The structure of the food matrix at different length scales drives the mechanism of digestion and the nutrient bioaccessibility and bioavailability

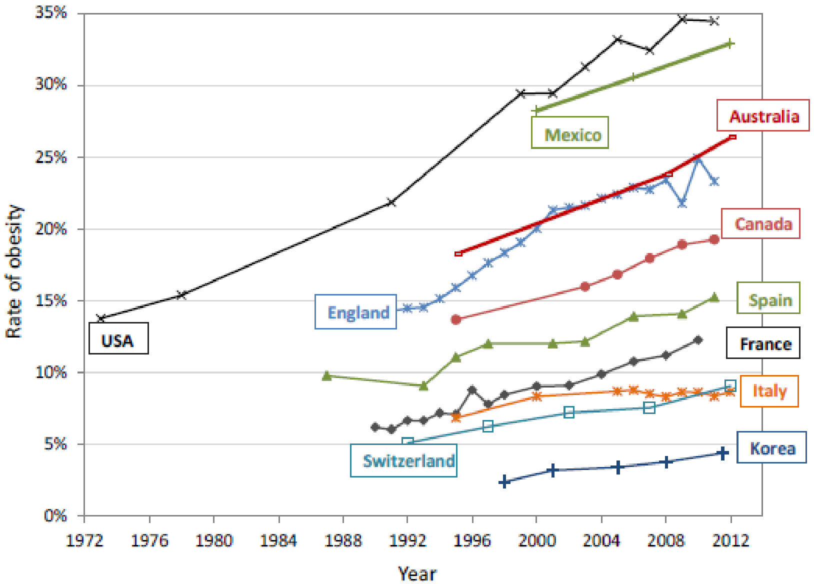
Didier Dupont

The structure of the food matrix at different length scales drives the mechanism of digestion and the nutrient bioaccessibility and bioavailability

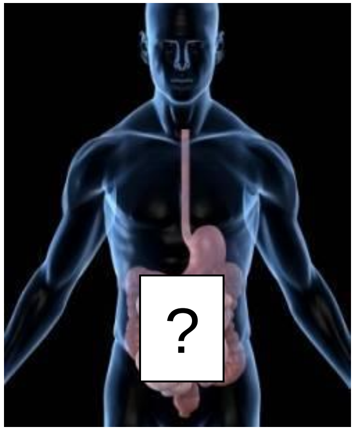
INRA
SCIENCE & IMPACT

Dr Didier DUPONT, INRAE, STLO, Rennes, France

Food and human health: the key role of digestion



Diet-related diseases ↑
Prevent these pathologies rather than cure them

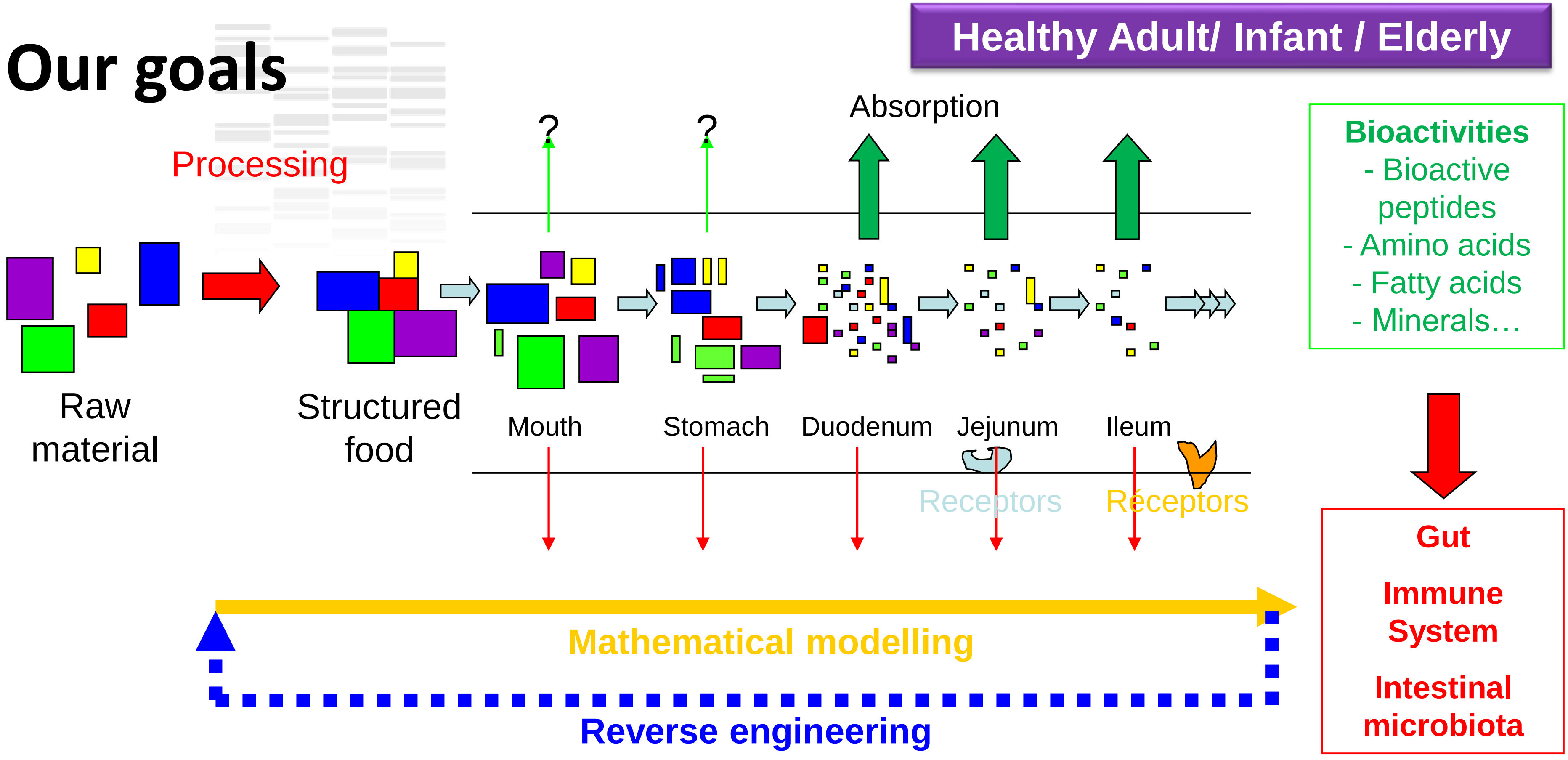


Gut = interface between food and human body
Digestion releases food components that can have a beneficial or a deleterious effect on human health

… 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

Our goals

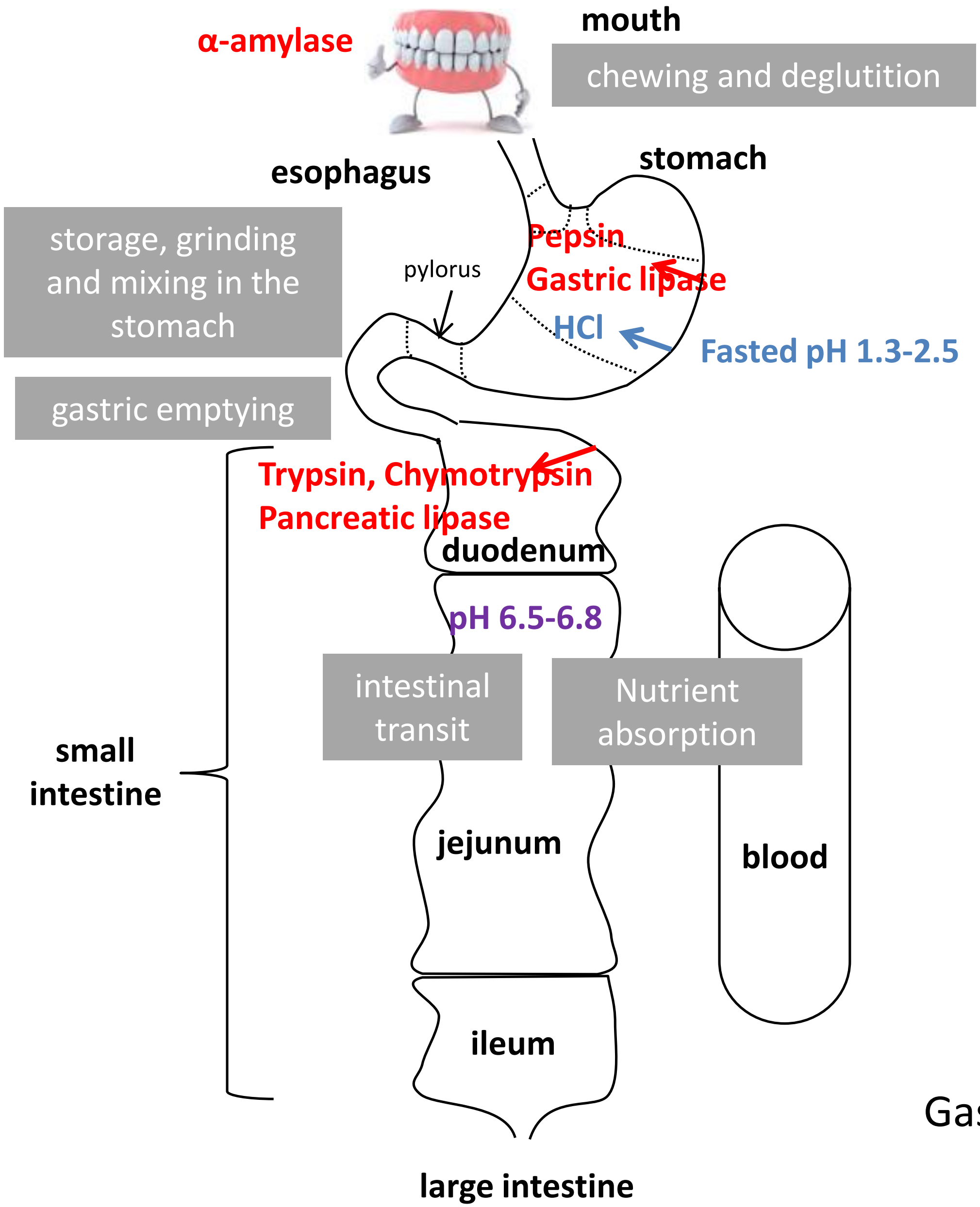


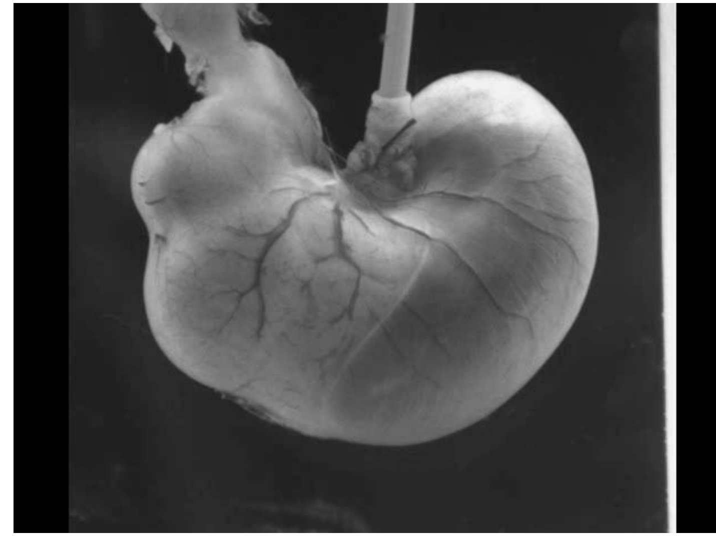
☞To understand the mechanisms of breakdown of food matrices and their constituents in the gut and identify the beneficial/deleterious food components released during digestion

☞To determine the impact of the structure of food matrices on nutrient bioavailability

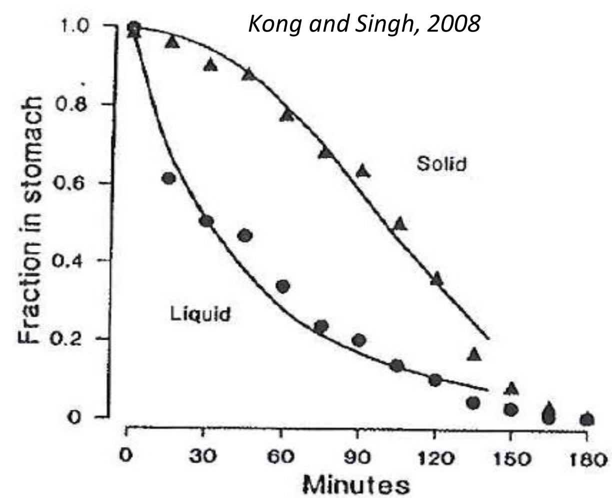
☞To model these phenomena in order to develop a reverse engineering approach

The digestive process





From Roger Lentle, Massey Univ. NZ



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

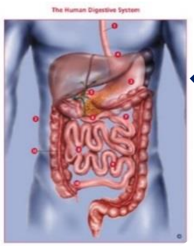
Models available at INRAE for simulating digestion

Menard et al. 2018, 2023
Wang et al. 2022

In vitro static models (infant, adult, elderly)

Peng et al. 2021
Halabi et al. 2021
Giribaldi et al. 2021
Chauvet et al. 2023
Nebbia et al. 2022, 2023

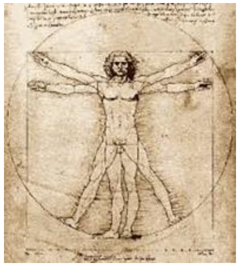
In vitro dynamic models (infant, adult, elderly)



Le Feunteun et al. 2014, 2020

In silico models

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



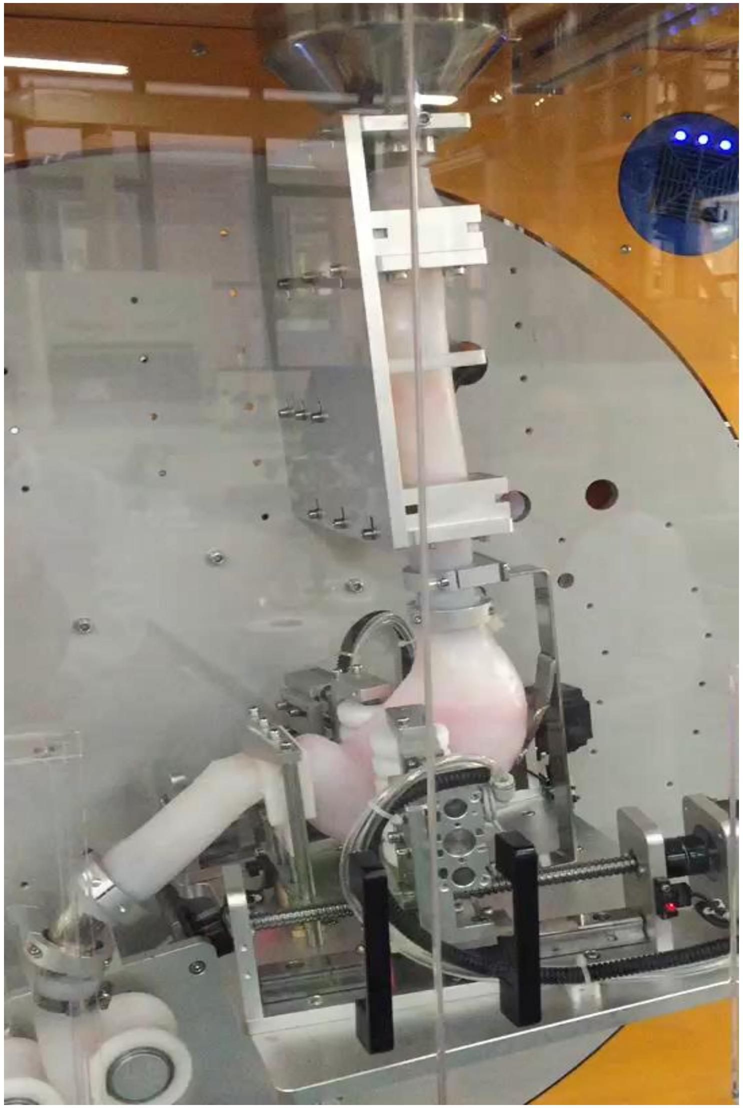
Human models

De Oliveira et al. 2016
De Oliveira et al. 2017
Buffière et al. 2020
Boulier et al. 2023

Animal models

Lemaire et al. 2021
Nau et al. 2022
Jimenez-Barrios et al. 2023
Charton et al. 2022, 2023

NERDT™ : the NEar Real Digestive Tract



Xiao Dong Pro-Health
Smart Digestion
Suzhou University

SOOCHOW UNIVERSITY
UNTO A FULL GROWN MAN

INRAE

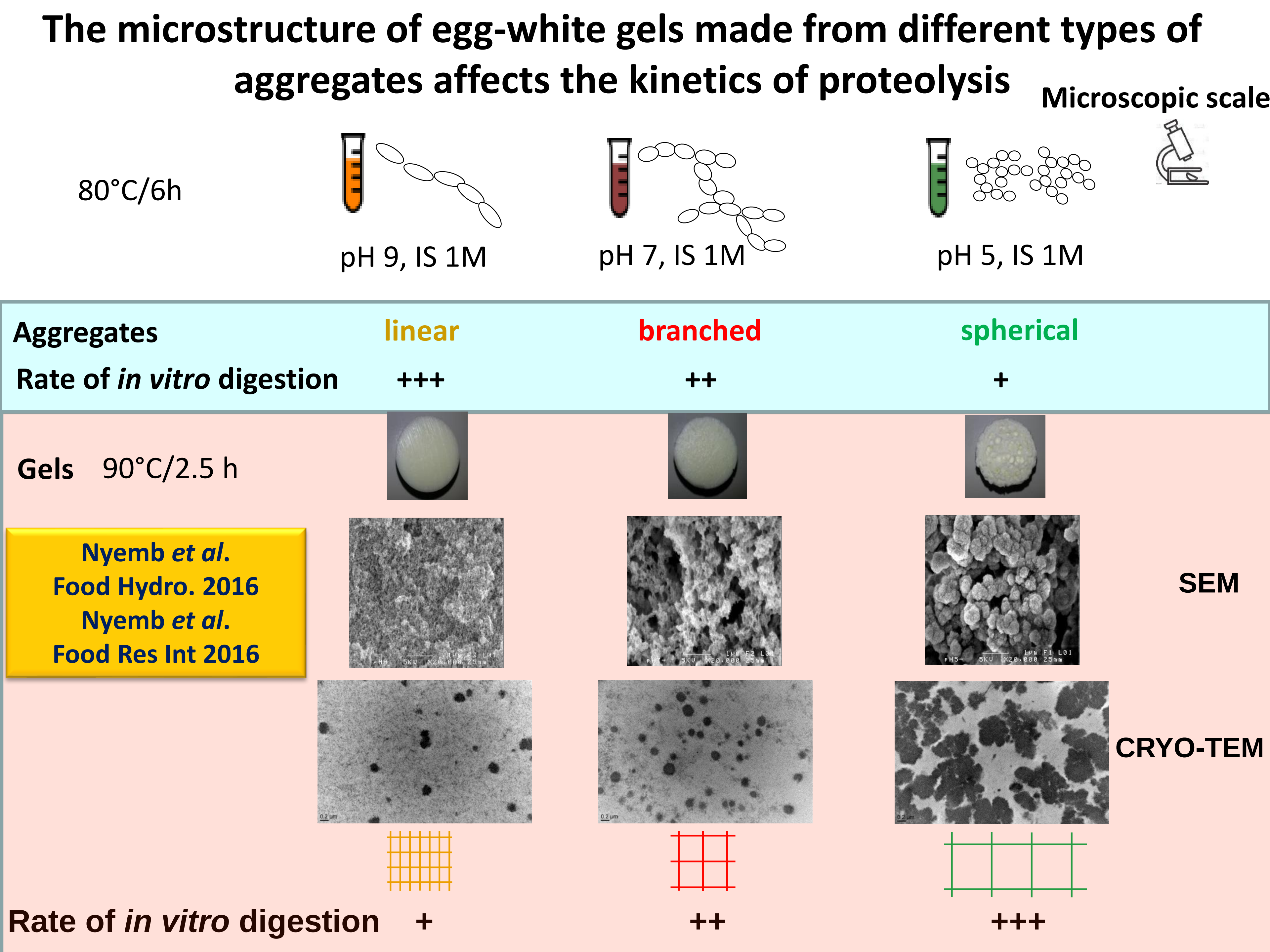
L'INSTITUT agro Rennes Angers

INSTITUT CARNOT QUALIMENT

STOMACH

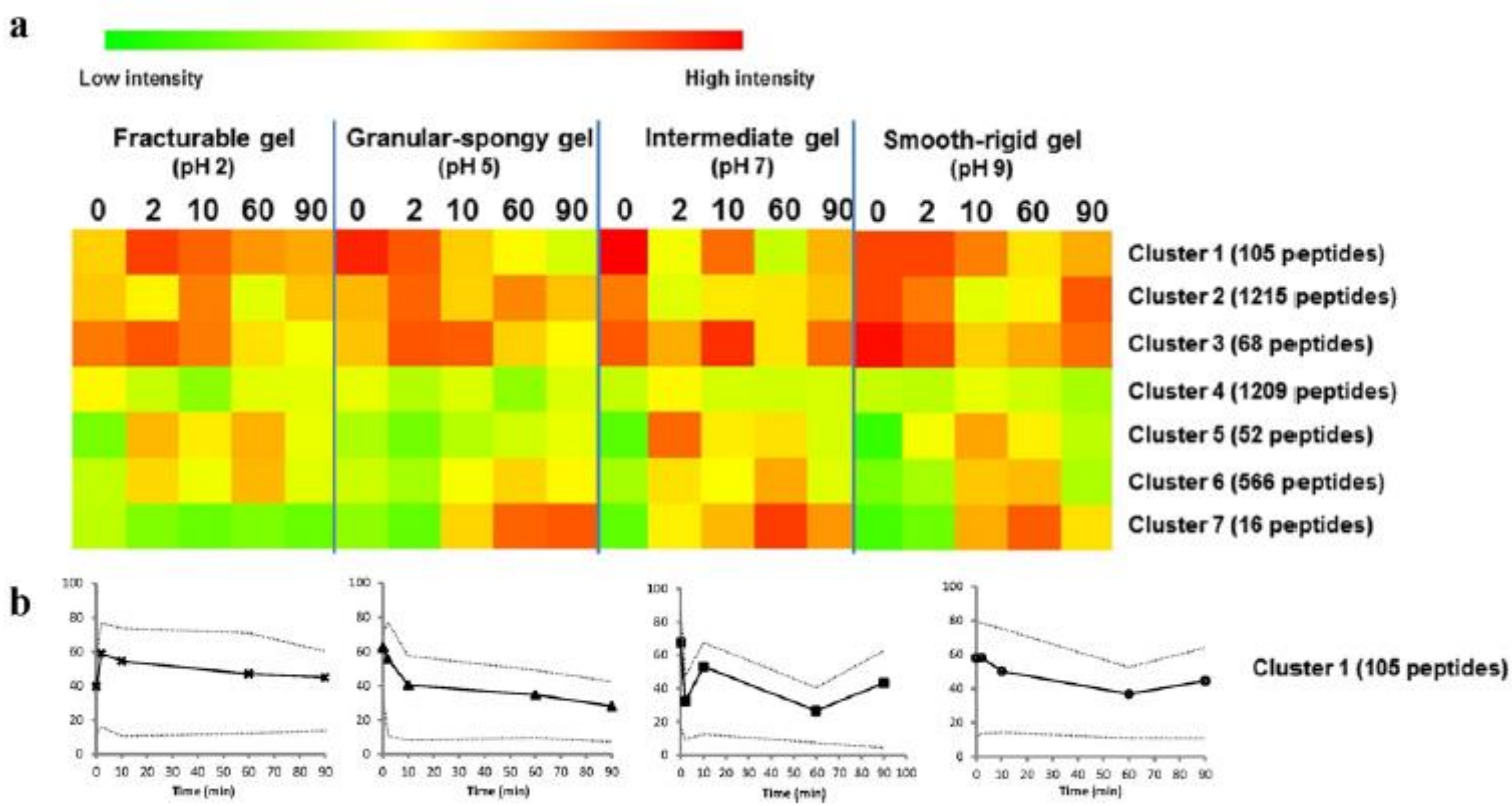
From the protein molecular structure to the the food microstructure:
The case of egg white gels

Nau F, & Dupont D.
INRAE, Rennes, France


The microstructure of egg-white gels made from different types of aggregates affects the kinetics of proteolysis
Microscopic scale
80°C/6h
pH 9, IS 1M
pH 7, IS 1M
pH 5, IS 1M
Aggregates
linear
branched
spherical
Rate of in vitro digestion
+++
++
+
Gels 90°C/2.5 h
Nyemb et al.
Food Hydro. 2016
Nyemb et al.
Food Res Int 2016
SEM
CRYO-TEM
Rate of in vitro digestion
+
++
+++

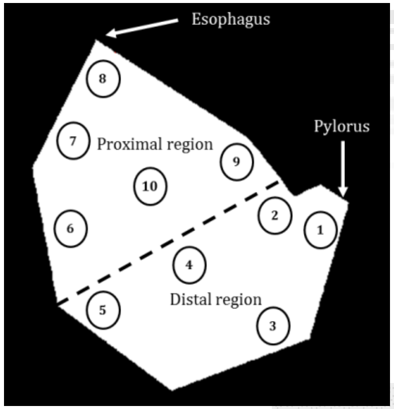
Peptidomics reveals significant differences in the peptide pattern released during digestion

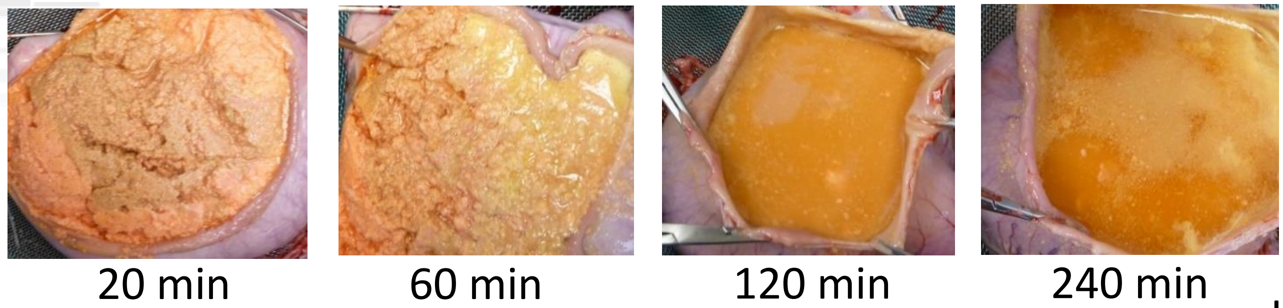
More than 3200 individual peptides identified



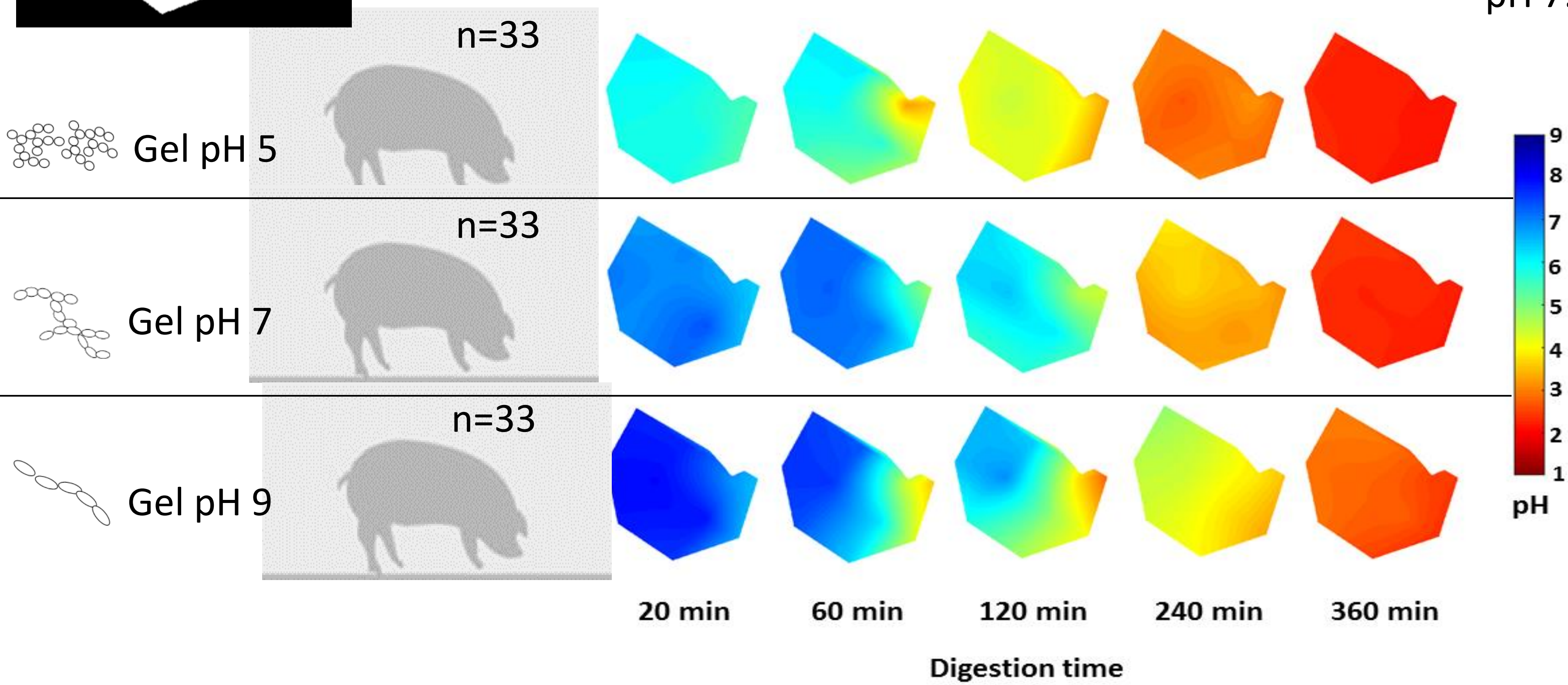
Spatial-temporal evolution of pH during an *in vivo* digestion

Microscopic scale



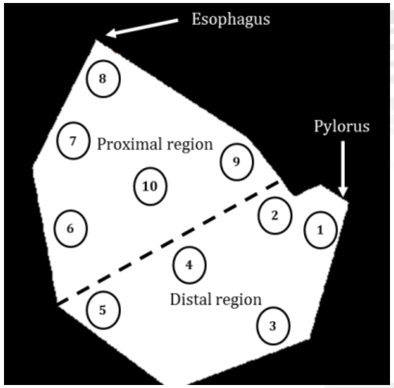


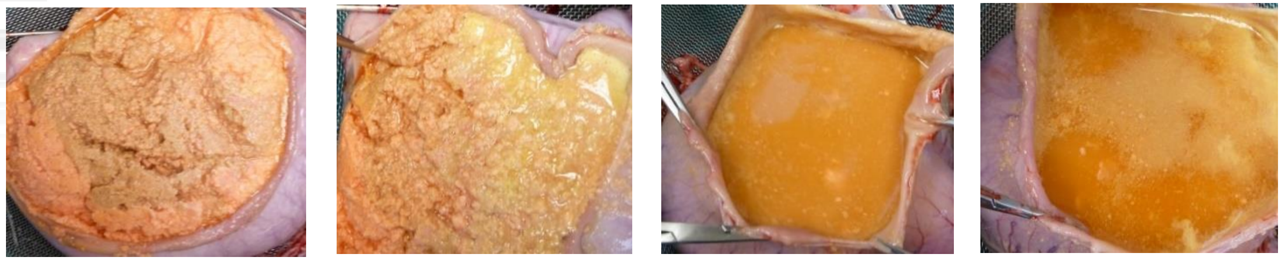
pH 7.0



Nau *et al.*
Food Chem. 2019

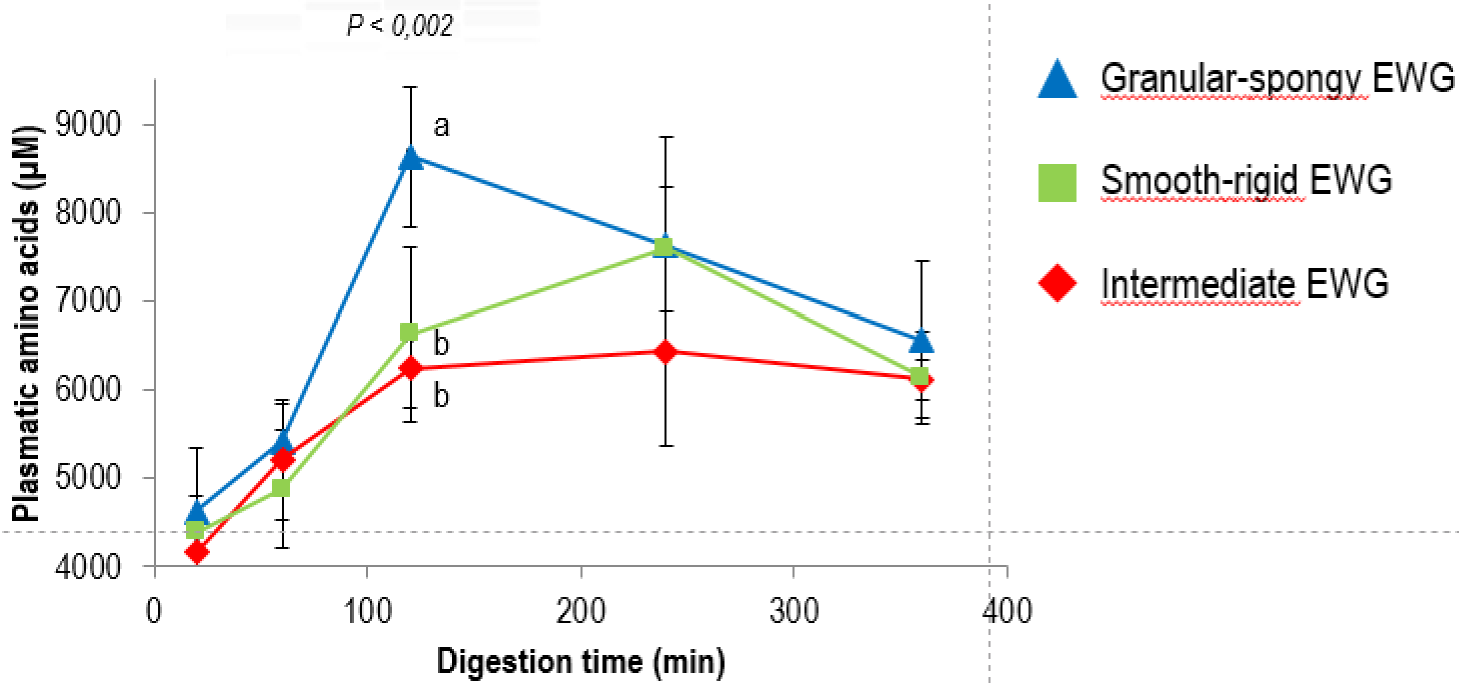
Spatial-temporal evolution of pH during an *in vivo* digestion

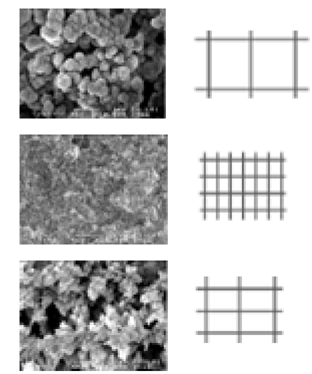




20 min | 60 min | 120 min | 240 min

pH 7.0



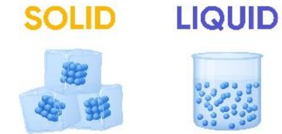


Microscopic scale

Food structure as modified by processing affects the kinetics of food digestion

Le Feunteun S, Menard O, Dupont D.
INRAE, Rennes, France

Comparison of 6 dairy products of identical composition but different structure



Macroscopic scale

Ultra Low Heat powder

rehydration in water 14.5%

unheated milk (“raw” milk)

heat treatment 90°C-10 min

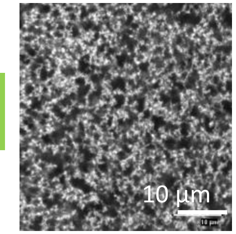
microstructure

heated milk

macrostructure

24h-20°C, rennet 0.003 % v/w

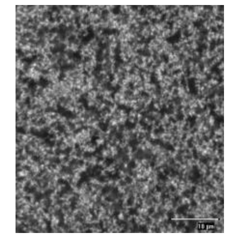
rennet gel



pH 6.6

24h-20°C, rennet 0.3 % v/w

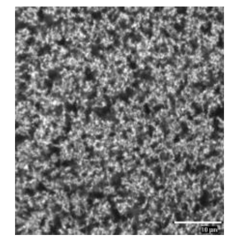
rennet gel



pH 6.6

24h-20°C, GDL 3 % w/w

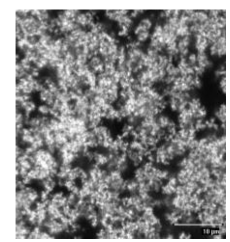
acid gel



pH 4

24h-20°C, GDL 3 % w/w + mixer 2 min

stirred acid gel



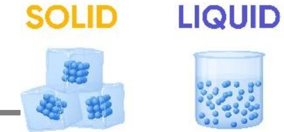
pH 4

Fat-free matrices:

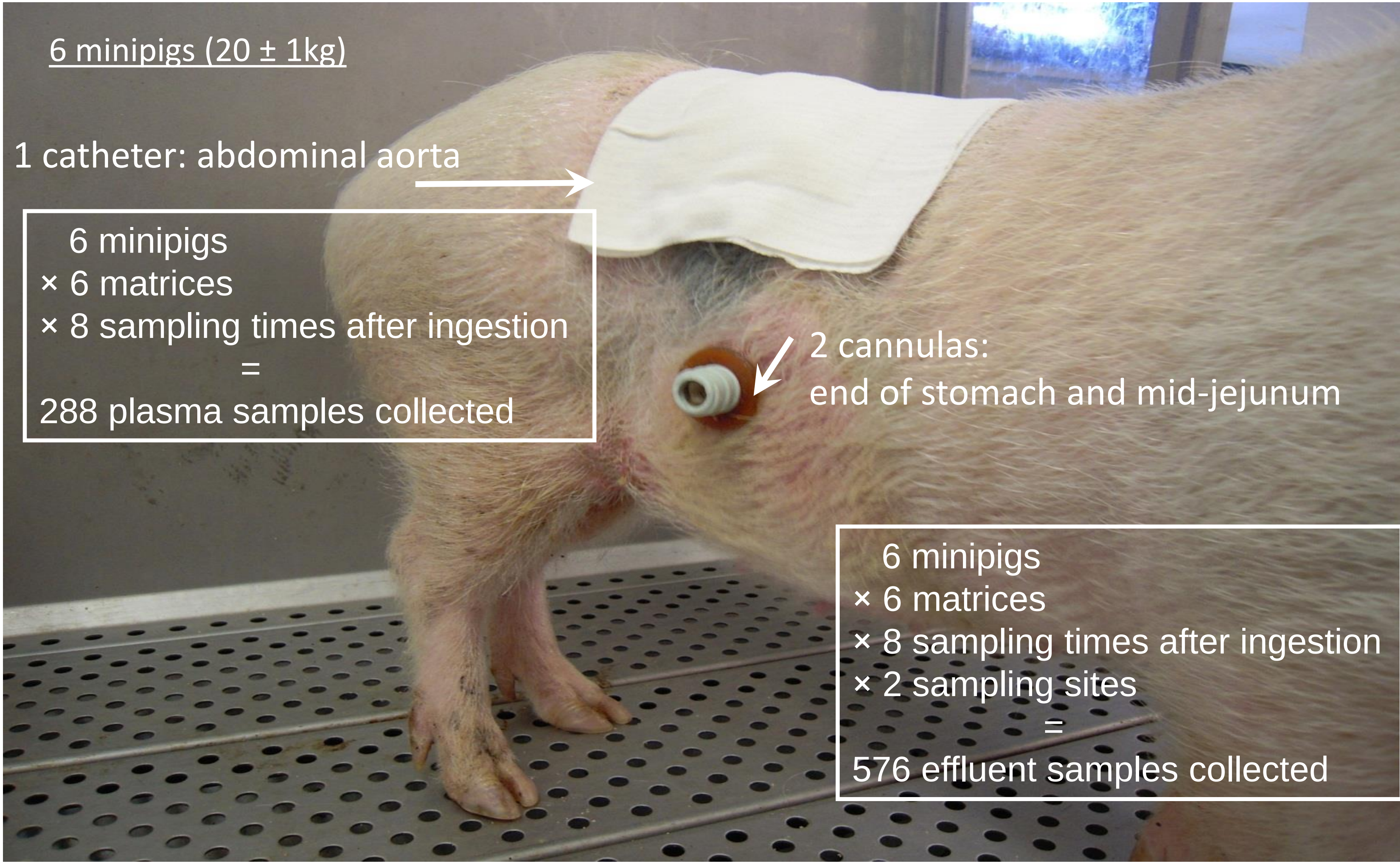
40 g/L caseins, 10 g/L whey proteins, 95 g/L lactose and minerals

+ marker of the meal transit (Cr^{2+}-EDTA) → Gastric emptying half-time

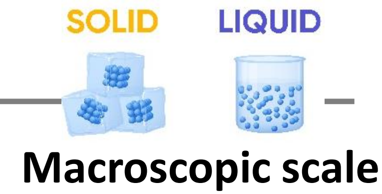
The multi-canulated mini-pigs

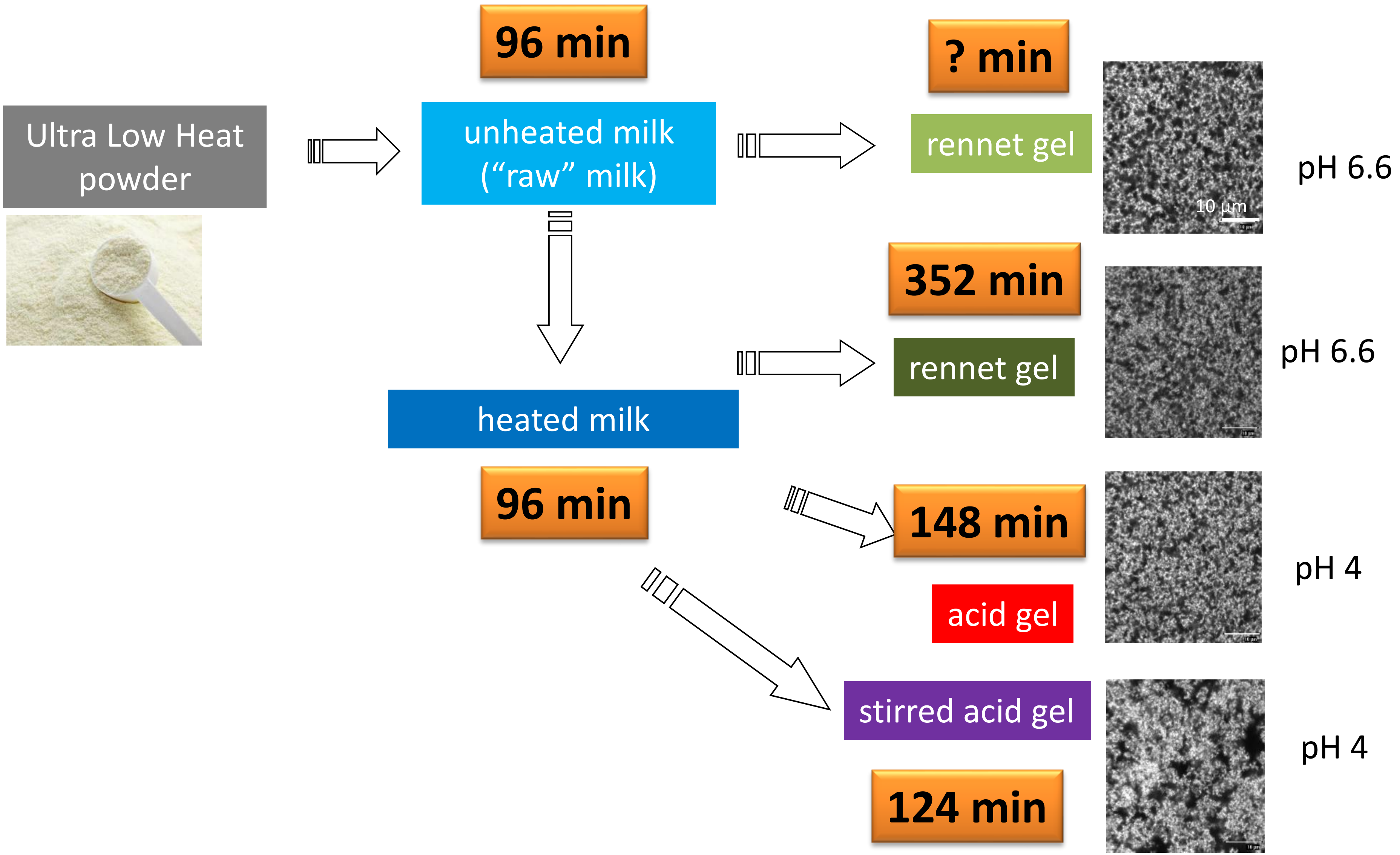


Macroscopic scale



Gastric emptying half time





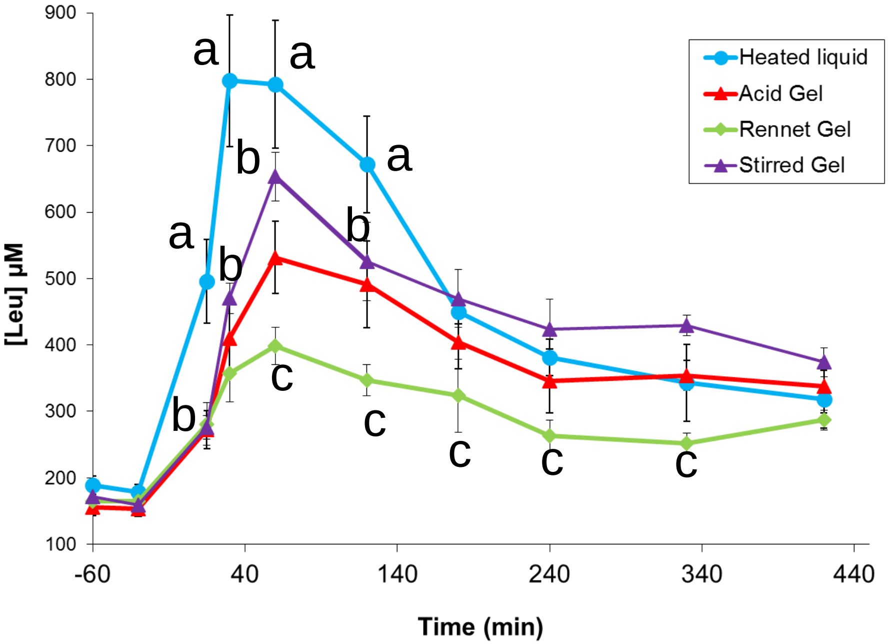
The liquid-gel transition

Barbé *et al.* Food Chem 2013

SOLID LIQUID

Macroscopic scale

Effect on absorption



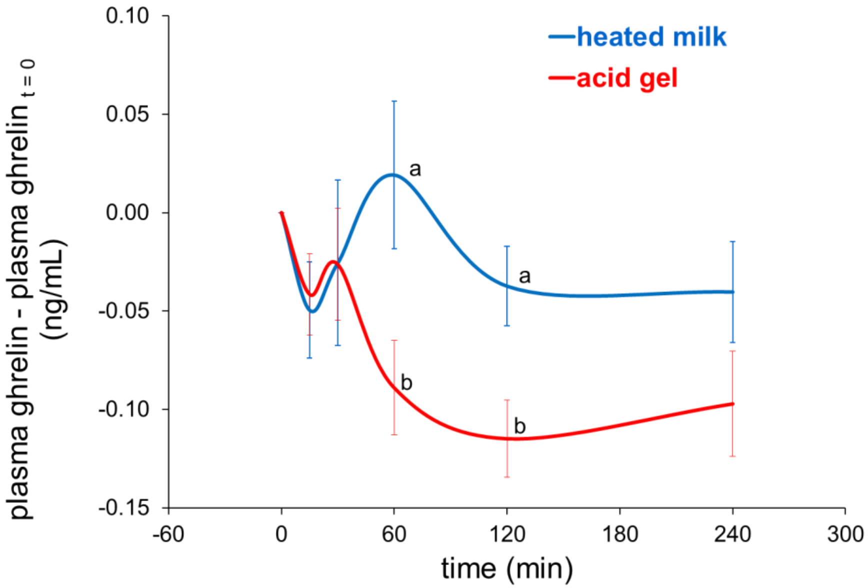
milk gelation:

→ delayed proteins transit → delayed AA absorption

↘ maximal AA concentration in the plasma

Potential effect on satiety

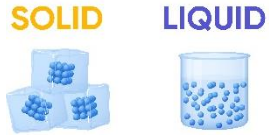
ghrelin (gastrointestinal hormone → appetite stimulation)



milk gelation:

↘ postprandial ghrelin concentration = ↗ satiety ?

Bioactive peptides released during digestion differ from one matrix to another



Macroscopic scale

More than 16000 peptides identified by LC-MS-MS in the jejunum

Protein	Sequence	Activity	Reference	4	20	50	105	165	225	315
αs1	1-23	EMUL	Shimizu et al. (1984)							
αs1	23-34	HYP	Maruyama & Suzuki (1982)							
αs1	30-45	MB	Meisel et al. (1991)							
αs1	40-52	MB	Adamson & Reynolds (1996)							
αs1	43-58	MB	Meisel et al. (1991)							
αs1	91-100	STRE	Miclo et al. (2001)							
αs1	99-109	MIC	McCann et al. (2006)							
αs1	167-180	MIC	Hayes et al. (2006)							
αs1	180-193	MIC	Hayes et al. (2006)							
αs2	1-24	MB	Miquel et al. (2005)							
αs2	124-146	MB	Miquel et al. (2005)							
αs2	183-206	TRAN	Kizawa et al. (1996)							
αs2	183-207	MIC	Recio & Visser (1999)							
αs2	189-197	HYP	Maeno et al. (1996)							
αs2	190-197	HYP	Maeno et al. (1996)							
β	1-24	MB	Bouhallab et al. (1999)							
β	33-52	MB	Miquel et al. (2005)							
β	60-80	OPI	Jinsmaa & Yoshikawa (1999)							
β	98-105	OXI	Rival et al. (2001)							
β	114-119	OPI	Jinsmaa & Yoshikawa (1999)							
β	132-140	HYP	Robert et al. (2004)							
β	192-209	IMM	Coste et al. (1992)							
β	193-202	IMM	Kayser & Meisel (1996)							
β	193-209	IMM	Coste et al. (1992)							
κ	18-24	HYP	Lopez-Exposito et al. (2007)							
κ	106-116	THR	Jolles et al. (1986)							
β–lg	32-40	HYP	Pihlanto-Leppala et al. (2000)							
β–lg	92-100	MIC	Pellegrini et al. (2001)							
β–lg	142-148	HYP	Mullally et al. (1997)							

Acid Gel

Protein	Sequence	Activity	Reference	4	20	50	105	165	225	315
αs1	40-52	MB	Adamson & Reynolds (1996)							
αs1	43-58	MB	Meisel et al. (1991)							
αs1	99-109	MIC	McCann et al. (2006)							
αs1	167-180	MIC	Hayes et al. (2006)							
αs1	180-193	MIC	Hayes et al. (2006)							
αs2	1-24	MB	Miquel et al. (2005)							
αs2	189-197	HYP	Maeno et al. (1996)							
β	33-52	MB	Miquel et al. (2005)							
β	166-175	HYP	Hayes et al. (2007)							
β	193-202	IMM	Kayser & Meisel (1996)							
β–lg	92-100	MIC	(8))							
β–lg	142-148	HYP	(9))							

Rennet Gel

- More bioactive peptides identified during digestion of acid gel than rennet gel
- Nature of peptides is identical (clearly defined by the digestive enzyme specificity)
- Kinetics of release are different

Barbé et al. 2014
Food Res Int

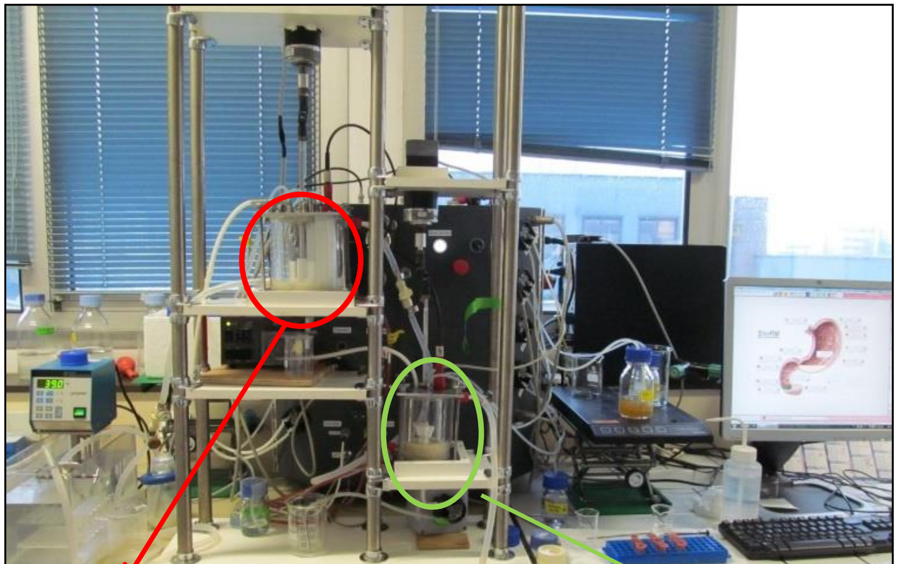
Differential behaviour of acid/rennet gels in gastric conditions

☞ Acid/Rennet gel: identical composition, similar pore size

☞ ≠ Time of residence in the stomach (Acid 148 min /Rennet 352 min)

☞ How can we explain this difference? Dynamic *in vitro* digestion of the 2 gels

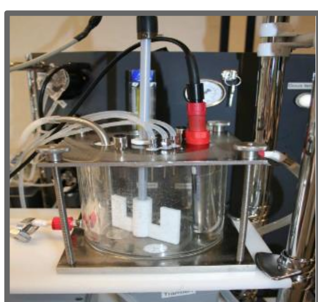
Ménard *et al.*
Food Chem 2014



DIDGI®

StoRM® software

Stomach



- Pepsine
- Gastric lipase
- Simulated gastric fluid
- HCl

Emptying : Elashoff's model

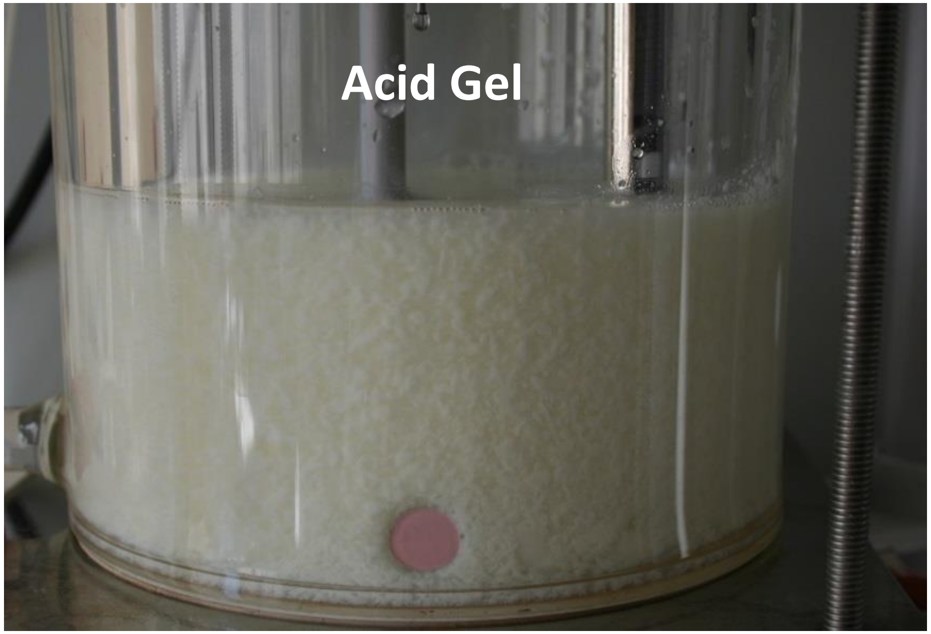
Small intestine

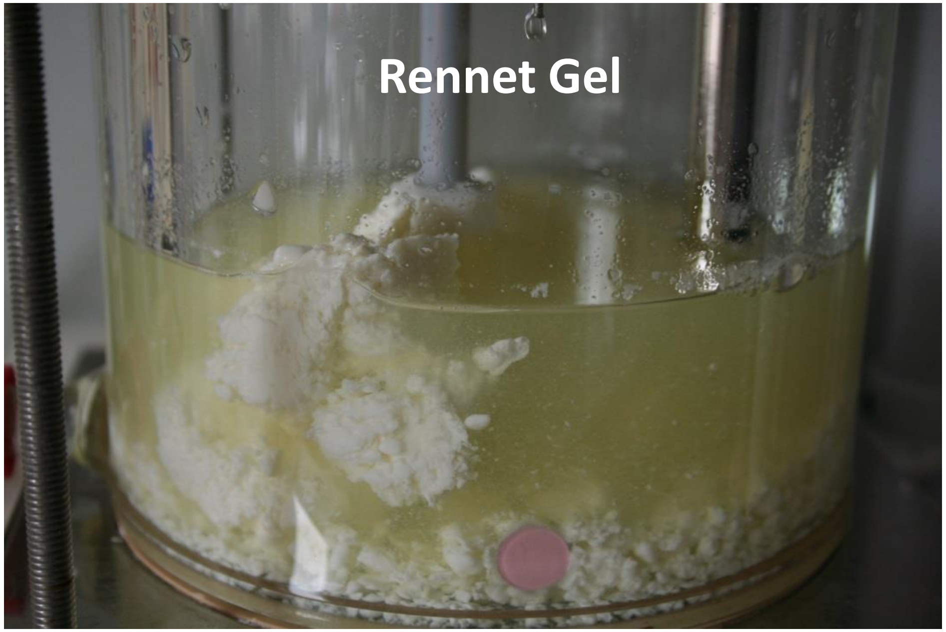
- Pancreatin
- Bile
- Simulated intestinal fluid
- $NaHCO_3$

Emptying : Elashoff's model

Behaviour of acid and rennet gels in the stomach during *in vitro* dynamic digestion

**Barbé et al.
Food Chem. 2014**





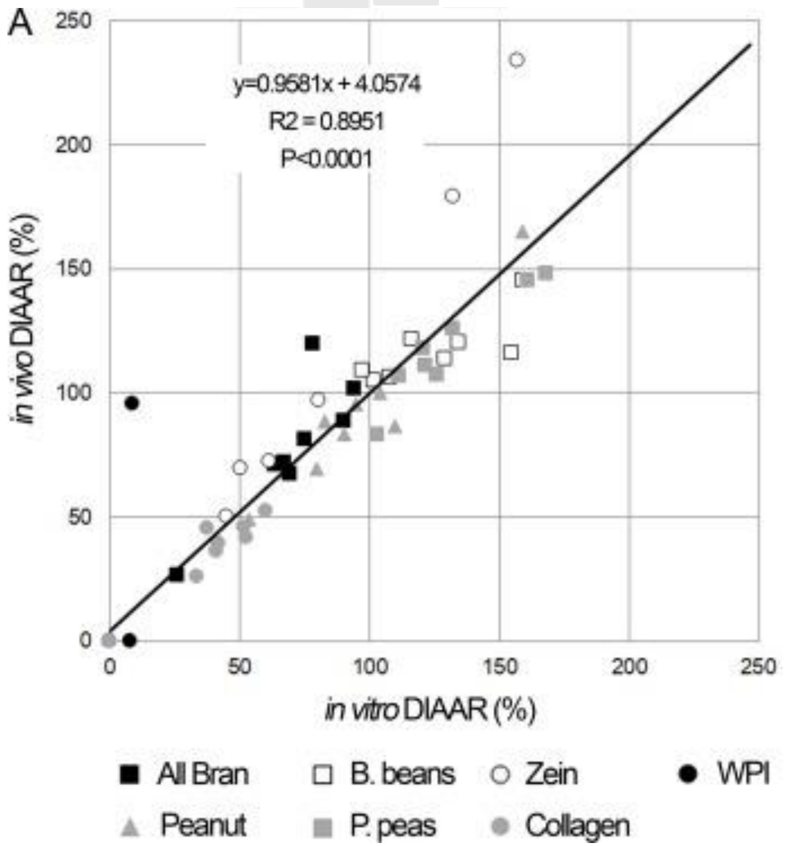
Formation of a strong coagulum with rennet gel → slow down the gastric emptying of caseins

The structure that a food adopts in the stomach is essential to understand its digestion

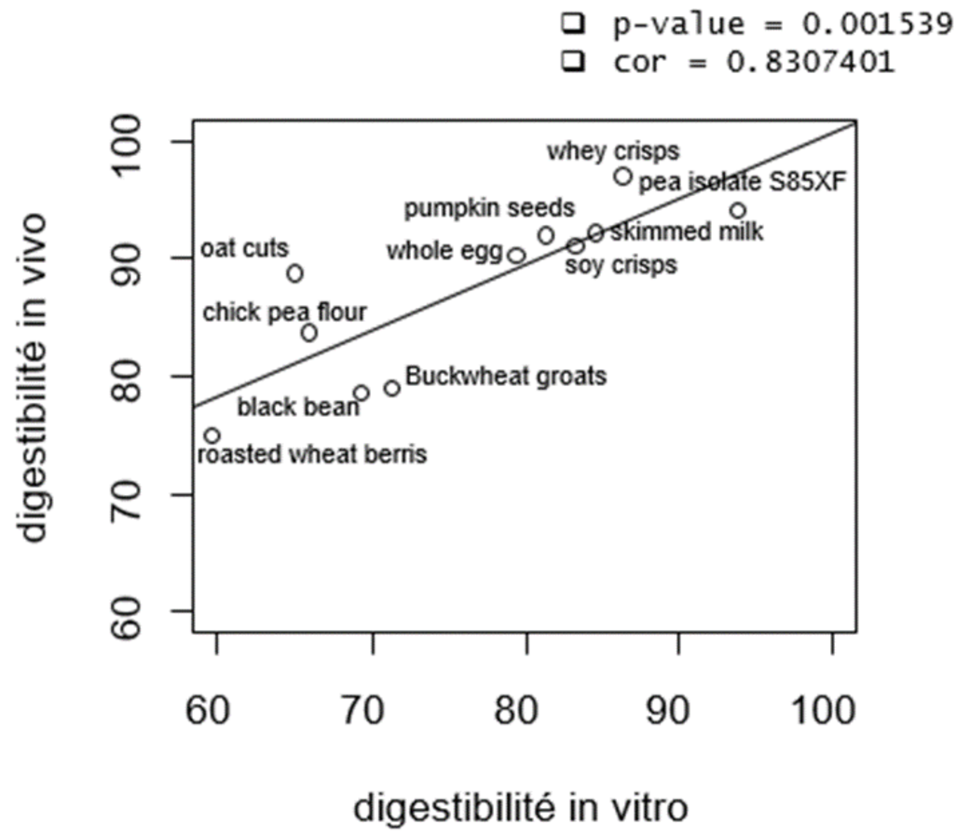
Can we estimate plant protein digestibility with *in vitro* digestion models?

Le Feunteun S, Menard O, Dupont D.
INRAE, Rennes, France

In vitro/ in vivo correlation for protein digestibility measurement



Sousa et al. 2023



Nau et al. unpublished

Overall, good correlation are observed but some differences between studies persist

Protein digestibility with a dynamic *in vitro* digestion model

Study of 4 plant-based foods: 2 solids / 2 liquids

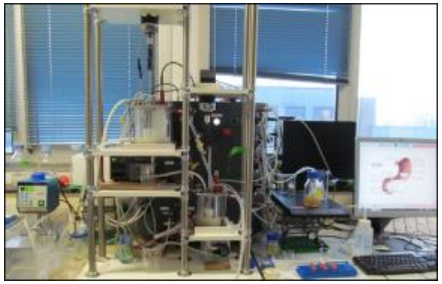
Reynaud et al. 2021
Food Chem. 341

Tofu

Soymilk

Seitan

Pea Emulsion

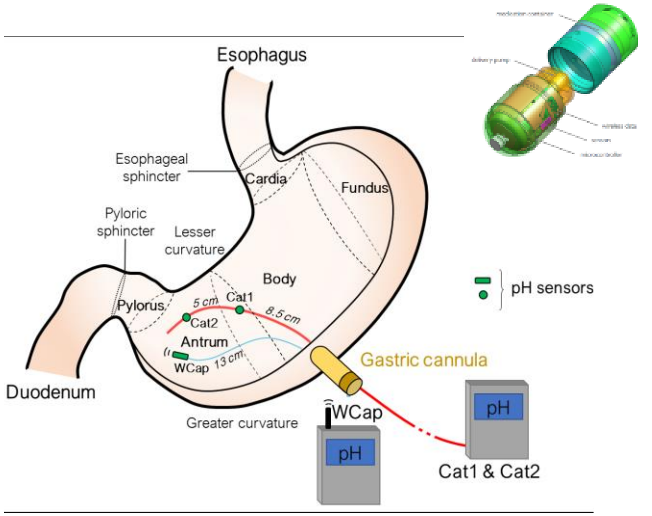


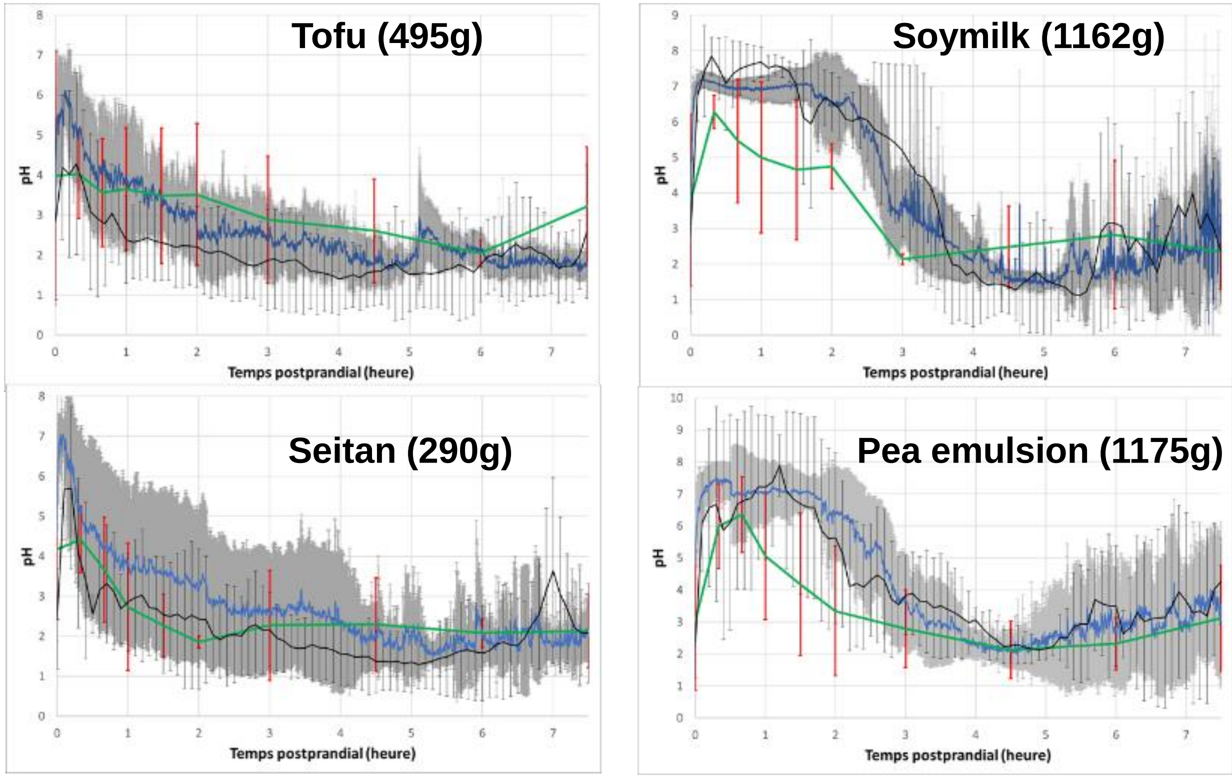
Dynamic *in vitro* digestion DiDGi®

In vitro digestibility (%)

In vivo data are needed to program the digestion simulator

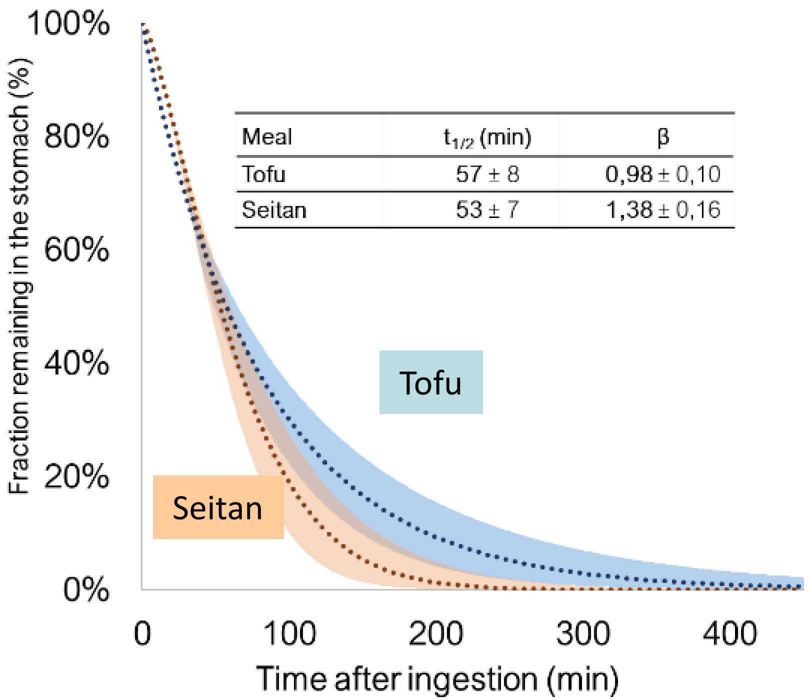
Evolution of gastric pH



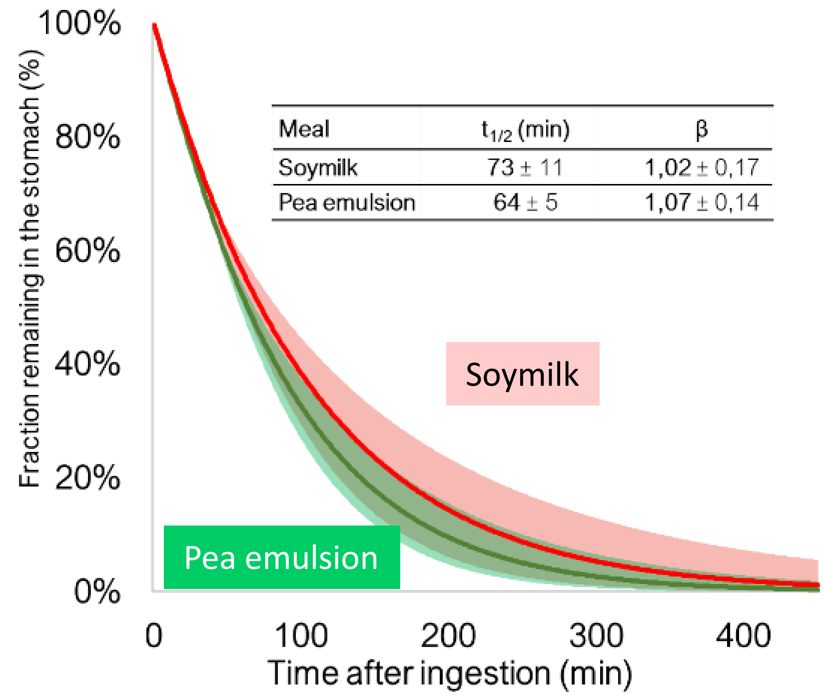


In vivo data are needed to program the digestion simulator

Gastric emptying



Meal	$t_{1/2}$ (min)	β
Tofu	57 ± 8	0,98 ± 0,10
Seitan	53 ± 7	1,38 ± 0,16



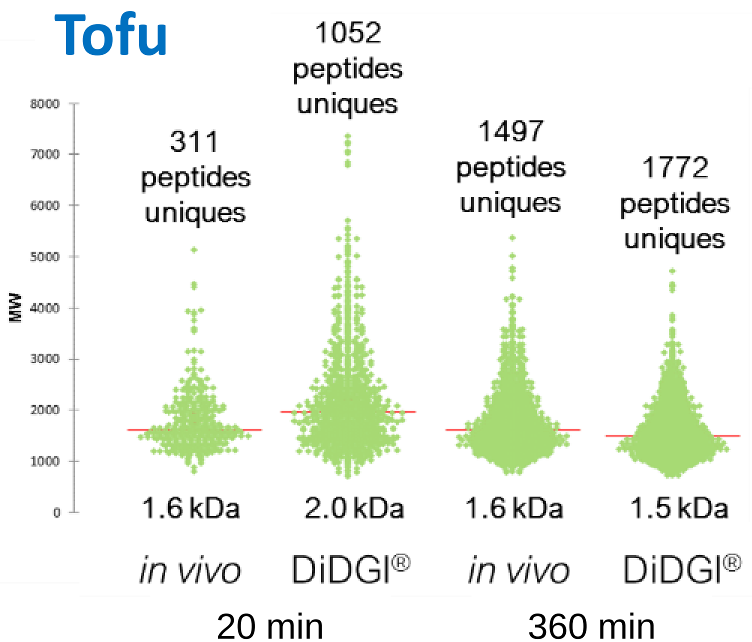
Meal	$t_{1/2}$ (min)	β
Soymilk	73 ± 11	1,02 ± 0,17
Pea emulsion	64 ± 5	1,07 ± 0,14

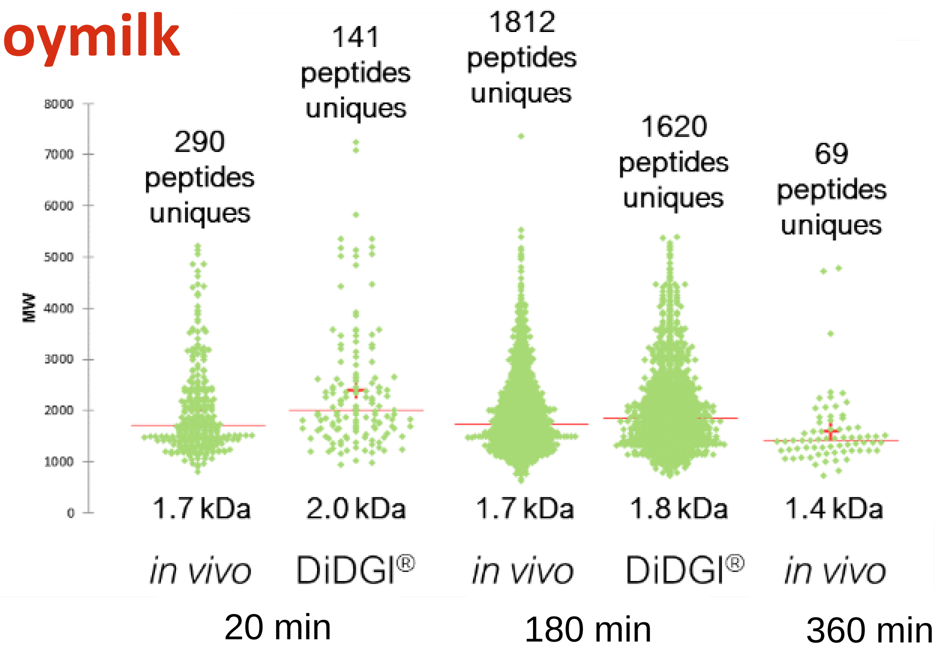
Reynaud et al. 2020
Food Res Int, 128

Comparison between pig and *in vitro* data

Model	Digestibility	Tofu	Soymilk
in vivo	True	97.1 ± 4.8%	99.4 ± 2.2%
	Apparent	**56.5 ± 7.8%[b]**	**71.3 ± 2.5%[a]**
in vitro	**Apparent simulated**	**63.7 ± 3.5%[b]**	**72.7 ± 1.4%[a]**

Comparison of the gastric peptidome





Improving DHA delivery by encapsulation and design of functional foods

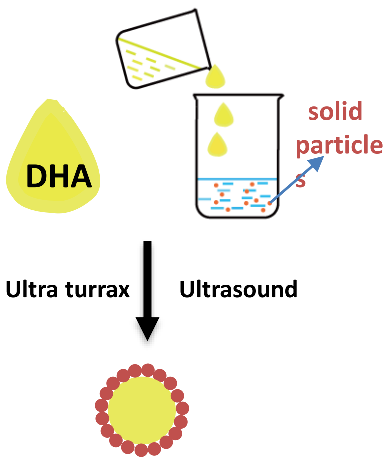
Wang J, Pedrono F, & Dupont D.
INRAE, Rennes, France

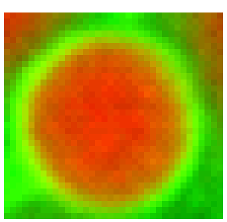
General strategy

DHA oil encapsulation

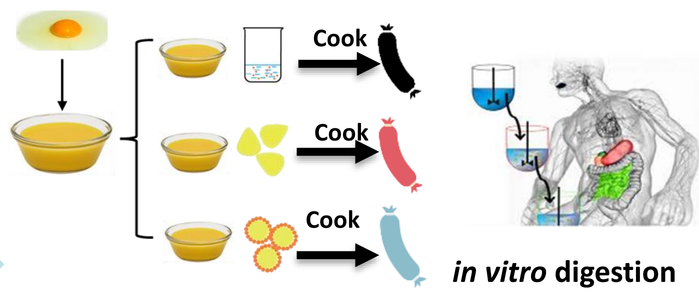
DHA bioaccessibility

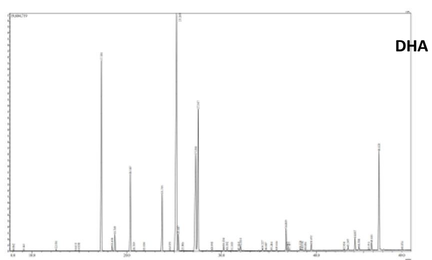
DHA bioavailability, accretion and metabolism



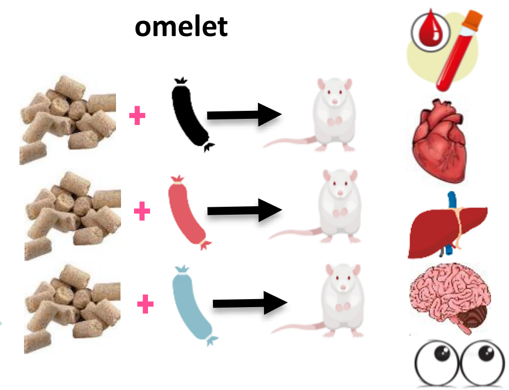


Characterization by CLSM



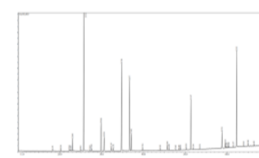


GC-MS

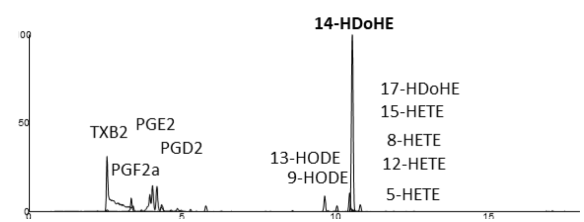


in a rat model

GC-MS



LC-QQQ



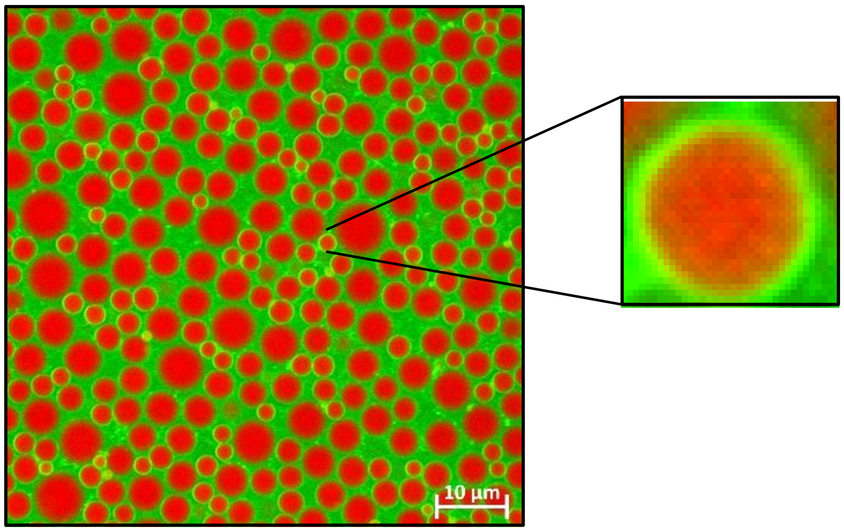
Wang *et al.*
Food Res Int 2022

Wang *et al.*
Frontiers in Nutr 2022

Wang *et al.*
Nutrients 2023

DHA oil in emulsion and omelet

In emulsion



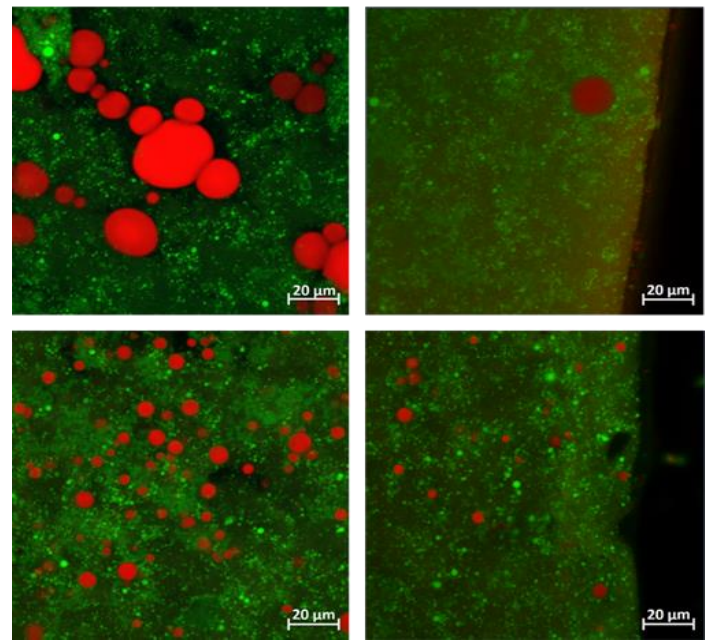
Encapsulated DHA oil with heat-denatured WPI

DHA oil stained with Nile Red and proteins stained with Fast Green.

The particle size of heat-denatured WPI on average is 42 nm.

In omelet

Center | Edge

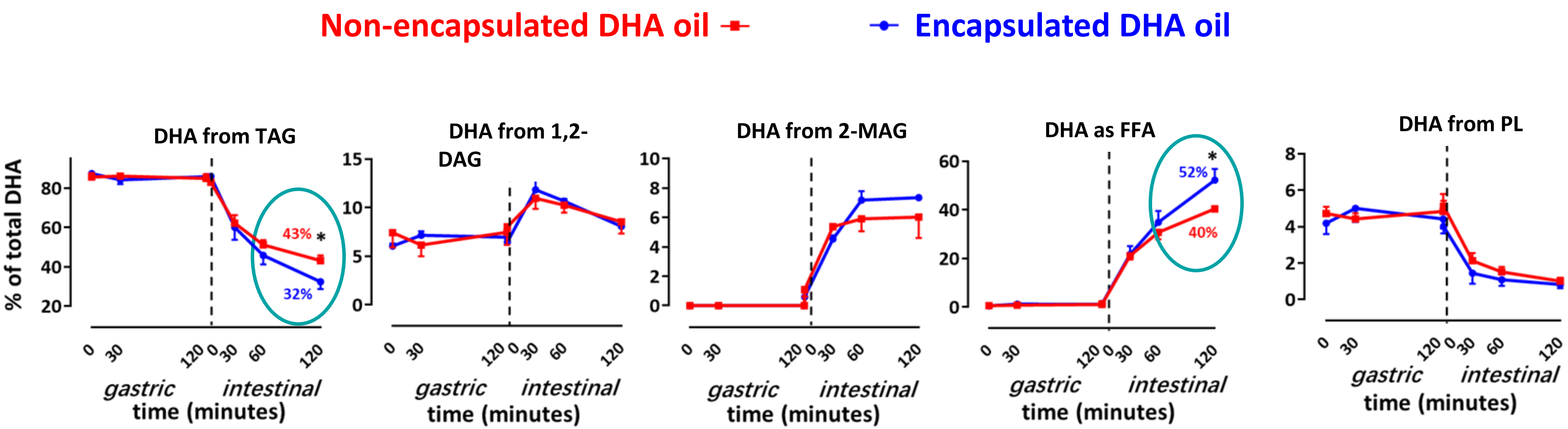


Non-encapsulated DHA oil

Encapsulated DHA oil

Distribution of non-encapsulated and encapsulated DHA oil in omelets.

Lipidomics allows to assess the bioaccessibility of DHA from different lipid species during digestion



The evolution DHA from different lipid species during digestion.

In gastric phase (pepsin and RGE):

- ☐ DHA oil was not hydrolyzed in gastric phase.

In intestinal phase (bile salt and pancreatin):

- ☐ Hydrolyzed TAG and released FFA encapsulation > unencapsulation

- ☐ **Larger interaction area between DHA oil and pancreatic lipase made by emulsification** *(Maljaars, 2012).*

- ☐ Around **10-25%** and 40-70% of ingested **TAG** can be hydrolyzed **in gastric** and intestinal phase, respectively *(Bauer et al; Carriere et al., 1993).*

Understanding human milk digestion to design new infant formulas that will have the same behaviour in the GI tract

INRA
SCIENCE & IMPACT

Deglaire A., Menard O., De Oliveira S., Bourlieu C.
& Dupont D.

INRAE, Rennes, France

Human/ bovine milk / Infant Formula

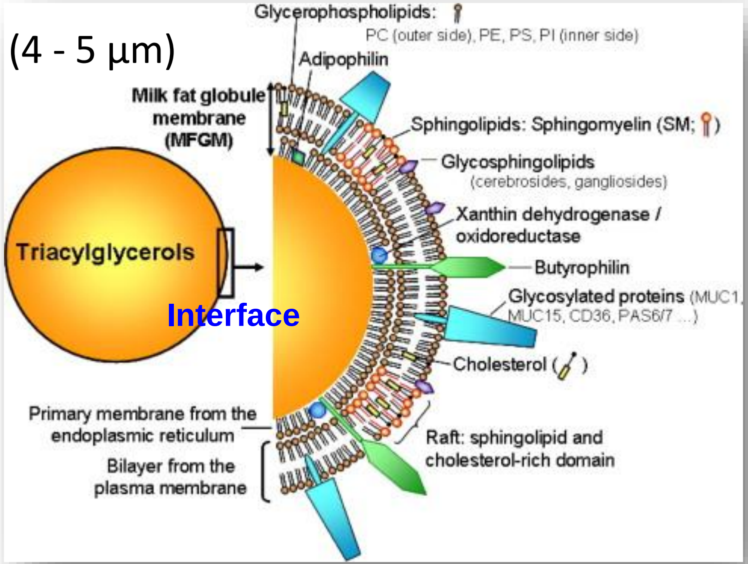
Lipid globule structure

Human milk

Bovine milk

Infant Formula

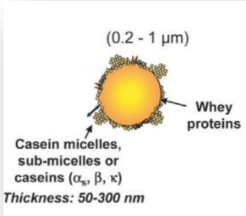
Native milk fat globule



(Lopez, 2010)

Lipid droplets

Triacylglycerols

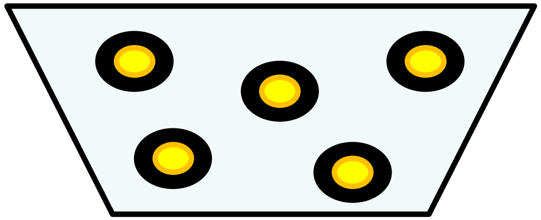


(0,2 - 1 µm)

(Lopez and Briard-Bion, 2007)

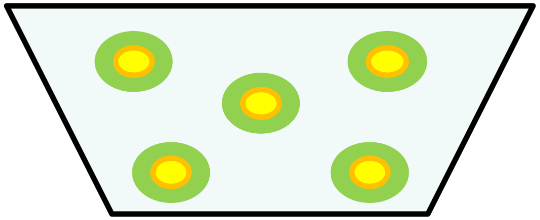
Infant formulas: can we create lipid structures biomimetic on the native fat globule?

Formula T1



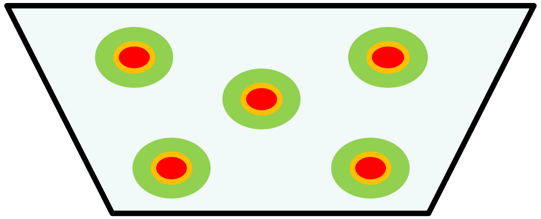
Interface 100 % Proteins
100% vegetable oil

Formula T2

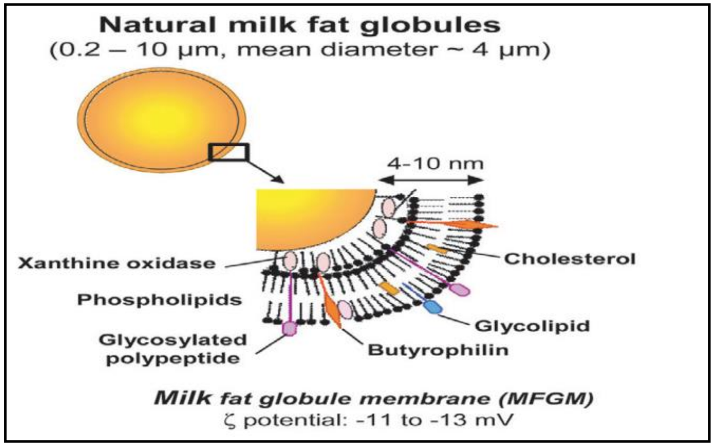


Interface 100 % phospholipids
100% vegetable oil

Formula T3

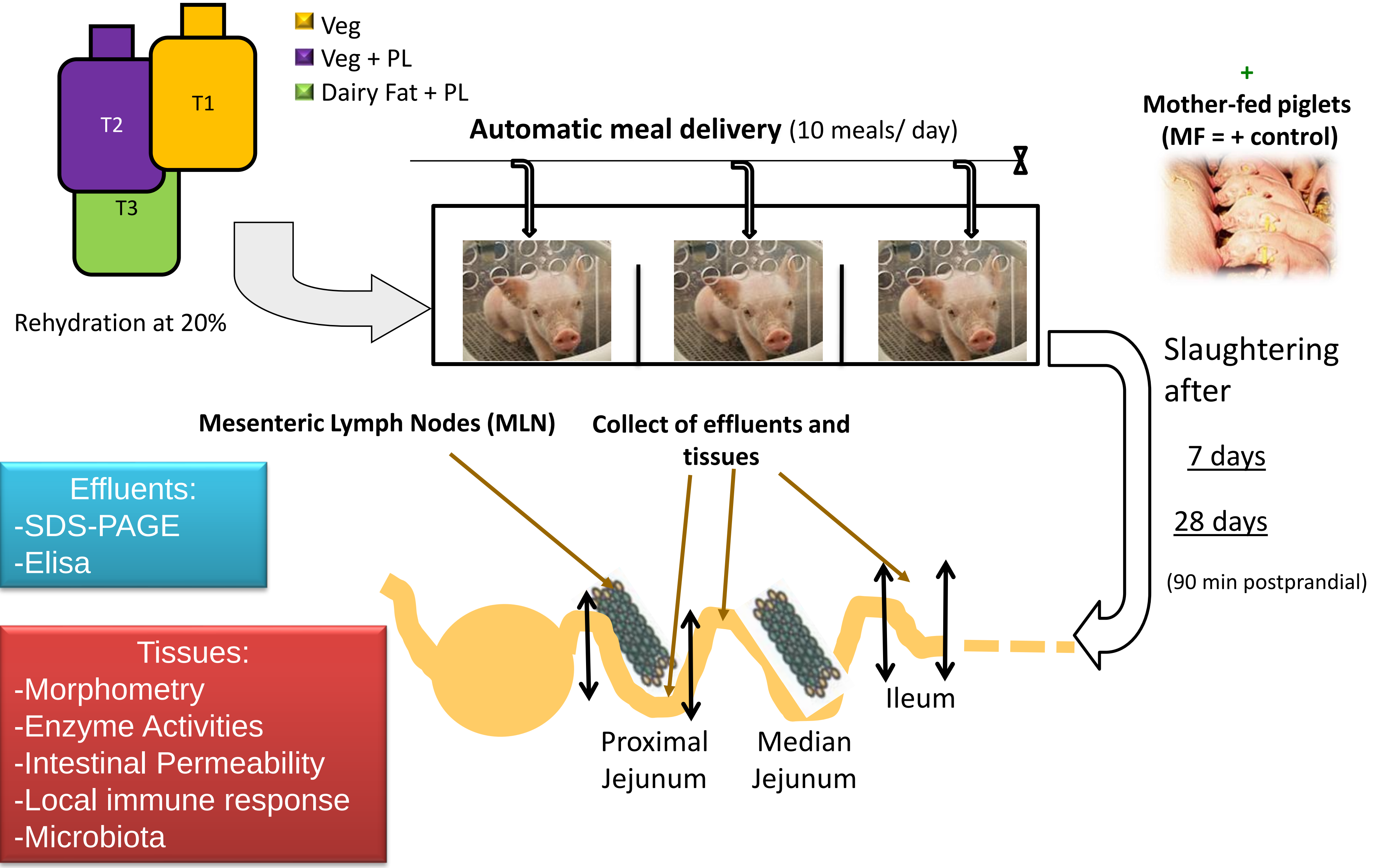


Interface 100 % phospholipides
40% vegetable oil + 60% milk fat

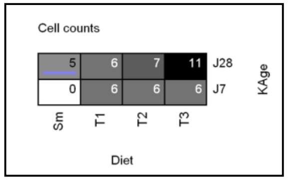


Lopez, (2007)

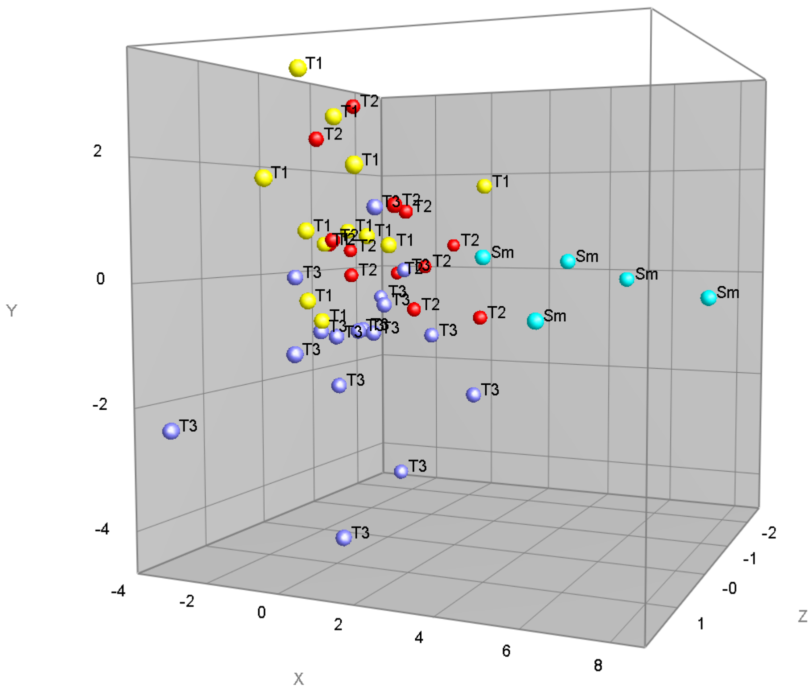
Can the composition of infant formula modulate the physiological response of the neonate?



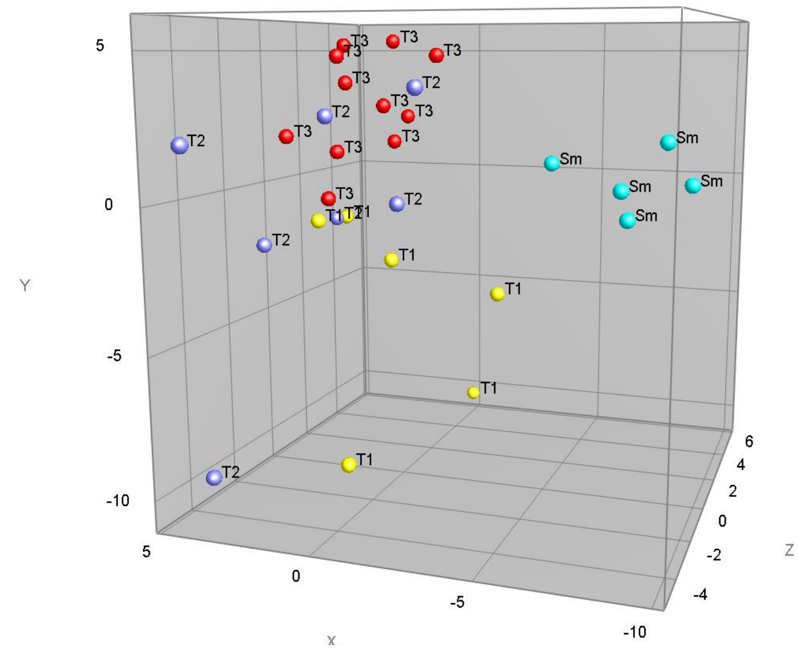
Microbiota by DHPLC



D7 & D28

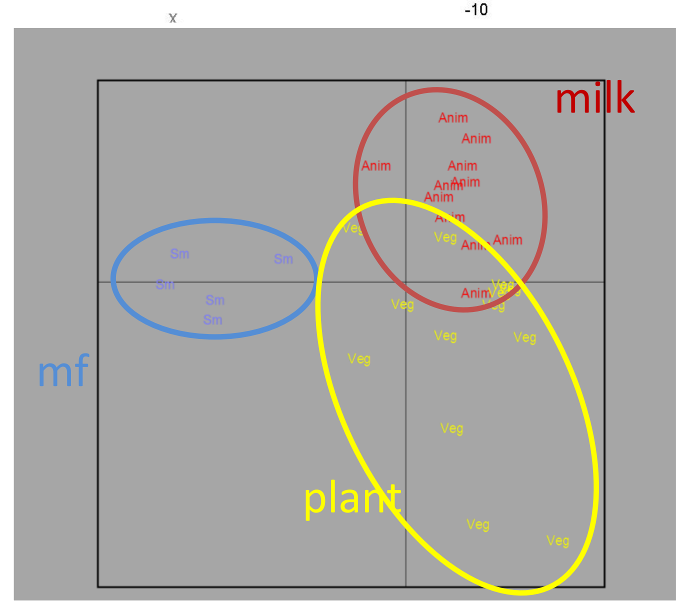


D28



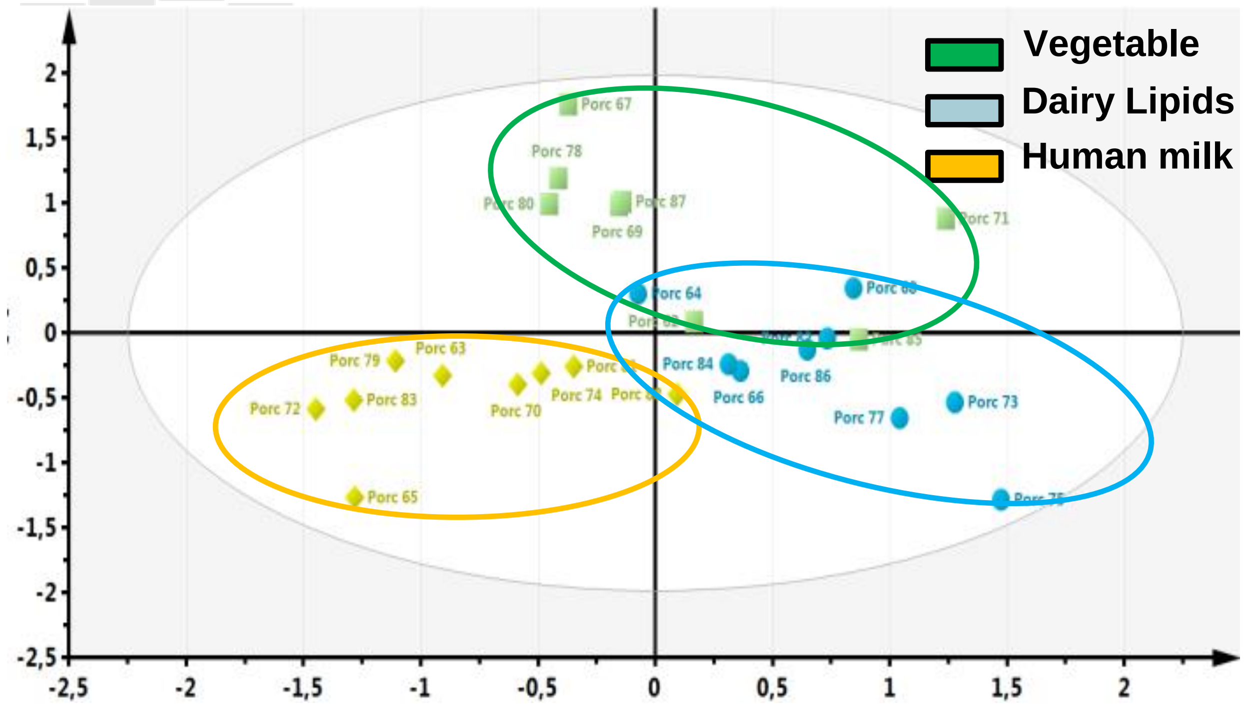
The composition/structure of the infant formula « orientates » the microbiota

More Proteobacteria with milk fat / More Firmicutes with plant oil



Bourlieu et al. Eur J Lipid Sci Technol 2016

What happens when they become older (140 d)?



If animals are submitted to a nutritional stress (high fat/sugar diet), some differences remain in:

* the microbiota composition
* the fecal metabolome with different metabolites (including propionate)
* the immune system with a reduced susceptibility to inflammation with milk lipids

Conclusion

The structure/composition of food regulate the kinetics of protein digestion in the gastrointestinal tract and the release of amino acids in the bloodstream

Omic technologies (proteomics, peptidomics, lipidomics…) are great tools to identify the molecules that are released in the gut during digestion and assess the bioaccessibility of nutrients

Being able to design food structures for controlling the kinetics of hydrolysis of macronutrients will allow to obtain food particularly adapted to specific population

The Bioactivity & Nutrition team at INRAE Rennes

Scientists

Amélie DEGLAIRE – *Lecturer*
Didier DUPONT- Senior Scientist
Catherine GUERIN - *Lecturer*
Steven LE FEUNTEUN – *Senior Scientist*
Vincent Mathieu – Senior Scientist
Martine MORZEL – *Senior Scientist*
Françoise NAU – *Professor*
Frédérique PEDRONO – *Lecturer*

Isuri JAYAWARDA– *Post-doc*
Mathilde CANCALON- *Post-doc*
Rozenn LE FOLL – *Post-doc*
Anaïs LAVOISIER – *Post-doc*

PhD students

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Eleonora LOFFREDI
Vibhu MISHRA
Simon POGU
Tanguy SAVIARD

Technicians

Severine CHEVALIER
Gwenaële HENRY
Yann LE GOUAR

Engineers

Marie-Françoise COCHET
Julien JARDIN
Olivia MENARD
Jordane OSSEMOND

Masters students

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

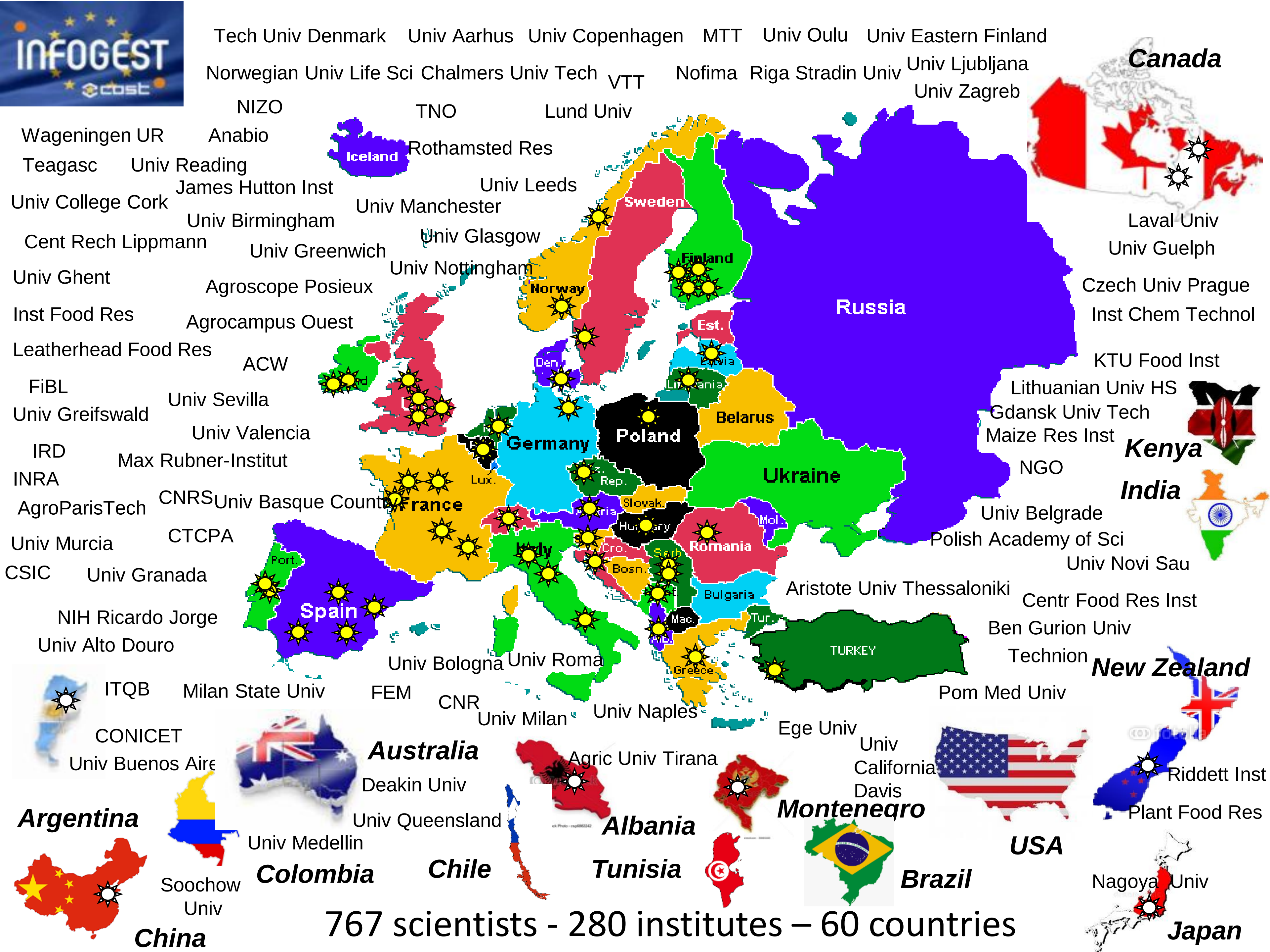
International Network

Dr. Didier DUPONT, Senior Scientist, INRAE, France

INFOGEST

Main objective: understanding the mechanisms of food digestion

- Develop new *in vitro*, *in vivo* and *in silico* digestion models including some for specific populations (infant, elderly)
- 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



767 scientists - 280 institutes – 60 countries

Industry involvement

☞ ~ 60 private companies are following INFOGEST

7 Working Groups running in parallel

INFOGEST

Chair
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didier.dupont@inrae.fr

www.cost-infogest.eu

Vice-chair
Alan Mackie - UK

In vitro models of digestion
WG1

Food interaction – meal digestion
WG2

Absorption models
WG3

Digestive lipases and lipid digestion
WG4

Digestive amylases and starch digestion
WG5

In silico models of digestion
WG6

Imaging Technologies applied to digestion
WG7

Isidra Recio

Pasquale Ferranti

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Steven Le Feunteun

Paul Smeets

Andre Brodkorb

Lotti Egger

Uri Lesmes

Brigitte Graf

Marion Letisse

Leslie Couedelo

Marilisa Alongi

Choi-Hong Lan

Luca Marciani

Some outputs

In vitro gastrointestinal digestion
Consensus INFOGEST protocol

Minekus et al. 2014
Food & Function, 5, 1113-1124
3125 citations

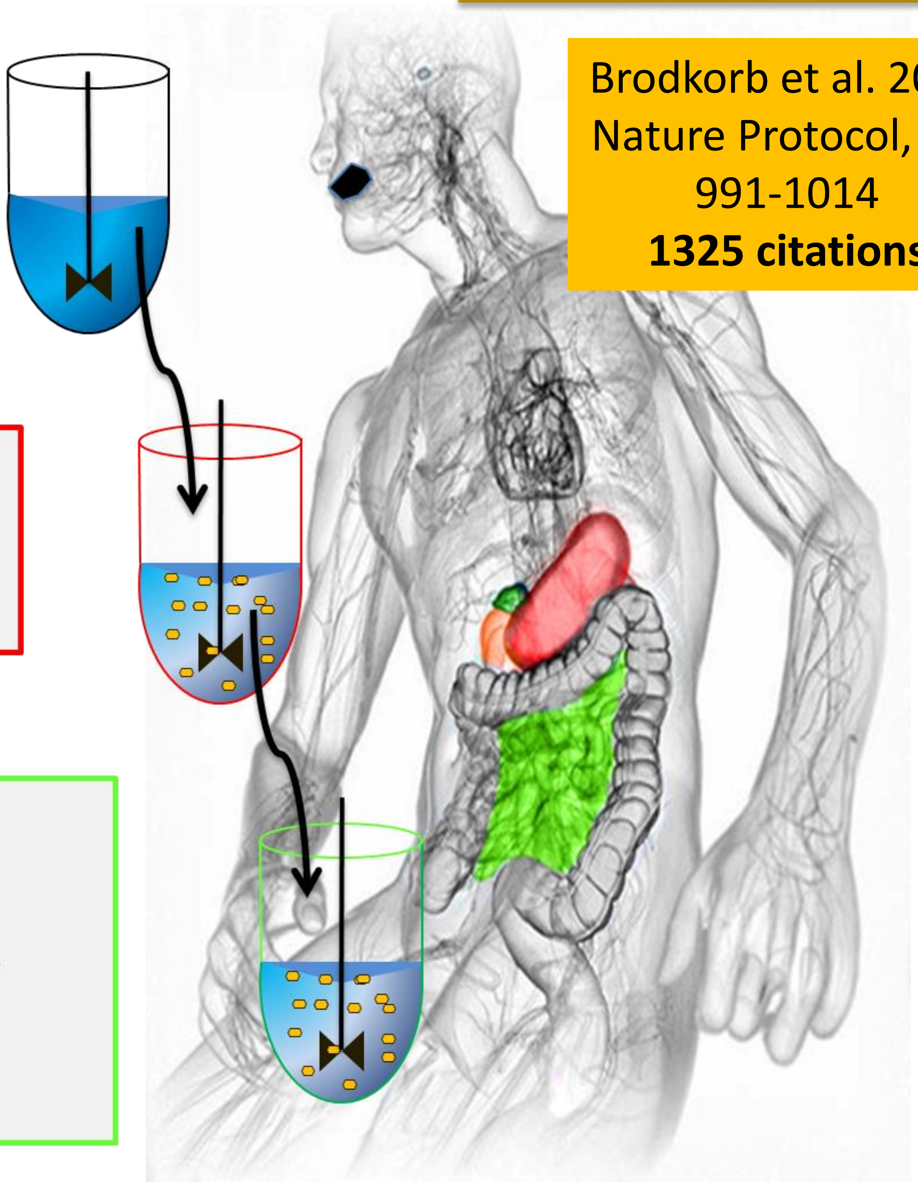
Brodkorb et al. 2019
Nature Protocol, 14, 991-1014
1325 citations

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



INFOGEST
International Conference
Porto, Portugal
2024

We are pleased to announce the next

8th International Conference on Food Digestion

in Porto, Portugal, 9-11 April 2024