboratory study, involving more than 30 laboratories around the world is undergoing. This involves determining and statistically evaluating the protein digestibility and DIAAS values of five dairy products and two plant sources.

### **Inter-laboratory Study: Substrates**



Fig. 1 Inter-laboratory study for the analysis of in vitro digestibility based on the INFOGEST protocol, including five dairy-, two plant-based food matrices, and as well a protein-free blank (cookie)

## **Experimental Procedure**

good food, healthy environment

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/D intestinal endpoint	Tested food	—	F <sub>s</sub>	Тс	In vitro DIAAS			
				ΤN	R-NH <sub>2</sub>	ר	ĀA	
				Supernatant				
	MeOH Precipitation 80%, -20° C, 1h				Hydrolysis 6 mol/L HCl, 110°C, 15 h			
				Kjeldahl	OPA	HPLC		
				Pellet				
	Protein-free				Hydrolysis 6 mol/L HCl, 110°C, 15 h			
	cookie		C <sub>s</sub>	Kjeldahl	OPA	ŀ	IPLC	
				Calculation				
2			C <sub>P</sub>	$in \ vitro \ digestibility[\%] = \frac{F_S - C_S}{(F_S - C_S) + max(0; F_P - C_P)} \times 100$				

Fig. 2 The seven substrates plus the protein-free enzyme blank (cookie) were in vitro digested according to the draft ISO/IDF method which is based on the INFOGEST static protocol<sup>[1]</sup> and the digestibility workflow published by Sousa et al.<sup>[2]</sup>

<sup>[1]</sup>Brodkorb et. al. Nat Protoc. https://doi.org/10.1038/s41596-018-0119-1 <sup>[2]</sup>Sousa et. al. Food Chem. 134720.

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Co-authors: Abbühl Lychou, Brügger Cédric, Isidra Recio, Marta Santos Hernández, Cristina Gómez-Marín, Adam Macierzanka, Patrycia Szumała, Addepalli Rama, Ana Isabel Mulet-Cabero, Natalia Perez-Moral, Senem Suna, André Brodkorb, Shannon Gwala, Conor Fitzpatrick, Anne Rieder, Leehen Mashiah, Catarina Gonçalves, Isabel Rodriguez Amado, Clarisse Nobre, Ana C Pinheiro, Elena Arranz, Francesca Bot, James A. O'Mahony, Myriam Grundy, Igor Pacheco Cruz, Ailynne Sepúlveda-González, Elva Gonzales, Irene Comi, László Abrankó, Judit Tormási, Felipe González, Patriciaas-Madiedo, Lorena Ruiz, Miguel Ângelo Faria, M. Madalena C. Sobral, Luis Miguel Jiménez-Muno, Milena Corredig, Muleya Molly, Natalia Rosa-Sibakov, Kerensa Broersen, Soenita Goerdayal, Olivia Menard, Vibeke Orlien, Ourani Gouseti, Iben LykkePetersen, Pasquale Ferranti, Raquel Olias, Cristina Delgado-Andrade, Ricardo Assunção, Ruben Lopez Nicolas, Sebnem Simsek, Sedef Nehir El, Sibel Karakaya, Serge Rezzi, Stéphane Canarelli, Delphine Cuenod, Juriaan Mes, Anouk Boudewijn, Anna Ogrodowczyk, Sébastien Dubois



Fig. 3 Comparison of total digestibilities between the first and the second ringtria (RT) (primary amines, RH<sub>2</sub> (A) and between the three analytical methods: primary amines (RH<sub>2</sub>, with OPA method), total nitrogen (TN), and total amino acids (TAA).

			SMP	WMP	Gru	WPI	Yog	SPI	ChickPea
2nd RT		Average		80.6	85.2	94.0	83.1	95.9	86.1
	N		11	11	11	12	11	12	10
	SD	6.9	6.0	7.1	6.4	5.4	9.2	4.8	9.7
	SEM	2.1	1.8	2.1	1.9	1.5	2.8	1.4	3.1
	s <sub>r</sub>	8.6	9.5	11.7	10.3	6.2	7.7	4.7	10.1
	S <sub>rRel</sub>	9.9	10.4	14.5	12.1	6.6	9.3	4.9	11.7
	ľ (sr*2.8)	24.1	26.5	32.7	28.9	17.3	21.6	13.1	28.3
			SMP	WMP	Gru	WPI	Yog		
1rst RT		Average	91.5	85.9	82.8	95.3	85.0		
	N		12	12	12	12	12		
	SD	13.4	9.4	13.1	16.0	10.1	18.3		
	SEM	3.9	2.7	3.8	4.6	2.9	5.3		
	s <sub>r</sub>	10.9	9.0	11.7	16.4	4.1	13.3		
	S <sub>rRel</sub>	12.7	9.8	13.6	19.8	4.4	15.7		
	ľ (sr*2.8)	30.9	25.4	33.0	46.4	11.7	37.7		

Table 1 Preliminary data from so far 14 received data sets, for the two RTs performed with over 30 participating laboratories, within the INFOGEST network.

#### **Next Steps**

2022-23: Inter-laboratory study, second round following the detailed protocol with trained participants

2023: ISO/IDF- Analytical Week (AW) October: Draft ISO protocol (DIS) and presentation of PT results and precision data.

2023: Vote and approval DIS (comments from countries)

2024: Final DIS (minor comments), formal approval of FDIS

2025: Publication of ISO/IDF standard

Revision can be undertaken at any time thereafter  $\rightarrow$  e.g. improvements in protocol, inclusion of other matrices

A systematic review takes place every 5 years

#### Summary

- The recently published protocol for the assessment of protein digestibility in foods, based on the INFOGEST static protocol, is undergoing standardization within ISO/IDF.
- Preliminary data obtained in two inter-laboratry studies within the INFOGEST network show a high comparability of digestibility results.
- Improved precision in the 2<sup>nd</sup> inter-laboratory study was achieved thanks to a more precise protocol and a training school for the participants.

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