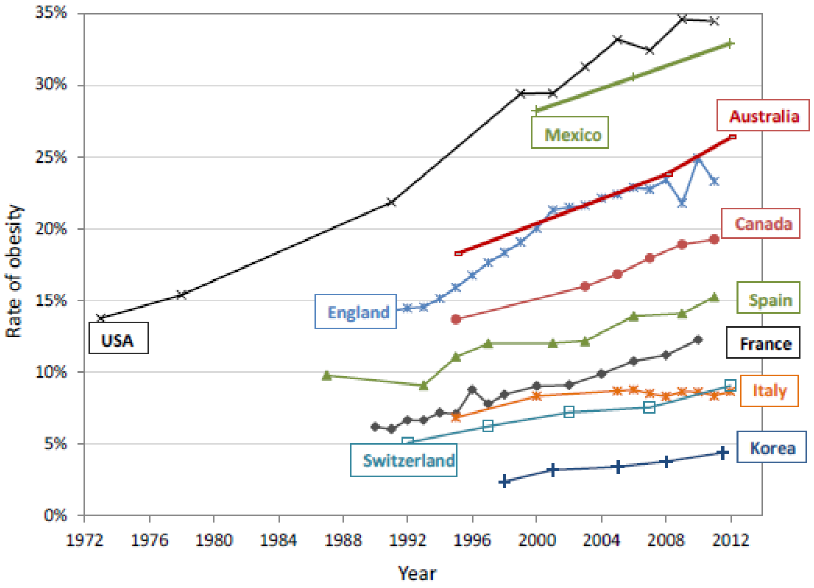
The structure of the food matrix at different length scales affects the mechanisms of digestion and the nutrient bioavailability

Didier Dupont

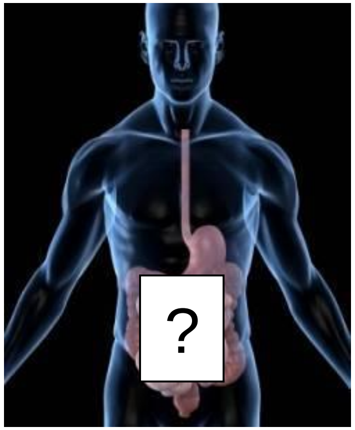
The structure of the food matrix at different length scales affects the mechanisms of digestion and the nutrient bioavailability

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



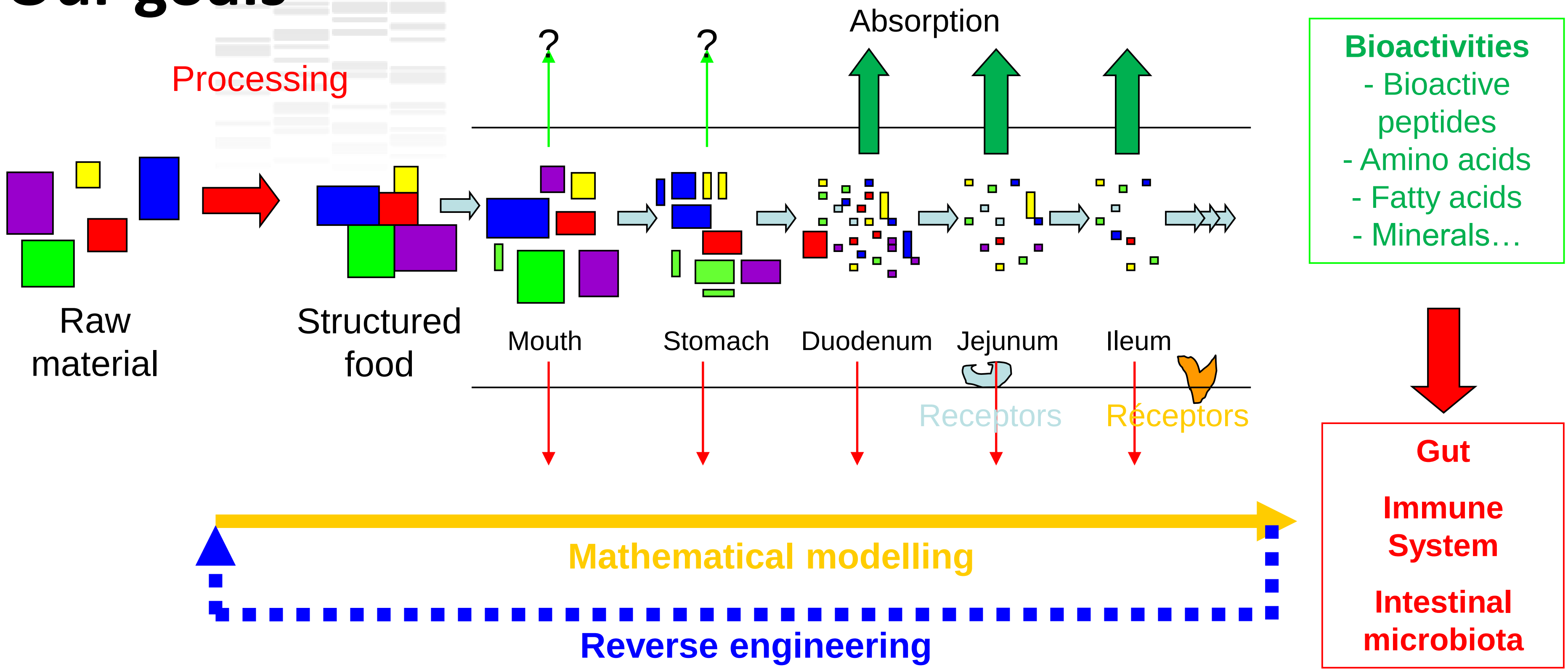
Gut = interface between food and human body
Digestion releases food components that can have a beneficial or a deleterious effect on human health

… 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

Healthy Adult/ Infant / Elderly

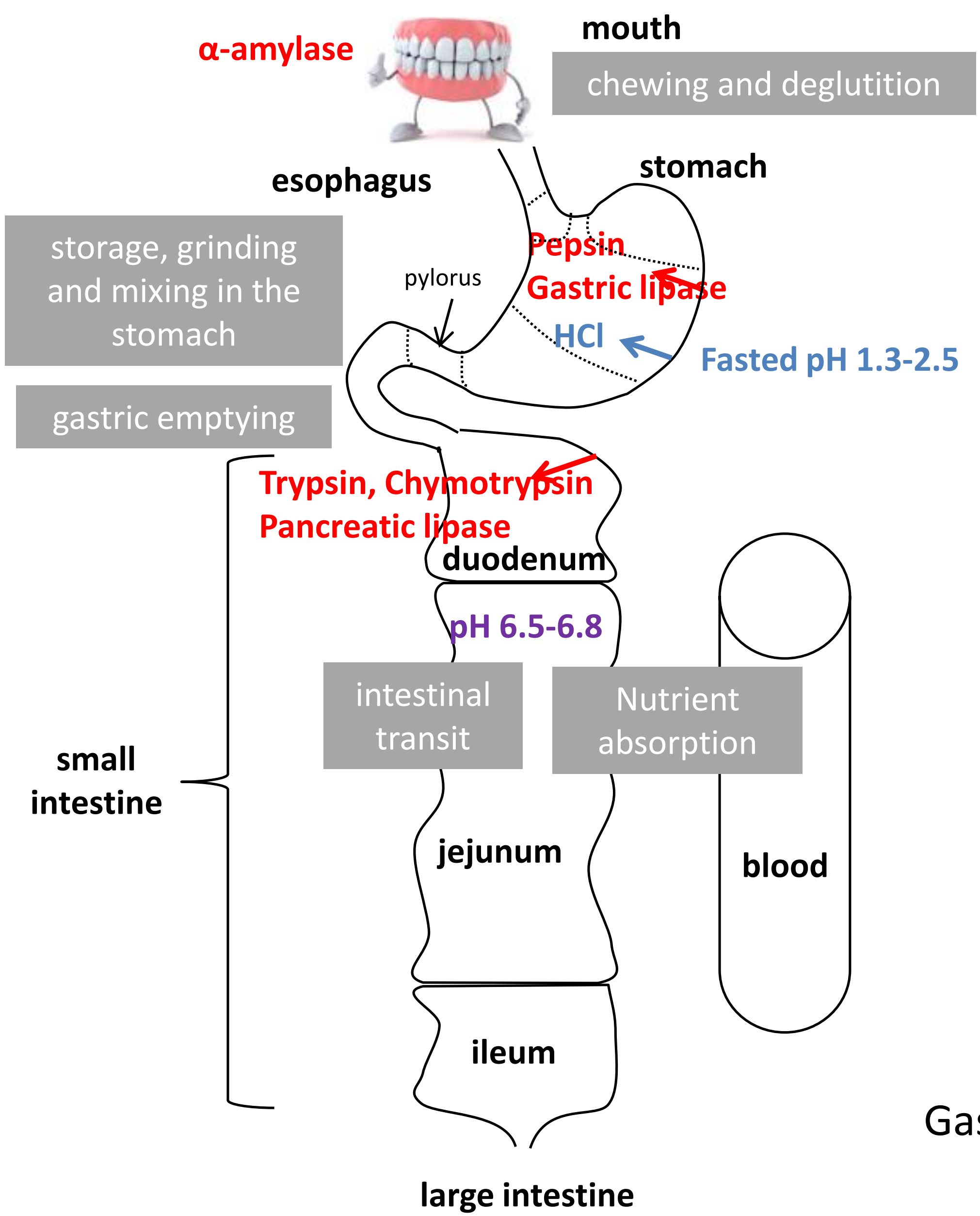


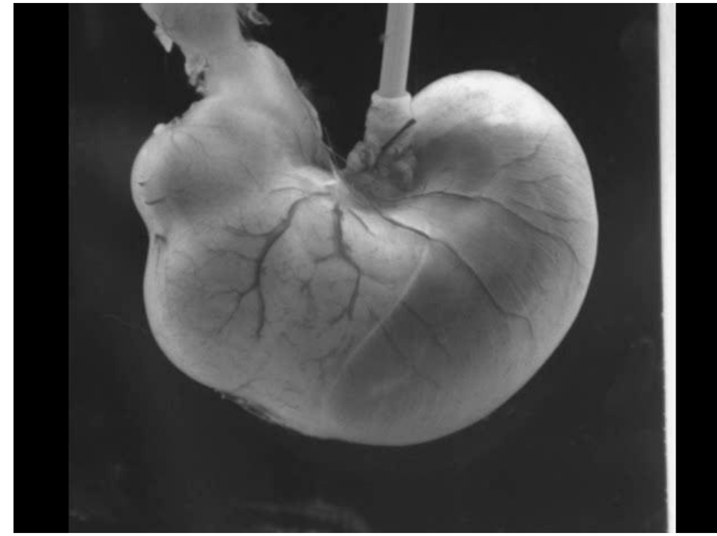
☞To understand the mechanisms of breakdown of food matrices and their constituents in the gut and identify the beneficial/deleterious food components released during digestion

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

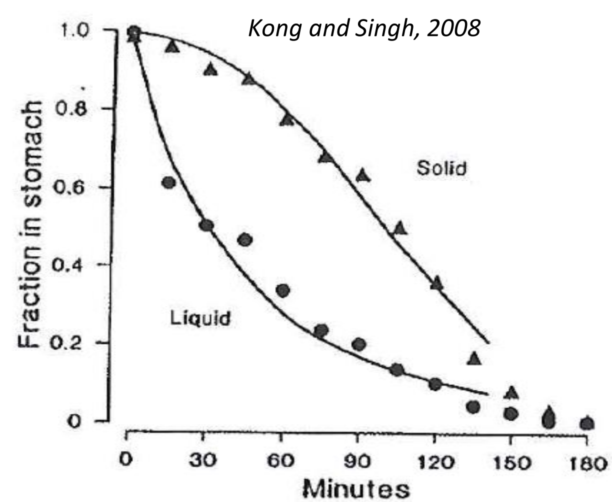
☞To model these phenomena in order to develop a reverse engineering approach

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)

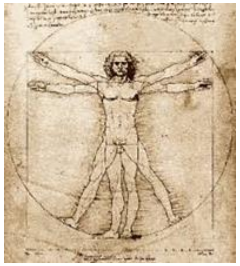
Peng et al. 2021
Halabi et al. 2021
Giribaldi et al. 2021
Chauvet et al. 2023
Nebbia et al. 2022, 2023

In vitro dynamic models (infant, adult, elderly)

Le Feunteun et al. 2014, 2020

In silico models

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



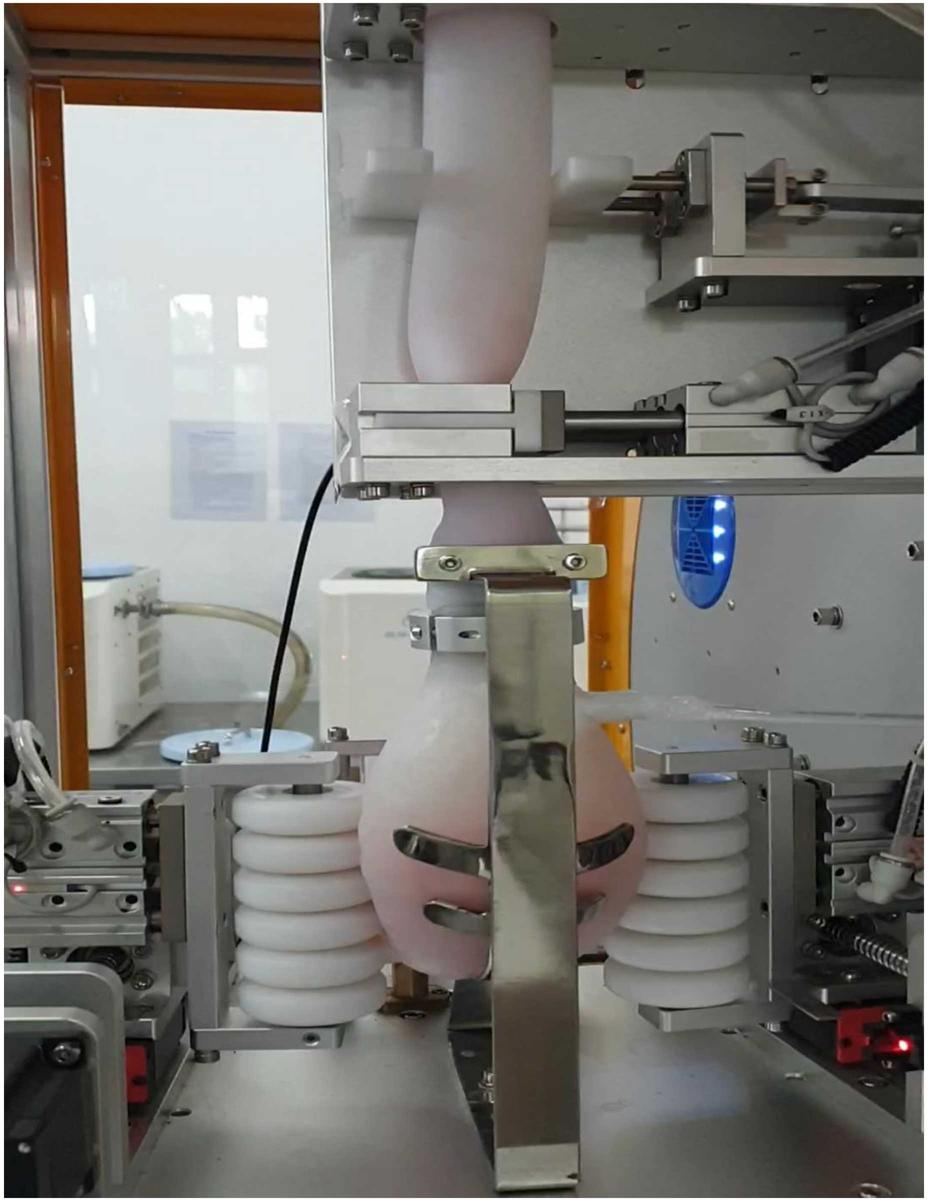
Human models

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



STOMACH

Xiao Dong Pro-Health
Smart Digestion
Suzhou University

SOOCHOW UNIVERSITY
UNTO A FULL GROWN MAN

INRAE

L'INSTITUT agro Rennes Angers

INSTITUT CARNOT QUALIMENT

The molecular structure of food protein affects the kinetics of digestion

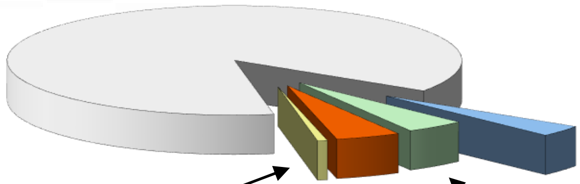
INRA
SCIENCE & IMPACT

Dupont D.

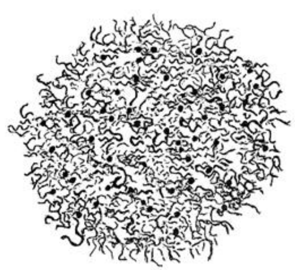
INRAE, Rennes, France

Milk

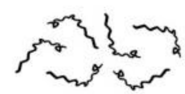
Molecular scale



ø~200 nm
(Holt, 1994)



Casein organized into a supramolecular structure: the **casein micelle (CM)**

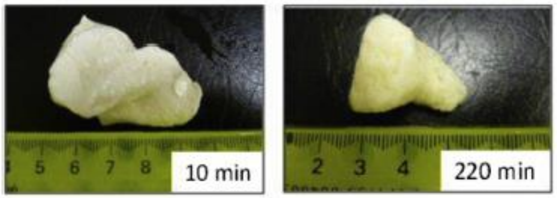


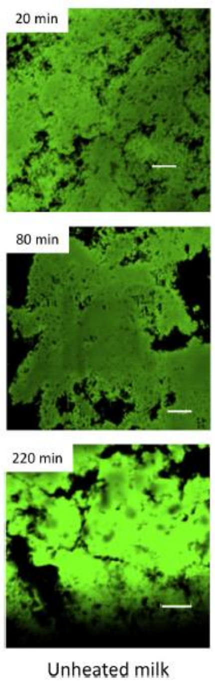
ø~11 nm
~15 casein molecules;
(Thomar *et al*. 2013)

Casein can also be extracted after acidification followed by neutralization: the **caseinate (CS)**

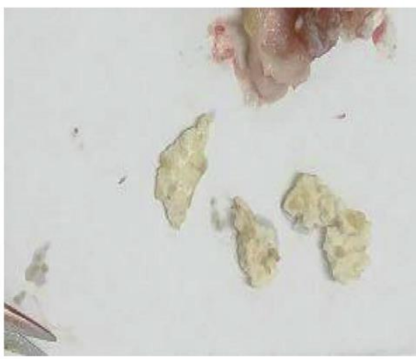
Milk coagulates in the stomach

Molecular scale

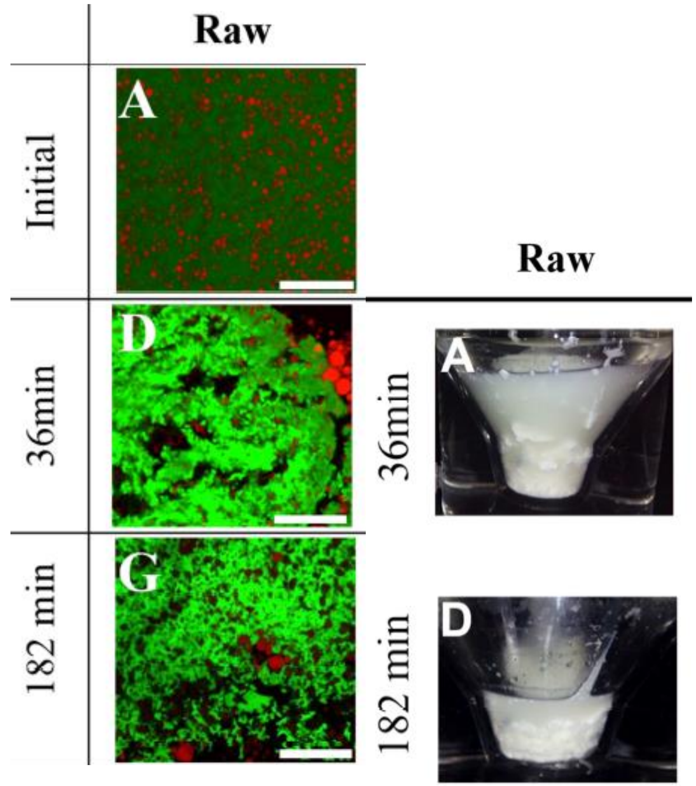




In vitro demonstration using the HGS
Ye *et al*. 2016



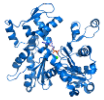
Ye *et al*. 2019
In vivo evidence
using a rat model



Mulet-Cabero *et al*. 2019
In vitro semi-dynamic model

Casein micelles and sodium caseinate form different coagulums
Wang *et al*. 2018

Objective

Molecular scale 

The objectives of the study were to:

1 Determine whether gastric emptying of an isoproteic solution of CM and CS are different or not (exp. 1)

2 Characterize the structure of the resulting chyme and determine if CM and CS are differently metabolized (exp. 2)

Experiment 1 – Determination of Gastric Emptying

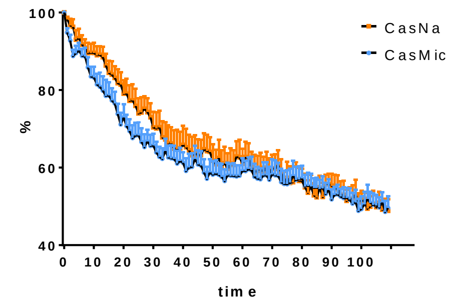
γ-scintigraphy over 120 min

96 g of CM or CS rehydrated in 800 ml of water

+12 g of glucose

+ ^{99m}Tc-colloidal (25Mbq)

9 pigs (20-25 kg)

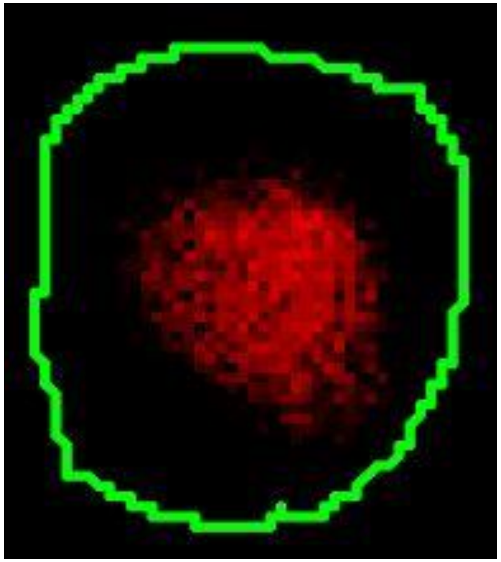


Gastric emptying half-time ($T_{1/2}$) and shape of the curve (β)

But a differential behaviour of CS and CM in the stomach

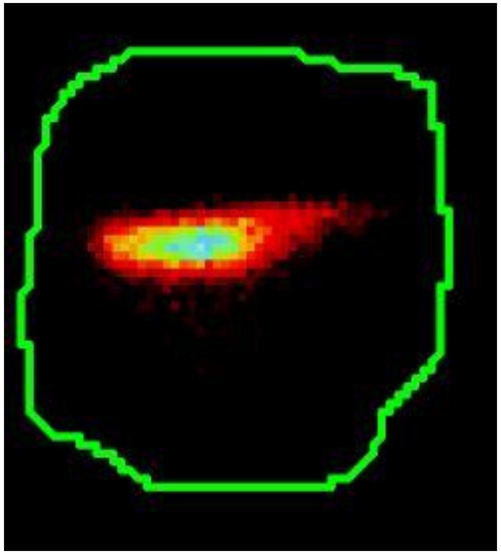
Exemple scintigraphic images at the beginning of gastric emptying (5-10 min after ingestion)

CM



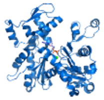
Radioactivity fully fills the stomach

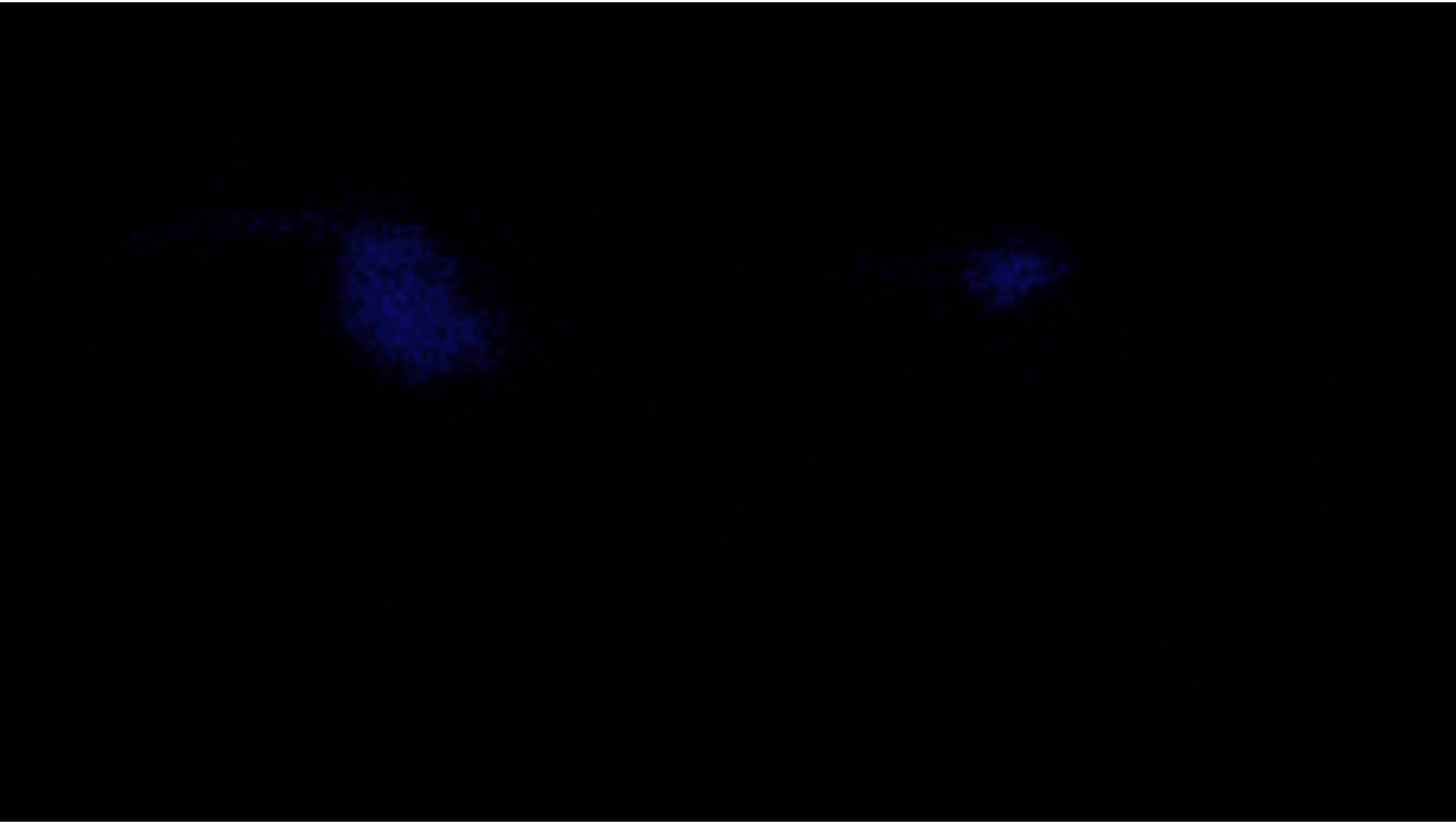
CS



Radioactivity is highly concentrated in the proximal part of the stomach

Gastric emptying

Molecular scale 



Objective

Molecular scale

The objectives of the study were to:

1 Determine whether gastric emptying of an isoproteic solution of CM and CS are different or not (exp. 1)

2 Characterize the structure of the resulting chyme and determine if CM and CS are differently metabolized (exp. 2)

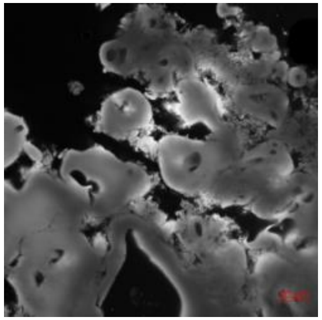
Experiment 2 – Chyme structure and protein metabolism

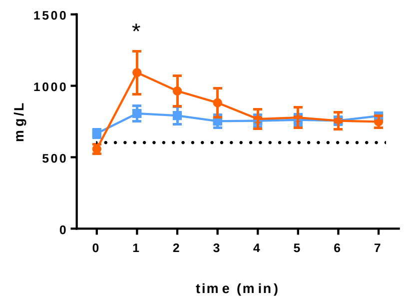
96 g of CM or CS rehydrated in 800 ml of water

+12 g of glucose

10 catheterized pigs (20-25 kg)

Characterization of the chyme structure (slaughtering after 10 min, n=4)





Free plasma amino acids over 7h n=6

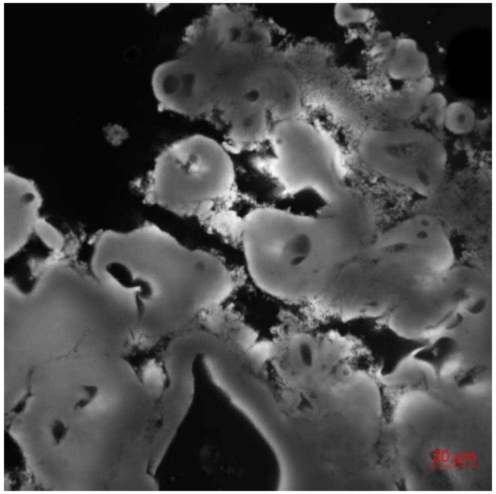
Collection of the stomach contents

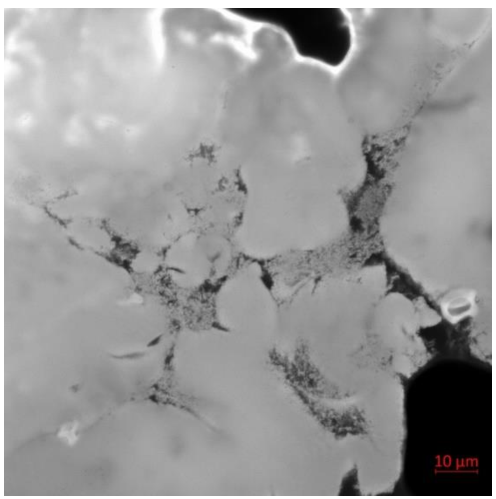
CM forms a large coagulum in the stomach whereas CS mainly remains in the liquid form

Microstructure of gastric chymes

Molecular scale

CM





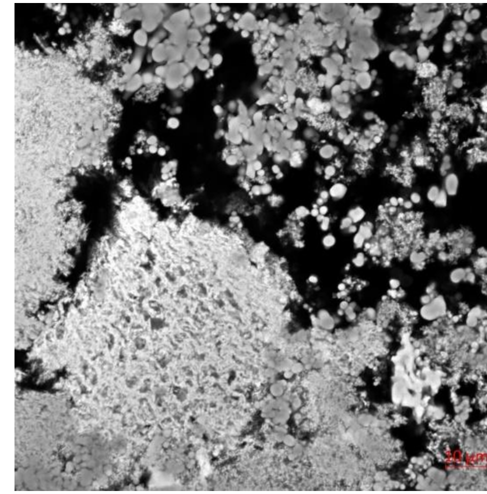
CM Gels (left) are compact and dense =

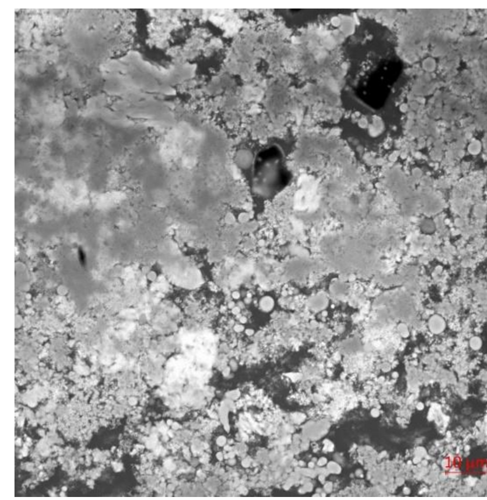
Strong coagulum

CS Gels (right) are an agglomerate of spherical particles that can easily dissociate. The gel have a very « loose » structure =

Protein precipitate

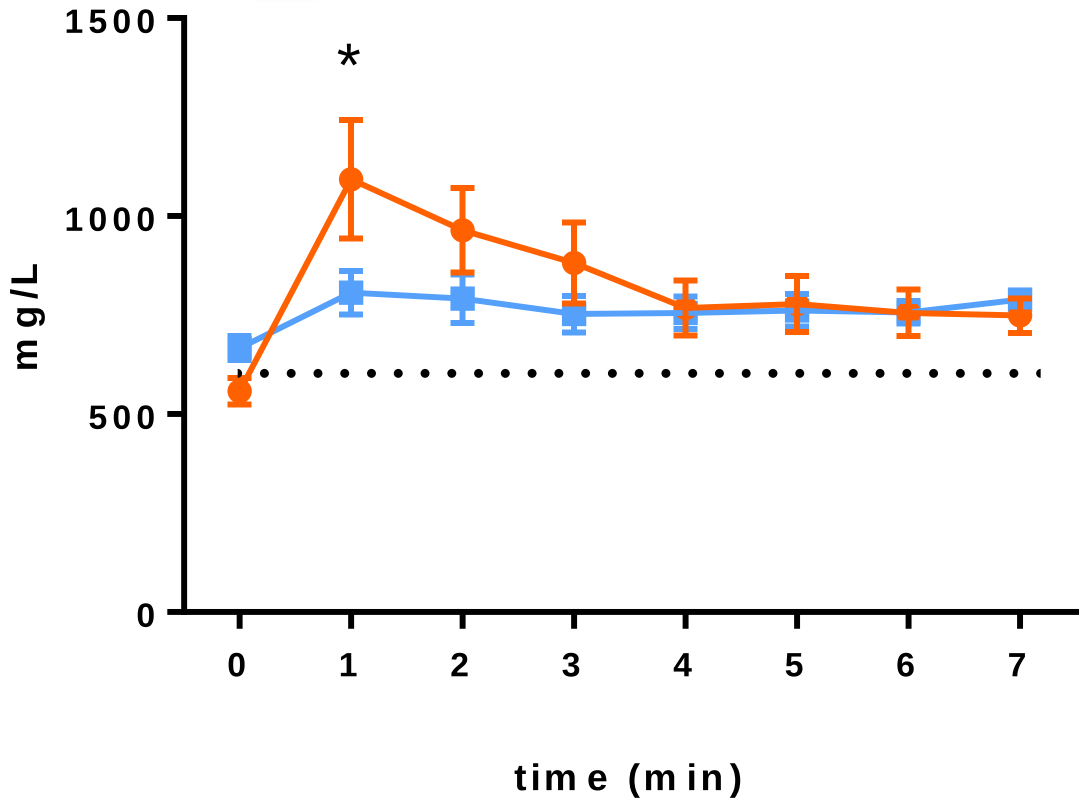
CS





Plasma amino acids

Molecular scale



casein x time
P=0.0007

Boulier *et al.* Food Chem. 2023

AA peak after 1h for CM whereas the concentration remains stable for CS

Conclusion

* CM form a strong coagulum in the stomach leading to a slow and constant release of plasma amino acids up to 7 h

* CS form a loose precipitate in the proximal part of the stomach but most of the caseins remain solubilized in the liquid fraction

* CS are rapidly metabolized in the small intestine leading to the appearance of a peak of plasma amino acids one hour after protein ingestion

CM = slow caseins, CS = fast caseins

Perspectives

Do some *in vivo* kinetics experiments
Use ^{15}N labelled-caseins to differentiate endo/exogenous proteins

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

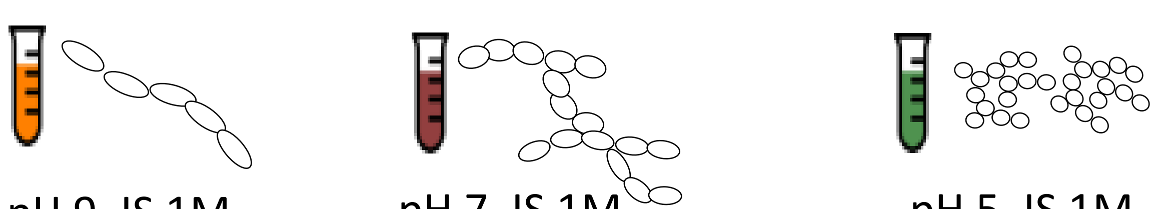
Dupont D.

INRAE, Rennes, France

The microstructure of egg-white gels made from different types of aggregates affects the kinetics of proteolysis

Microscopic scale

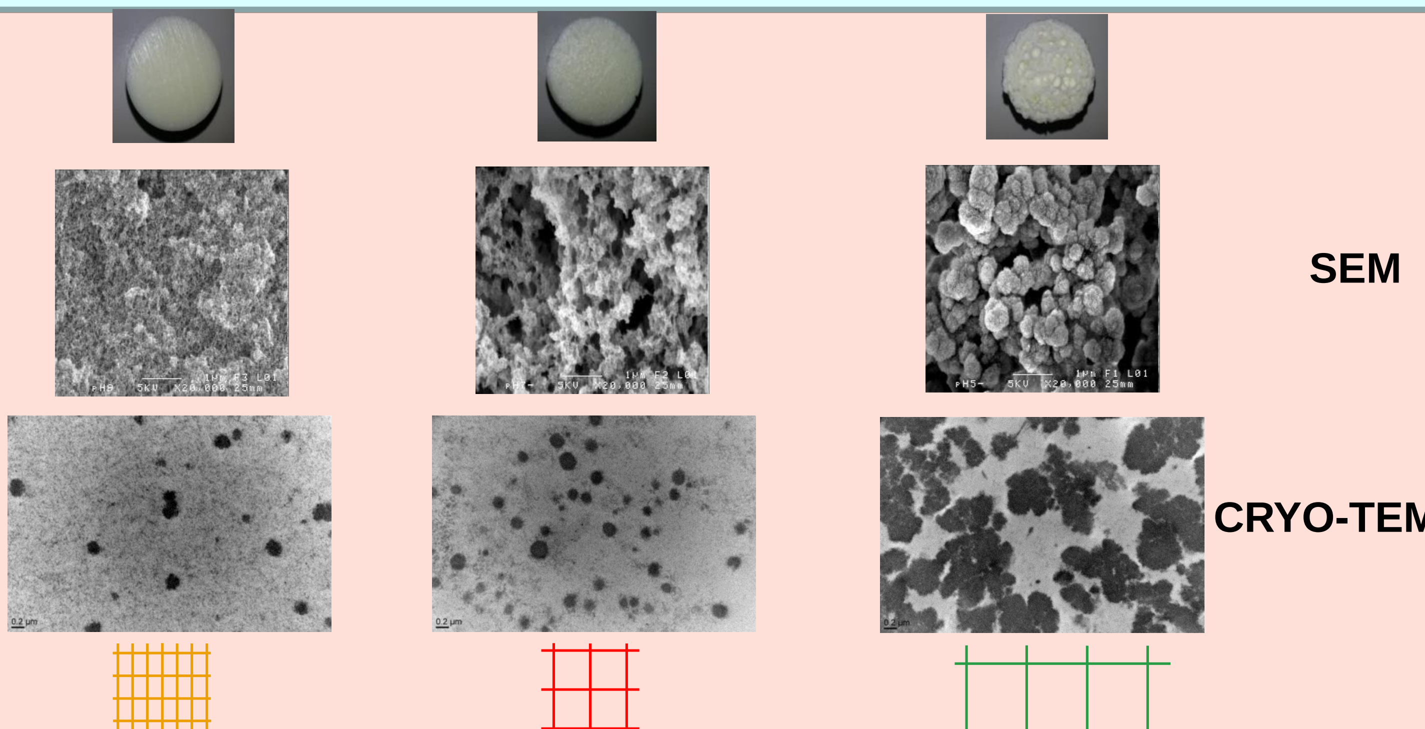
80°C/6h



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

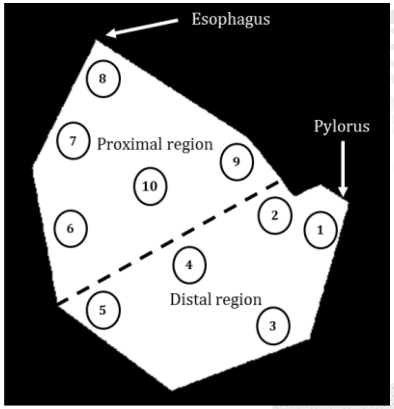


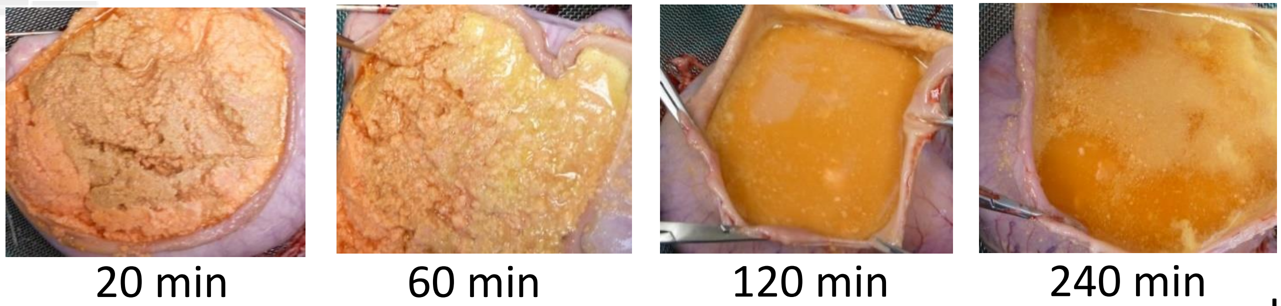
Nyemb *et al.*
Food Hydro. 2016
Nyemb *et al.*
Food Res Int 2016

Rate of *in vitro* digestion	+	++	+++

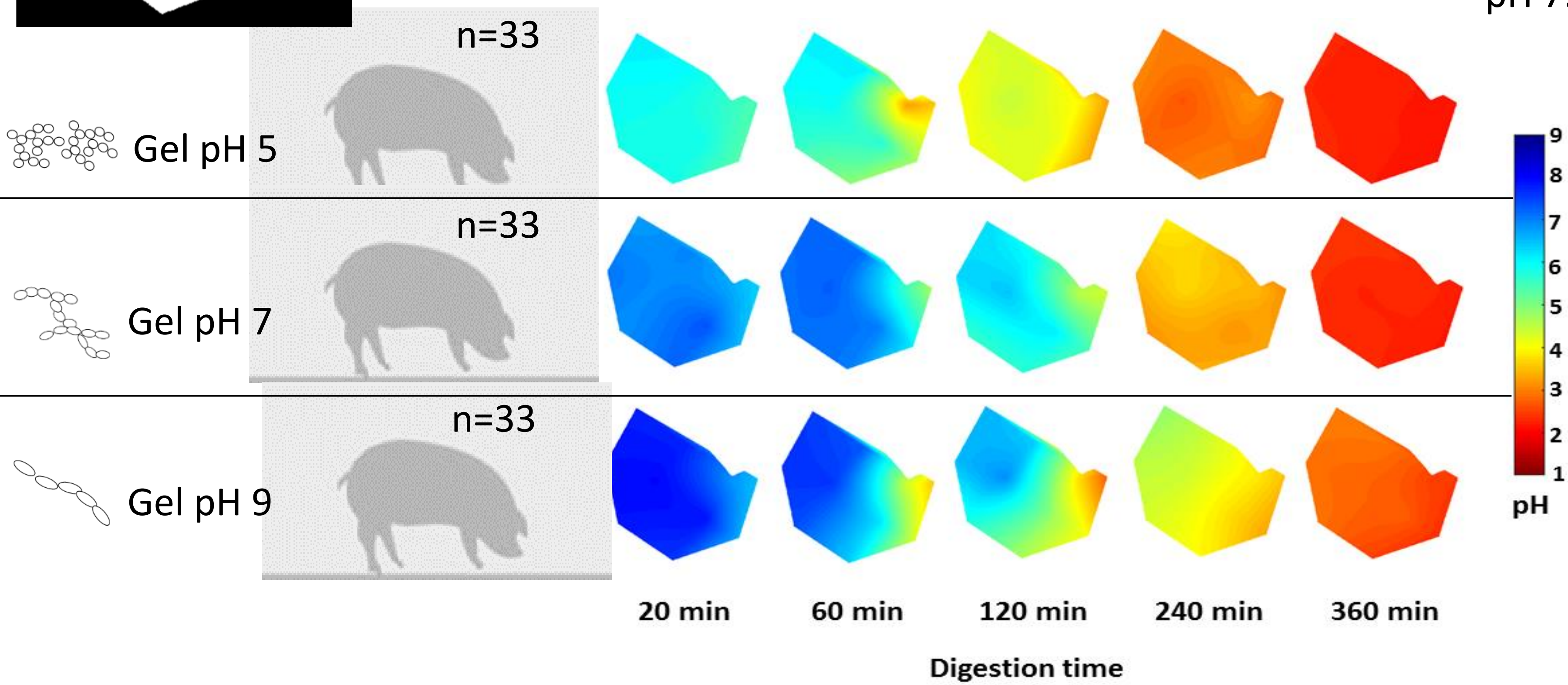
Spatial-temporal evolution of pH during an *in vivo* digestion

Microscopic scale



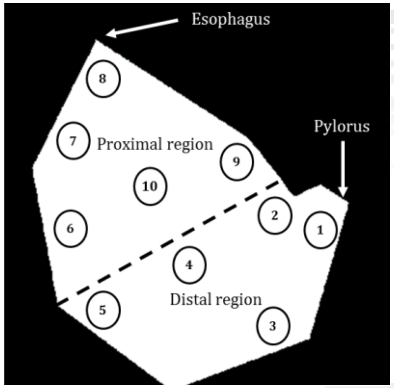


pH 7.0

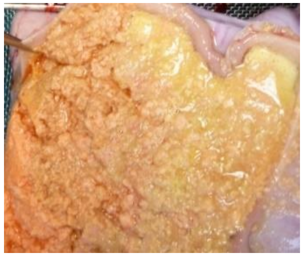


Nau *et al.*
Food Chem. 2019

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



20 min

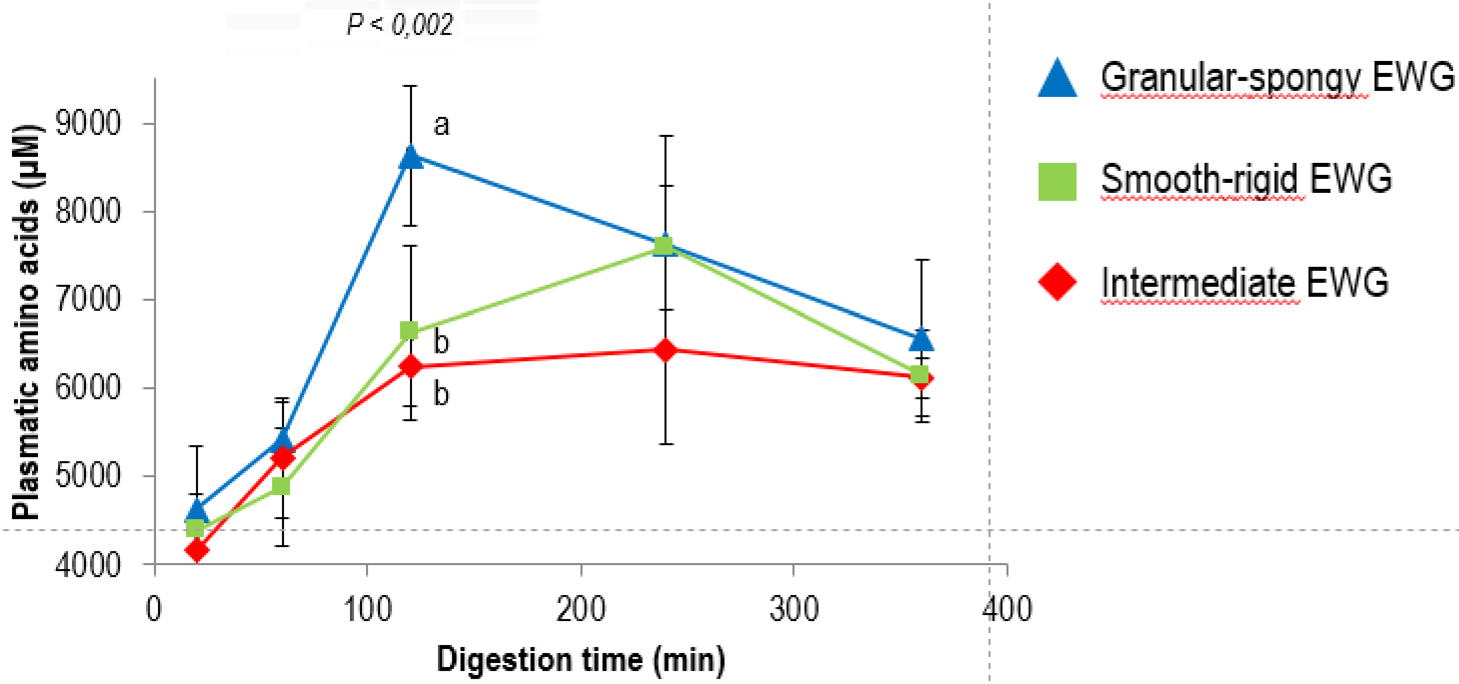


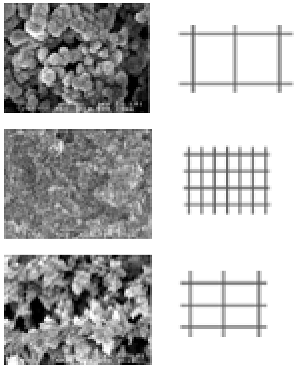
60 min

120 min

240 min

pH 7.0





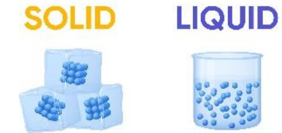
Microscopic scale

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

Dupont D.

INRAE, Rennes, France

Comparison of 6 dairy products of identical composition but different structure



Macroscopic scale

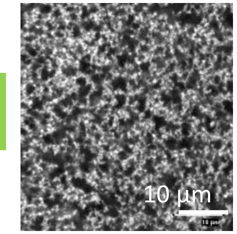
Ultra Low Heat powder

rehydration in water 14.5%

unheated milk (“raw” milk)

macrostructure

24h-20°C, rennet 0.003 % v/w → rennet gel



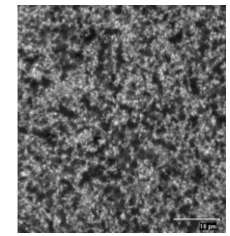
pH 6.6

heat treatment 90°C-10 min

microstructure

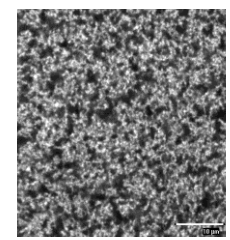
heated milk

24h-20°C, rennet 0.3 % v/w → rennet gel



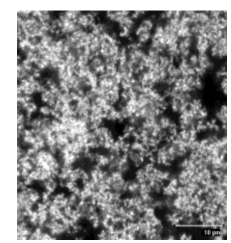
pH 6.6

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:

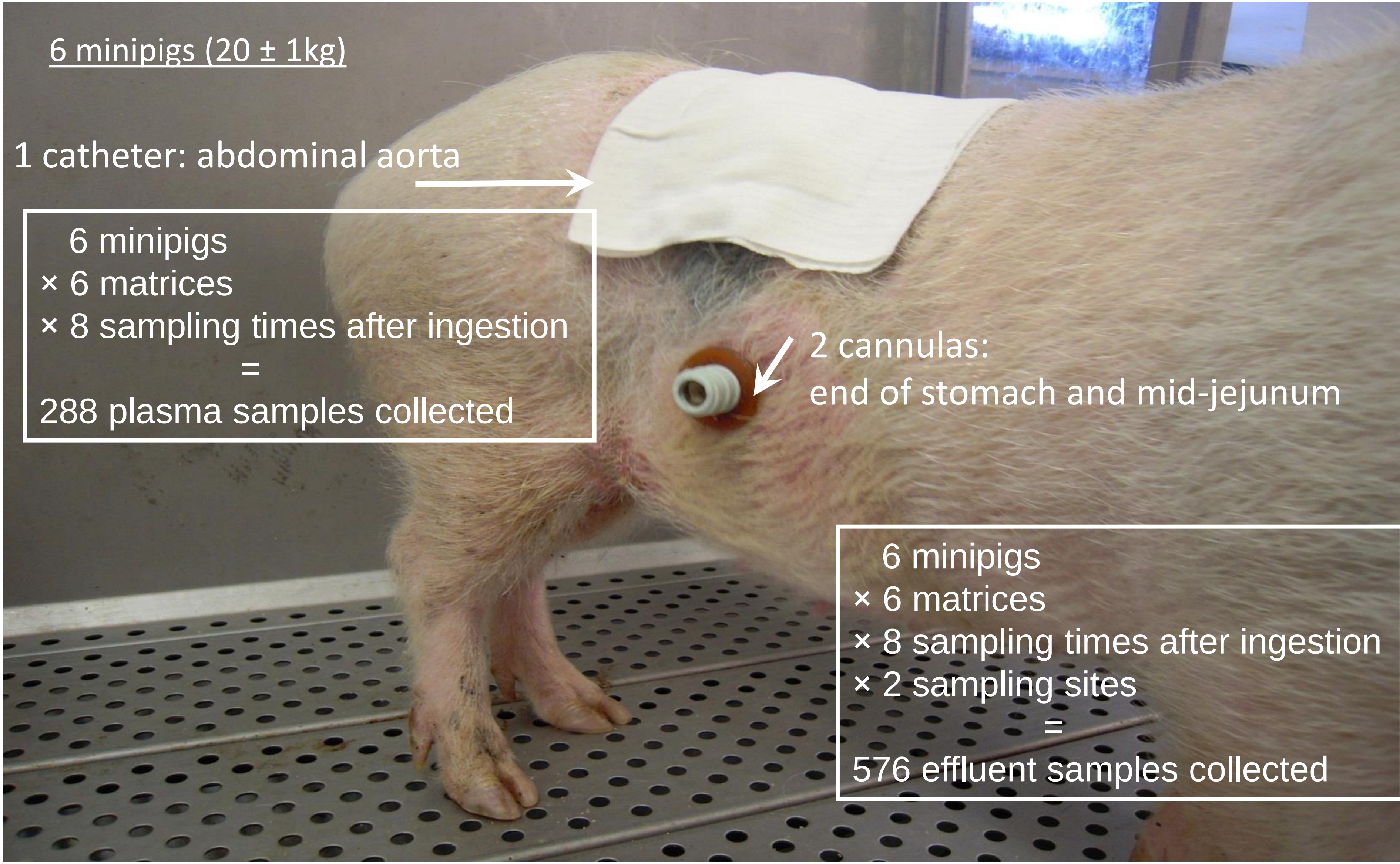
40 g/L caseins, 10 g/L whey proteins,
95 g/L lactose and minerals

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

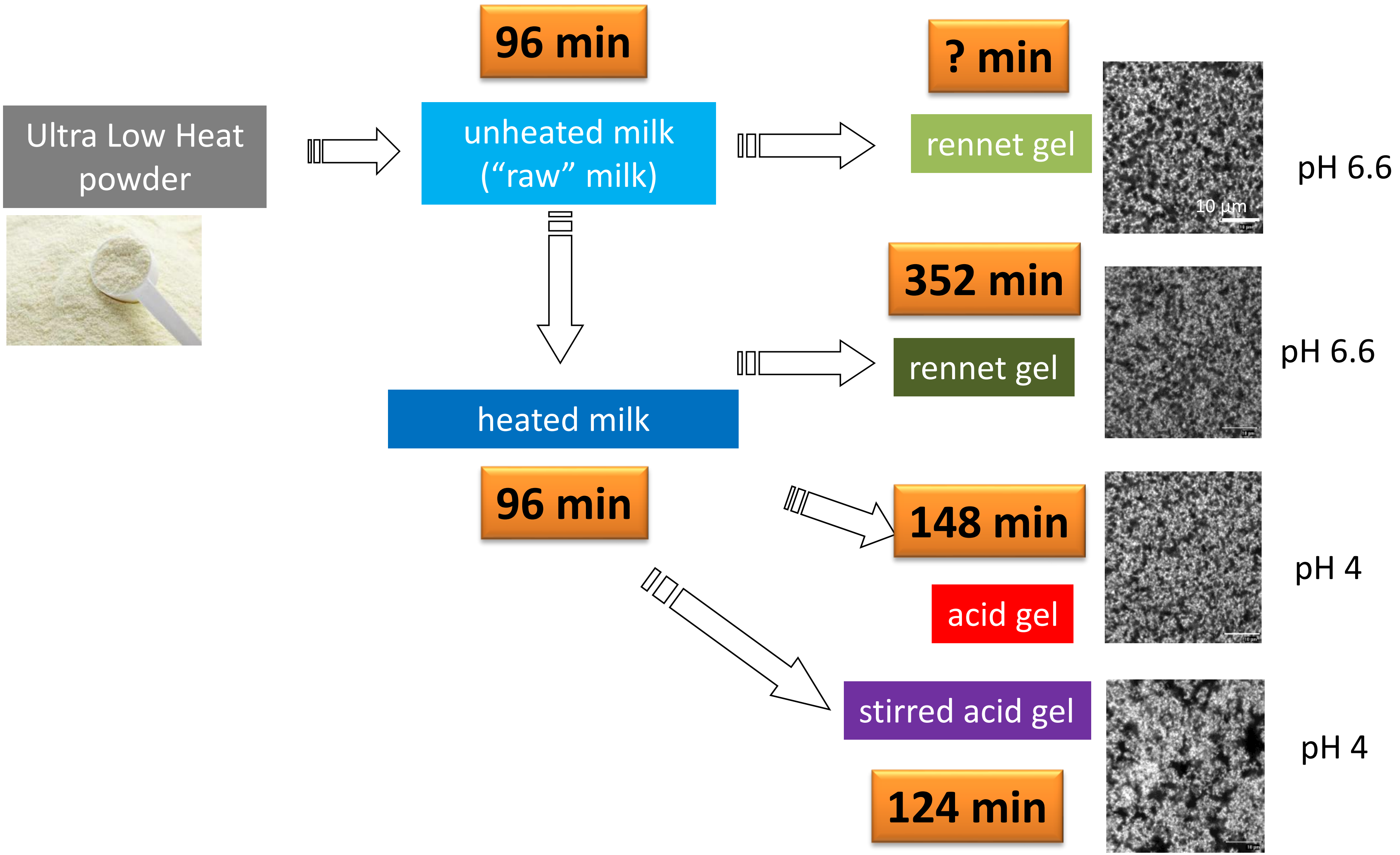
The multi-canulated mini-pigs

SOLID LIQUID

Macroscopic scale



Gastric emptying half time



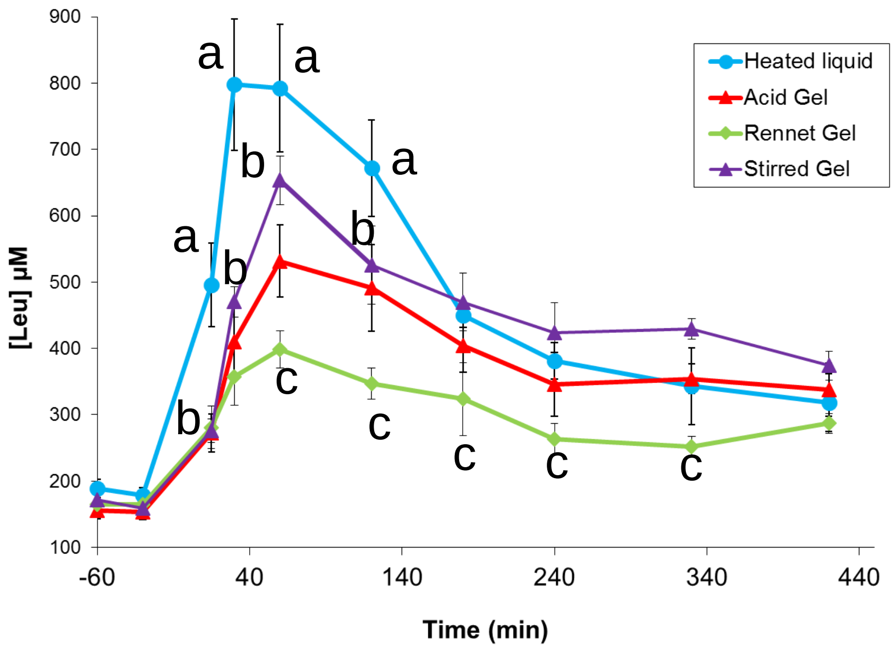
The liquid-gel transition

Barbé *et al.* Food Chem 2013

SOLID LIQUID

Macroscopic scale

Effect on absorption



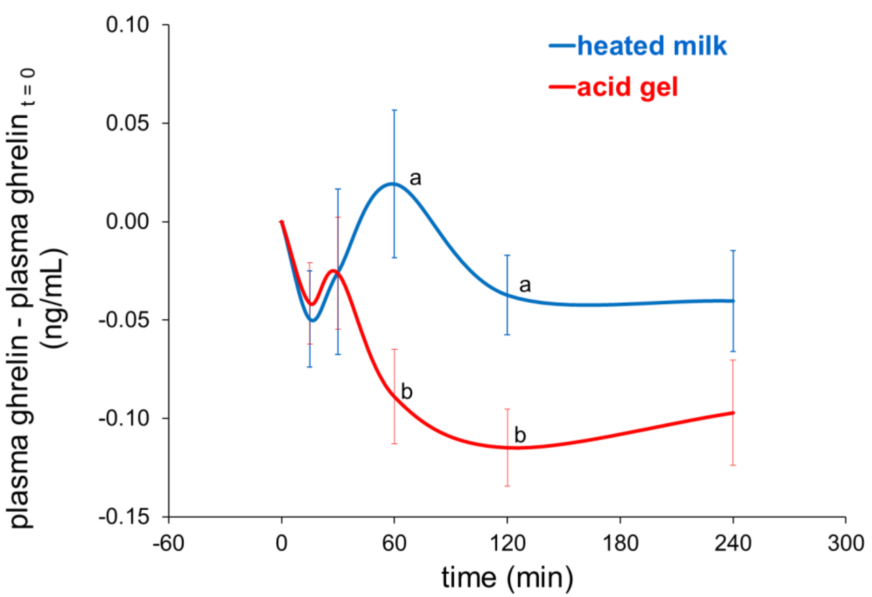
milk gelation:

→ delayed proteins transit → delayed AA absorption

↘ maximal AA concentration in the plasma

Potential effect on satiety

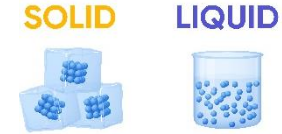
ghrelin (gastrointestinal hormone → appetite stimulation)



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

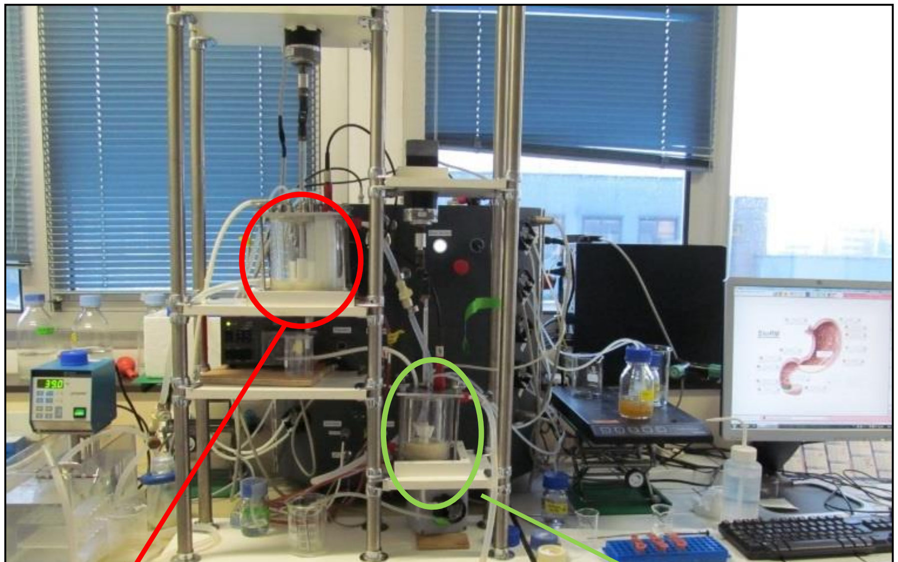
- More bioactive peptides identified during digestion of acid gel than rennet gel
- Nature of peptides is identical (clearly defined by the digestive enzyme specificity)
- Kinetics of release are different

Barbé et al. 2014
Food Res Int

Differential behaviour of acid/rennet gels in gastric conditions

- ☞ Acid/Rennet gel: identical composition, similar rheological properties and 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

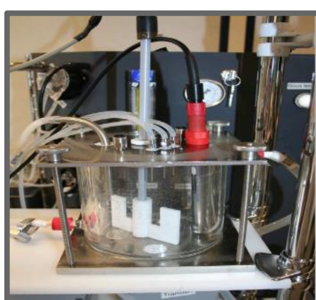


DIDGI®

StoRM® software

Stomach

- Pepsine
- Gastric lipase
- Simulated gastric fluid
- HCl



Emptying : Elashoff's model

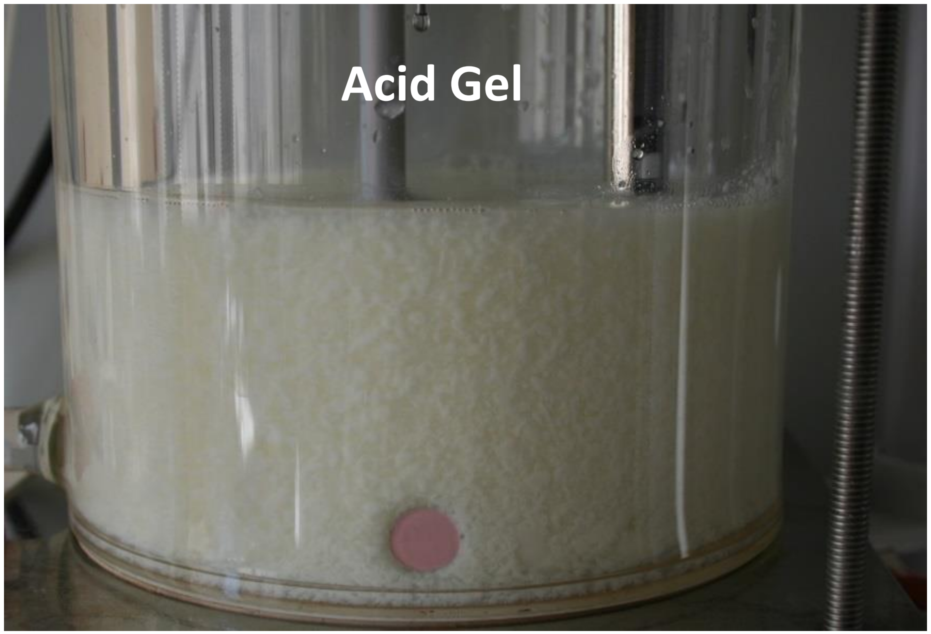
Small intestine

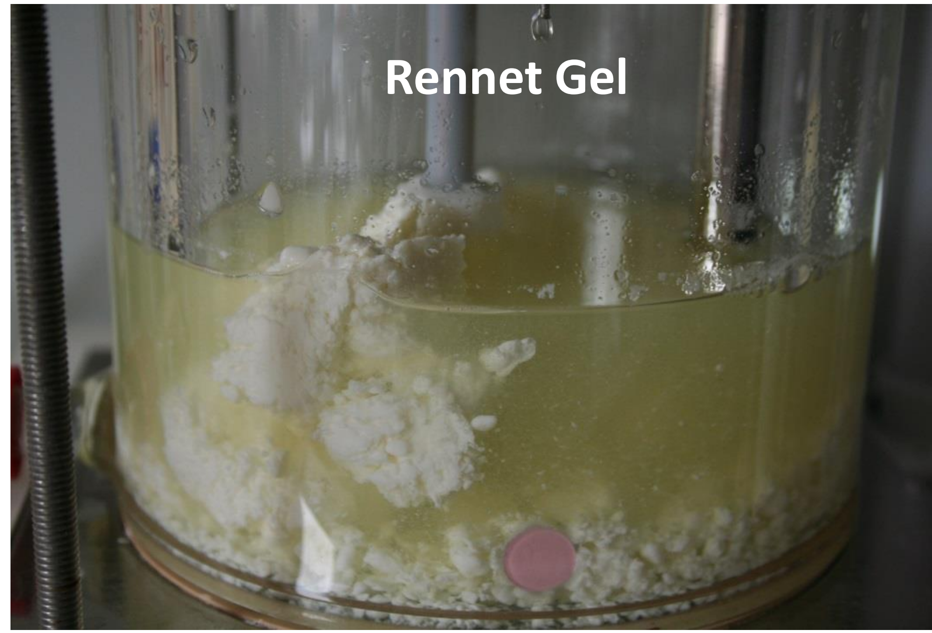
- Pancreatin
- Bile
- Simulated intestinal fluid
- $NaHCO_3$

Emptying : Elashoff's model

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

Barbé et al.
Food Chem. 2014

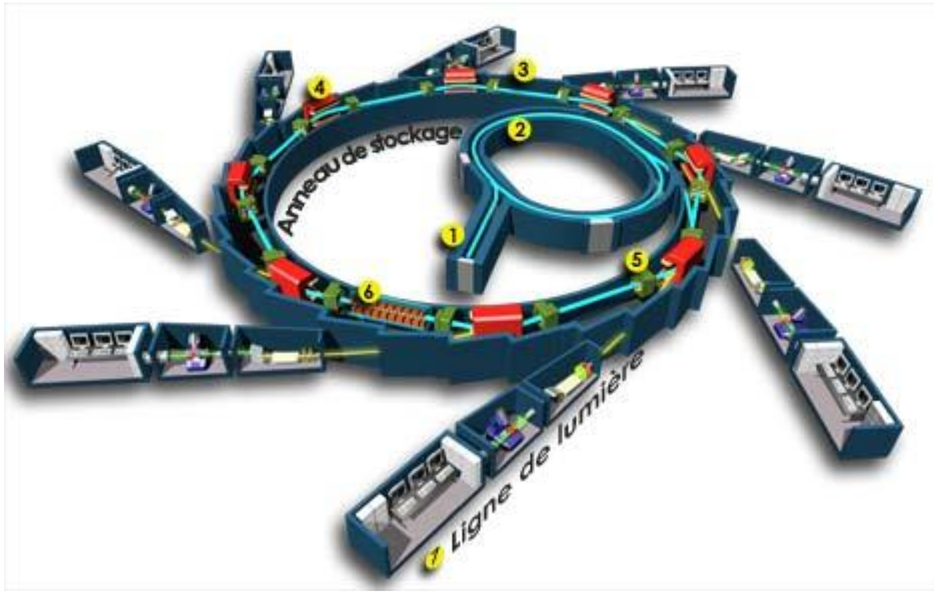




Formation of a strong coagulum with rennet gel → slow down the gastric emptying of caseins

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

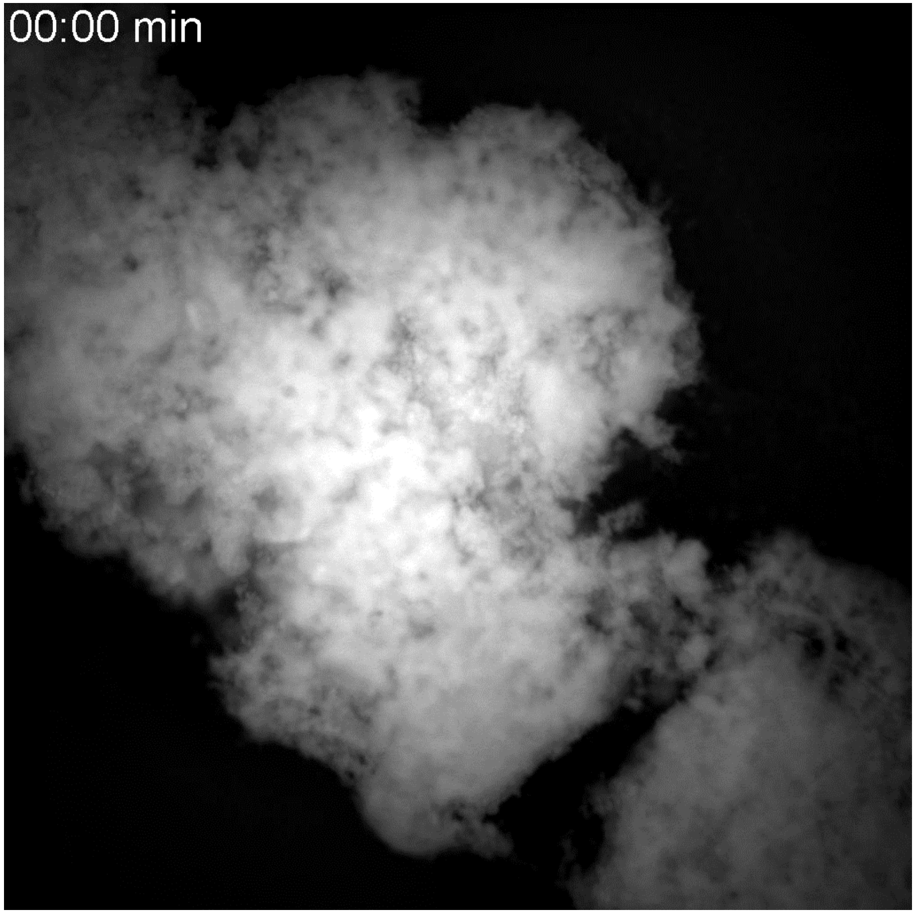
Soleil is a particle (electron) accelerator that produces the synchrotron radiation, an extremely powerful source of light that permits exploration of inert or living matter

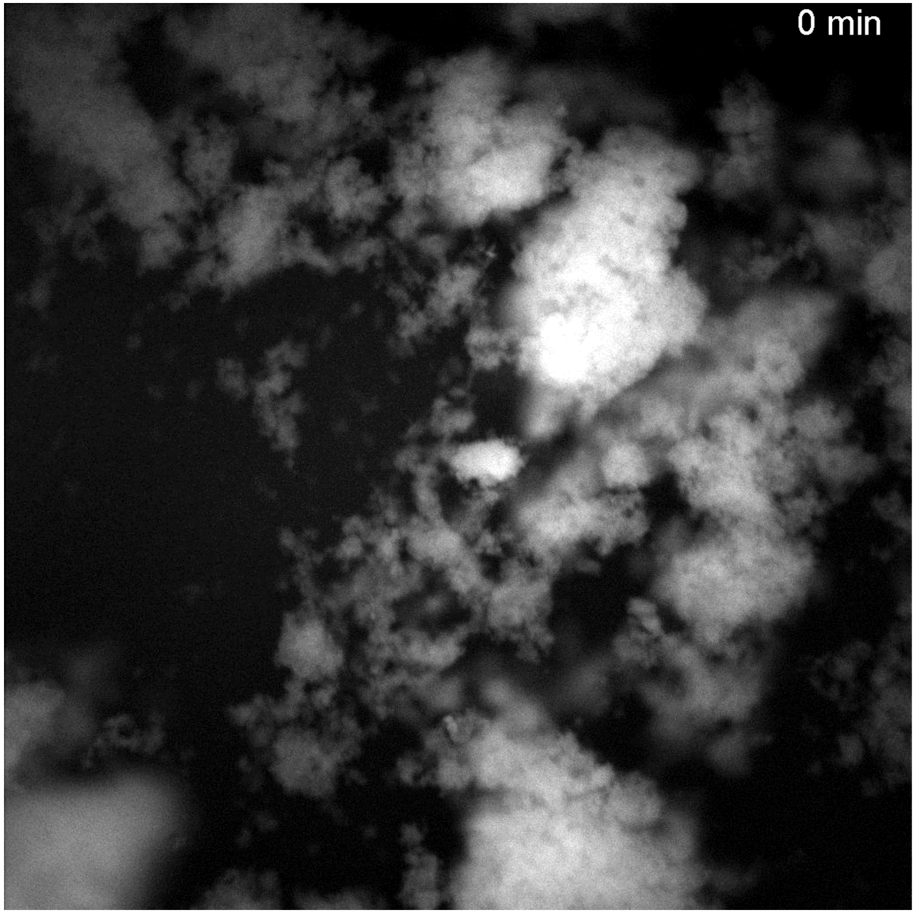


DISCO is a VUV to visible beamline dedicated to biochemistry, chemistry and cell biology. The spectral region is optimized between 60 and 700 nm with conservation of the natural polarization of the light

☞ Allow the imaging of protein intrinsic fluorescence with a UV microscope

Kinetics of gel particles disintegration





Rennet Gel

Acid Gel

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

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

INRAE, Rennes, France

Human/ bovine milk / Infant Formula

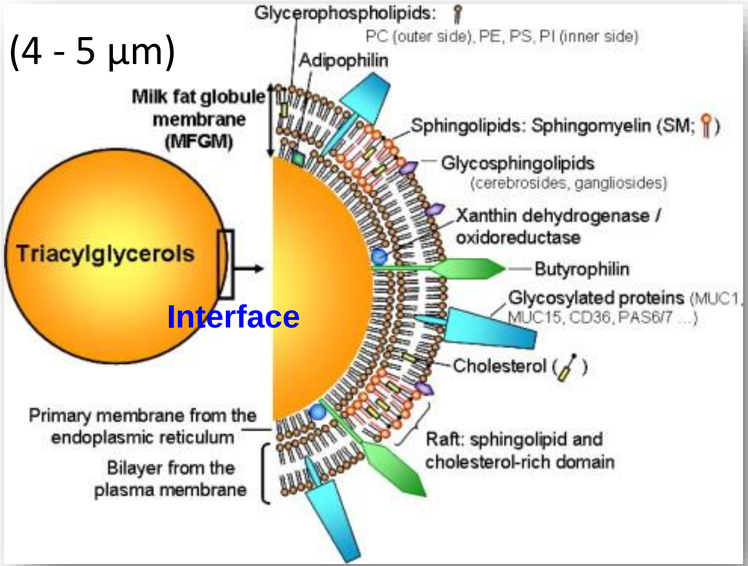
Lipid globule structure

Human milk

Bovine milk

Infant Formula

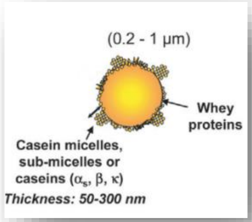
Native milk fat globule



(Lopez, 2010)

Lipid droplets

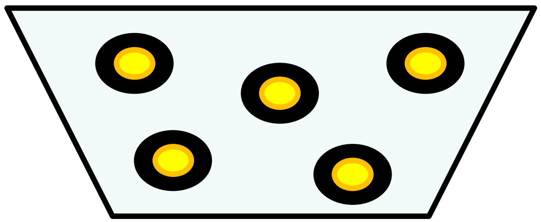
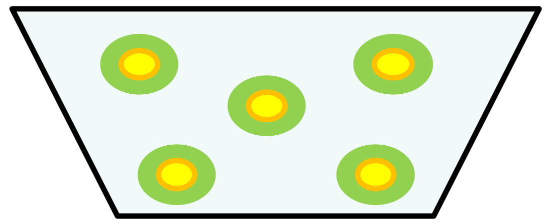
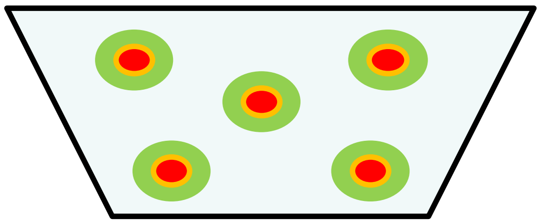
Triacylglycerols

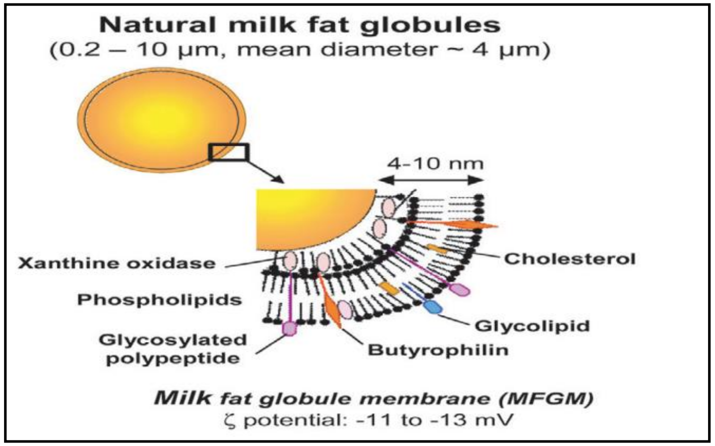


(0,2 - 1 µm)

(Lopez and Briard-Bion, 2007)

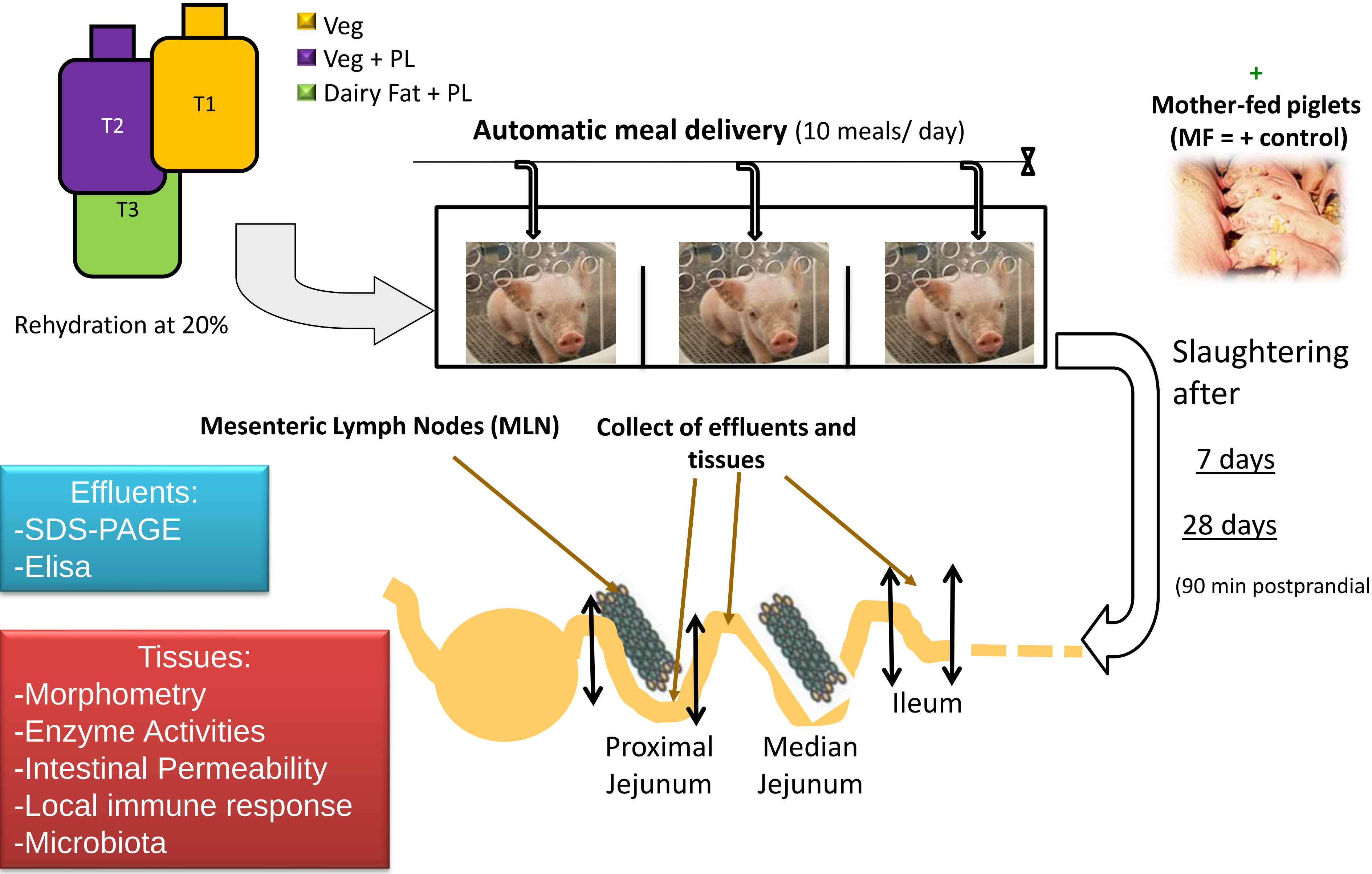
Infant formulas: can we create lipid structures biomimetic on the native fat globule?

Formula T1	Formula T2	Formula T3
		
Interface 100 % Proteins **100% vegetable oil**	**Interface 100 % phospholipids** **100% vegetable oil**	**Interface 100 % phospholipides** **40% vegetable oil + 60% milk fat**



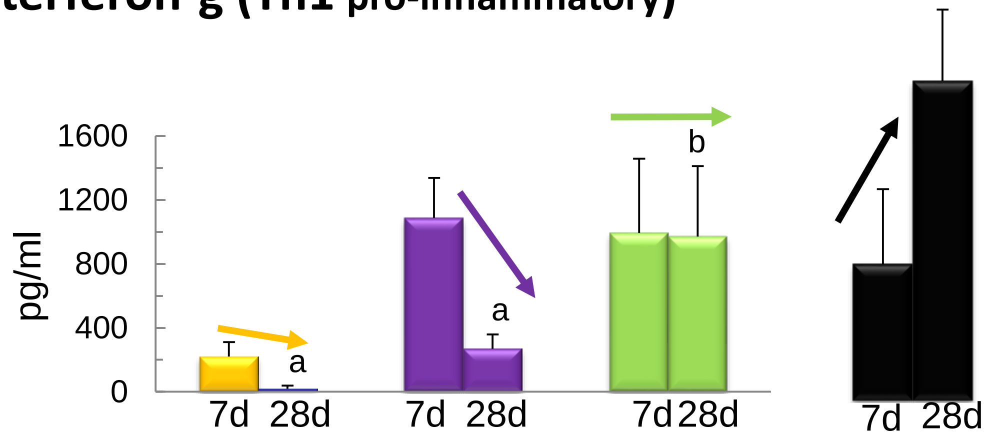
Lopez, (2007)

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

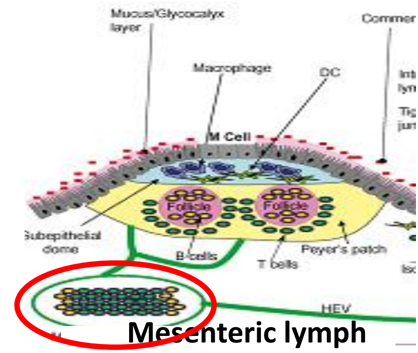


Secretory activity of MLN

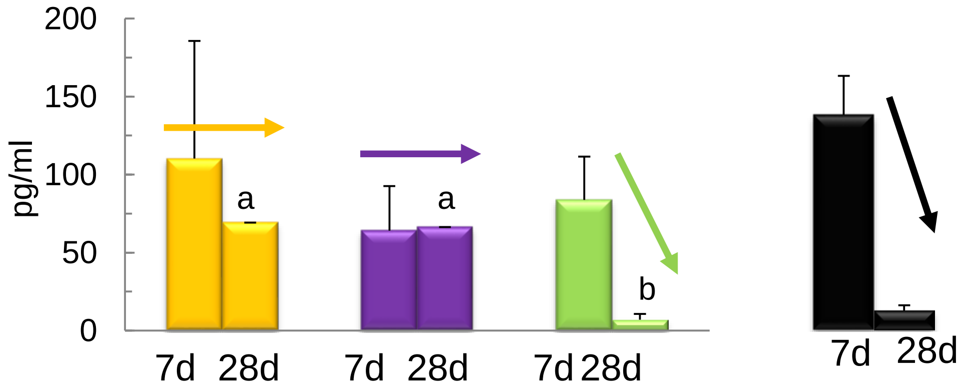
Interferon-g (Th1 pro-inflammatory)



Veg
Veg + PL
Dairy Fat + PL
Porcelets SM



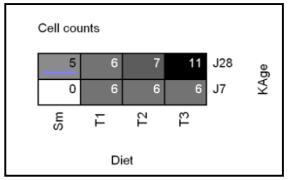
Interleukine-10 (Th2 anti-inflammatory)



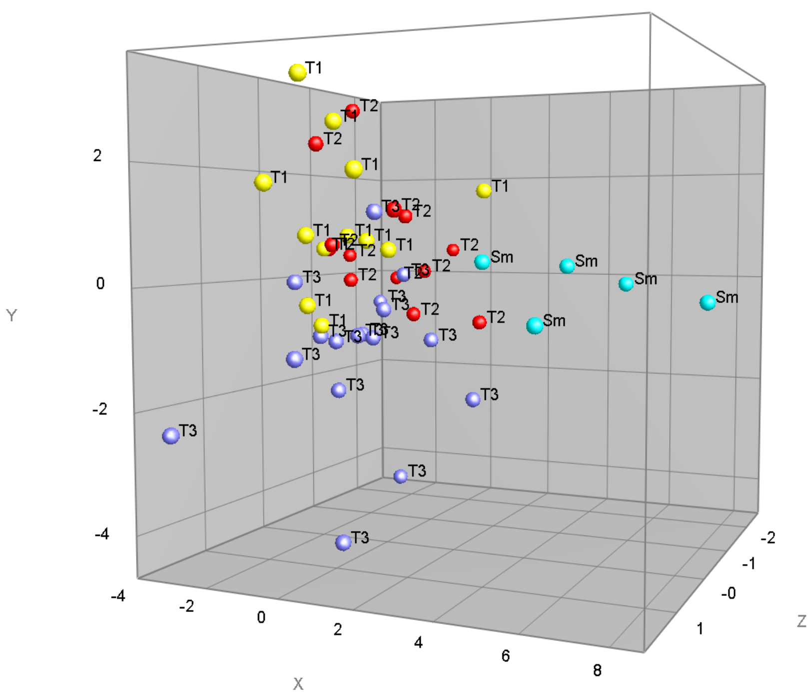
Milk lipids → maturation of the piglet's immune system more similar than with sow's milk

Le Huerou et al.
Eur J Nutr 2017

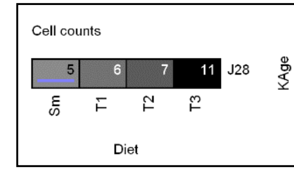
Microbiota by DHPLC

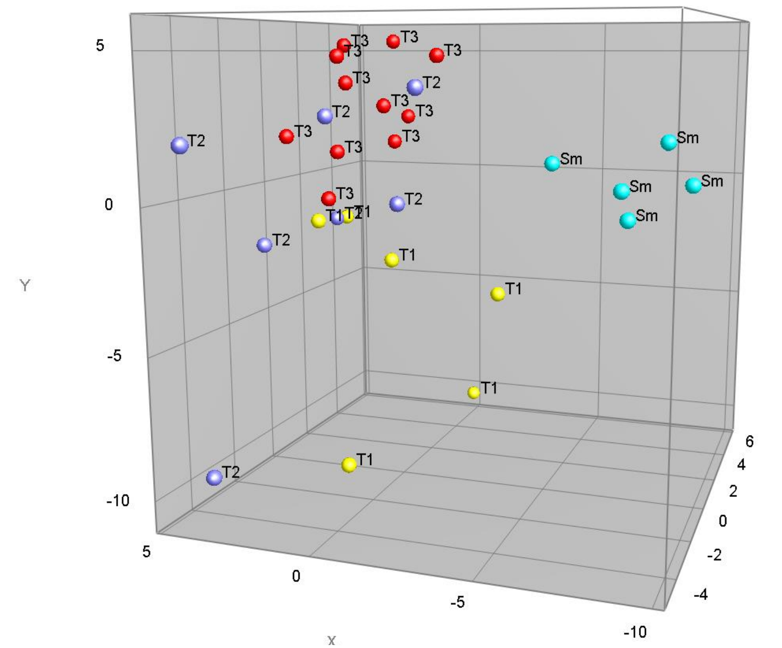


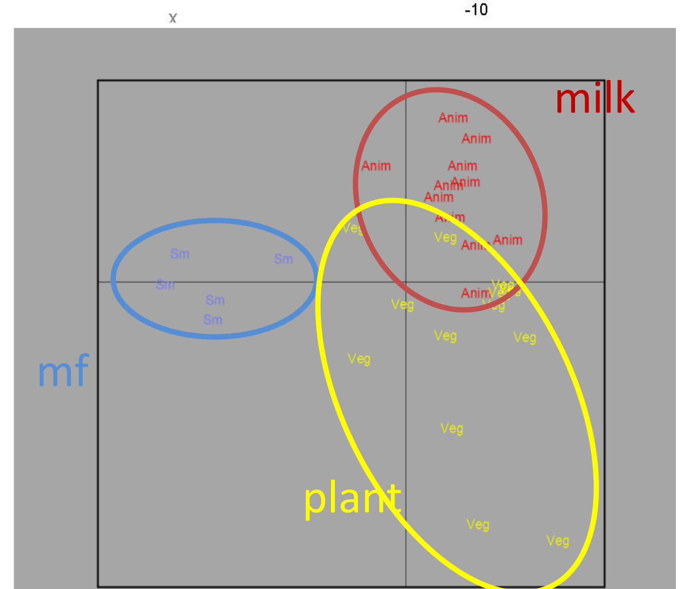
D7 & D28



D28





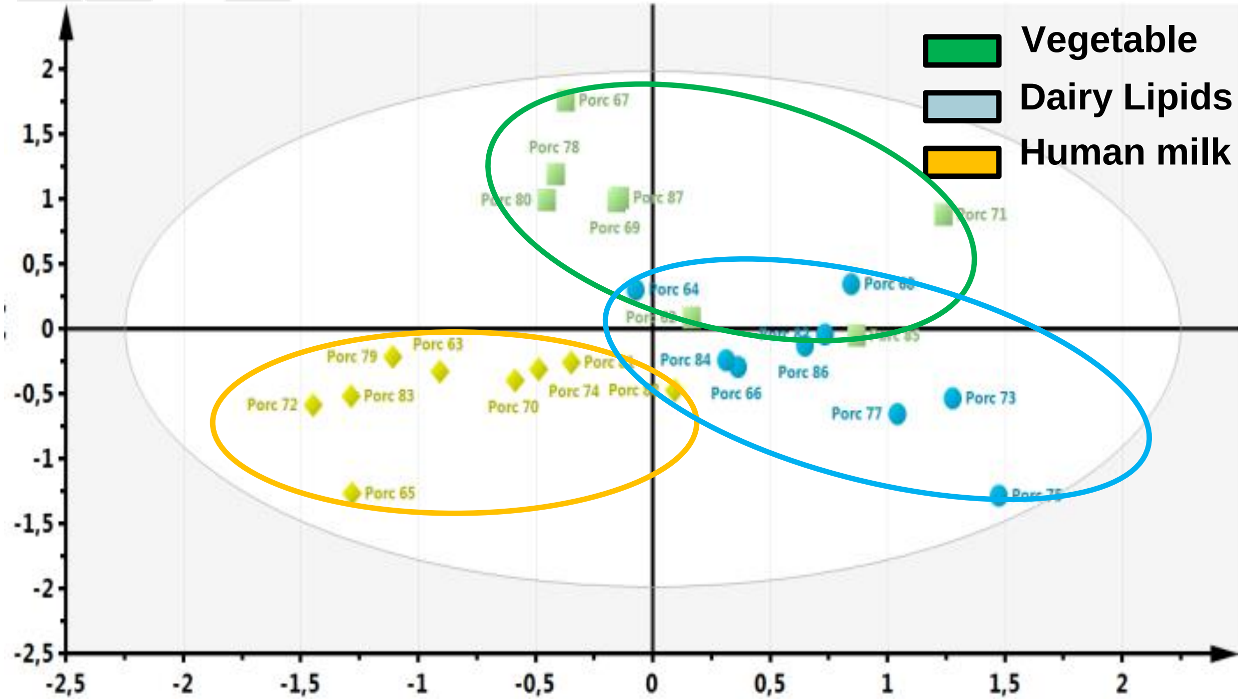


The composition/structure of the infant formula « orientates » the microbiota

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 and the release of amino acids in the bloodstream

Gastric emptying rate will highly depend on the structure that the product will adopt in the stomach cavity.

Understanding the mechanisms of food particle breakdown in the stomach is critical to control the structure a food will adopt in gastric conditions

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 INRA Rennes

Head
Didier DUPONT- Senior Scientist

Scientists
Amélie DEGLAIRE – *Lecturer*
Juliane FLOURY – *Lecturer*
Catherine GUERIN - *Lecturer*
Steven LE FEUNTEUN – *Senior Scientist*
Joëlle LEONIL – *Senior Scientist*
Martine MORZEL – *Senior Scientist*
Françoise NAU – *Professor*
Frédérique PEDRONO – *Lecturer*

Xiaoxi YU – *Post-doc*
Guilherme FURTADO – *Post-doc*

PhD students
Yohan REYNAUD *(2016-2019)*
Amira HALABI *(2017-2020)*
Jun WANG *(2018-2021)*
Lea SALLELES *(2018-2021)*
Elise CHARTON *(2019-2022)*
Lucile CHAUVET *(2019-2022)*
Ousmane SUWAREH *(2019-2022)*

Technicians
Gwenaële HENRY
Yann LE GOUAR
Nathalie MONTHEAN

Engineers
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

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Spain
Mac.
Tur.
TURKEY
Alb.
Greece
746 scientists - 250 institutes – 57 countries

Industry involvement

☞ ~ 60 private companies are following INFOGEST

Chair
Didier Dupont - France
didier.dupont@inrae.fr

INFOGEST

Vice-chair
Alan Mackie - UK

www.cost-infogest.eu

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

Isidra Recio

Pasquale Ferranti

Linda Giblin

Myriam Grundy

Daniela Freitas

Choi-Hong Lai

Andre Brodkorb

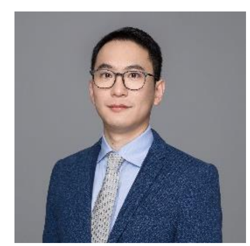
Lotti Egger

Uri Lesmes

Brigitte Graf

Frederic Carriere

Anabel Mulet-Cabero



Bin Zhang

Steven Le Feunteun

Some outputs

In vitro gastrointestinal digestion
Consensus INFOGEST protocol

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

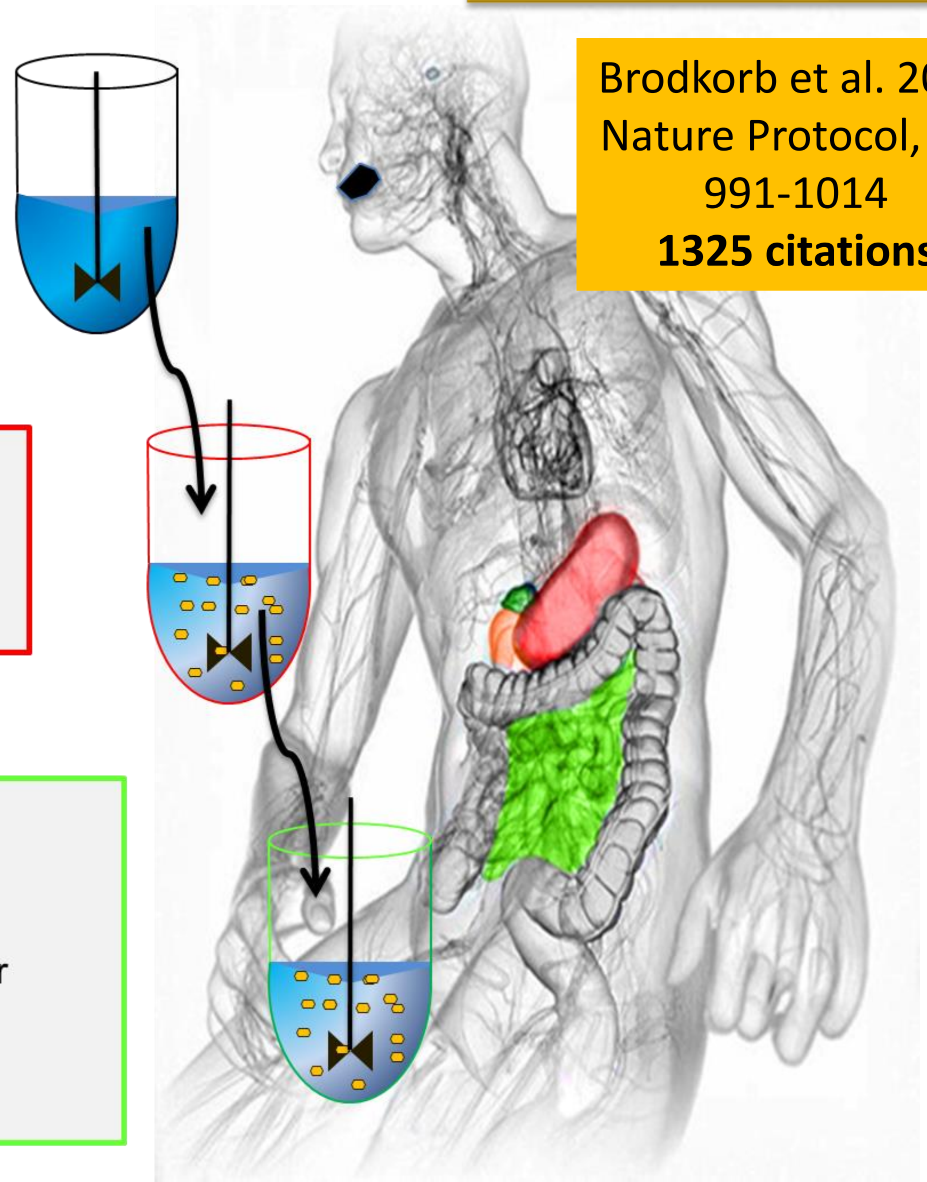
Brodkorb et al. 2019
Nature Protocol, 14, 991-1014
1325 citations

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