A standardised static *in vitro* digestion method suitable for food An international consensus

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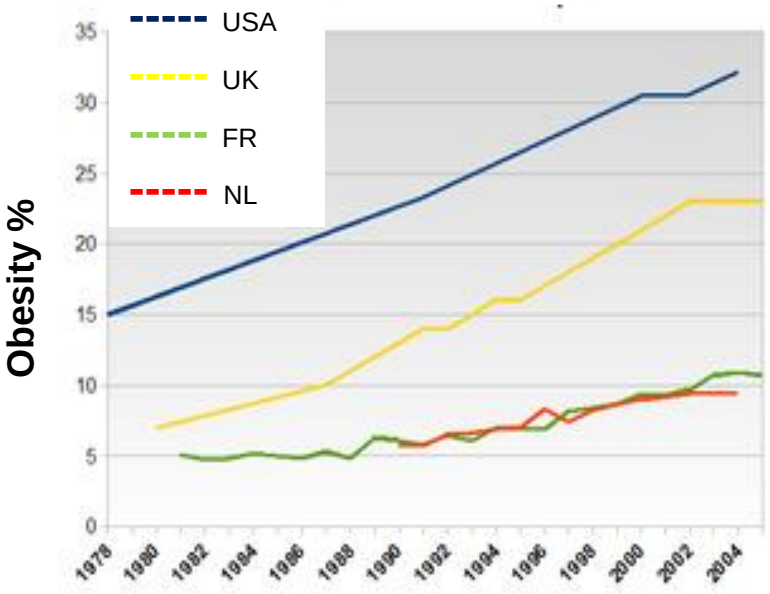
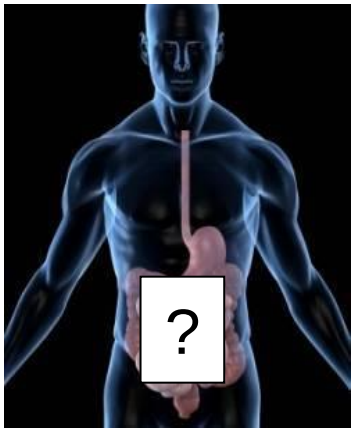
Scientific Context



Diet-related diseases ↑

↳ Prevent these pathologies rather than cure them



Gut = interface between food and human body

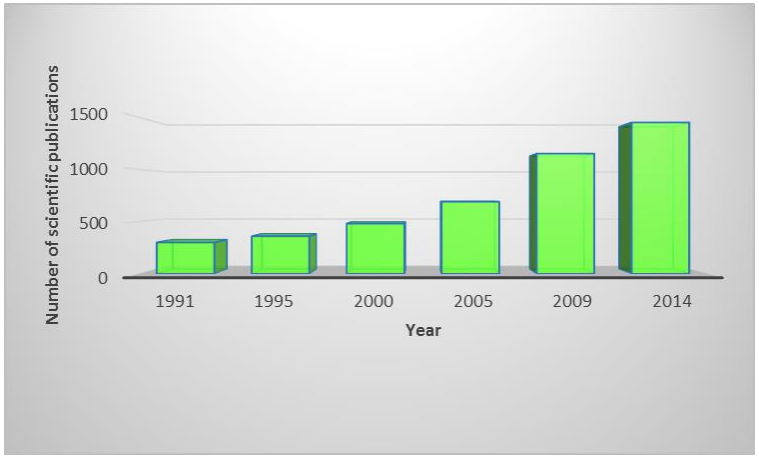
Digestion releases food components that can have a beneficial or a deleterious effect on human health

… but the mechanisms of food disintegration in the gastrointestinal tract remain unclear and the digestive process has been considered as a black box so far

By increasing our knowledge on food digestion, we will increase our knowledge on the effect of food on human health

However…

☞ During the last 10 y, ↑ in the number of publication on food digestion, associating food scientists, nutritionists and gut physiologists: a multidisciplinary new scientific community has been created



☞ This community is scattered: many ongoing projects at the national level but no current research action on this topic in Europe and no network for exchanges

☞ There is no scientific international congress on food digestion where scientists could have exchanges

☞ There were no scientific journal dedicated to food digestion before the creation of « Food Digestion » and «Food & Function » (2010)

☞ There is a dramatic lack of harmonization between the *in vitro* digestion models used throughout Europe and a real need of validation of these models

This is the perfect time for developing a trans-European network to improve dissemination of critical findings, develop truly multidisciplinary collaborations and harmonise approaches between groups and…

COST IS THE BEST MECHANISM FOR THAT

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

COST Action FA1005

Dr. Didier DUPONT, Senior Scientist, INRA, France

INFOGEST

June 2011 – May 2015

Objectives

- Compare the existing digestion models, harmonize the methodologies and propose guidelines for performing experiments
- Validate *in vitro* models towards *in vivo* data (animal and/or human)
- Identify the beneficial/deleterious components that are released in the gut during food digestion
- Determine the effect of the matrix structure on the bioavailability of food nutrients and bioactive molecules

But these goals can only be reached by…

- Gathering scientists from different disciplines (food science, nutrition, gastroenterology, immunology…) to share and improve our knowledge on food digestion

Chair
Didier Dupont - France

Administrative Manager

Nathalie Le Marre

II T

Vice-chair
Alan Mackie - UK

Dairy, Egg
Fruits & Vegetables
Meat, Cereals

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

BFC identification
Stability during processing
Food multi-scale characterization

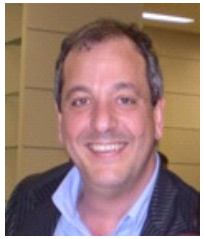
***In vitro*, *in vivo* and *in silico* models of mammalian gastrointestinal digestion**
WG2

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

Evaluation of the health effects
WG3

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

B. Murray
UK



F Capozzi
Italy

A. Brodkorb
Ireland

I. Recio
Spain

A. Bordoni
Italy

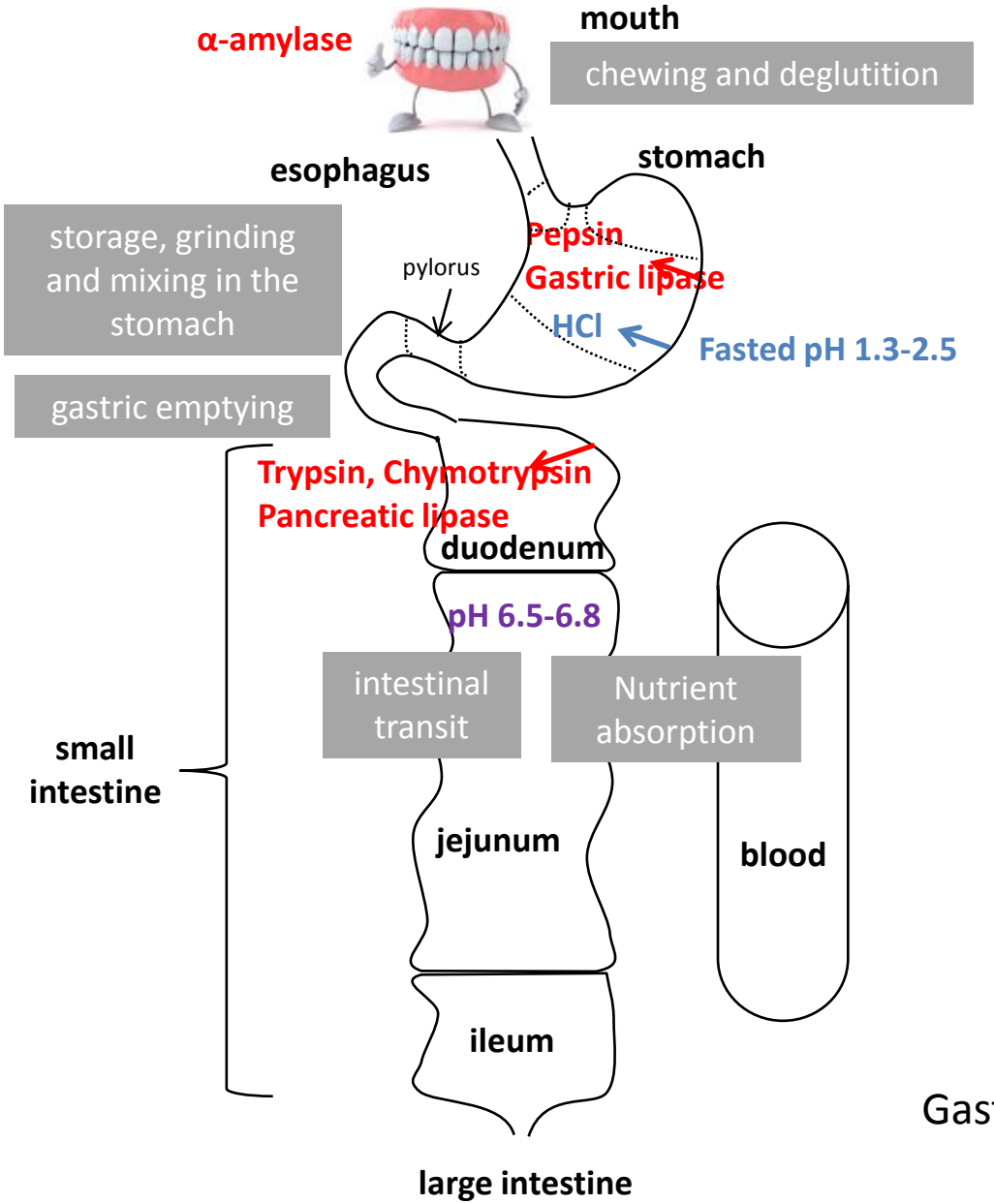
Tor Lea
Norway

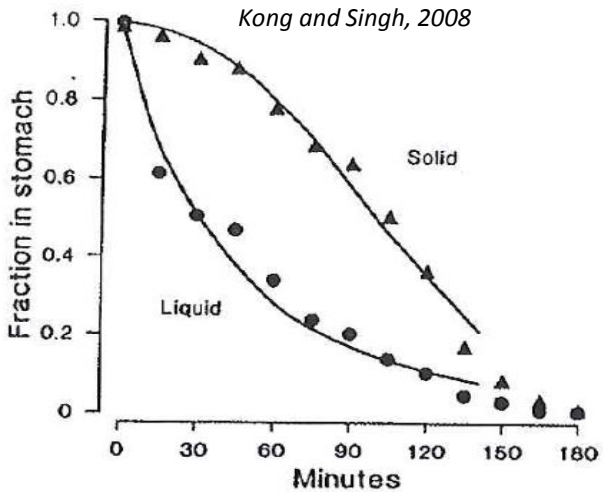
340 scientists - 130 institutes – 37 countries

Industry involvement

☞ ~ 40 European companies are involved in INFOGEST

The digestive process

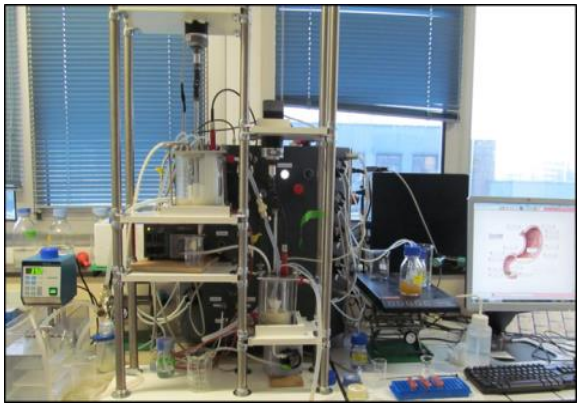




Gastric phase = a very complex but crucial step for the whole digestion process

Models for simulating digestion

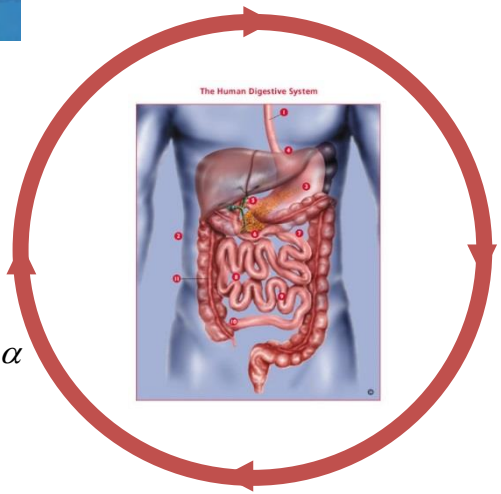
In vitro static models



In vitro dynamic models

In silico models

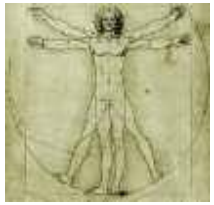
$$\Phi_{12} = k_{12whey} \times \left(V_1 - m_{caswpd1} \times \alpha\right) + k_{12aggr} \times m_{caswpd1} \times \alpha$$



Human models

Animal models

Static *in vitro* digestion models: pro's & con's



In vivo

In vitro

Main Reasons :

- Ethical - Technical – Financial

Advantages:

Standardisation of the experimental conditions
Good reproducibility and repeatability
Easy sampling, possibility to follow kinetics

Disadvantages:

You can't mimic the complexity of the GI tract in a test tube!!!
Needs harmonization

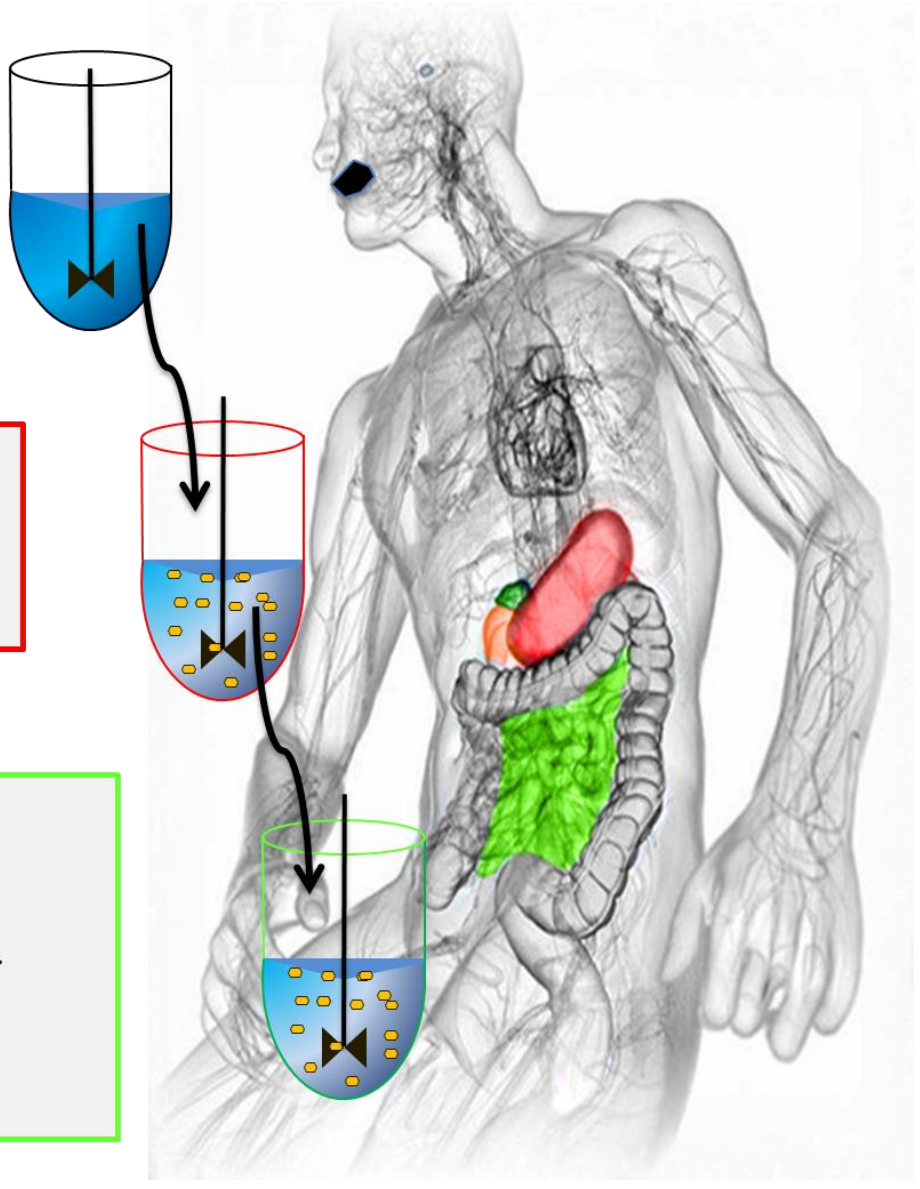
In vitro gastro-intestinal digestion
Consensus INFOGEST protocol

Oral phase
Mix 1:1 with Simulated Salivary Fluid (SSF)
salivary amylase (75 U/mL)
2 min, pH 7

Gastric Phase
Mix 1:1 with Simulated Gastric Fluid (SGF)
Pepsin (2000 U/mL)
2h, pH 3

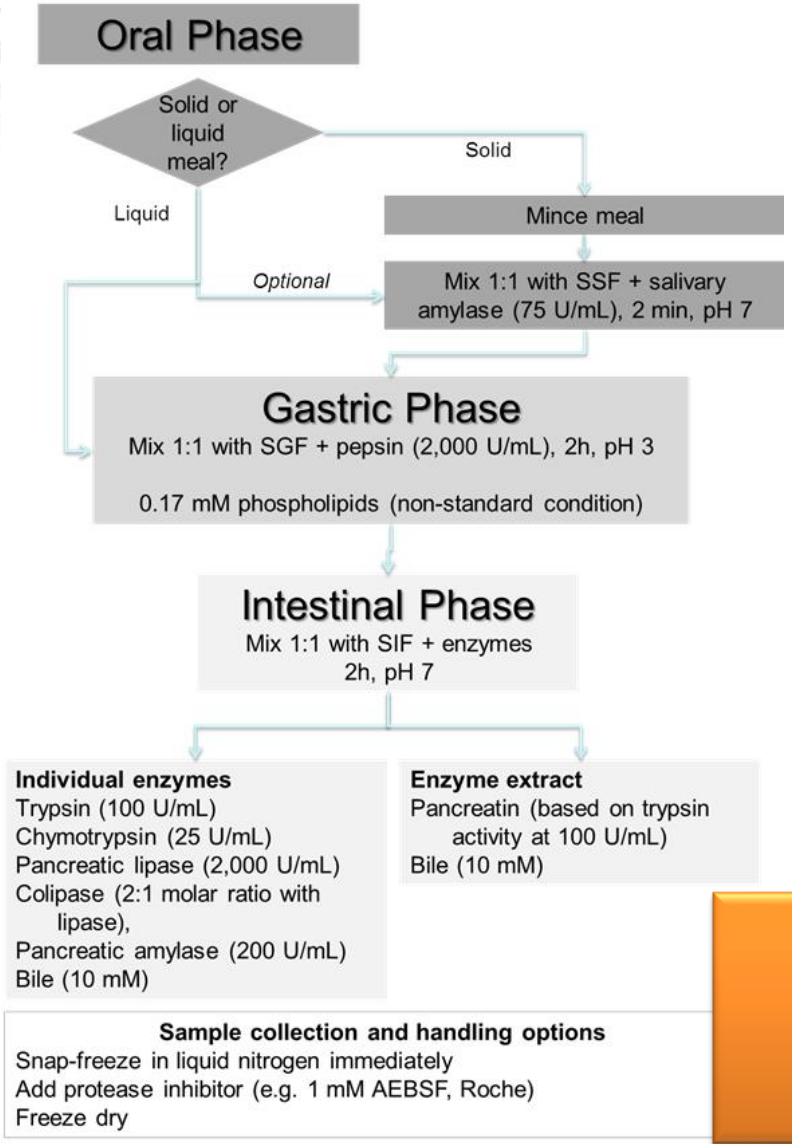
Intestinal Phase
Mix 1:1 with Simulated Intestinal Fluid (SIF)
Enzymes
- Pancreatin (based on trypsin 100 U/mL) or
- Pure enzymes

Bile (10mM)
2h, pH 7



The Infogest consensus *in vitro* digestion model

Consensus model based on available physiological data (*in vivo*)



OPEN ACCESS article

Calibration of the digestive enzymes, bile provided as supplementary material

29 authors

Minekus et al. 2014
Food Funct. 5, 1113-24
46 citations
Hot paper (0.1%)

Simulated digestion fluids

Table 2 Preparation of stock solutions of simulated digestion fluids. The volumes are calculated for a final volume of 500 mL for each simulated fluid. We recommend to make up the stock solution with distilled water to 400 mL instead, *i.e.* 1.25× concentrate, for storage at −20 °C. In the Experimental section, these 1.25× concentrates are referred to as Simulated Salivary Fluid (SSF), Simulated Gastric Fluid (SGF) and Simulated Intestinal Fluid (SIF) electrolyte stock solutions. The addition of enzymes, bile salts, Ca^{2+} solution *etc.* and water will result in the correct electrolyte concentration in the final digestion mixture. $CaCl_2(H_2O)_2$ is not added to the electrolyte stock solutions as precipitation may occur. Instead, it is added to the final mixture of simulated digestion fluid and food[a]

			SSF		SGF		SIF	
			pH 7		pH 3		pH 7	
Constituent	Stock conc.		Vol. of stock	Conc. in SSF	Vol. of stock	Conc. in SGF	Vol. of stock	Conc. in SIF
	g L^{-1}	mol L^{-1}	mL	mmol L^{-1}	mL	mmol L^{-1}	mL	mmol L^{-1}
KCl	37.3	0.5	15.1	15.1	6.9	6.9	6.8	6.8
KH_2PO_4	68	0.5	3.7	3.7	0.9	0.9	0.8	0.8
$NaHCO_3$	84	1	6.8	13.6	12.5	25	42.5	85
NaCl	117	2	—	—	11.8	47.2	9.6	38.4
$MgCl_2(H_2O)_6$	30.5	0.15	0.5	0.15	0.4	0.1	1.1	0.33
$(NH_4)_2CO_3$	48	0.5	0.06	0.06	0.5	0.5	—	—
For pH adjustment								
	mol L^{-1}		mL	mmol L^{-1}	mL	mmol L^{-1}	mL	mmol L^{-1}
NaOH	1		—	—	—	—	—	—
HCl	6		0.09	1.1	1.3	15.6	0.7	8.4
$CaCl_2(H_2O)_2$ is not added to the simulated digestion fluids, see details in legend								
	g L^{-1}	mol L^{-1}		mmol L^{-1}		mmol L^{-1}		mmol L^{-1}
$CaCl_2(H_2O)_2$	44.1	0.3		1.5 (0.75*)		0.15 (0.075*)		0.6 (0.3*)

[a] * in brackets is the corresponding Ca^{2+} concentration in the final digestion mixture.

The oral phase

- ☞ Always include an oral phase (± enzymes)
- ☞ Ratio Food / Simulated Salivary Fluid (SSF): **50/50 w/v**
- ☞ Time of chew: **2 min**

Add 5 g food + 5 mL SSF
Add Human salivary alpha amylase 150 IU/ mL in the SSF
Add 0,5 µL of CaCl2 (588 g/ L) per mL SSF

Simulate mastication

After addition of simulated salivary fluid (with salivary amylase)

The gastric phase

Ratio oral content / Simulated gastric fluid (SGF) : **50/50 w/v**
Porcine pepsin: **2000 U/mL**
Time of gastric digestion: **2 hours**
pH of the reaction: **3**

Why 2 hours?
Duration highly depends on the type of food/meal

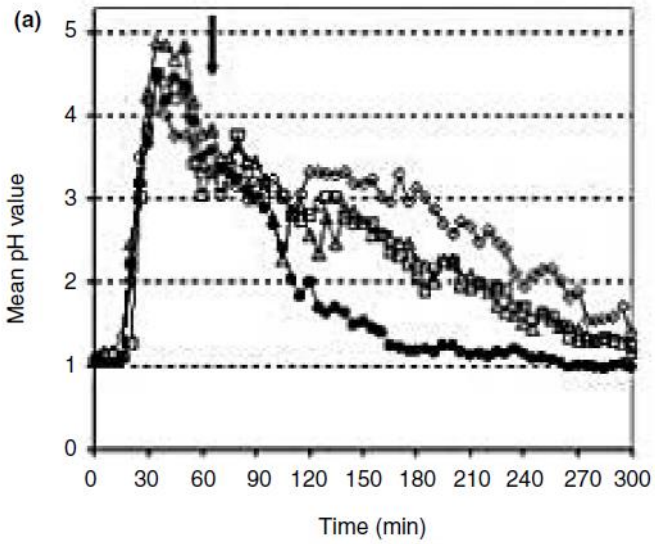
* Gastric emptying of a western type solid meal: 3-4h, of a liquid 0.5-1h
* Addition of nutrients to a liquid meal increases the transit time
* Strong inter and intra-individual variability

A time of 2h for gastric digestion represents the half emptying of a moderately nutritious and semi-solid meal

Why pH 3?
Fasted pH commonly found is around or below 2
pH increases to 5 and above because of the buffering capacity of the food/meal

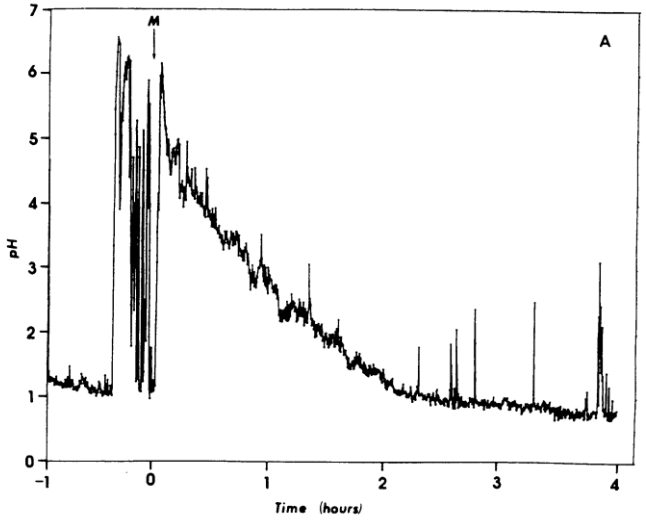
pH 3 represents the mean value for a general meal exhibiting a gastric emptying half-time of 2 h

pH and duration of the gastric phase



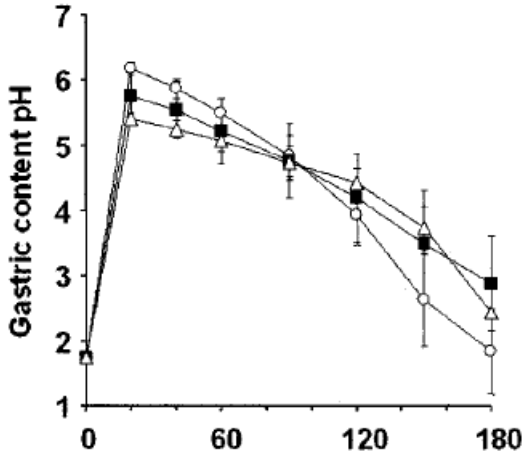
Gardner et al. 2002

125g steak, 200g boiled potatoes, 200g fresh vegetables, 50g salad, 200mL dessert, 200 mL water



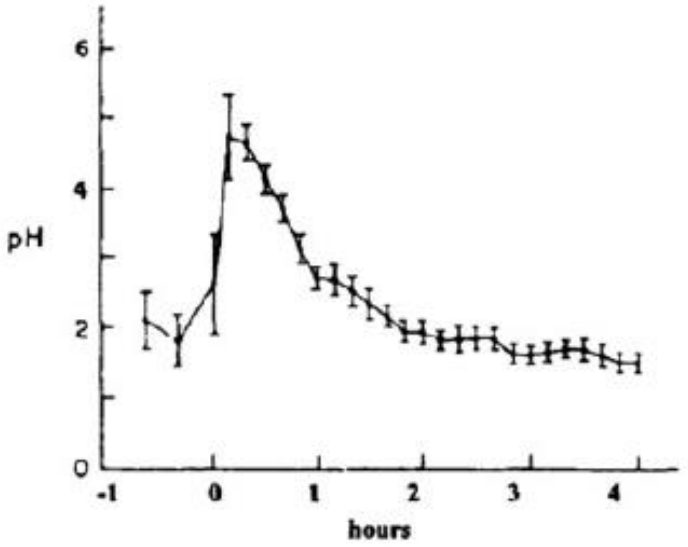
Dressman et al. 1990

6 oz hamburger, 2 slices bread, 2 oz potatoes, ketchup, mayonnaise



Tyssandier et al. 2003

Tomato puree, carrot puree or chopped spinach



Malagelada et al. 1976

Solid meal 400 mL 458 kcal pH 6

The intestinal phase

- ☞ Ratio Food (gastric content) / Simulated duodenal fluid (SDF): **50/50 w/v**
- ☞ Time of duodenal digestion: **2 hours**
- ☞ pH of the solution: **7**

20 mL gastric content
+ 3.0 µL of **CaCl2** (H2O)2 (588 g/L, w/v)
+ Bile: (final concentration in total fluid 10 mM). There are two options for bile for the duodenal stage, which is to use either:

Bile extract (e.g. B8631-100G from Sigma) or
Fresh porcine bile (available from several InfoGest members including IFR (160 mM stock). The SDF the concentration is made up to 20mM.

+ fill up to a final volume of 40 mL with SDF to reach the same volume as the gastric digesta (20mL).

At this point there are two options in how to proceed:

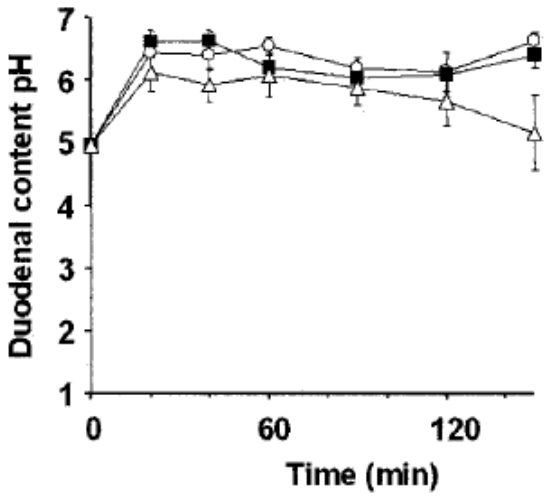
1. Use pancreatin: sufficient pancreatin to provide 100 U/ml of trypsin (TAME Units). The proteolytic, lipolytic and amylolytic activity should be determined

2. Use individual enzymes

pH and duration of the intestinal phase

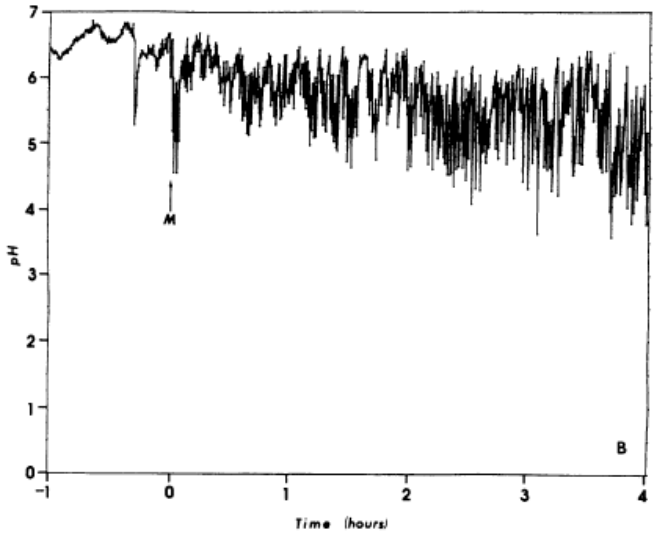
Why **pH 7**?

- ☞ pH measured in the duodenum is close to 6,5 (see below)
- ☞ In the small intestine, pH increases slightly over its length to a value of around 7,5 in the distal ileum



Tyssandier et al. 2003

Tomato puree, carrot puree or chopped spinach



Dressman et al. 1990

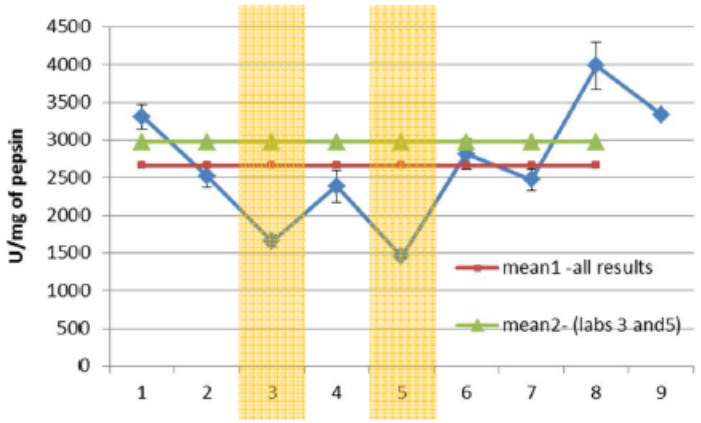
6 oz hamburger, 2 slices bread, 2 oz potatoes, ketchup, mayonnaise

Key points

The calibration of the digestive enzymes is crucial and not that easy to perform

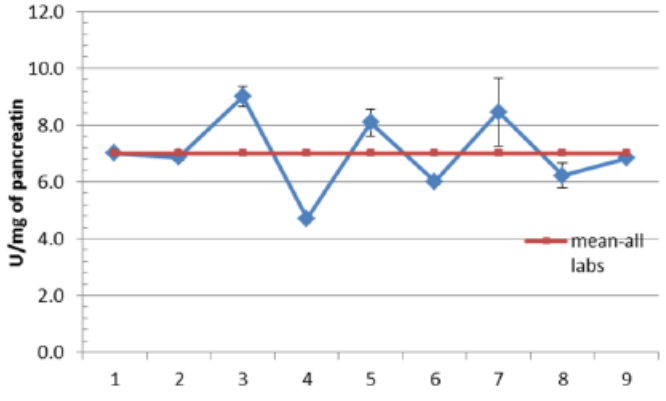
International inter-laboratory assay (7 labs in Europe)

Pepsin reference
From porcine gastric mucosa
P7012
Batch number Lot#SLBJ4999V



► 2976 U/mg of pepsin powder

Pancreatin reference
Porcin pancreatin
P7545
Batch number Lot#SLBJ7293V



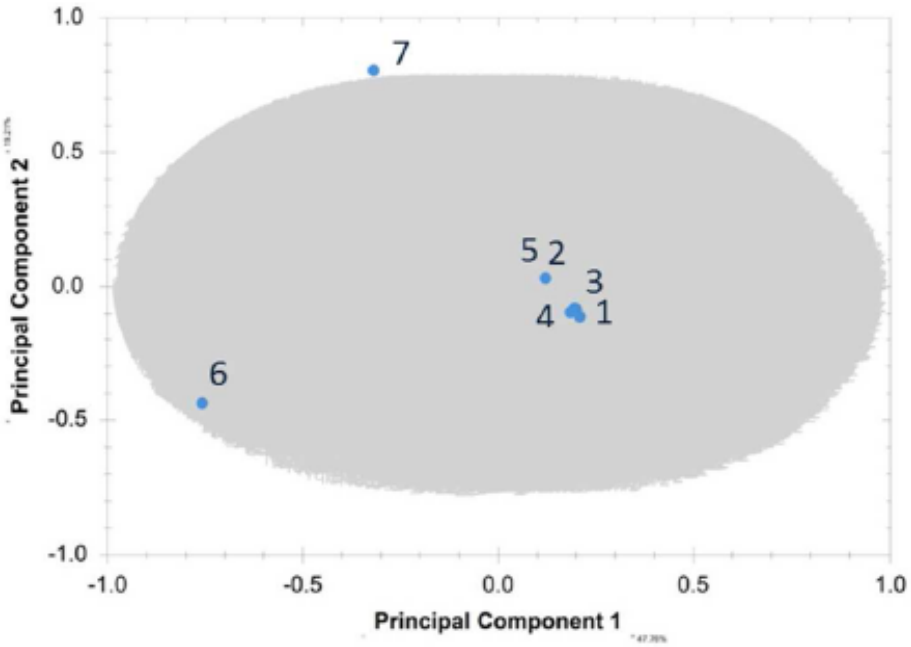
► 7.0 trypsin U/mg of pancreatin powder

Same batch of pepsin and pancreatin analyzed by trained people

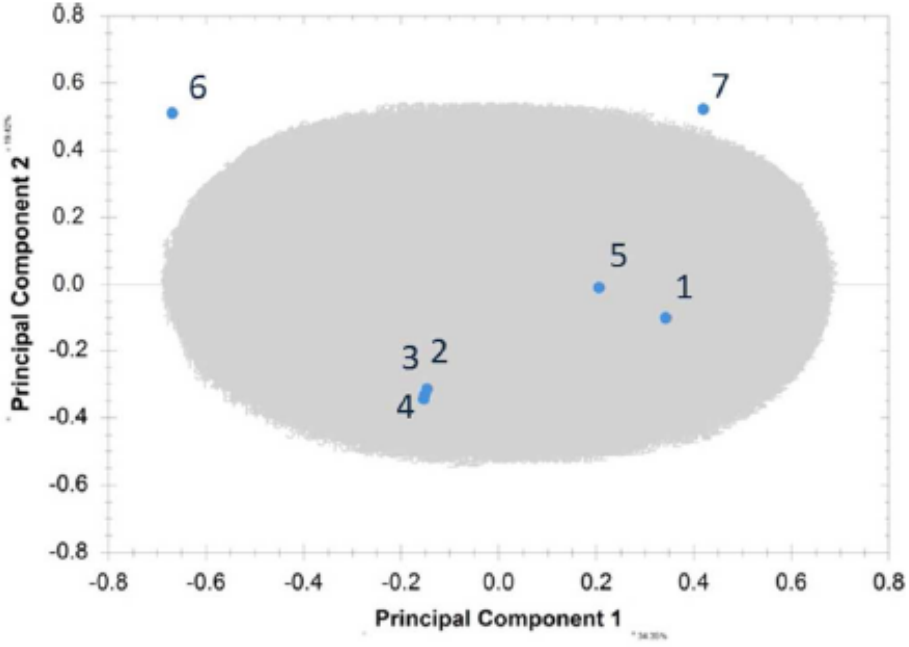
Key points

Differences in digestive enzymes calibration leads to different peptidomes

Gastric phase

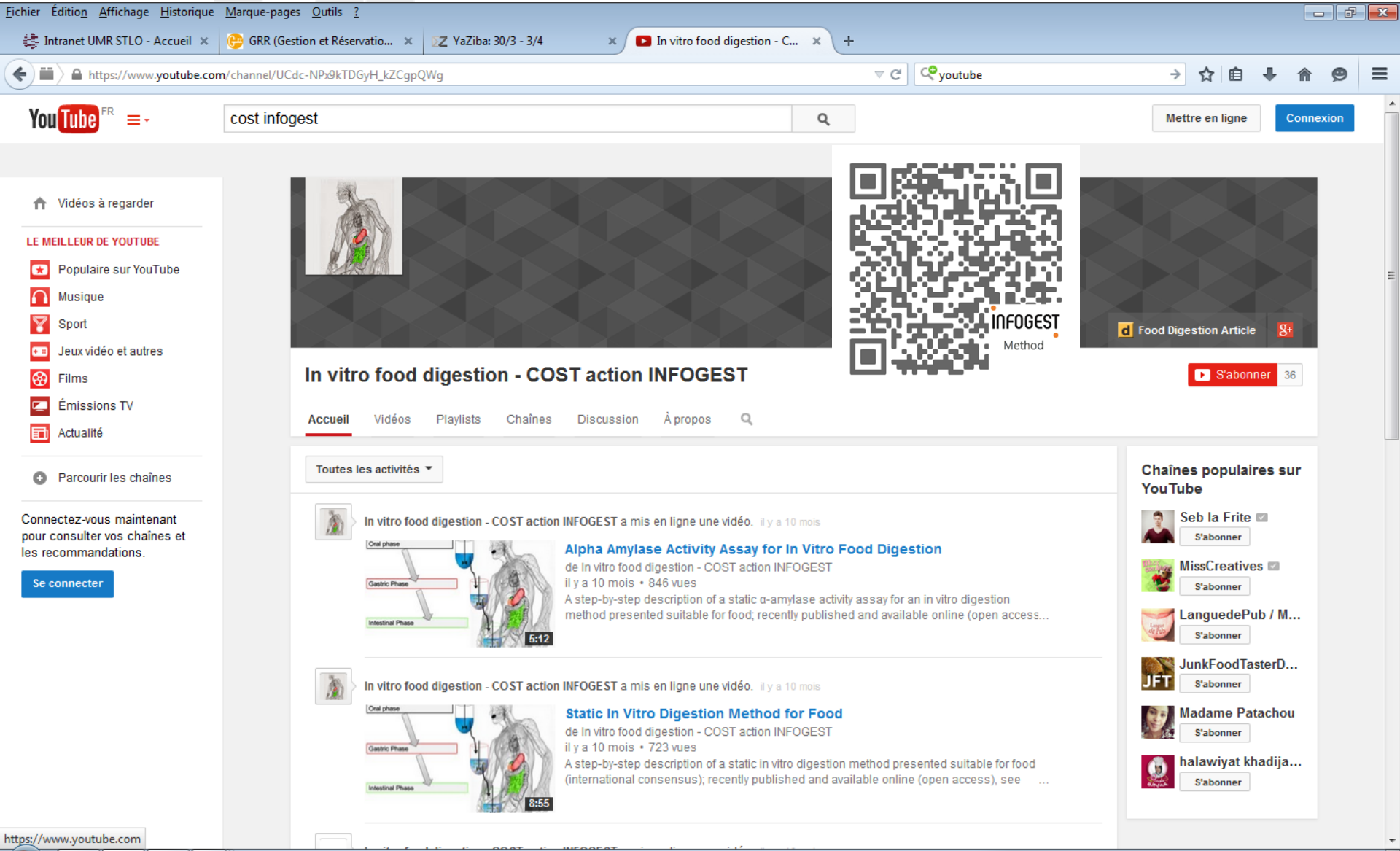


Intestinal phase



- The extremely sensitive metabolomics approach clearly discriminates laboratory 6 and 7 as being outliers during gastric as well as intestinal phase

The consensus model can be learned with videos on YouTube



Conclusion

☞ The Infogest model has been applied successfully to several foods like milk, meat, pasta, bread… and it works! (46 citations in 1.5 year)

☞ The Infogest *in vitro* digestion model is now used all over the world, in Europe, USA, Australia, New Zealand, Argentina…

☞ Interlaboratory trials have been performed at the European level on the digestion of milk and meat

☞ People can easily learn how to run the model, calibrate the digestive enzymes and the bile with the open access publication and the videos available on YouTube

☞ Validation towards *in vivo* data is under investigation. Data on pigs will be available before the end of 2015

INFOGEST
International Conference
Rennes, France
April 2017

We are pleased to announce the next

5th International Conference on Food Digestion

in Rennes, France, April 2017