In vitro digestibility testing – INFOGEST

Improving health properties of food by sharing our knowledge on the digestive process

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Characterization of raw materials and processed food matrices for optimized nutrient bioaccessibility
WG1

In vitro, in vivo and in silico models of mammalian gastrointestinal digestion
WG2

Evaluation of the health effects
WG3

BFC identification
Stability during processing
Food multi-scale characterization

Digestion models harmonization
Comparison in vitro / in vivo
Digestion products identification
BFC absorption /bioavailability

Immunomodulatory properties
Regulation of appetite and satiety
Effect of BFC on human microbiota

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UK

F Capozzi
Italy

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I. Recio
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Food Structure and Nutrient Release

Cognitive and sensory enhancement of satiety and enzyme secretion

Oral processing: aroma, taste and texture sensing starch hydrolysis and chewing

gastric processing: proteolysis, lipolysis, acidification and storage -> fullness

gastric processing: shearing, grinding

intestinal processing: proteolysis, lipolysis, amylolysis, mixing and absorption

intestinal processing: nutrient sensing, hormone secretion

hormone secretion: controlling GI motility, enzyme and bile secretion, appetite
The objective for Infogest

To produce a protocol to simulate human digestion that was:

– “Simple” and could be used in any laboratory
– Based on human physiology

Giving results that are:

– Reproducible
– Consistent with human data on the same samples
The PROCEDURE

**Oral Phase**
- pH=7.0, 2min

Is meal solid or liquid?
- Liquid
  - Mix 1:1 with SSF/SGF
  - Skip to gastric phase
- Solid
  - Mince meal then mix 1:1 with SSF + Salivary amylase (150 U/mL)

**Gastric Phase**
- Mix 1:1 with SGF + pepsin (2000 U/mL), pH 3.0, 2h
- OPTION: Add 0.17 mM phosphatidylcholine (PC) vesicles

**Intestinal Phase**
- Mix 1:1 with SIF, pH=7.0, 2h
- Individual enzymes
  - Trypsin (100 U/mL)
  - Chymotrypsin (25 U/mL)
  - Pancreatic lipase (2000 U/mL)
  - Collase 2:1 with lipase
  - Pancreatic amylase (200 U/mI)
  - Bile (10mM)
- Extract or individual enzymes?
- Extract
  - Pancreatin (based on trypsin at 100 U/mL)
  - Bile (10mM)

**Sample collection and handling options**
- Add protease inhibitor (1 mM AEBSF, Roche)
- Snap-freeze at -80°C immediately
- Freeze dry
Dissemination


Youtube: [https://www.youtube.com/channel/UCdc-NPx9kTDGyH_kZCgpQWg](https://www.youtube.com/channel/UCdc-NPx9kTDGyH_kZCgpQWg)

Dropbox folder: [https://www.dropbox.com/sh/kjjv365egc1be11/AAC5tJUYFWxnnJKyMokvzTYwa?dl=0](https://www.dropbox.com/sh/kjjv365egc1be11/AAC5tJUYFWxnnJKyMokvzTYwa?dl=0)
Harmonisation

Digestion of skimmed milk powder (SMP)
Harmonisation

Release of free amino acids from SMP after gastric and intestinal phases of *in vitro* digestion.

HPLC analysis of samples from inter-laboratory trials applying the harmonized protocol.
Pros and cons

Pros:
• Simple to use
• Has been used in different labs giving the same results
• The end points seem the same as *in vivo* (SMP in pigs)

Cons:
• Cannot be used for kinetics
• Only mimics adult conditions
• No gastric lipase included
Updates

• Semi-dynamic
  – Dilution in the oral phase to be based on dry weight
  – Inclusion of gastric emptying (based on caloric density), gradual secretion of simulated gastric fluid including acid and enzymes, inclusion of “gastric lipase”

• Infant conditions
  – tba

• Elderly conditions
  – tba
Semi-dynamic

A 500mL meal is assumed for calculating the emptying rate.
- Volumes are then scaled based on a smaller experimental sample (in this case 20g of food).
- The caloric density (0.72) gives 360 calories to empty @ 2 kcal/min = 180 mins
- Gastric secretion occurs over the same time.
Semi-dynamic

• Assuming 20g of food with a dry weight of 8g, the oral phase volume = 20+8 = 28g
• The final volume of gastric secretion = 28g, 10% is put in at the start
• Gastric lipase(rabbit) is included at 50 U/mL
• Intestinal digestion is in parallel. In this case 7g is emptied and diluted with 7g of simulated intestinal fluid
Pros and cons

Pros:
• More physiological simulation of the gastric phase
• Can be used to assess kinetics
• Still based on small volumes and simple apparatus
• Can be used in many labs

Cons:
• More complicated procedure
• The emptying may be difficult with some foods (solids)
• Sourcing a suitable gastric lipase