

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

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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 inter-laboratory 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 as a protein-free blank (cookie)



Preliminary Results

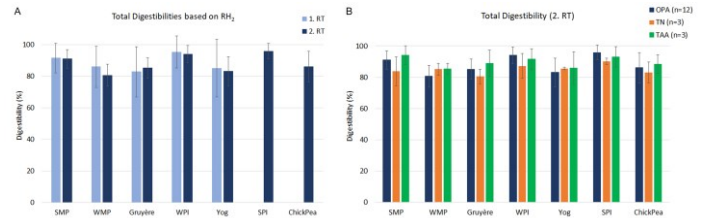


Fig. 3 Comparison of total digestibilities between the first and the second ringling (RT) (primary amines, RH_2) (A) and between the three analytical methods: primary amines (RH_2 , with OPA method), total nitrogen (TN), and total amino acids (TAA).

			SMP	WMP	Gru	WPI	Yog	SPI	ChickPea
2nd RT		Average	91.0	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
	SEM		2.1	1.8	2.1	1.9	1.5	2.8	1.4
	S_r		8.6	9.5	11.7	10.3	6.2	7.7	4.7
	S_{rRel}		9.9	10.4	14.5	12.1	6.6	9.3	4.9
	r ($sr^*2.8$)		24.1	26.5	32.7	28.9	17.3	21.6	13.1
1st 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_r		10.9	9.0	11.7	16.4	4.1	13.3	
	S_{rRel}		12.7	9.8	13.6	19.8	4.4	15.7	
	r ($sr^*2.8$)		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 → e.g. improvements in protocol, inclusion of other matrices
- A systematic review takes place every 5 years

Experimental Procedure

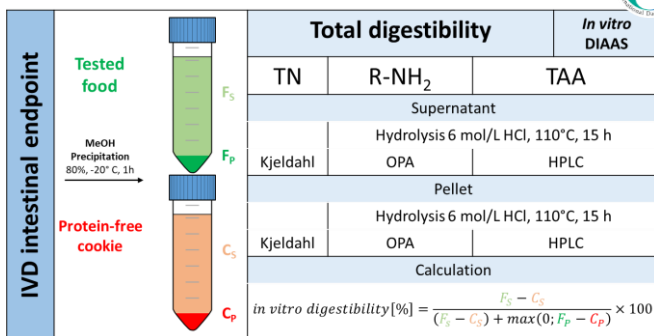
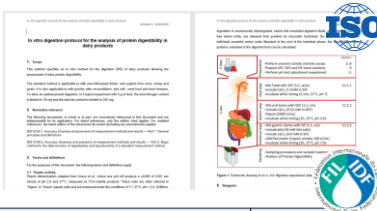


Fig. 2 The seven substrates plus the protein-free enzyme blank (cookie) were *in vitro* digested according to the draft ISO/IDF method which is based on the INFOGEST static protocol^[1] and the digestibility workflow published by Sousa et al.^[2]

^[1] Brodtkorb et. al. *Nat Protoc.* <https://doi.org/10.1038/s41596-018-0119-1>
^[2] Sousa et. al. *Food Chem.* 134720.
<https://doi.org/https://doi.org/10.1016/j.foodchem.2022.134720>

Summary

- The recently published HPLC 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-laboratory studies within the INFOGEST network show a high comparability of digestibility results.
- Improved precision in the 2nd inter-laboratory study was achieved thanks to a more precise protocol and a training school for the participants.