In vitro Digestion of Food: Validation of the INFOGEST consensus protocol

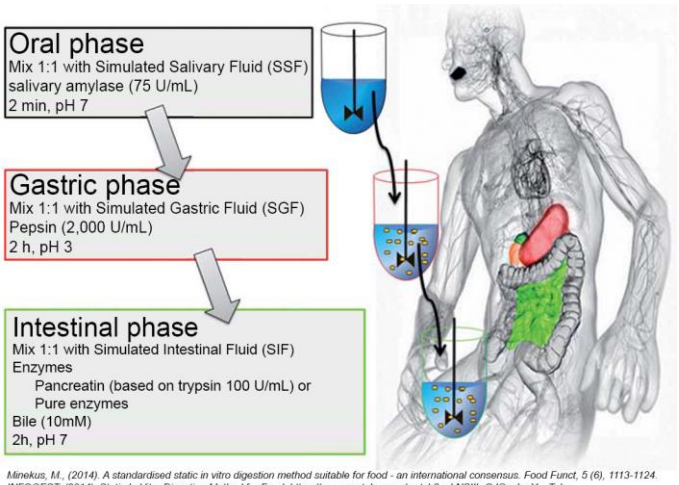
Lotti Egger, Helena Stoffers, Guy Vergères and Reto Portmann
Agroscope Institute for Food Sciences IFS, CH-3003 Bern; www.agroscope.ch

Abstract

The COST action INFOGEST (FA1005; http://www.cost-INFOGEST.eu/) has recently published a consensus *in-vitro* digestion (IVD) protocol, mimicking the human gastric system. One major goal of the work was to improve comparability of experimental IVD data between different laboratories. The conditions of the INFOGEST static IVD protocol, including an oral-, gastric-, and intestinal step, respectively, with the corresponding simulated fluids and fixed pH, were chosen based on published *in-vivo* data. For the validation of the protocol, a standardized skim milk powder (SMP) was *in-vitro* digested in different laboratories of the INFOGEST community. The digested samples were collected at Agroscope and analyzed for protein degradation by gel electrophoresis, appearance of peptides by mass spectrometry and total free amino acids by HPLC.

The analytical results showed that the consensus protocol indeed improved the harmonization of IVD and identified pepsin activity as the key factor for differences in protein degradation during the gastric phase between the different laboratories.

Consensus *in vitro* digestion method for food



Minekus, M., (2014). A standardised static in vitro digestion method suitable for food - an international consensus. Food Funct, 5 (6), 1113-1124. INFOGEST. (2014). Static In Vitro Digestion Method for Food, https://www.youtube.com/watch?v=LNSIlb-OJGc. In YouTube.

Figure 1. Workflow of the consensus IVD protocol published by the Cost Action INFOGEST

digestion phases was recently published within this network (Figure 1).

Experimental validation of the INFOGEST consensus protocol

Degradation of proteins was followed with gel electrophoresis (Figure 2). All samples were digested with the same enzyme batch, samples 1-5 show the expected pattern of SMP digestion after the gastric phase,-samples 6 and 7, however, still have intact caseins at this stage of digestion, pointing towards an impaired pepsin activity. Interestingly, after the intestinal phase, the differences observed for samples 6 and 7 had completely vanished.

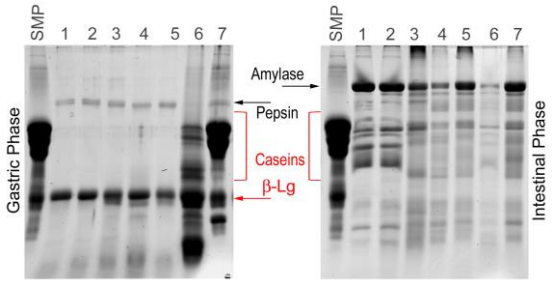


Figure 2. Protein degradation of SMP after the gastric and intestinal digestion visualized by SDS-Page

Conclusions

- The Harmonized system reduces interlaboratory variability
- Pepsin activity is the crucial factor leading to differences in protein degradation during the gastric phase.

Peptide Patterns

Protein degradation was analyzed by mass spectrometry. The peptide identifications of the individual caseins as well as β-lactoglobulin were color coded (Figure 3) making the decomposition of the proteins as well as the differences between the laboratories visible. Unlike β-lactoglobulin, the caseins were found to be mostly degraded after gastric digestion. After full digestion, only peptides from a few regions were identified. Peptides that were more resistant to digestion are shown in yellow to red color. The peptide patterns for samples 6 and 7 were clearly different, confirming the observation made with SDS-Page.

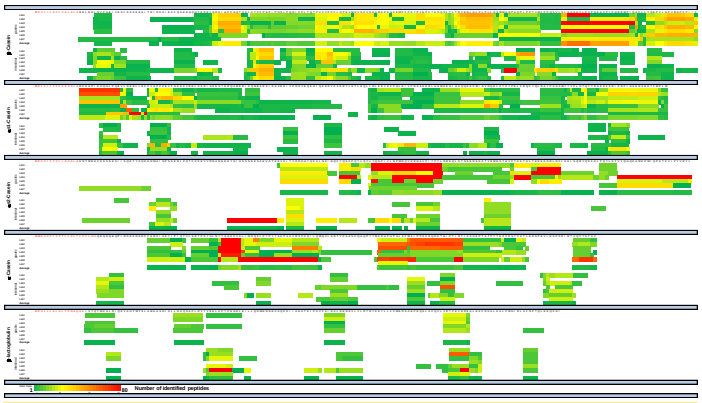


Figure 3. Peptide patterns of β-casein, αs1-casein, αs2-casein, κ-casein, and β-lactoglobulin after gastric and intestinal digestion.

Appearance of Free Amino Acids

During gastric digestion, the release of individual amino acids was marginal as compared to undigested SMP and no differences between samples could be observed. After full digestion, the proteins were almost completely degraded to the level of free amino acids (Figure 3, 4).

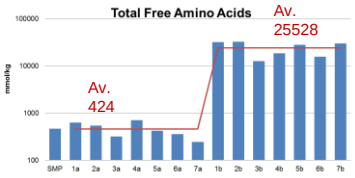
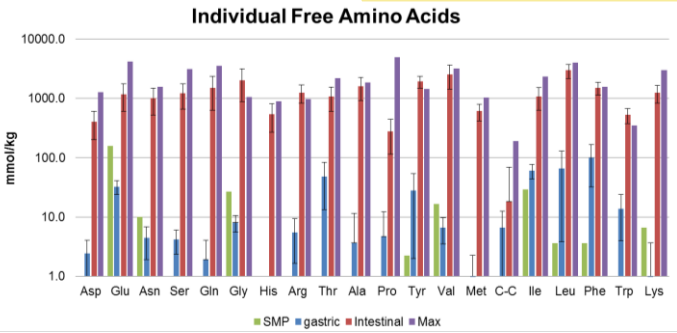


Figure 4. Presence of total free amino acids in SMP, after gastric digestion (a) and after full digestion (b) for samples 1-7.

Figure 5. Average amount of individual free amino acids (FAA) of undigested SMP, SMP after gastric phase, SMP after intestinal phase, and maximal content of FAA.



Comparability of IVD results between different labs

The degree of harmonization was visualized by a Spearman correlation matrix based on the peptidomics results obtained for β-casein (Figure 6). The previous observations that samples 6 and 7 were different, mainly during the gastric phase, could be confirmed. Samples from laboratories 1-5 showed a high degree of similarity for both digestion phases.

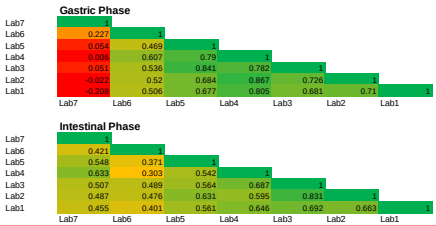


Figure 6. Spearman correlation matrix for peptide numbers of b-casein released during digestion (based on Figure 3). Red color indicates low and green color indicates high correlation.

Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra

Swiss Confederation

Federal Department of Economic Affairs, Education and Research EAER
Agroscope

Agroscope good food, healthy environment