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Food structure affects the kinetics of food digestion and the bioavailability of nutrients.

Didier Dupont

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Submitted on 5 Jun 2020

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Food structure affects the kinetics of food digestion and the bioavailability of nutrients



Dr Didier DUPONT

INRA, Rennes, France



Portrait

The National Institute for Agronomic Research

- Set up in 1946
- A public, scientific and technological establishment
- Under the joint authority of the Ministries of Agriculture and Research
- Second largest French public research organisation with a staff of nearly 13000 and a budget of 850 millions euros
- Largest European organisation for agricultural research



DIET
AGRICULTURE
ENVIRONMENT

INRA

Three major fields

Diet Agriculture Environment

... in **six** research areas:

- > Environment and rural areas
- > Human nutrition and food safety
- > Quality of agricultural products
- > Knowledge of the living world
- > Agricultural practices and systems
- > Social sciences

STLO ID card

Joint research unit between **INRA** and **Agrocampus Ouest**

Keywords

Egg products, cheese technology, milk processing (spray-drying, membrane filtration, heat treatment), dairy products

Nutrition, digestion, bioactivity

Probiotics, lactic acid bacteria, propionic acid bacteria, food spoiling, mastitis.

Facilities

Milk processing platform (PFL/800m²), spray-drying (Bionov/industrial scale), Strain library (CIRM-BIA), L2-level labs, cell culture labs

Workforce

83 permanent staff (39 scientists and professors; 44 technicians and engineers)

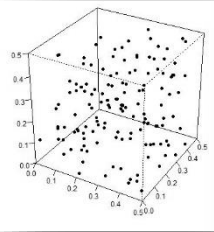
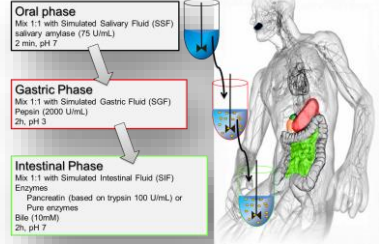
Total workforce: 130-140 persons.

~**25** PhD and post-docs /year

~**20** students (shorter training periods)

16 staff from industrial R&D teams

Organization



Vice directors

Director



Microbiology



Interaction
 Structure/
 Function



Bioactivity
 & Nutrition



Filtration
 process

Spray-drying
 process



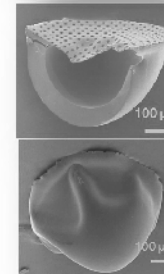
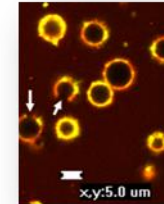
Centre de ressource biologique
 CIRM-BIA



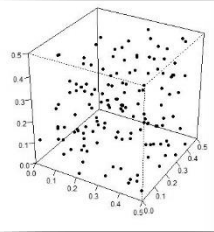
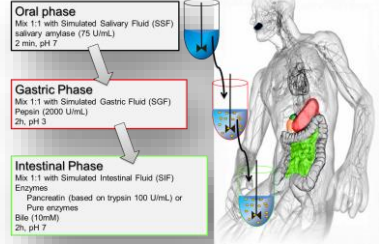
Plateforme Lait
 PFL






SAPHIRE
 Administration,
 Informatics & Infrastructure, Doc & Com



Organization



Vice directors Director





MICROBIO ISF BM



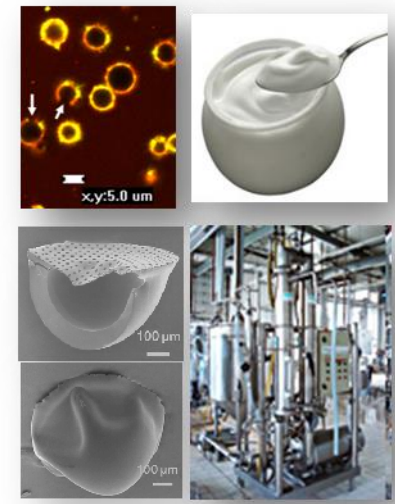

SMCF




Centre de ressource technologique CIRM-BIA Plateforme Lait PFL



SAPHIRE
 Administration,
 Informatics & Infrastructure, Doc & Com



2 transverse axes:
 - optimisation of infant formulas
 - animal-plant mix

A 800 m² Dairy Platform



The Bioactivity & Nutrition group at INRA in Rennes



Head

Didier DUPONT - *Senior Scientist*

Scientists

Rachel BOUTROU – *Junior Scientist*

Amélie DEGLAIRE – *Lecturer*

Juliane FLOURY – *Lecturer*

Catherine GUERIN - *Lecturer*

Joëlle LEONIL – *Senior Scientist*

Steven LE FEUNTEN – *Junior Scientist*

Françoise NAU – *Professor*

Frédérique PEDRONO – *Lecturer*

Guilherme FURTADO – *Post-doc*

Xaoxi YU – *Post-Doc*

PhD students

Linda LEROUX (2016-2019)

Manon HIOLLE (2016-2019)

Yohan REYNAUD (2016-2019)

Amira HALABI (2017-2020)

Léa SALELLES (2018-2021)

Jun WANG (2018-2021)

2-3 Masters students

Technicians

Gwenaële HENRY

Yann LE GOUAR

Nathalie MONTHEAN

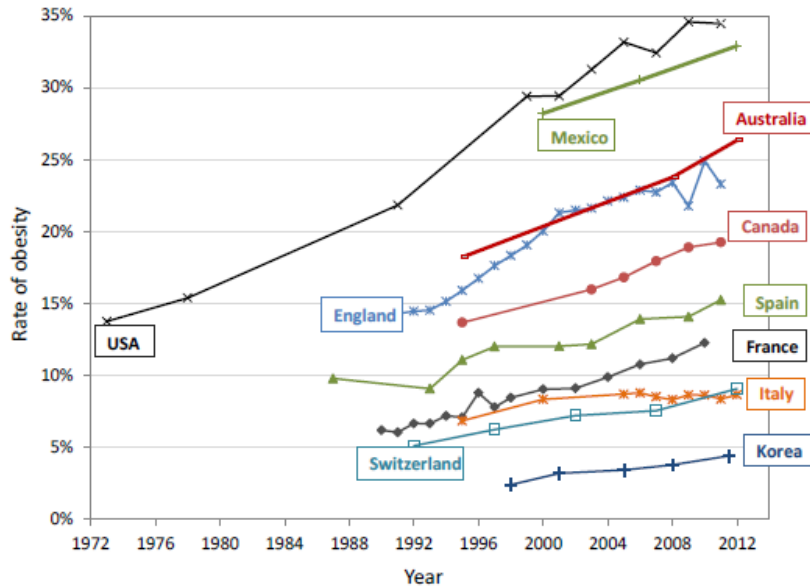
Engineers

Julien JARDIN

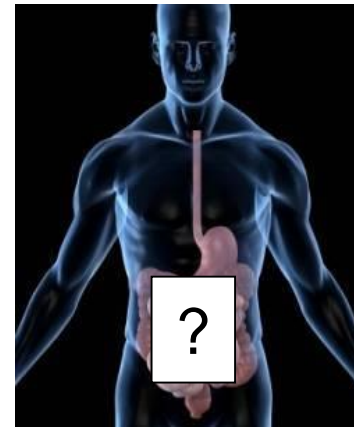
Olivia MENARD

Jordane OSSEMOND

Food and human health: the key role of digestion



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



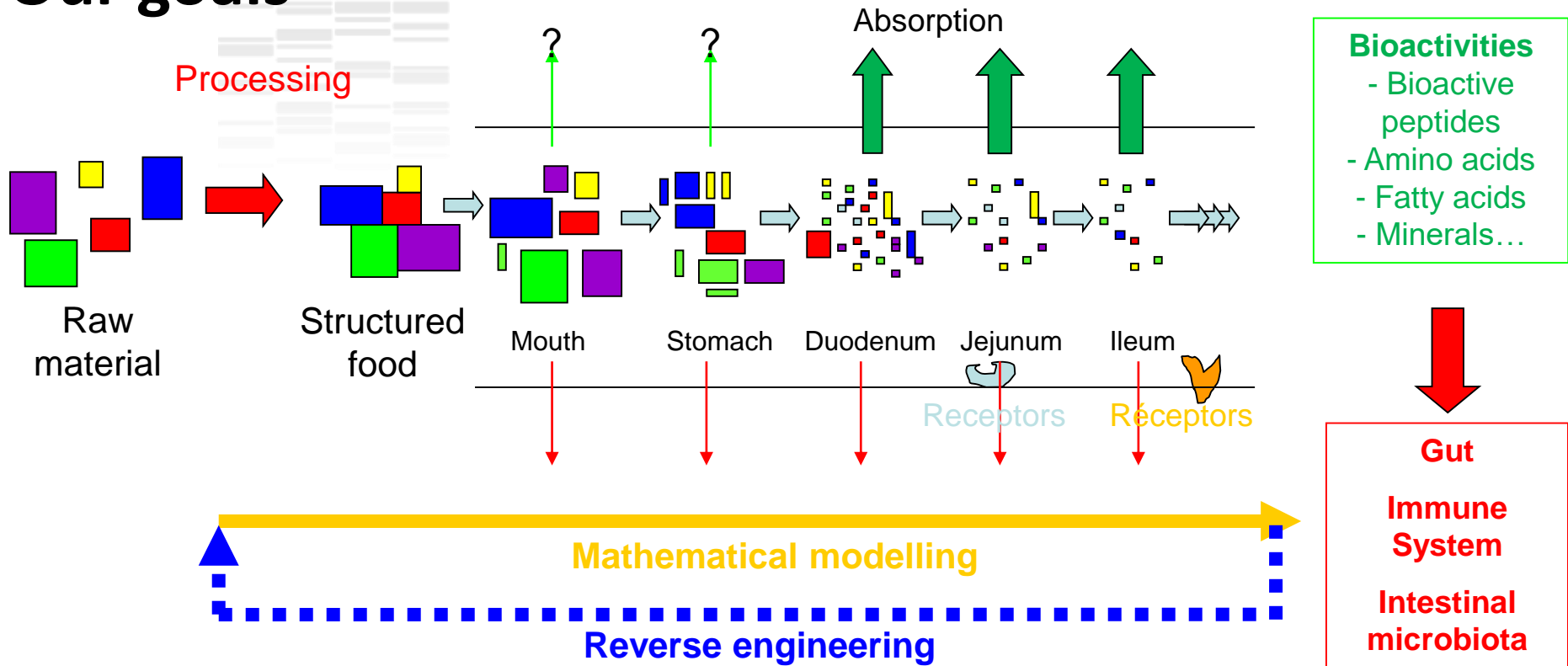
Gut = interface between food and human body

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

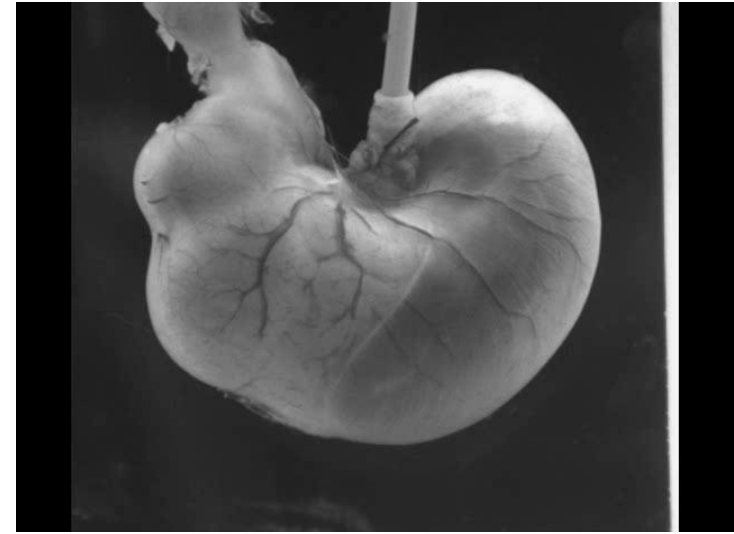
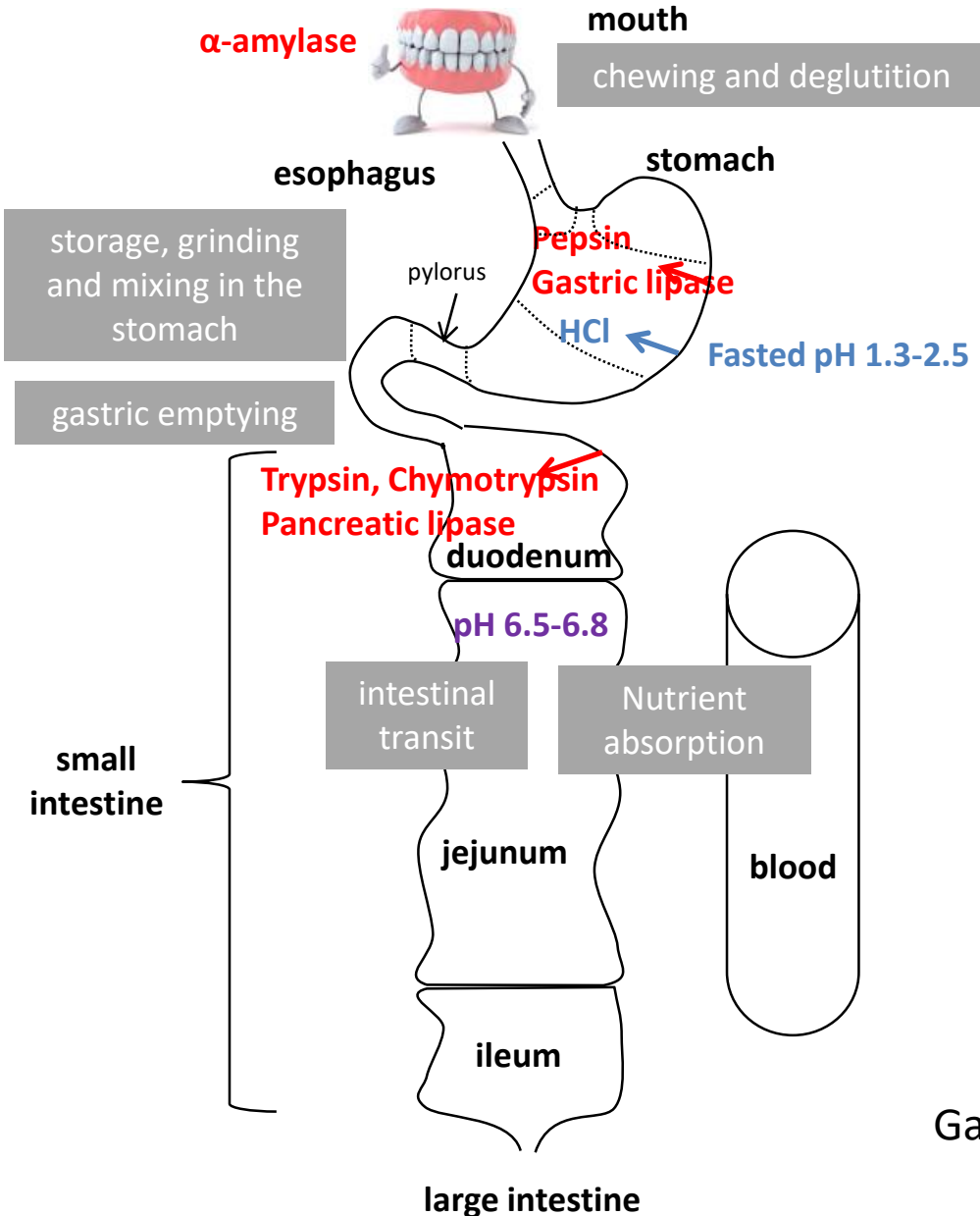
By increasing our knowledge on food digestion, we will increase our knowledge on the effect of food on human health

Our goals

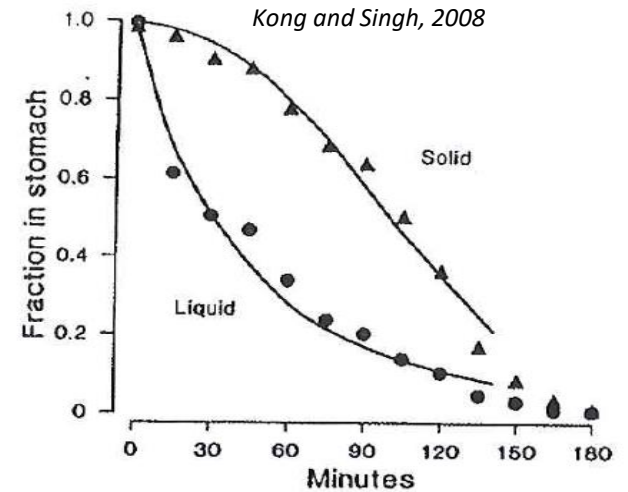


- ☞ To understand the mechanisms of breakdown of food matrices and their constituents in the gut and identify the beneficial/deleterious food components released during digestion
- ☞ To determine the impact of the structure of food matrices on nutrient bioavailability
- ☞ To model these phenomena in 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 INRA for simulating digestion

Menard et al. 2014,
Food Chem
Sanchez et al. 2015
Food Res Int

Dupont et al.
2010ab,
Mol Nutr Food Res



*In vitro static models
(infant, adult)*

Le Feunteun et al.
2014
Food Bioprocess
Tech

*In silico
models*



*In vitro dynamic models
(infant, adult, elderly)*



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



*Human
models*



Animal models



Barbé et al. 2013, 2014
Food Chem
Le Huerou-Luron et al.
2016 Eur J Nutr

De Oliveira et al. 2016
Am J Clin Nutr
De Oliveira et al. 2017
Clin Nutr

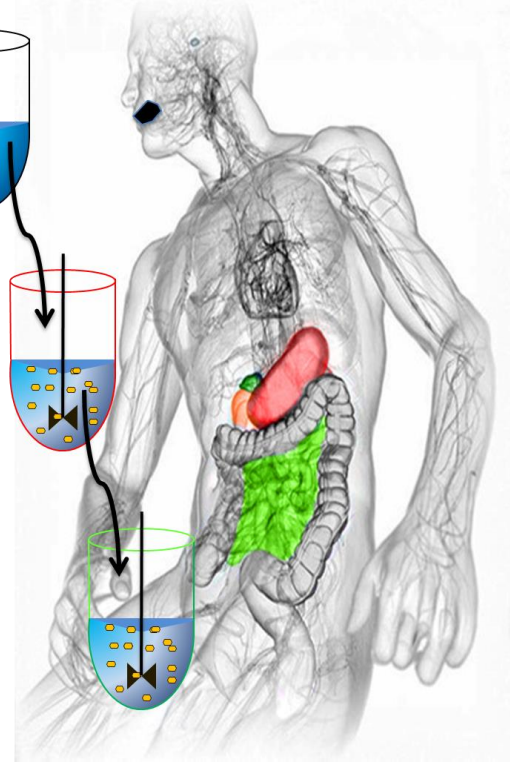
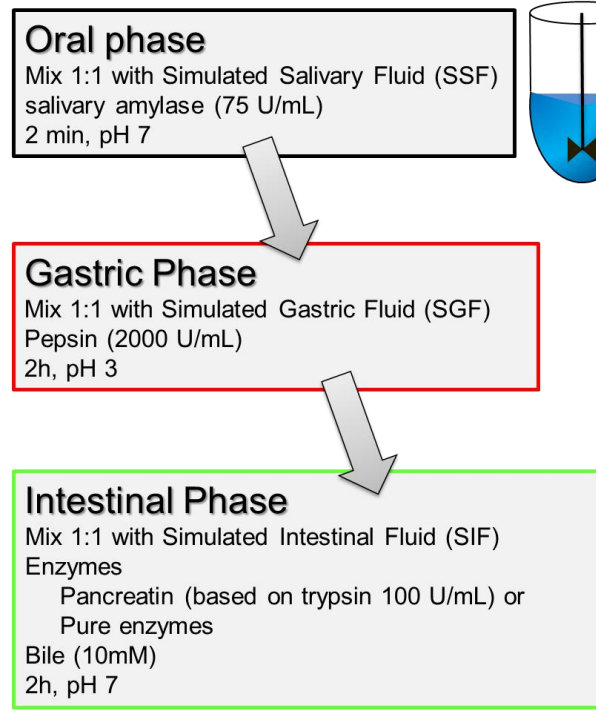
Static *in vitro* digestion models

- ☞ Huge diversity between the models mimicking the GI tract of the adult (E/S, pH...)
- ☞ Impossible to compare results from one study to another – Need for harmonization

Consensus *in vitro* digestion method for food

INFOGEST

International network
– 410 researchers
– 150 institutes
– 45 countries



A standardised static *in vitro* digestion method suitable for food- an international consensus, Food & Function, Minekus et al. 2014 – Highly Cited Paper 800 citations in 4 years!

The INFOGEST model can be learned through videos on YouTube

The screenshot shows a web browser window displaying a YouTube channel page. The browser's address bar shows the URL: https://www.youtube.com/channel/UCdc-NP9kTDGyH_kZCgpQWg. The page title is "In vitro food digestion - COST action INFOGEST". The search bar contains the text "cost infogest". The channel banner features a diagram of the human digestive system. Below the banner, the video title "In vitro food digestion - COST action INFOGEST" is displayed, along with a "S'abonner" (Subscribe) button showing 36 subscribers. The navigation menu includes "Accueil", "Vidéos", "Playlists", "Chaînes", "Discussion", and "À propos". The main content area shows a list of videos, with the first one titled "Alpha Amylase Activity Assay for In Vitro Food Digestion" (5:12) and the second titled "Static In Vitro Digestion Method for Food" (8:55). Both videos include a thumbnail with a diagram of the digestive system and a "S'abonner" button. The right sidebar lists popular channels such as "Seb la Frite", "MissCreatives", "LanguedePub / M...", "JunkFoodTasterD...", "Madame Patachou", and "halawiyat khadija...".

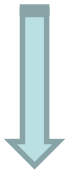
Static *In vitro* digestion models: pro's & con's



Main Reasons:

Ethical - Technical – Financial

in vivo



in vitro

Advantages:

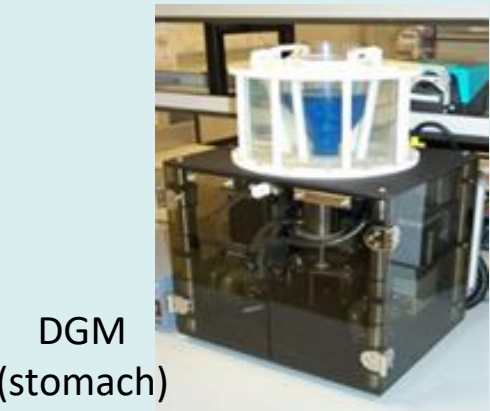
Standardisation of the experimental conditions
Good reproducibility and repeatability
Easy sampling, possibility to follow kinetics

Disadvantages:

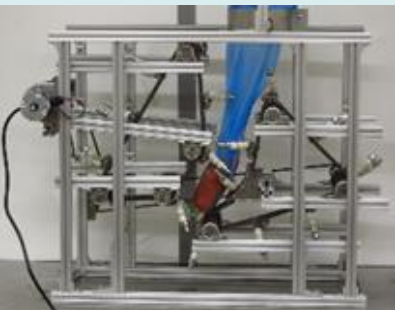
You cannot mimic the complexity of the GI tract in a test tube!!!

Bohn et al. 2017
Crit Rev Food Sci
Nutr

Dynamic *in vitro* digestion models



DGM
(stomach)



HGS
(stomach)



ARCOL
(colon)

Mono-compartmental models

DIDGI (stomach + small intestine)



Dupont et al. 2017
Crit Rev Food Sci
Nutr

TIM
(TIM1 =
duod+jej+ile
TIM2 = colon)



Multi-compartmental models

SIMGI (whole GI tract)



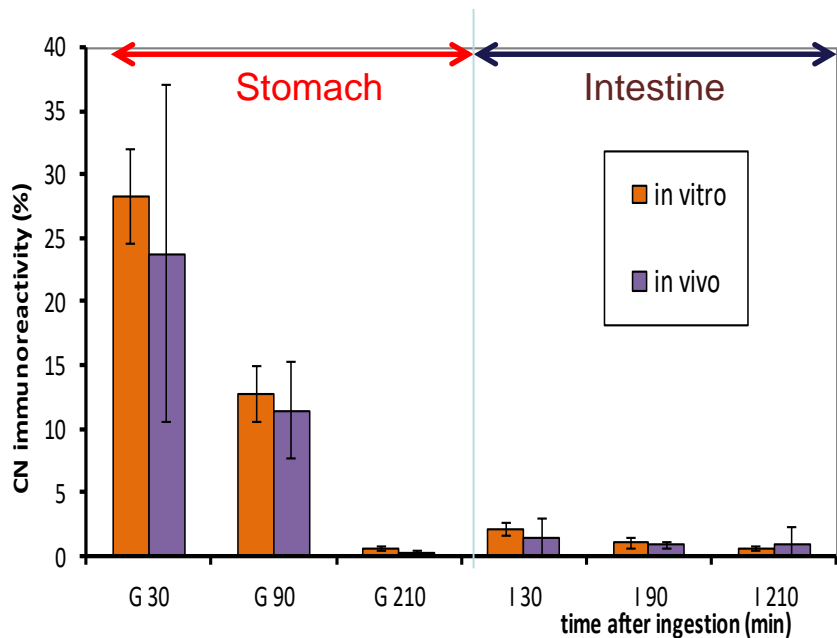
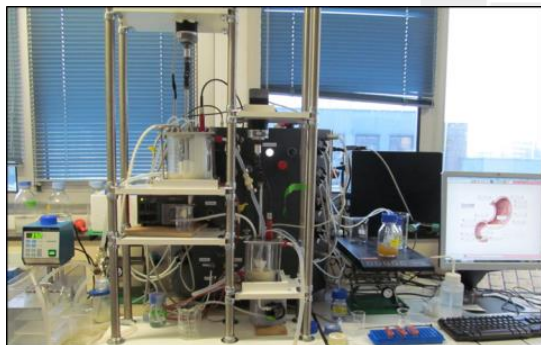
SHIME (whole GI tract)



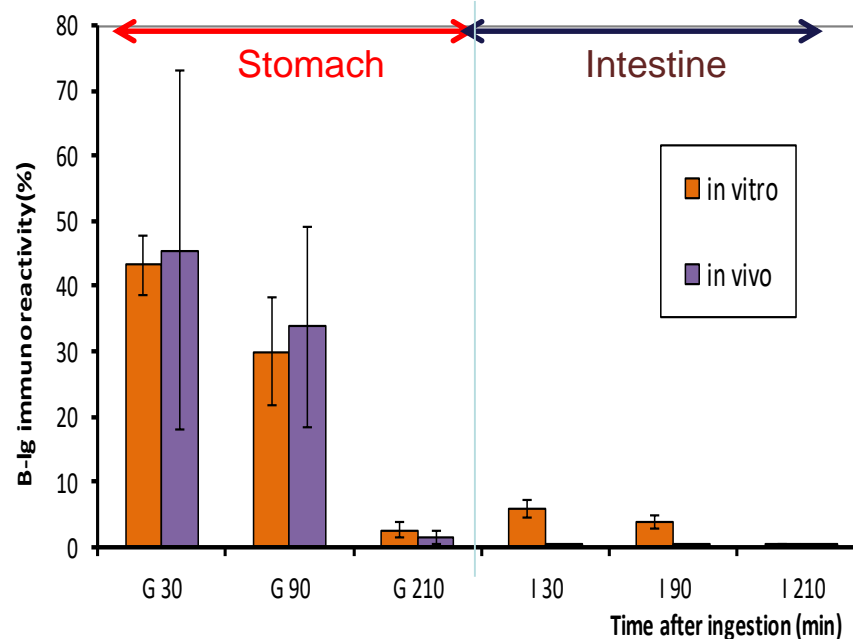
ESIN
(stomach
+ small
intestine)



Validation of the dynamic *in vitro* digestion system



Caseins



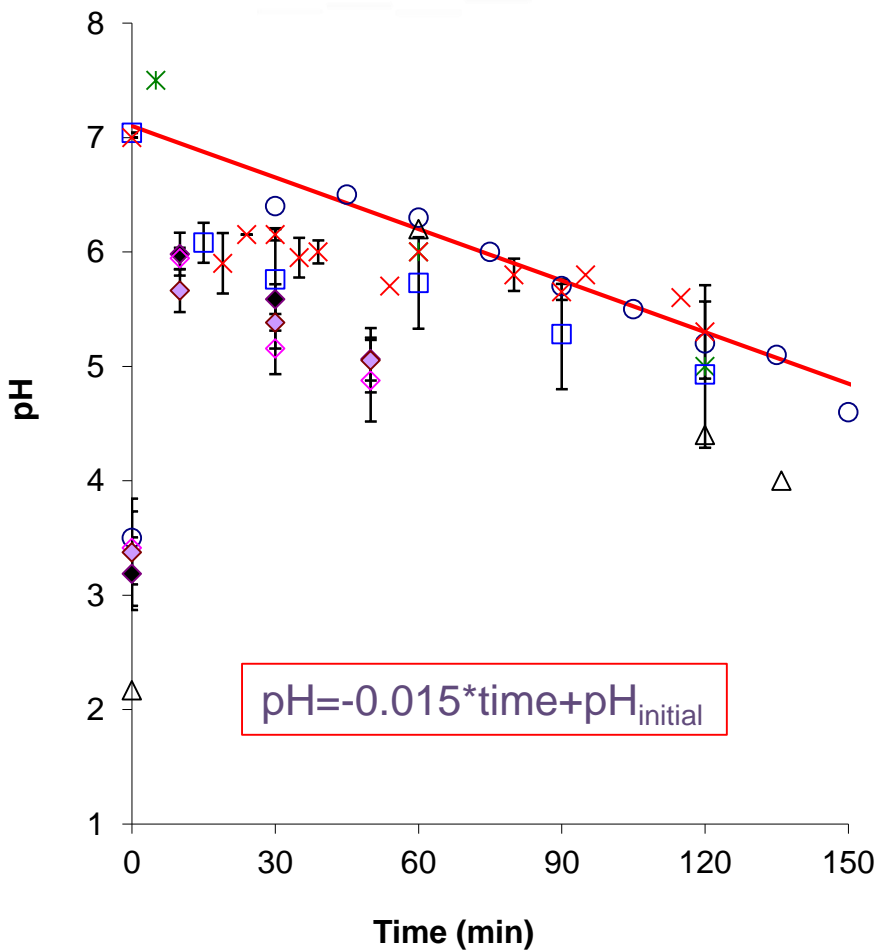
β-lactoglobulin

Menard et al.
2014
Food Chem

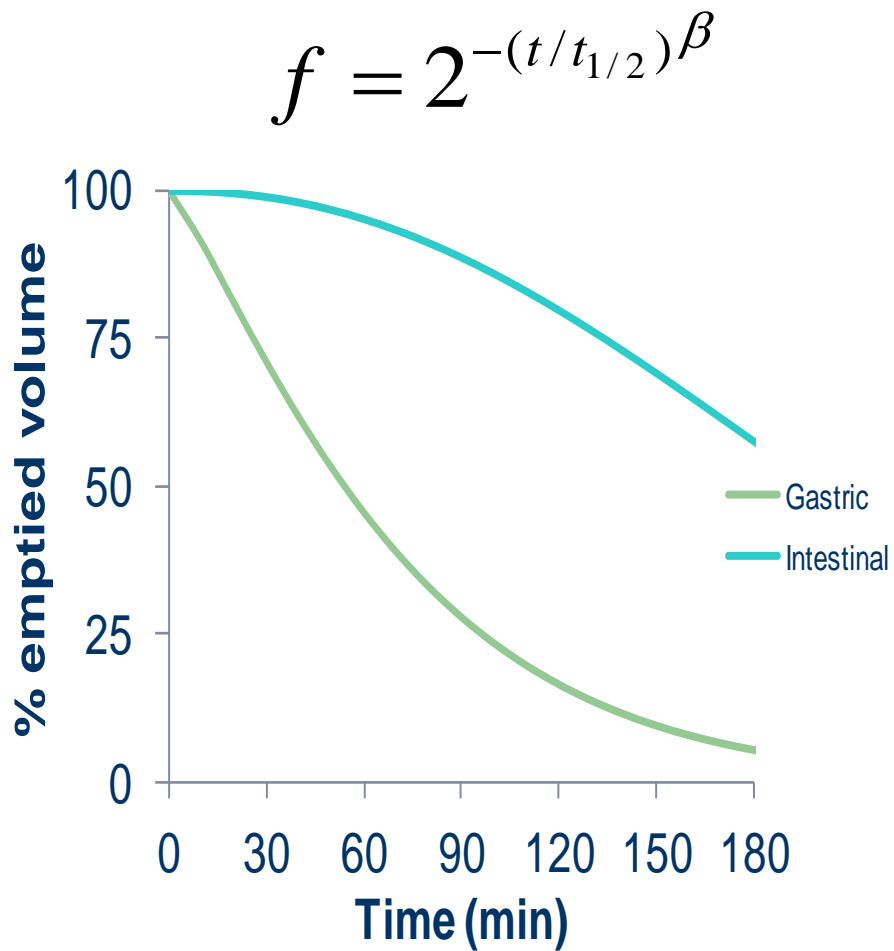
Need parameters to set up the simulator

Parameters taken from previous *in vivo* studies

pH evolution in the stomach



Gastric emptying rate

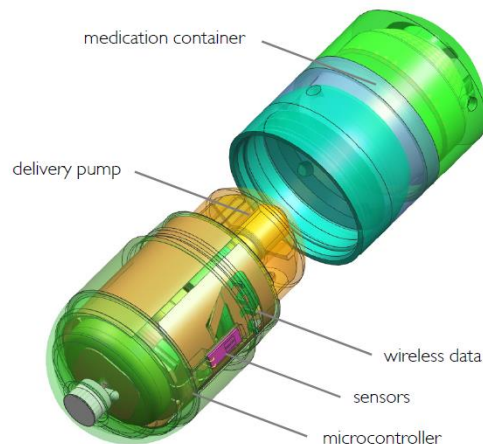


When parameters are not available, you need to determine them

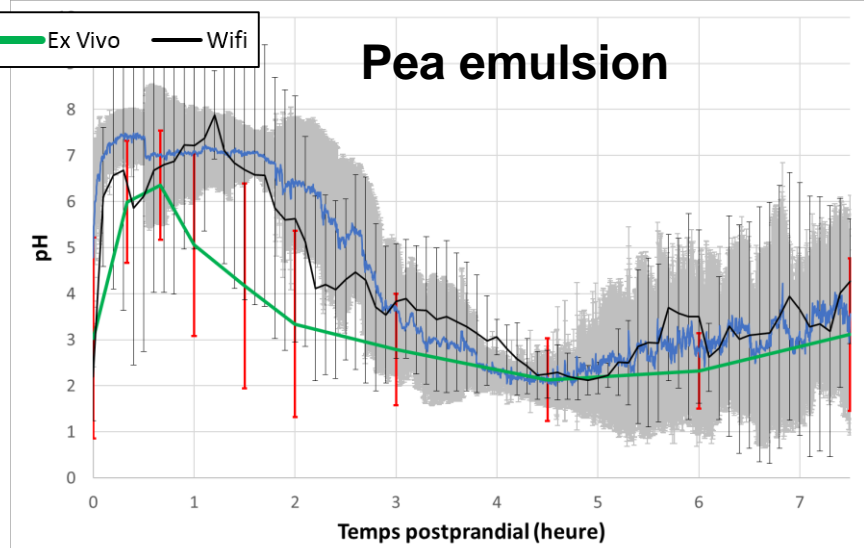
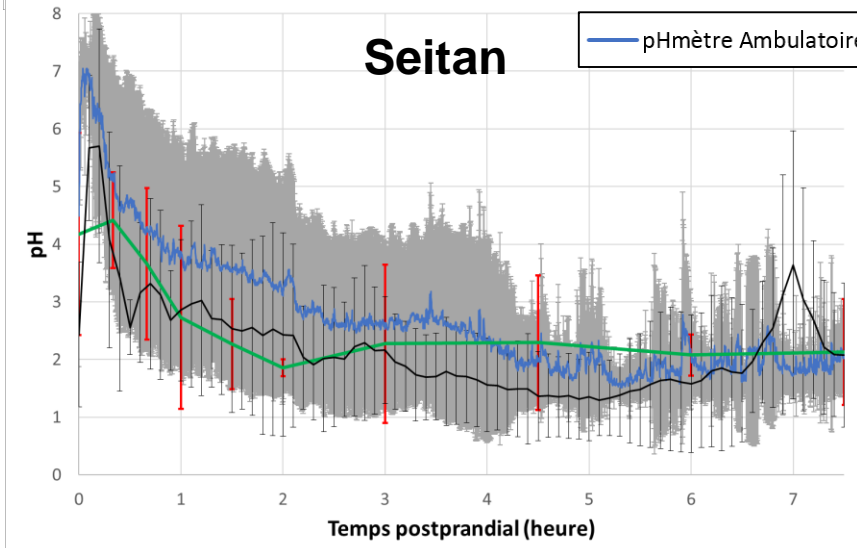
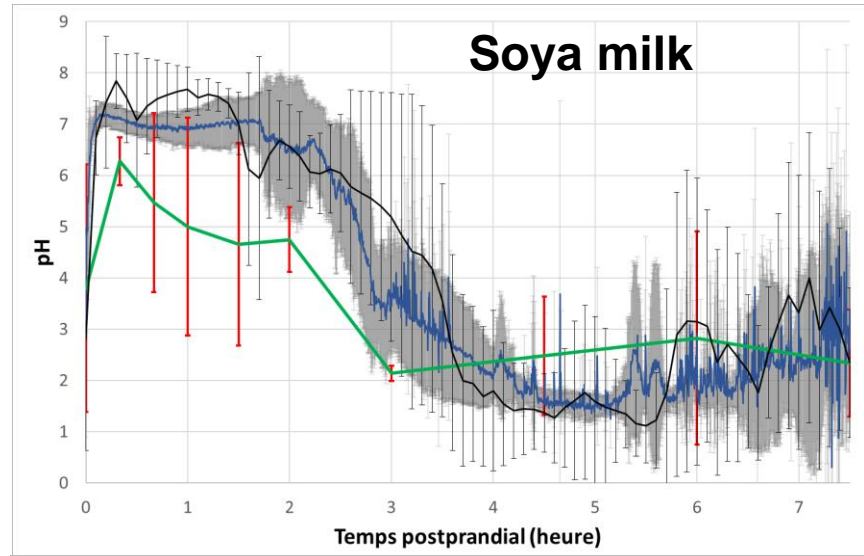
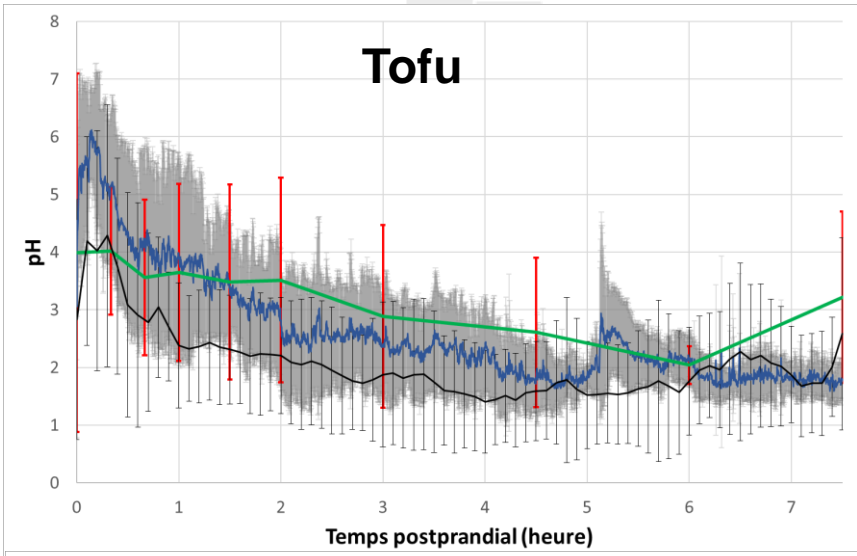
For example, for the pH, the Intellicap[®] System



- Ingestible capsules
- Real-time monitoring of physiological parameters
- Controlled drug delivery unit
- Size: 11 x 26 mm
- IntelliCap[®] measurements:
 - pH (relative accuracy: ± 0.3 pH units)
 - Temperature (relative accuracy: ± 0.1 °C)



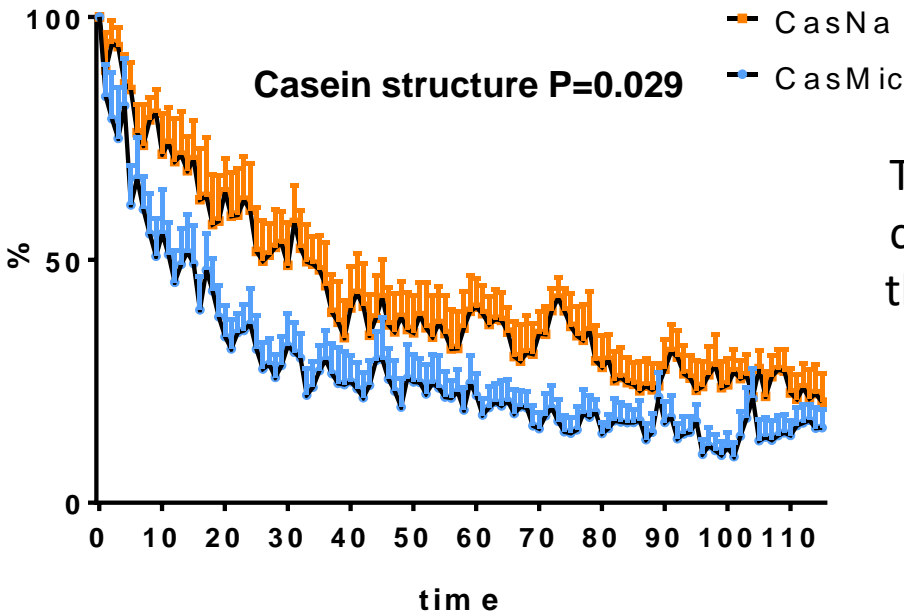
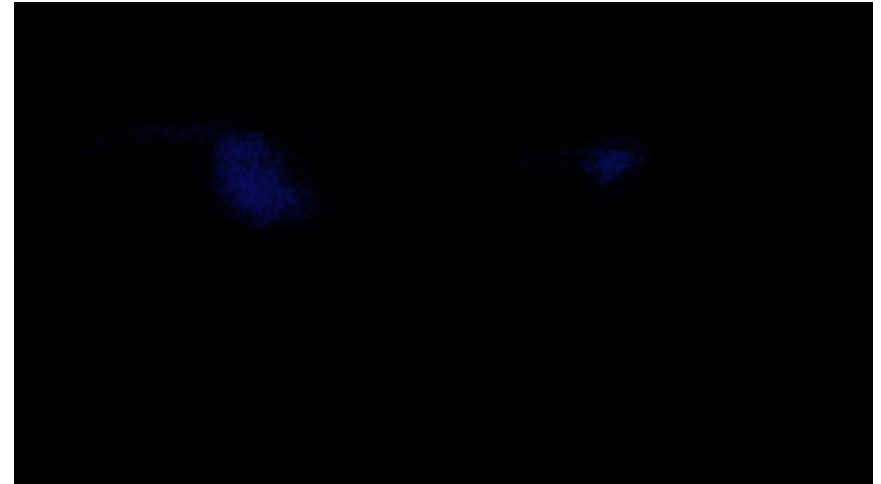
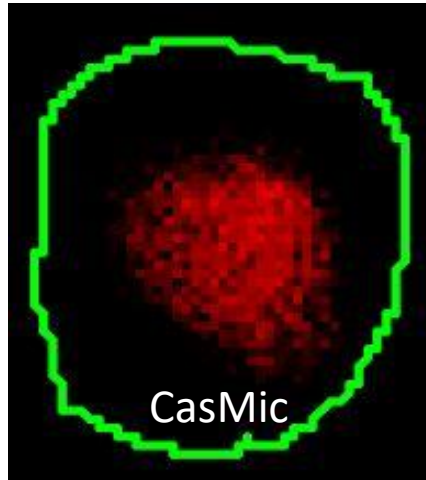
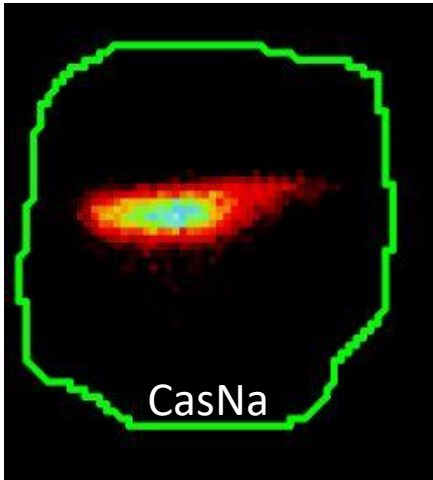
Evolution of gastric pH in mini-pigs during digestion of plant matrices comparison of 3 measurement systems



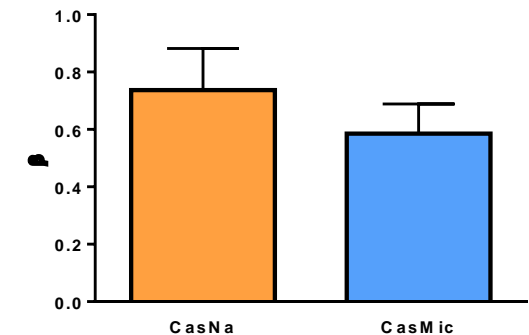
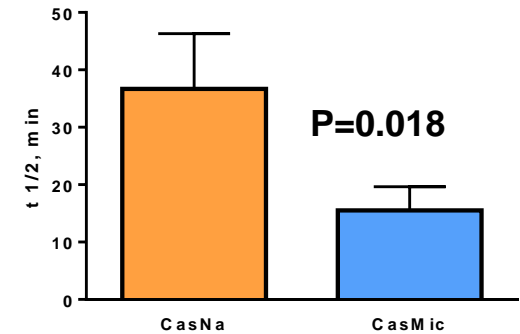
— pHmètre Ambulatoire — Ex Vivo — Wifi

Gastric emptying by gamma-scintigraphy on pigs

Comparison of sodium caseinate/ casein micelles



The structure of caseins regulate their behavior in the stomach



Dynamic *in vitro* digestion models: Pro's & con's

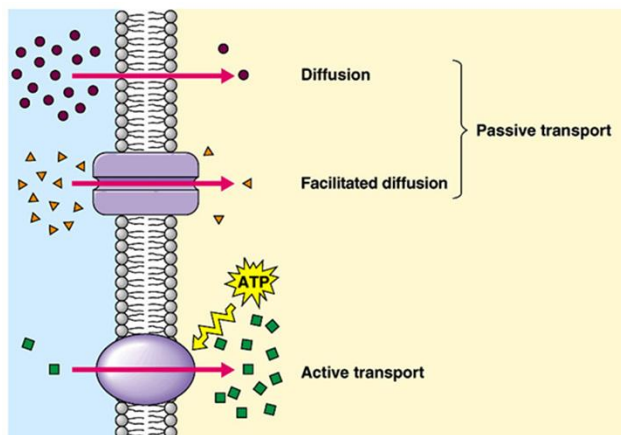


- Highly reproducible
- Physiologically relevant if parameters are available

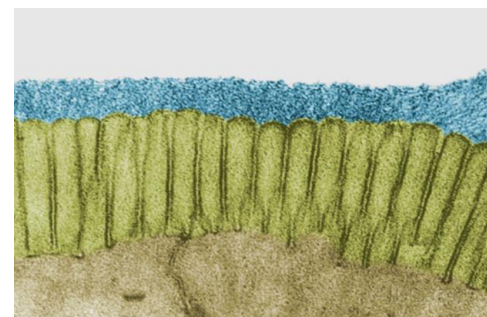


- More expensive than static models
- Too « large » to use pure enzymes (gastric lipase)
- Low throughput

Limitations



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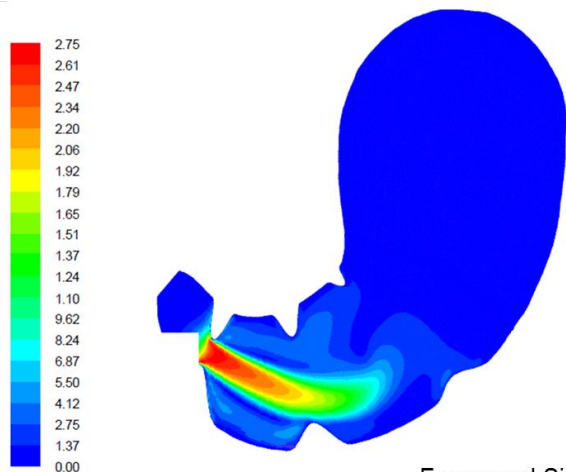


Role of the brush border

Porcine Intestinal Mucus



Role of the mucus layer



Ferrua and Singh, 2010

Shape of the stomach

Absorption is usually poorly mimicked

***In vivo* (animal) models**

Rodents



Probably not the best model to mimic the upper part of the human GI tract
Gnotobiotic animals (« humanized » mice) are of interest to study the effect of food on the microbiota

Pigs



Piglets



Mini-pigs



Multi-canulated and catheterized pigs

Pretty good model of the upper GI tract
Canulas and catheters allow to collect effluent and plasma throughout digestion (kinetics)

Human Models

Patients with ileostomy



Naso-gastric or naso-jejunal probes





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

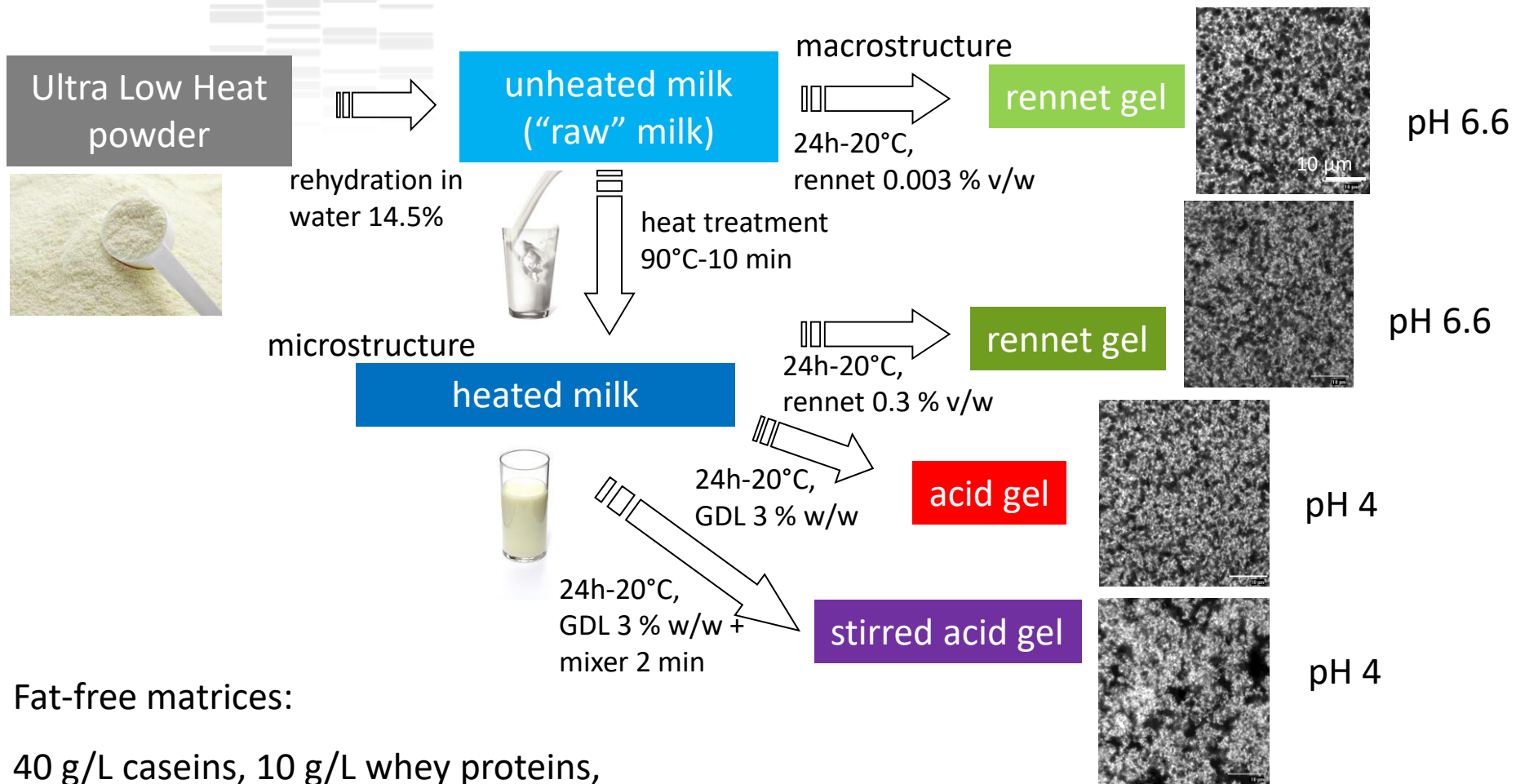


Dupont D.

INRA, Rennes, France



Comparison of 6 dairy products of identical composition but different structure



Fat-free matrices:

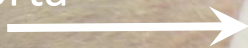
40 g/L caseins, 10 g/L whey proteins,
95 g/L lactose and minerals

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

The multi-cannulated mini-pigs

6 minipigs (20 ± 1kg)

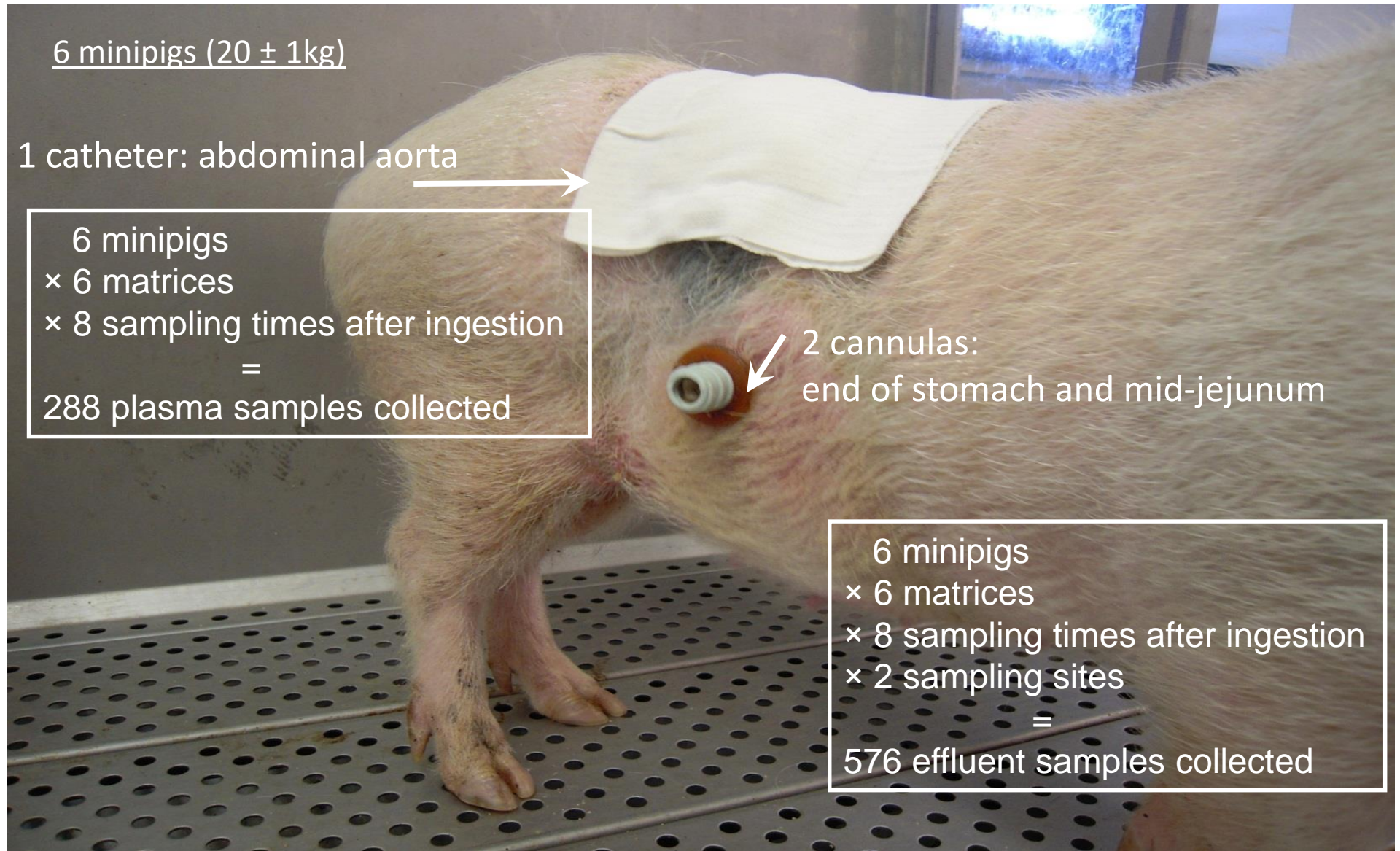
1 catheter: abdominal aorta



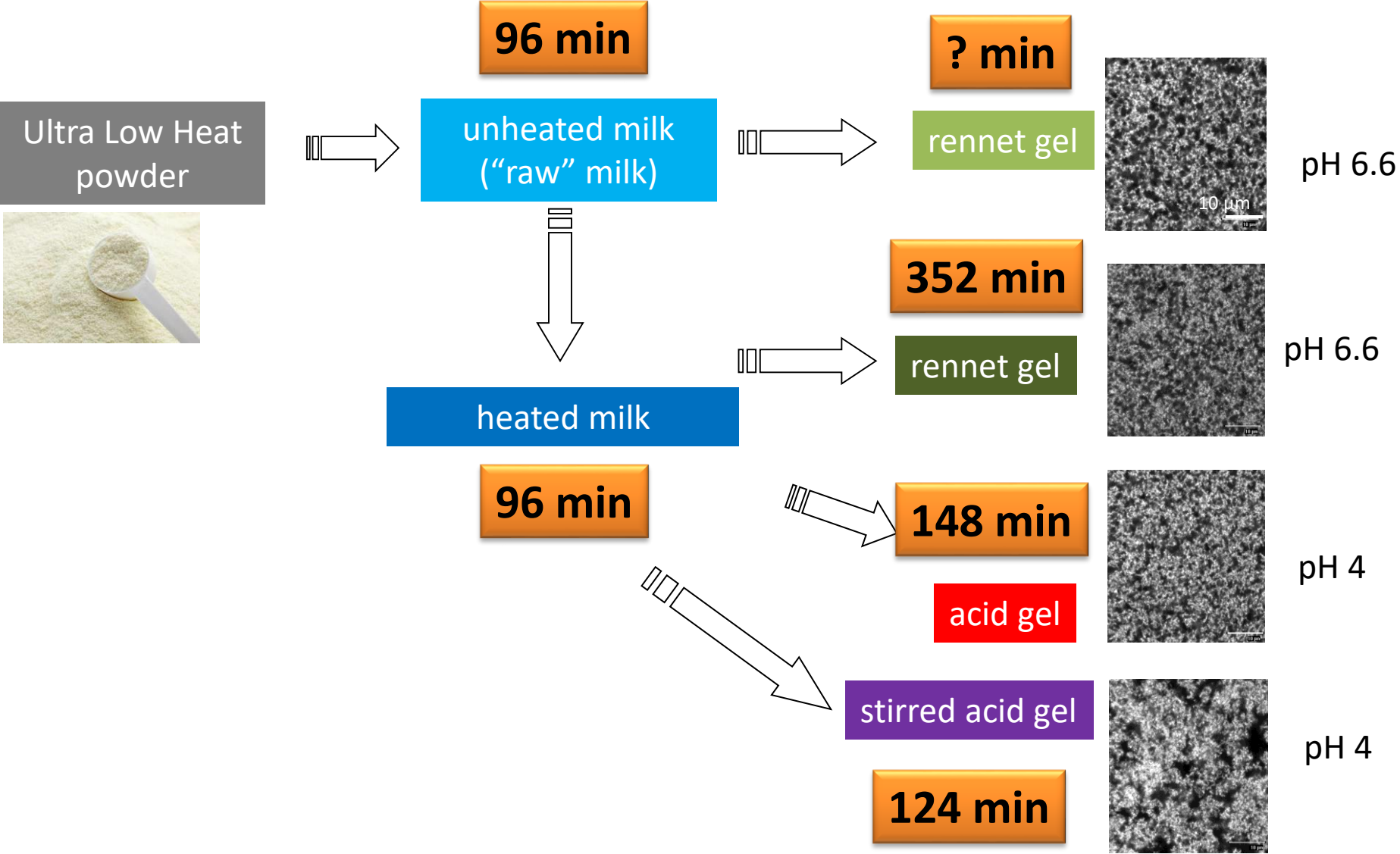
6 minipigs
× 6 matrices
× 8 sampling times after ingestion
=
288 plasma samples collected

2 cannulas:
end of stomach and mid-jejunum

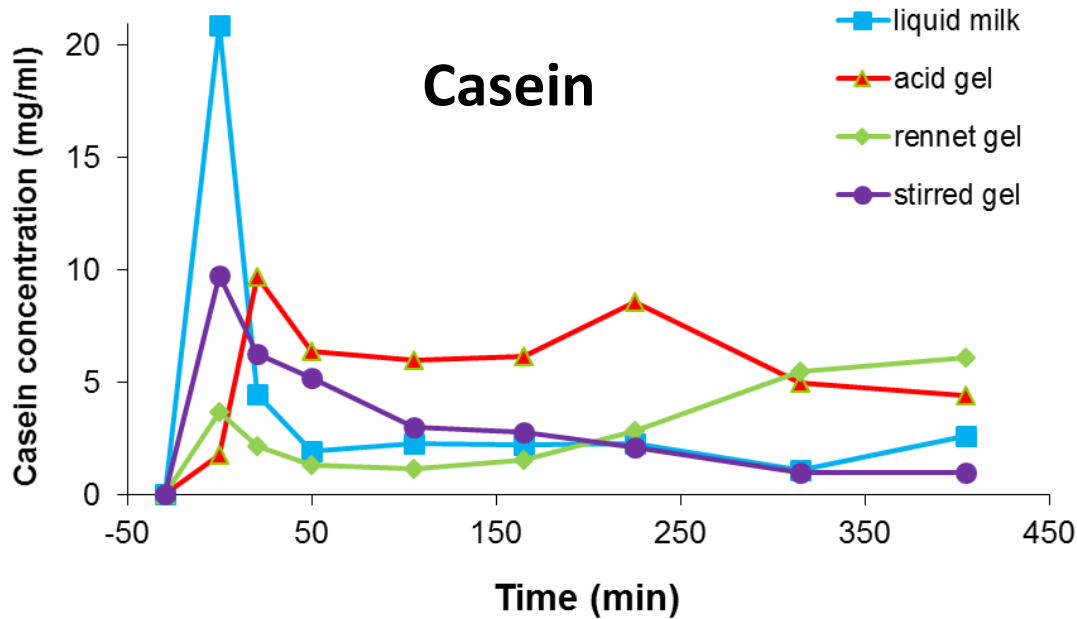
6 minipigs
× 6 matrices
× 8 sampling times after ingestion
× 2 sampling sites
=
576 effluent samples collected



Gastric emptying half time

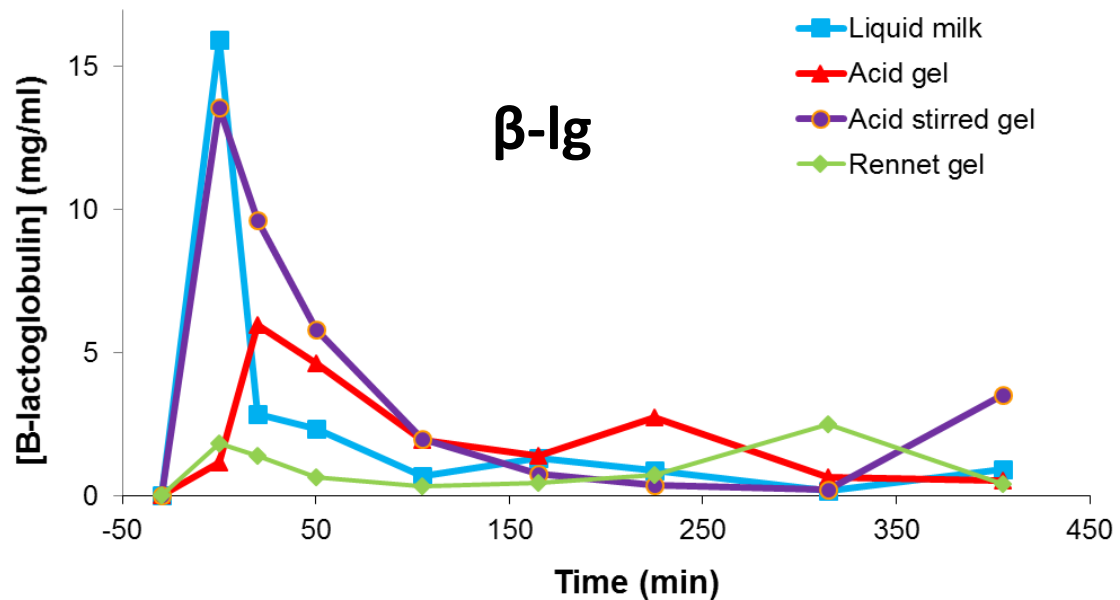


Milk proteins in the duodenum (ELISA)



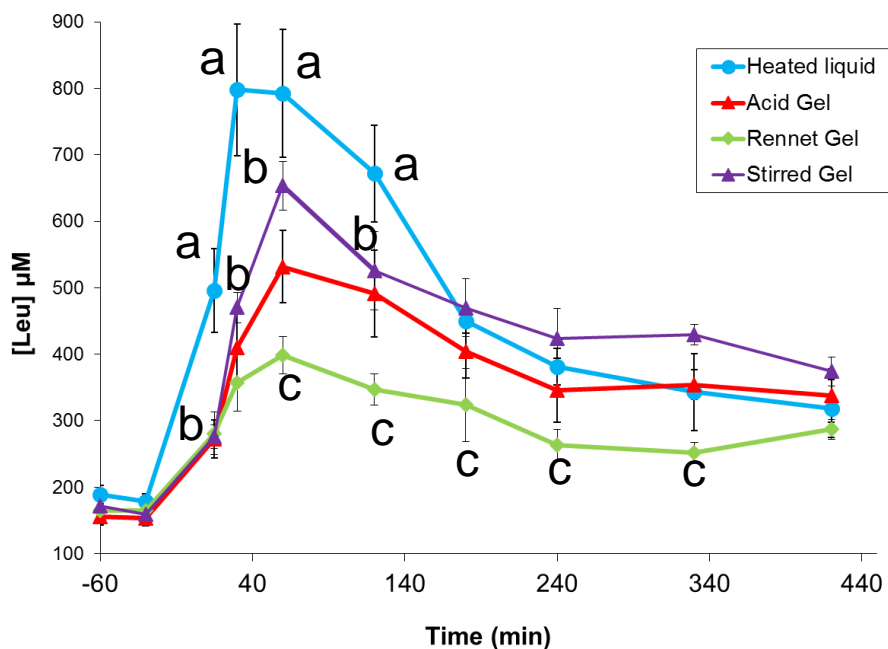
- Intense and early peak with milk
- Lower and delayed with gels
- Intermediate behaviour with stirred gel
- Low concentrations with rennet gel but casein release tends to increase over time

- Only traces of milk proteins found in the jejunum
- Dairy products remain highly digestible



The liquid-gel transition

Effect on absorption



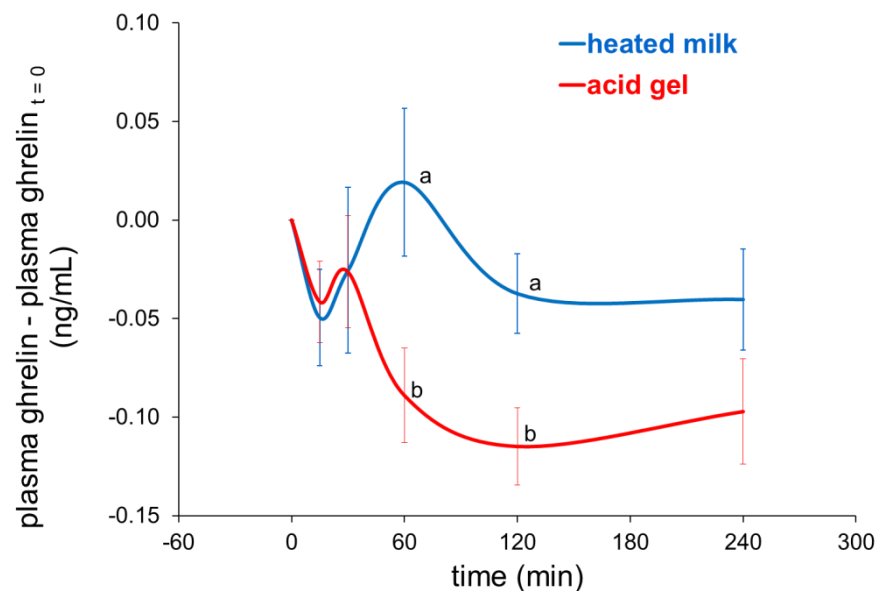
milk gelation:

→ delayed proteins transit → delayed AA absorption

↘ maximal AA concentration in the plasma

Potential effect on satiety

ghrelin (gastrointestinal hormone → appetite stimulation)

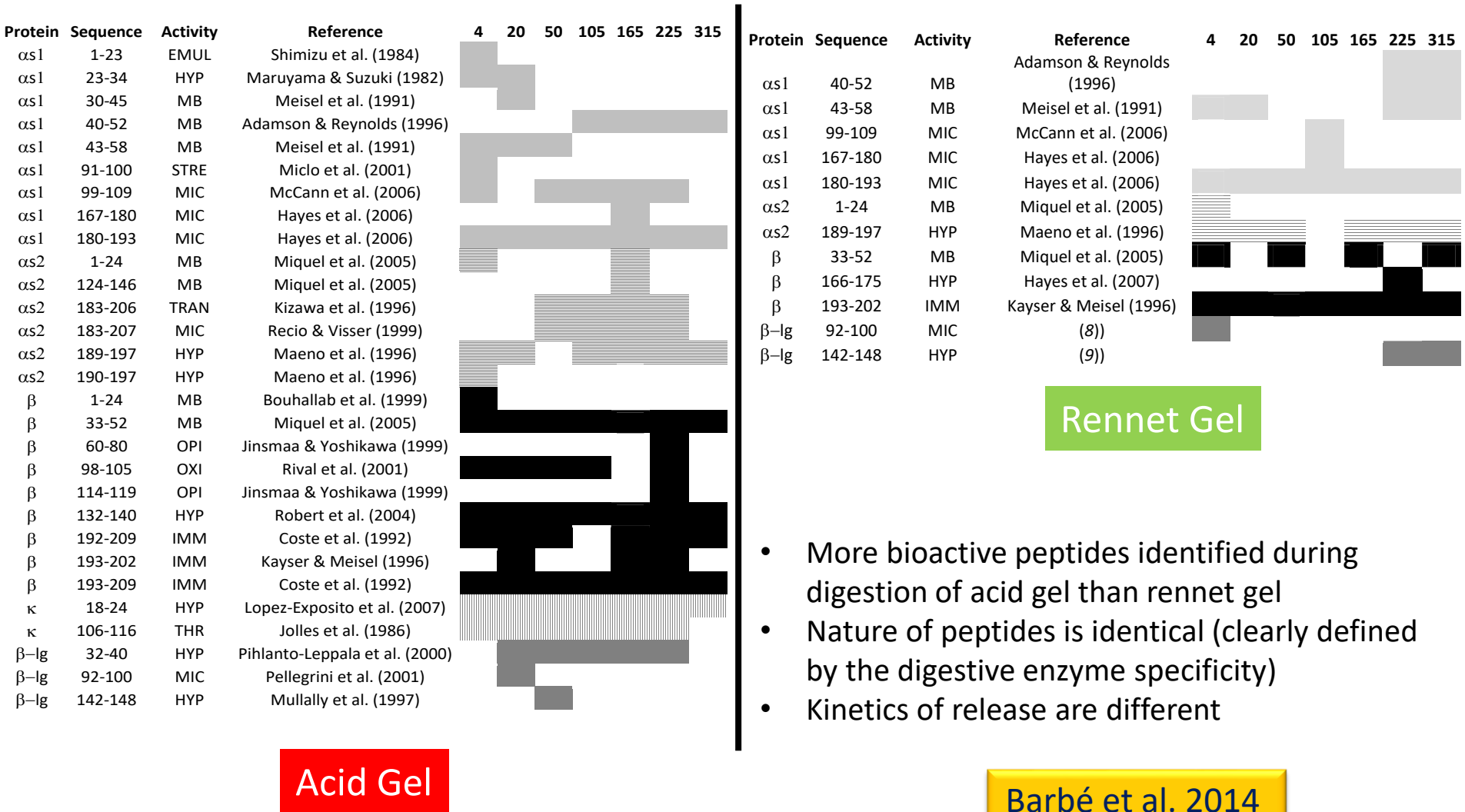


milk gelation:

↘ postprandial ghrelin concentration =
↑ satiety ?

Bioactive peptides released during digestion differ from one matrix to another

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



Rennet Gel

Acid Gel

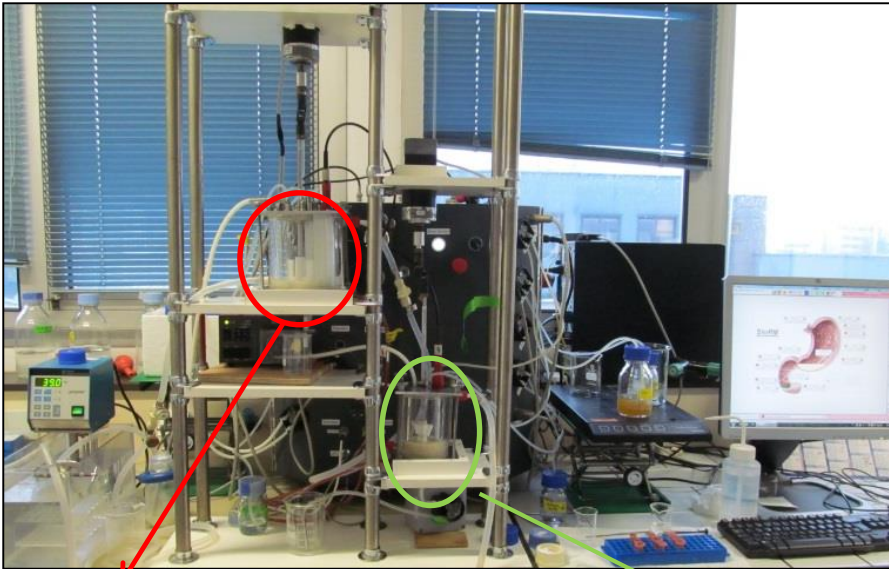
Barbé et al. 2014
Food Res Int

- 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

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



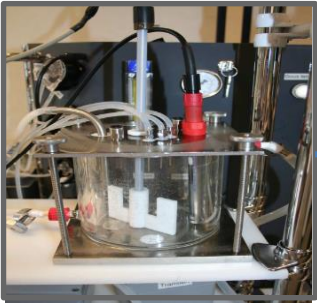
DIDGI®

StoRM® software

Stomach

Small intestine

- Pepsine
- Gastric lipase
- Simulated gastric fluid
- HCl



Emptying :
Elashoff's model



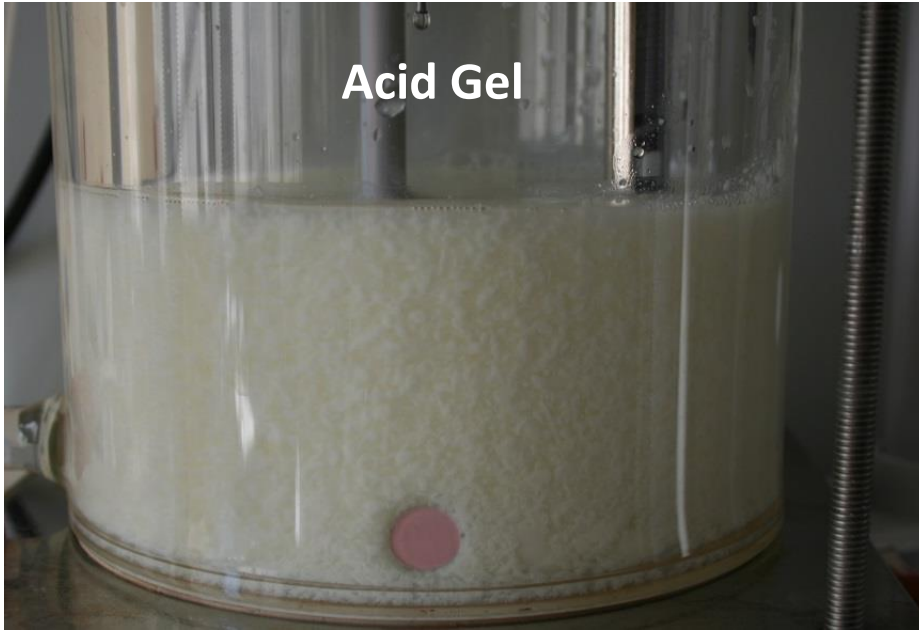
- Pancreatin
- Bile
- Simulated intestinal fluid
- NaHCO₃

Emptying :
Elashoff's model

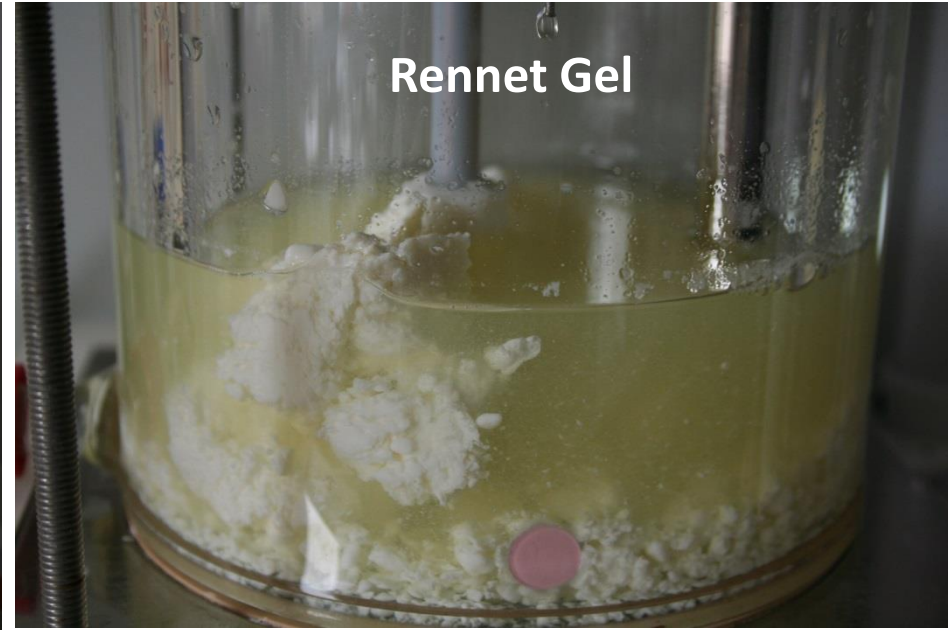
Behaviour of acid and rennet gels in the stomach during *in vitro* dynamic digestion

Barbé et al.
Food Chem. 2014

Acid Gel



Rennet Gel



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

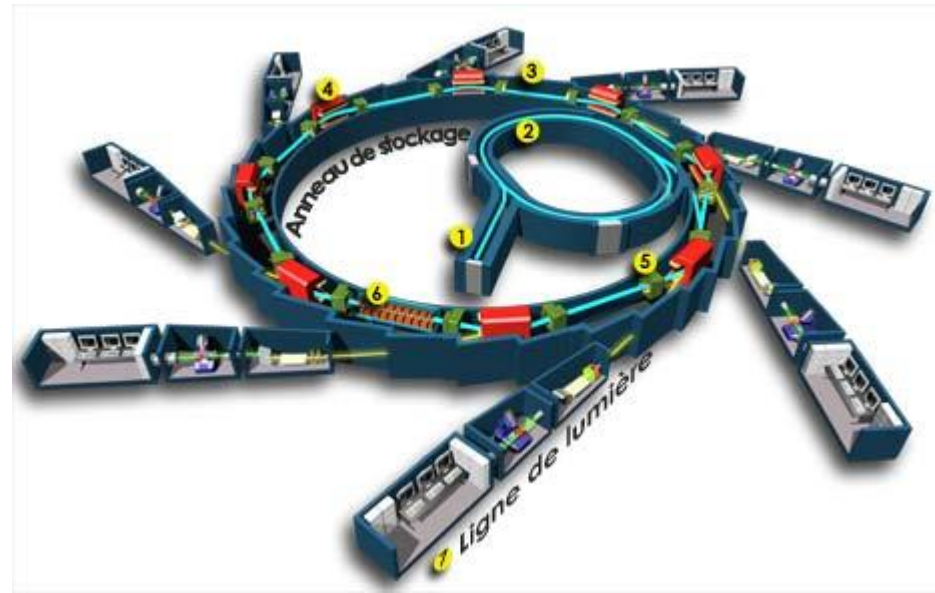


Soleil is a particle (electron) accelerator that produces the synchrotron radiation, an extremely powerful source of light that permits exploration of inert or living matter

DISCO

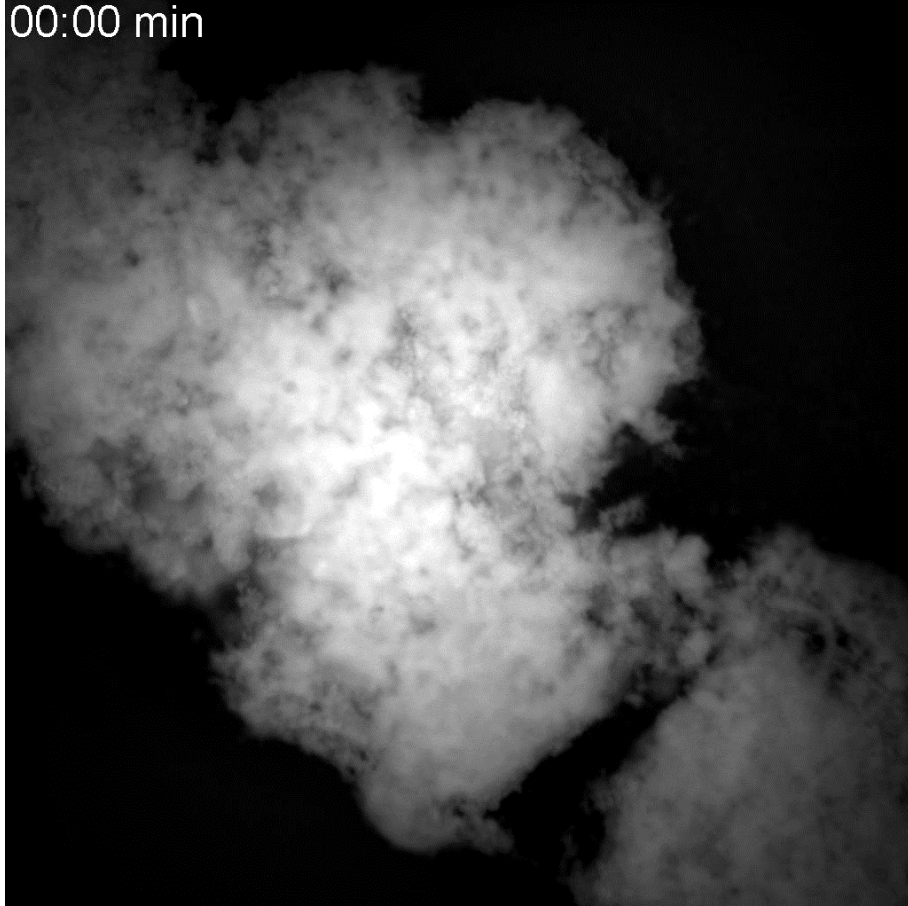
DISCO is a VUV to visible beamline dedicated to biochemistry, chemistry and cell biology. The spectral region is optimized between 60 and 700 nm with conservation of the natural polarization of the light

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

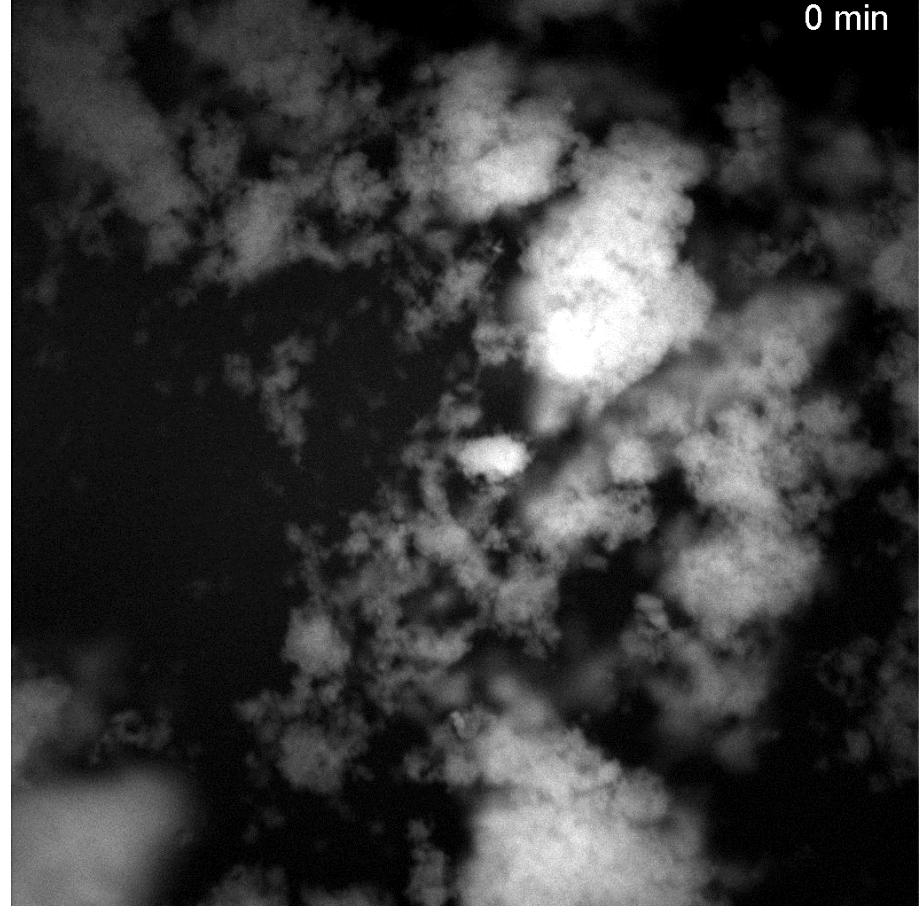


Kinetics of gel particles disintegration

00:00 min



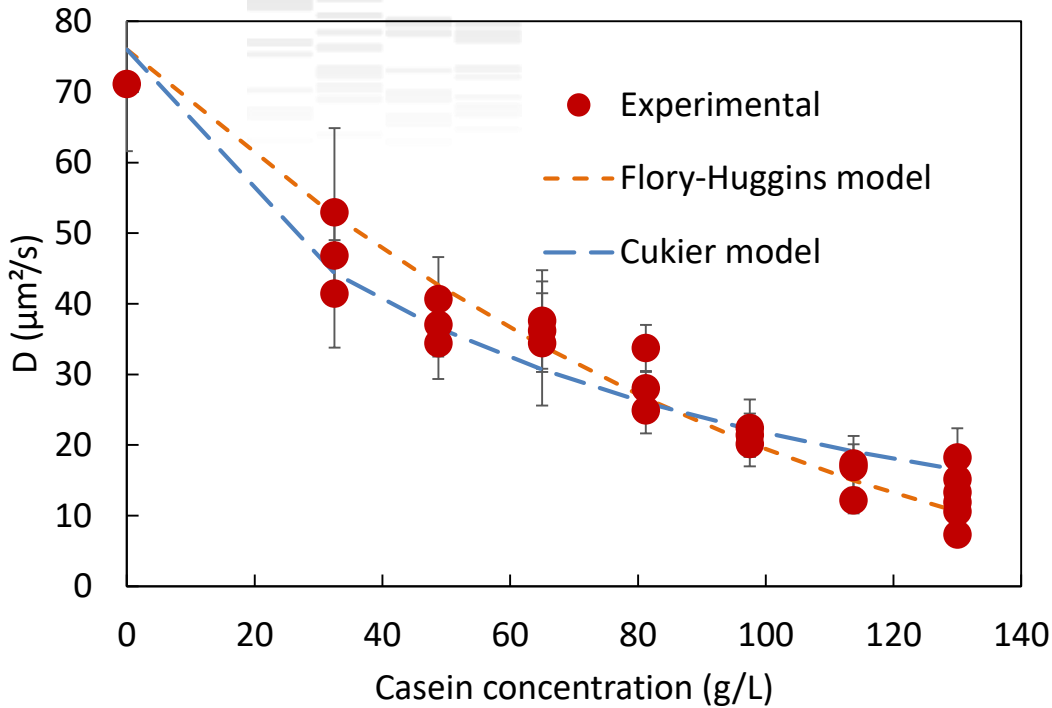
0 min



Rennet Gel

Acid Gel

Diffusion of FITC-pepsin in Rennet gel of increasing casein concentration (37°C)

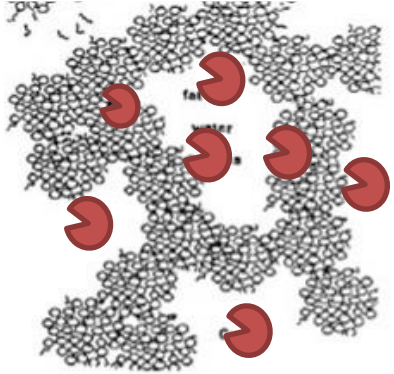


Thevenot et al. Food Chem 2017

Pepsin diffusion of enzyme through the protein network is possible



Food proteins can effectively be hydrolyzed by pepsin from the interior of the gel pores.



What happens when food have the same macrostructure but different microstructures? The case of egg white gels



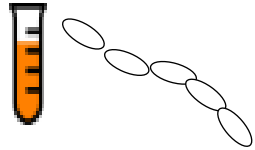
Dupont D.

INRA, Rennes, France

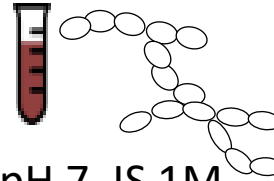


The microstructure of egg-white gels made from different types of aggregates affects the kinetics of proteolysis

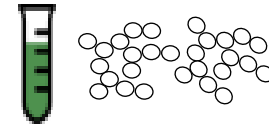
80°C/6h



pH 9, IS 1M

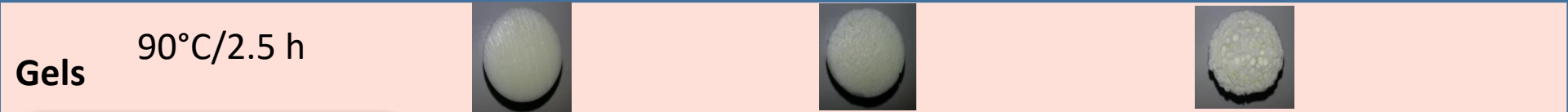


pH 7, IS 1M



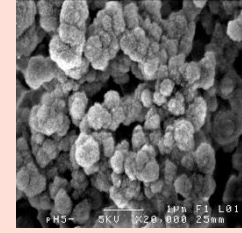
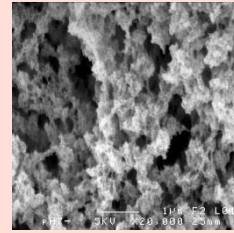
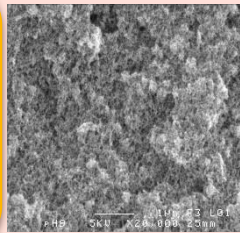
pH 5, IS 1M

Aggregates	linear	branched	spherical
Rate of <i>in vitro</i> digestion	+++	++	+

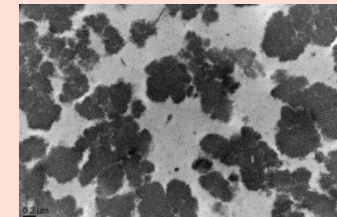
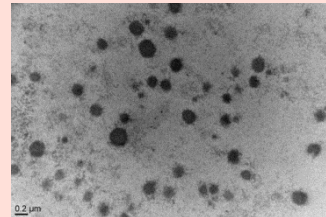
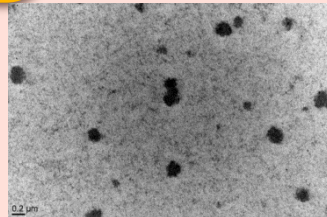


Nyemb et al., 2016. Food Hydrocolloid, 54, 315-27

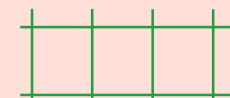
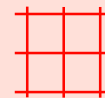
Nyemb et al., 2016. Food Res. Int., 88, 302-9



SEM



CRYO-TEM



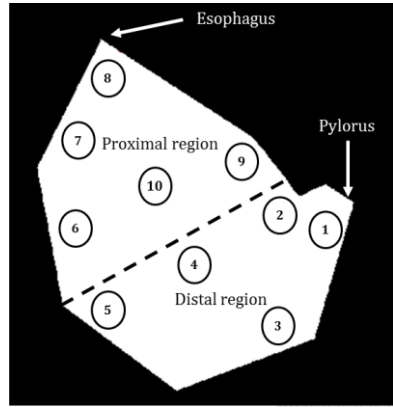
Rate of *in vitro* digestion

+

++

+++

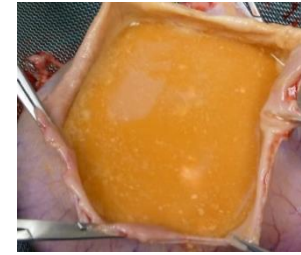
Spatio-temporal evolution of pH during an *in vivo* digestion



20 min



60 min

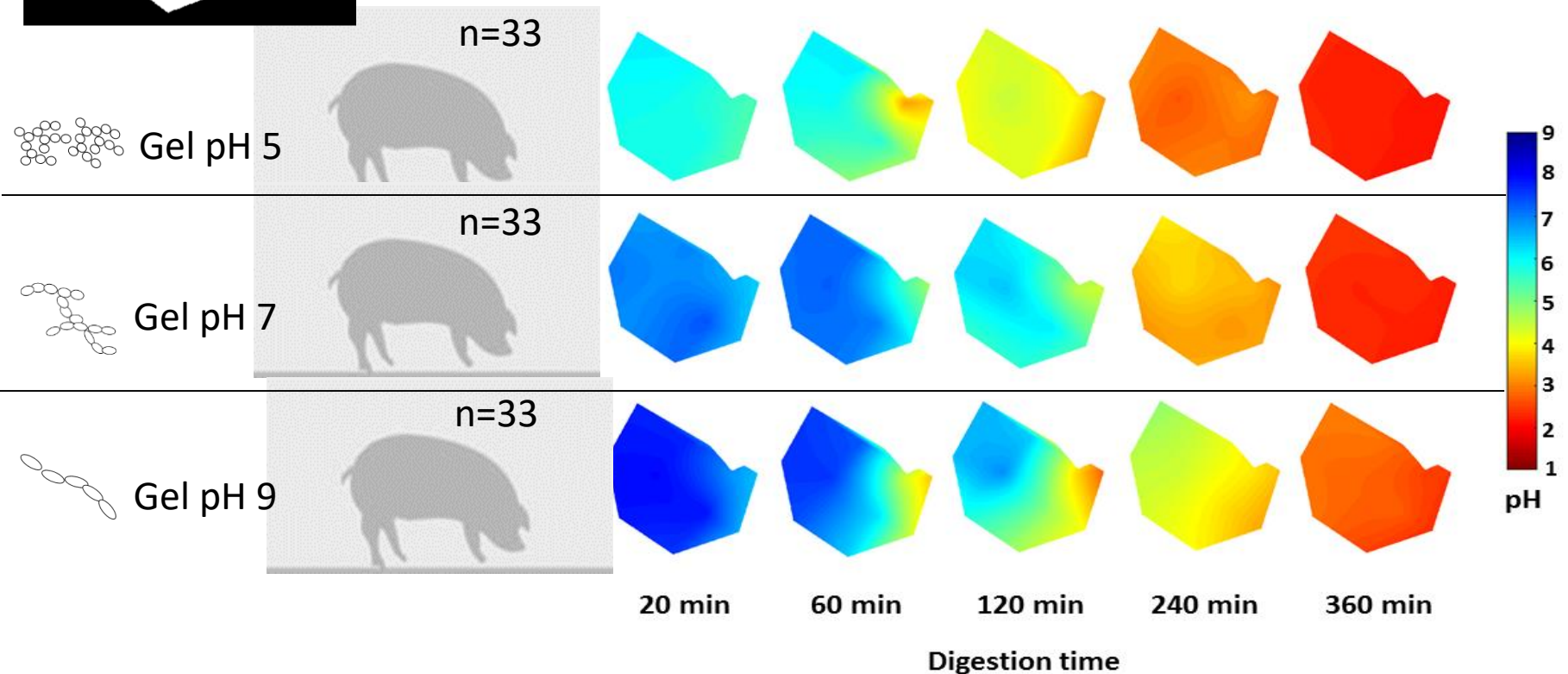


120 min

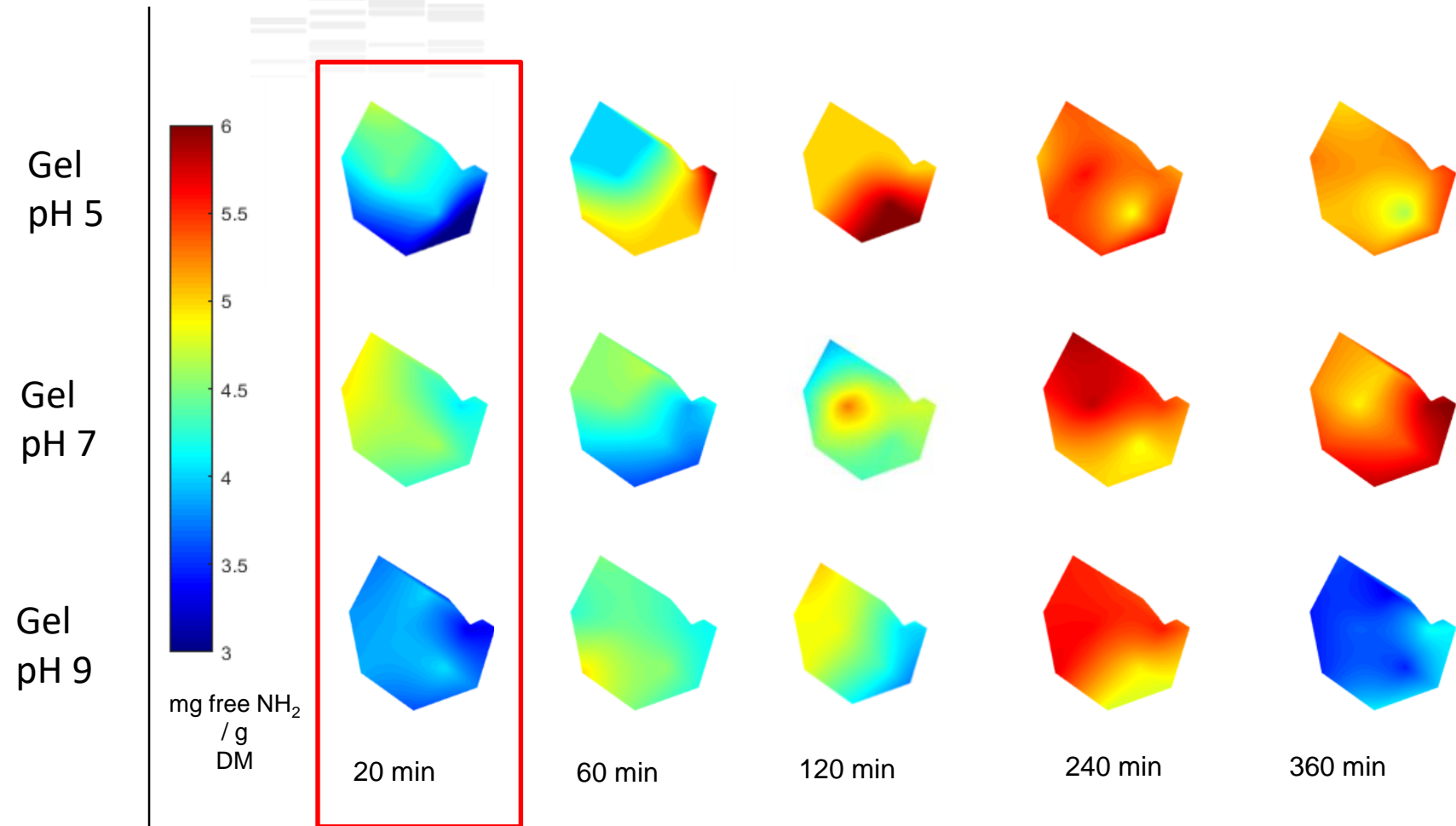


240 min

pH 7.0



Kinetics of proteolysis



Proteolytic activity even at pH 7 and 9: probably driven by local pH

Digestion and oral comfort of cheese developed to meet the sensory and nutritional needs of the elderly



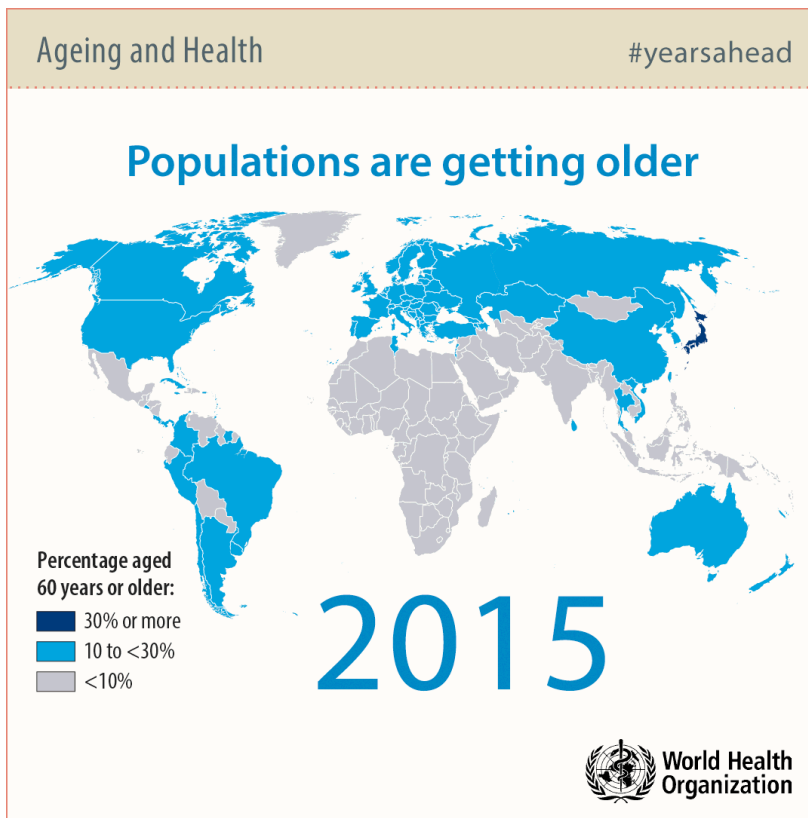
L Lorieau, J Flourey & D Dupont

STLO, UMR 1253, INRA, Agrocampus Ouest, 35000 Rennes, France



AGEING = a world problem...

The EU population over 65
2015 : **1/5** of the population
2050 : **1/3** of the population

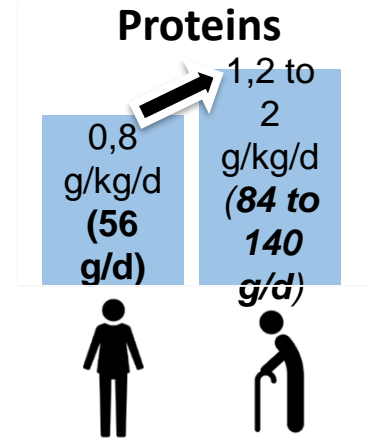
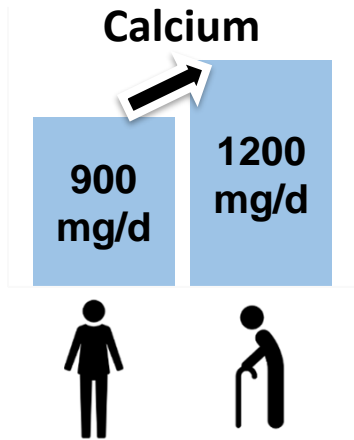


Challenge : healthy ageing

=

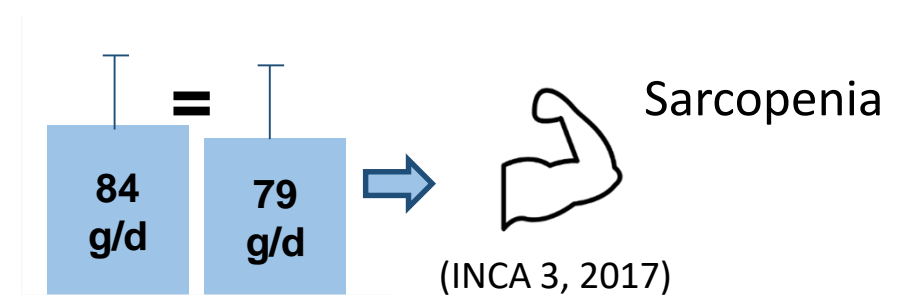
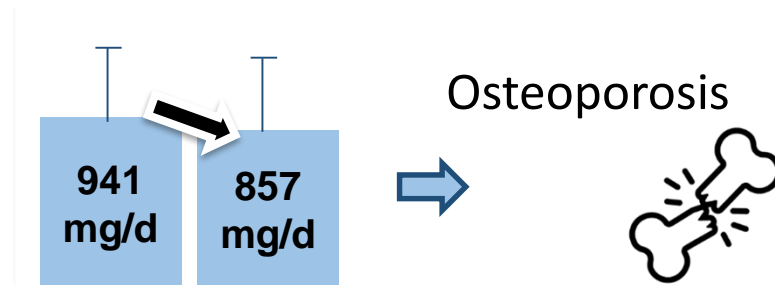
- Keeping people autonomy
- Offering them physical and social activities
- Preventing the development of pathologies
- **Offering them an adequate diet**

The nutritional needs of elderly people



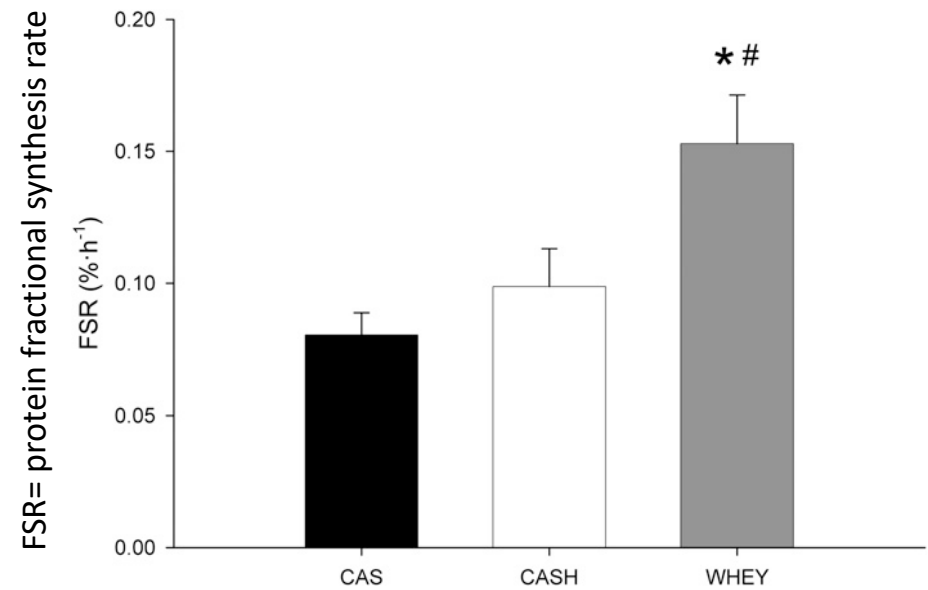
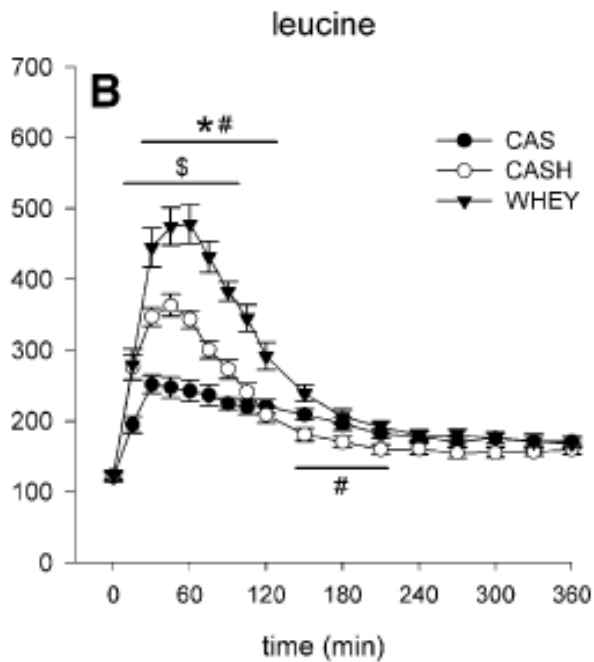
(Baum et al. 2017)

Mean daily intake



(INCA 3, 2017)

Muscle protein synthesis can be restored when providing high amounts of Leucine through whey proteins (10 g) but not caseins



Pennings et al. 2013

Objectives of the project

To **design a range of cheeses** with identical composition (n=4):

- ☞ exclusively made of whey proteins to provide 10 g of protein/portion and high intake of Leucine

- ☞ enriched in calcium to provide 280 mg of calcium (25% of the daily intake)









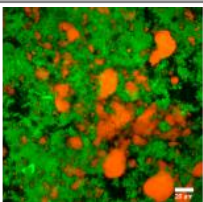
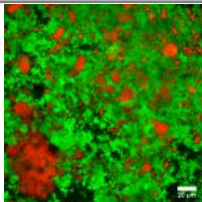
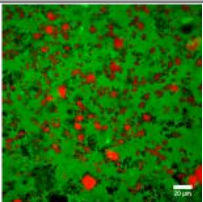
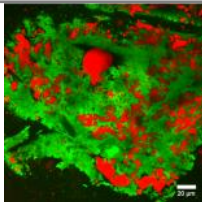
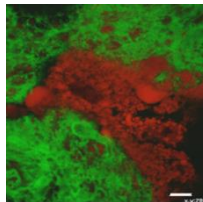
To **assess the sensory perception of these cheeses** by elderly people with impaired or intact dental health (n=76)

To **assess the calcium and amino acid bioavailability** in conventional pigs



Design of the cheeses



	Liquid (24%) 	Soft 	Processed 	Hard 	Whipped 	Mozzarella 
Texture perception		Soft and melting	Sticky and pasty	Firm and slightly dry	Dry and granular	Elastic
pH	6.4 ± 0.05	6.4 ± 0.05	6.0 ± 0.05	6.4 ± 0.05	5.6 ± 0.05	5.3 ± 0.05
Hardness		16 ± 8	9 ± 4	108 ± 20	6 ± 1	95 ± 1
Type of protein	Whey proteins					Caseins
[Leucine] g/kg	4.70 ± 0.41					3.61 ± 0.01
Microstructure confocale x60 scale: 20 μ m  Proteins  Lipids						

Which textures of cheeses are comfortable for older people with or without oral health impairment?



76 volunteers + 65 y.o
(Dentition and Flow saliva)
x
4 cheese models



Perception

Food comfortability
Questionnaire
(Vandenberghé *et al*, 2017)



- Q1. Comfort
- Q2. Bolus formation
- Q3. Pain
- Q4. Product texture
- Q5. Flavor



Bolus formation

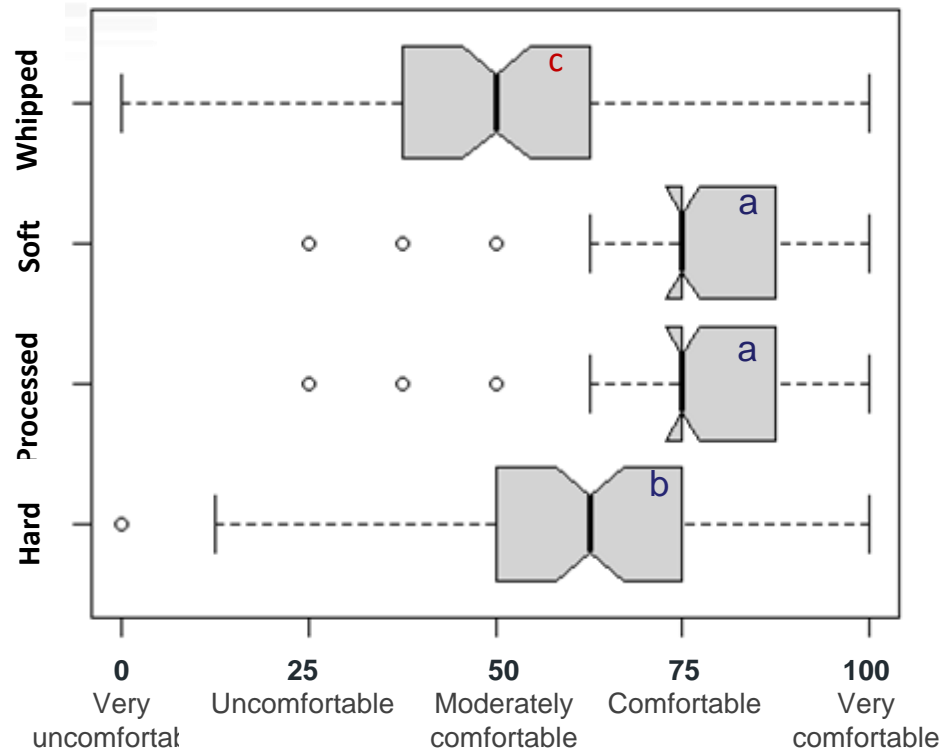
- **Chewing behavior :**
Video

Cycle number
Chewing time

- **Food bolus**
Rheology - *Compression*
Quantity of saliva - *Dry extract*
(Drago *et al*, 2011)



The food comfortability is mainly impacted by the type of product consumed

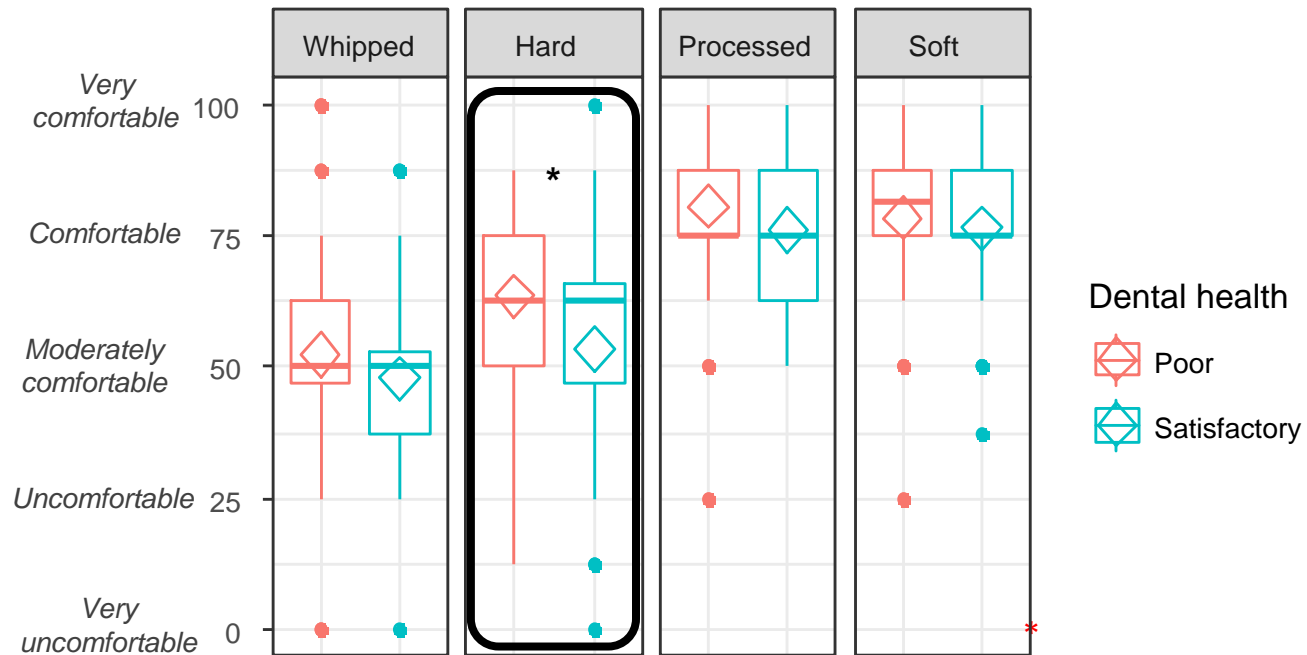


ANCOVA : Variable~ Product + Error(Subject)



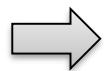
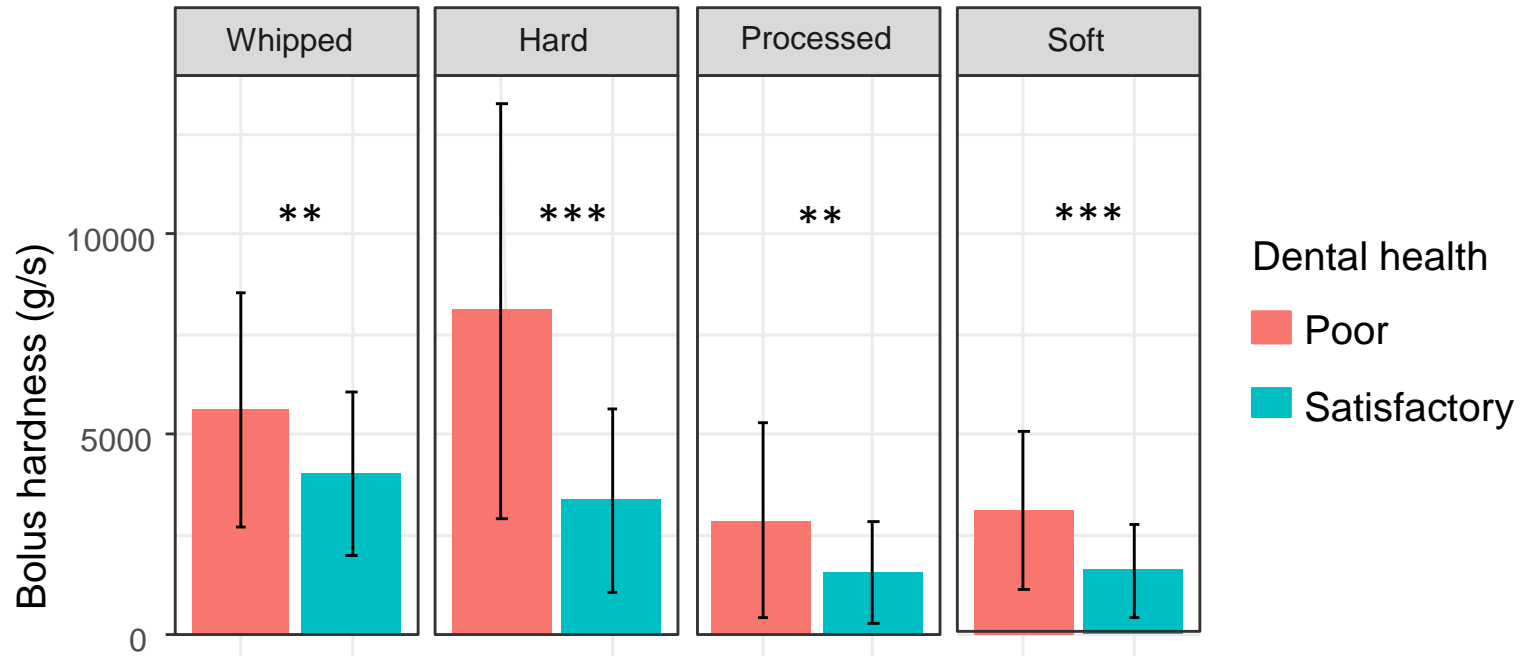
The most comfortable products are the Soft and Processed products

The food comfortability is not negatively impacted by the dental health



Elderly people with a poor dental health considered the Hard product more comfortable than elderly people with a satisfactory dentition

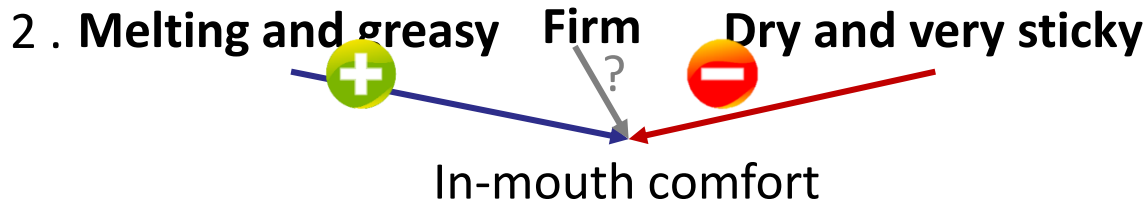
Elderly persons with a poor dental health made harder bolus than elderly persons with a satisfactory one



Decrease in food bolus disintegration by denture wearers observed by Mishellany-Dutour *et al.* (2008), Woda *et al.* (2006), Yven *et al.* (2006)

Which textures of cheeses are comfortable for older people with or without oral health impairment?

1 . A product is comfortable when the bolus is easy to form



3 . Oral health alterations do not negatively impact the in-mouth comfort
Vandenberghé-Descamps et al (2017), Assad-Bustillos et al. (2017), Laguna (2016)

But the bolus formed are different (hardness and humidity)

In vivo digestion

Whey-based
Soft cheese
CaCO₃

Whey-based
Soft cheese
CaP

Mozzarella
Milk Calcium

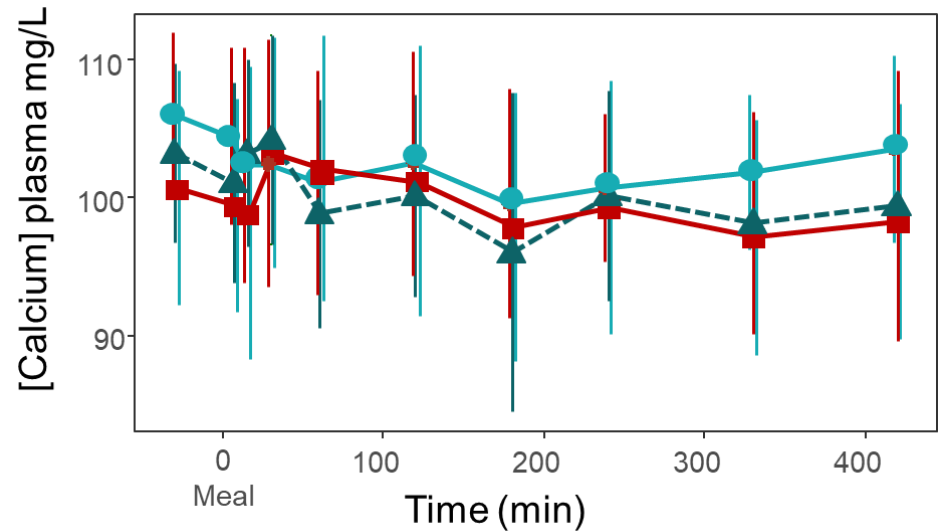
Iso-calcium : 0,7%
Isocaloric ≈
280 kcal /100g

Test meals



Blood samples

from 30 min before to 7h after meal ingestion



6 castrated and
catheterized
pigs

[Calcium] in plasma is highly stable

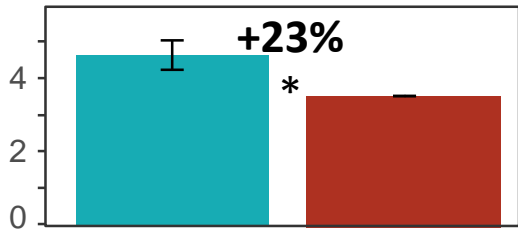
Both calcium sources allow to provide bioavailable calcium

Needs long-term studies for possible consequences on bone health

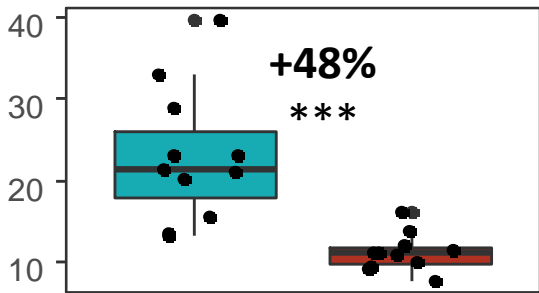
Leucine « bioavailability »



Cheese leucine content (g/Kg)

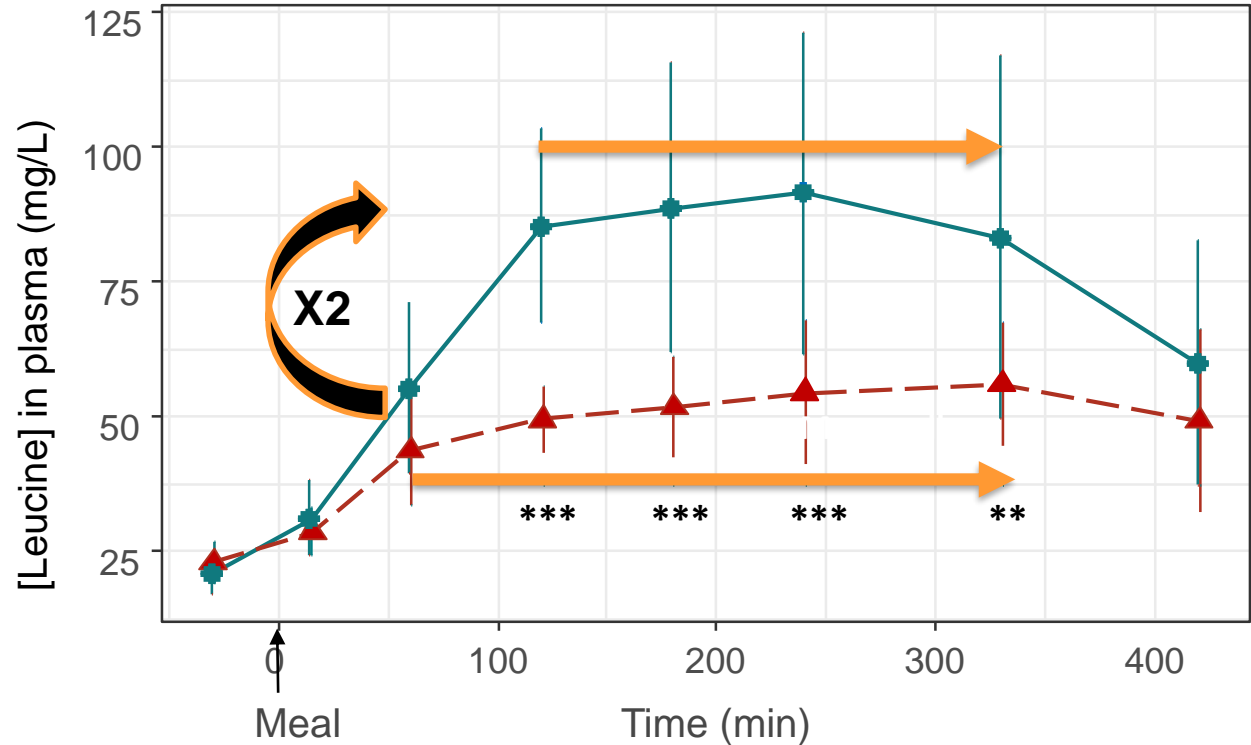


Area under the curve (g/L/min)



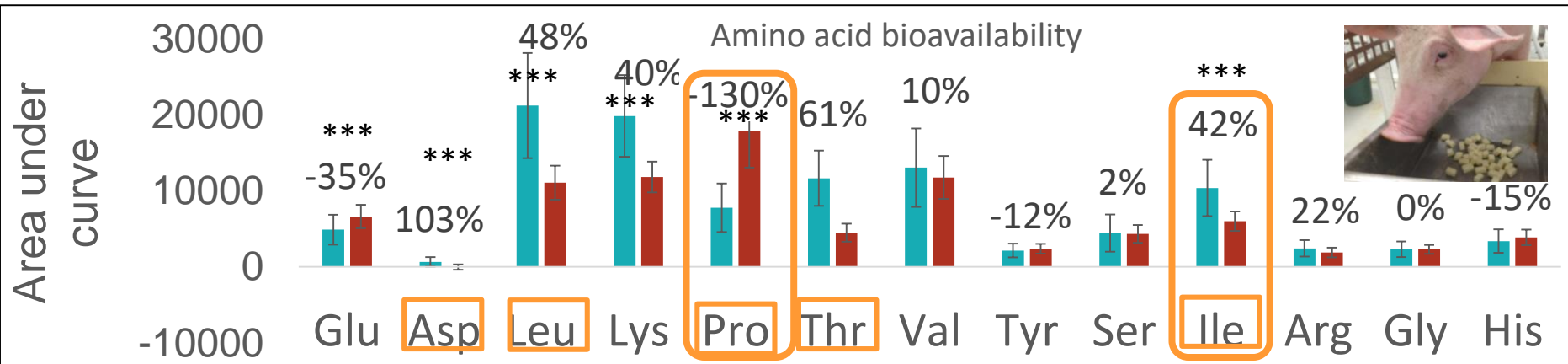
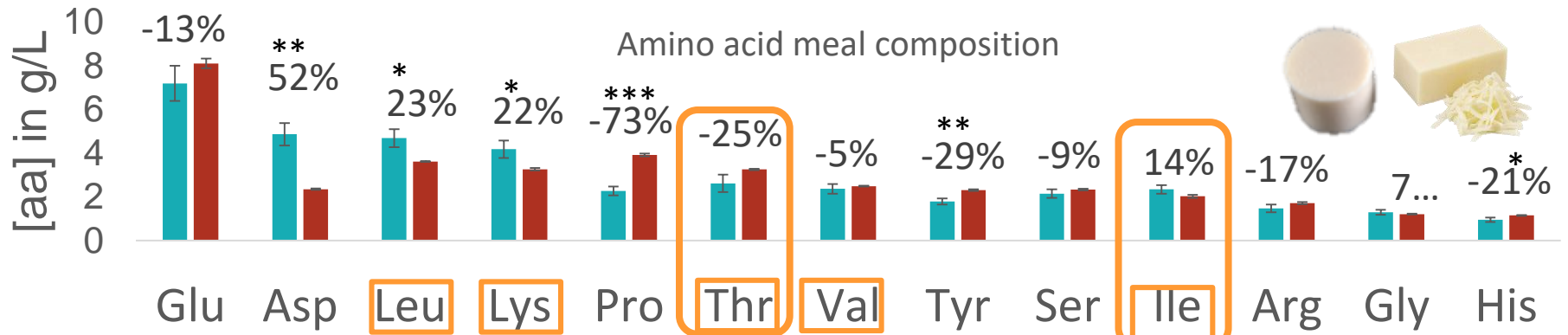
Whey-based cheese

Casein-based mozzarella



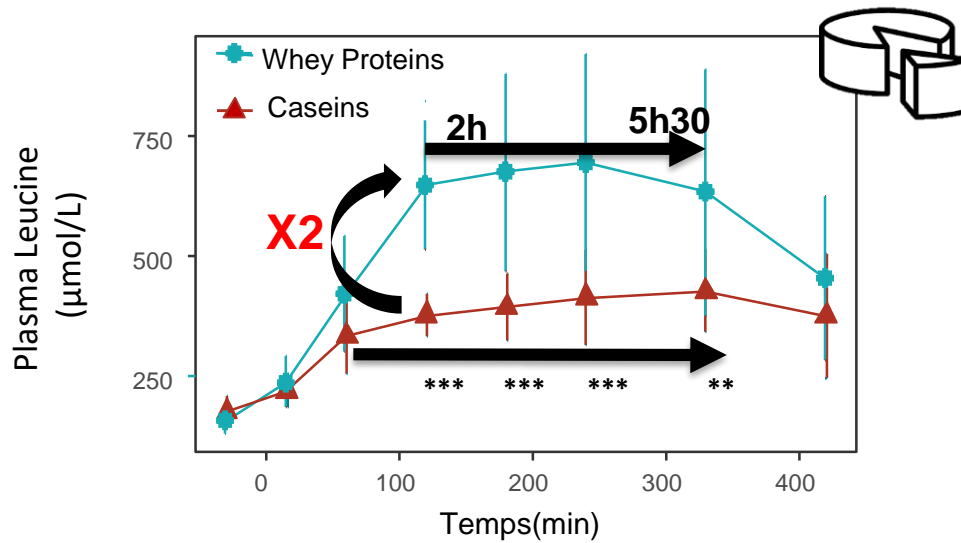
Leucine « Bioavailability »
Whey-based cheese > Casein-based Mozzarella

What about the other amino acids?

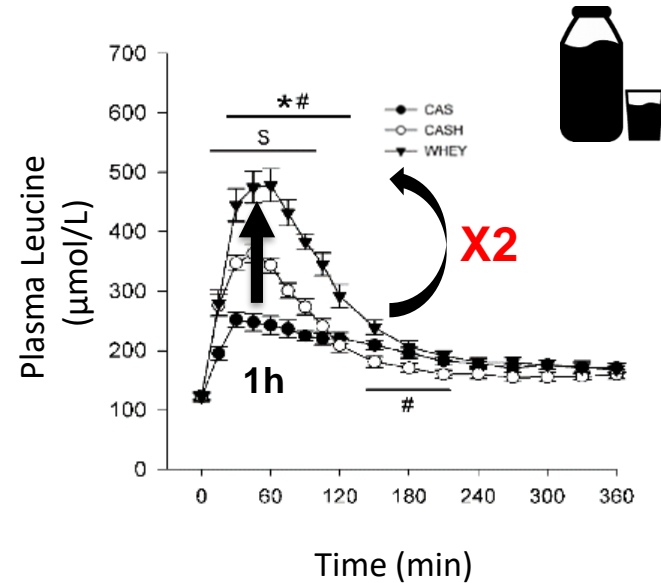


■ Whey-based cheese
 ■ Casein-based mozzarella
 Essential amino acids

Amino acid bioavailability for solid foods



Lorieau et al. 2018



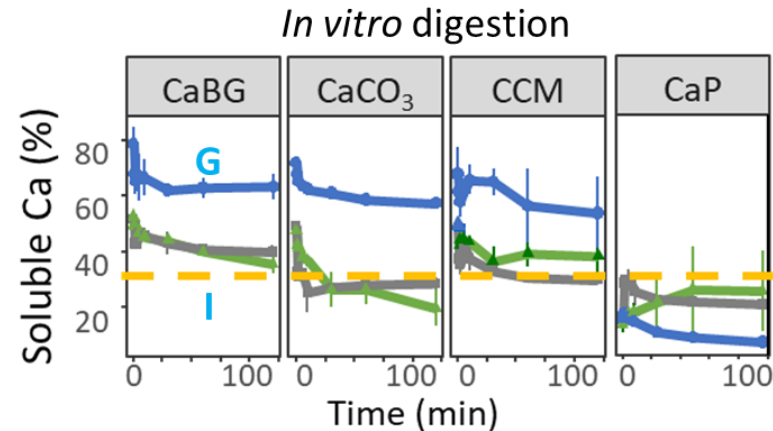
In elderly people (~74 y)
Pennings et al, 2011

👉 Our data confirm those previously obtained on elderly for liquid samples

Conclusion & perspectives

No differences in Ca bioavailability were seen during the *in vivo* study on pigs

Whey-based cheeses allowed to increase Leu bioavailability



The differences between casein and whey-based cheeses could be due difference in kinetics of digestion. This could result in the selective sequestration of some AA by the splanchnic area ? Or be due to oxidation of some AA before and after absorption???


In the future, we would like to determine:

if other sources of calcium can increase its bioavailability

how the hardness and humidity of the bolus will affect digestion in elderly

if the whey-based cheeses really allow to restore muscle synthesis in elderly





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.

INRA, Rennes, France



Human/ bovine milk / Infant Formula

Protein structures

Human milk



Bovine milk



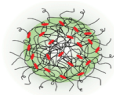
Infant Formula



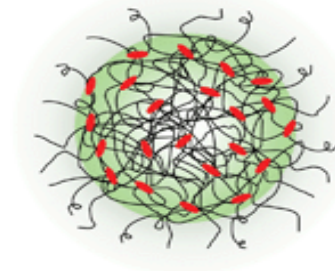
(Turck *et al*, 2010)

$\varnothing = 64 \text{ nm}$
(β , κ -casein)

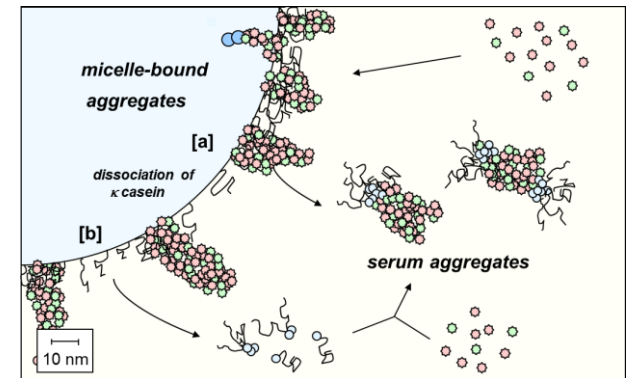
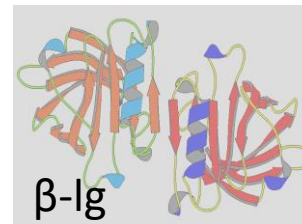
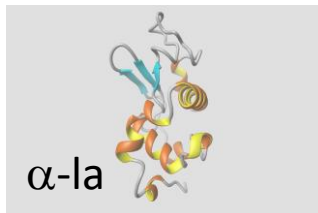
Casein
micelle



$\varnothing = 182 \text{ nm}$
(α 1, β -casein)



Whey
Proteins



Human/ bovine milk / Infant Formula

Lipid globule structure

Human milk

Bovine milk

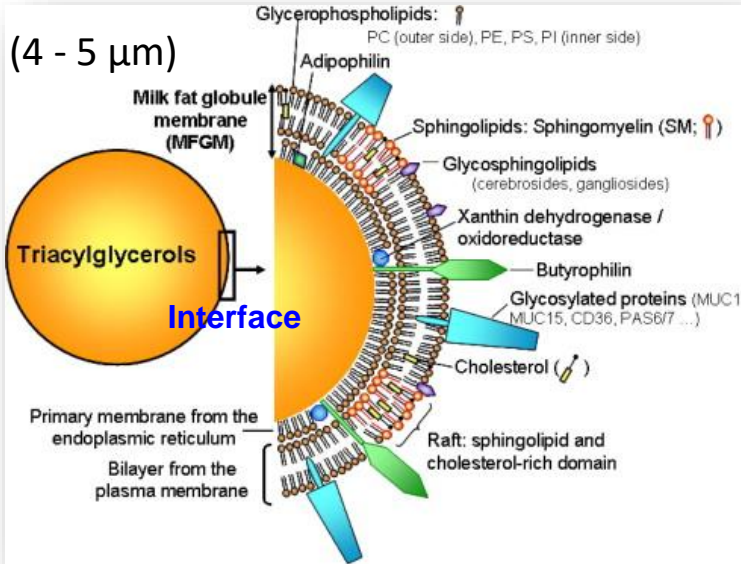
Infant Formula



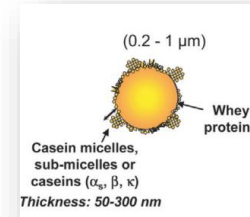
Native milk fat globule

Lipid droplets

Triacylglycerols




(Lopez, 2010)



(0,2 - 1 μm)

(Lopez and Briard-Bion, 2007)



Preterm hospitalized infants
Feeding nasogastric tube
Fed every 3 hours

Aim: to compare meals with similar composition but different structure

Human milk from their **own mother**

Human milk from **anonymous donor**

GROUP A

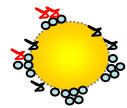
GROUP B



Raw human milk

Pasteurized human milk

Pasteurized human milk



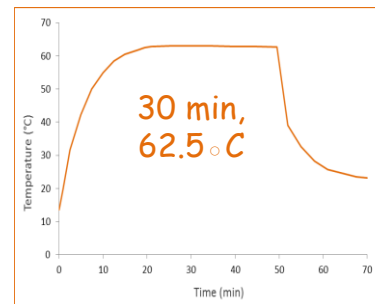
Pasteurized-homogenized human milk

24h before feeding (4°C)

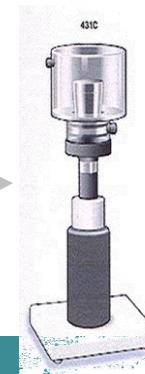
Stored at -20°C
(bank milk)

Stored at -20°C (bank milk)

Bank milk



Homogenization
by sonication

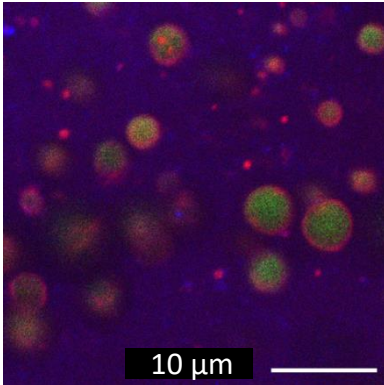


Pasteurization affected the initial structure and the emulsion disintegration of HM

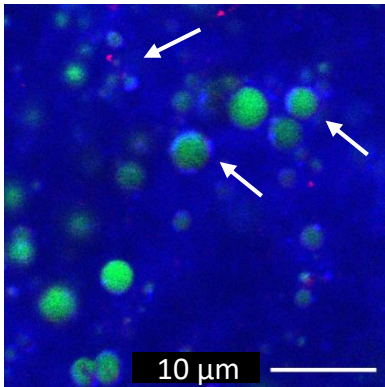
(n = 6 infants)

Initial structure

Raw HM



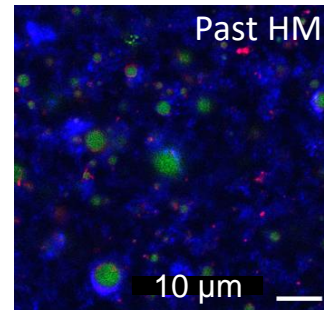
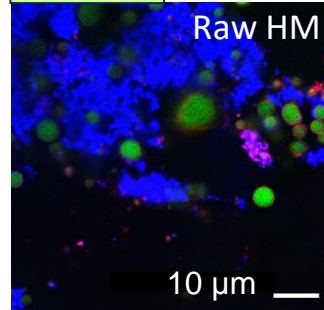
Past HM



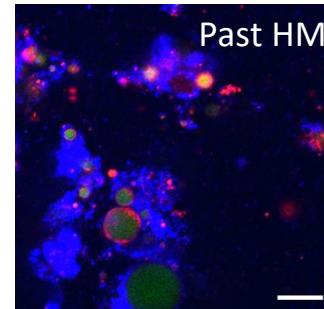
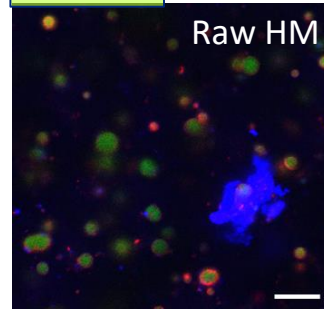
→ Protein heat-induced aggregation

Gastric disintegration

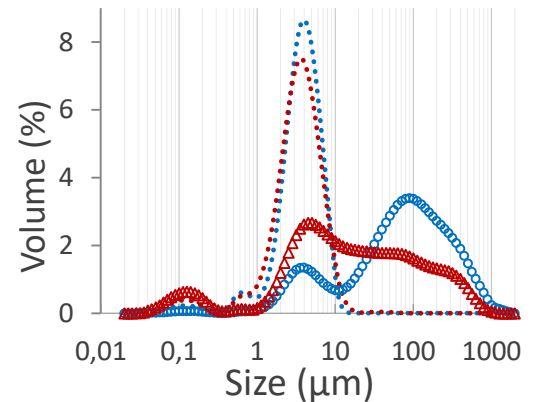
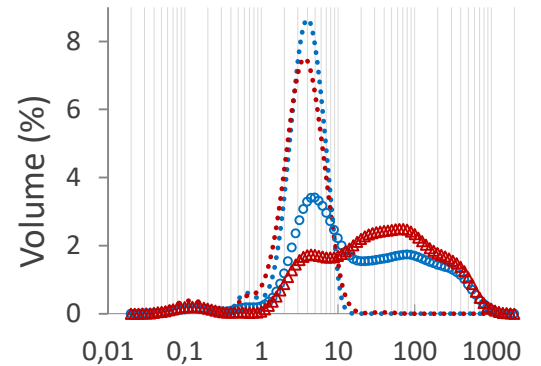
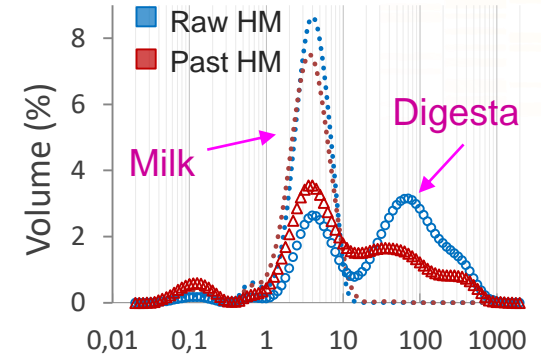
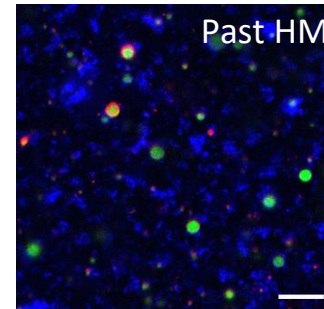
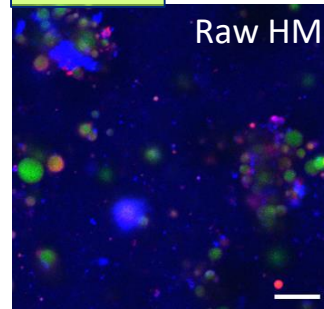
35 min



60 min



90 min

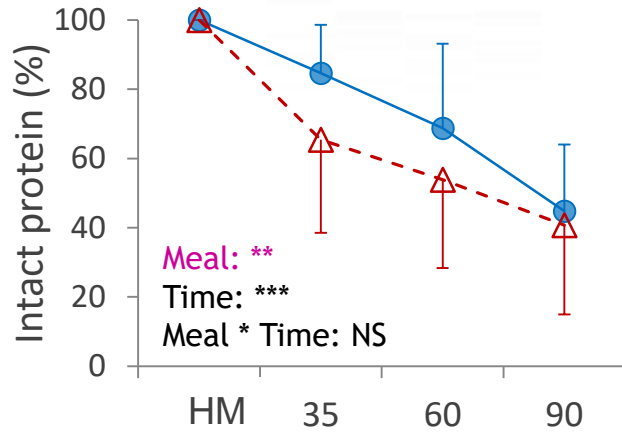


■ Apolar lipids ■ Amphiphiles ■ Proteins

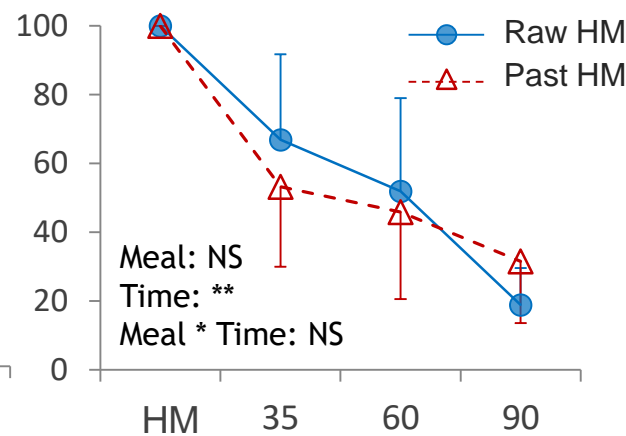
Pasteurization selectively impacted gastric proteolysis



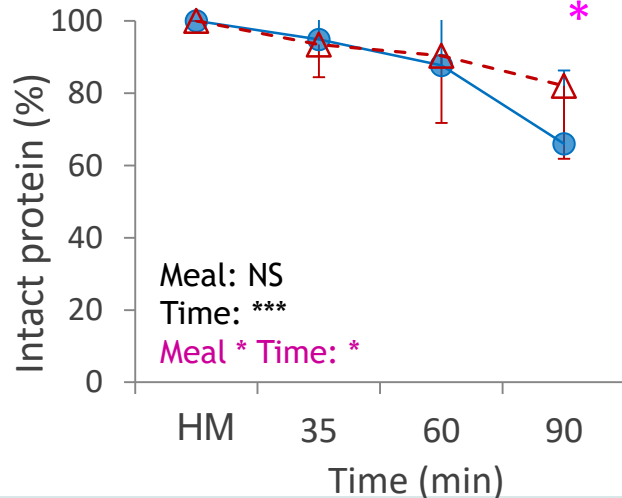
Lactoferrin



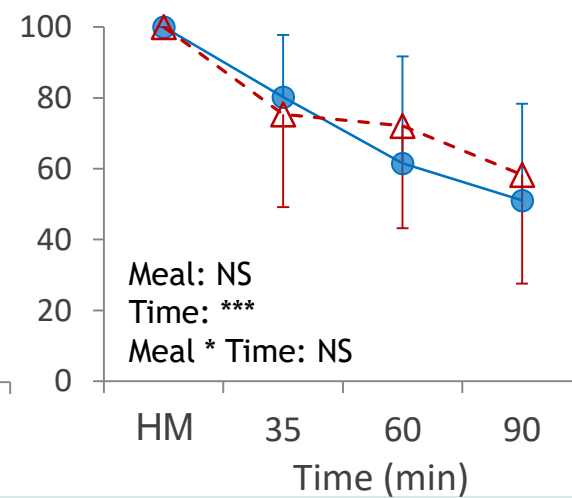
Serum albumin



α -lactalbumin



β -casein



→ Faster decrease of lactoferrin in Past HM

→ Slower decrease of α -lactalbumin in Past HM (only at 90 min)

→ α -lactalbumin was the most resistant to gastric digestion

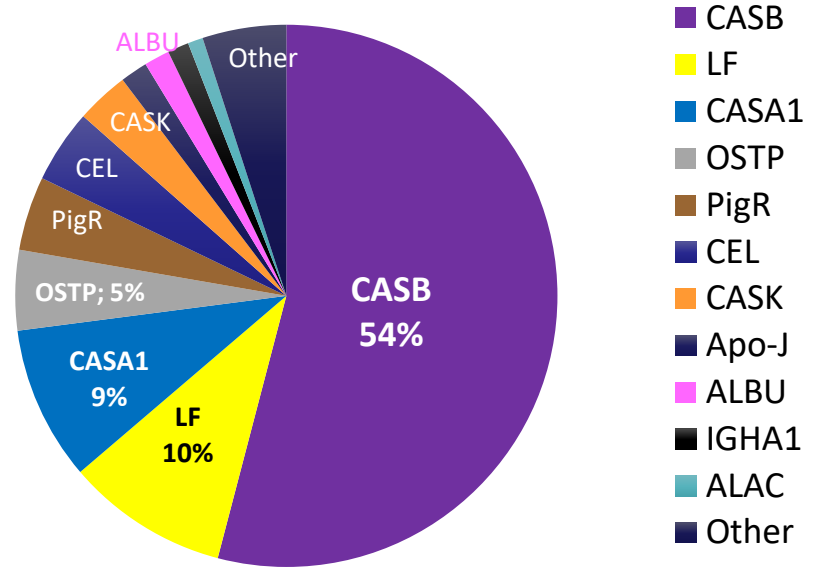
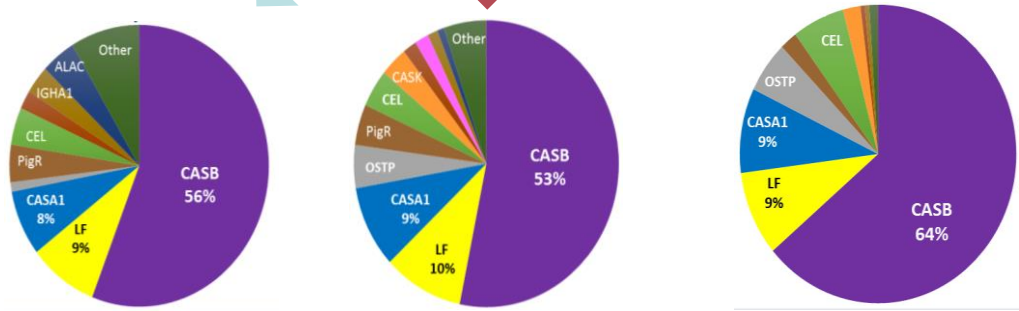
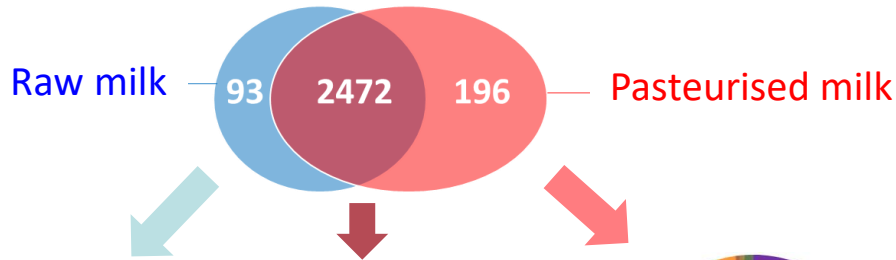
Impact on protective qualities of the native lactoferrin?

De Oliveira et al.,
Am J Clin Nutr 2017

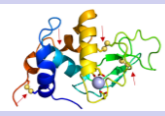
Effect of pasteurisation on gastric peptidome

→ 2761 peptides in milk and digestas

*Originating from 18 proteins
with 7 proteins contributing
to less than 1% of the peptides*



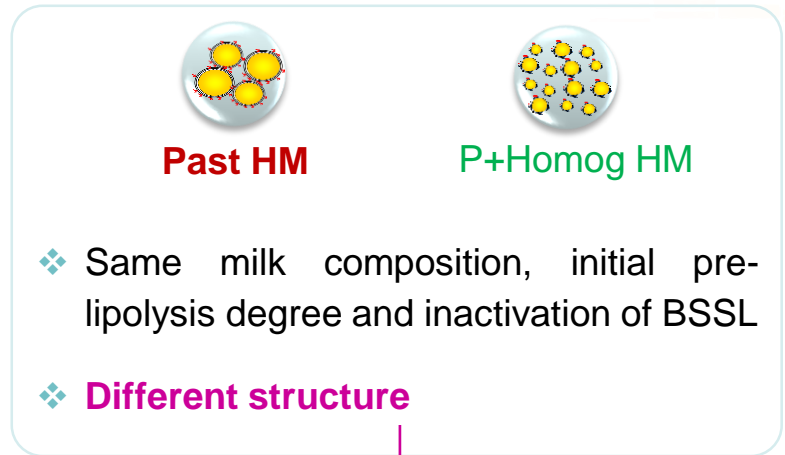
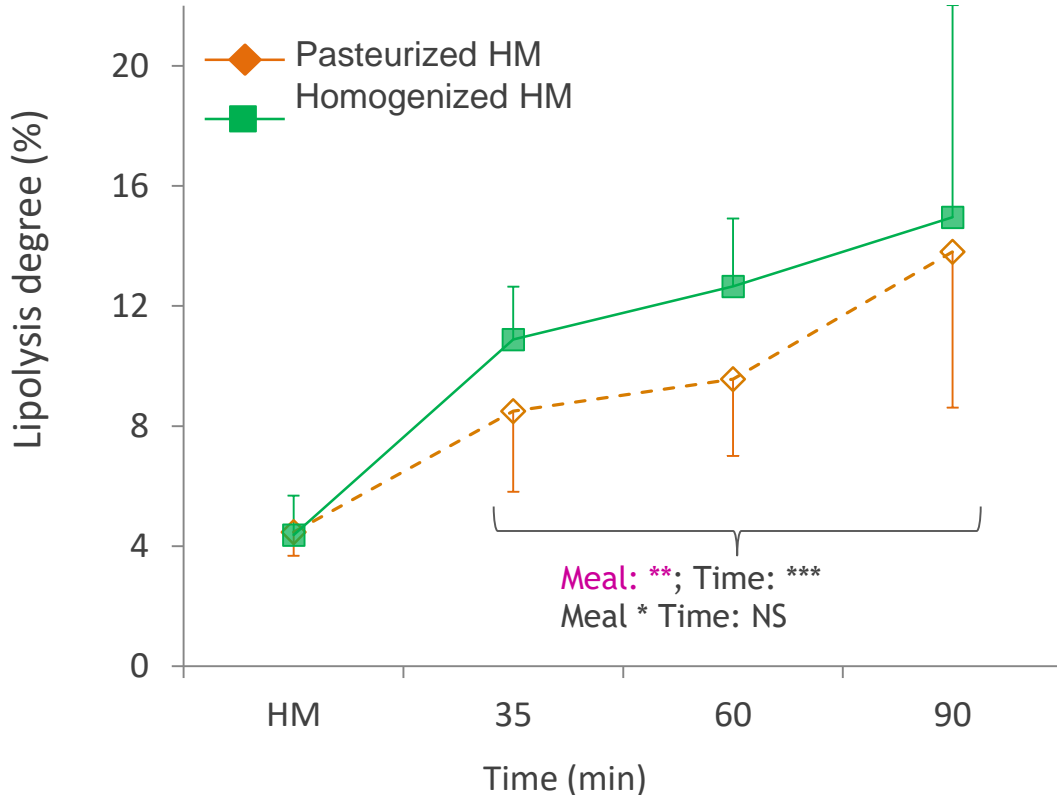
- Peptides essentially come from β -casein
- L' α -lactalbumin (ALAC) : 1% of peptides
 - → Resistant to gastric digestion
 - → 4 disulphide bridges
- More peptides specific of pasteurised milk



Homogenization accelerates gastric lipolysis

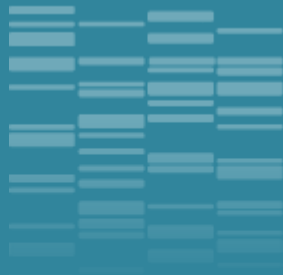


Instantaneous lipolysis level



➤ Increase of specific surface of droplets facilitating HGL adsorption

De Oliveira et al.,
Clin Nutr 2017



Effect of heat treatment on casein *in vitro* digestion in model infant formula



Objective

☞ Does processing affect protein hydrolysis during milk powder digestion?



Step 1 : Dissolution in milliQ water

Skimmed ultra-low-heat powder

25% DM

35% DM

ULH Milk

ULH Milk

80°C/20 s

105°C/60 s

80°C/20 s

105°C/60 s

85°C / 3 min

85°C / 3 min

LA

LB

LC

LE

LF

LG

Step 2 :
Heat
treatment

Step 3 :
Spray drying

A

B

C

E

F

G

T

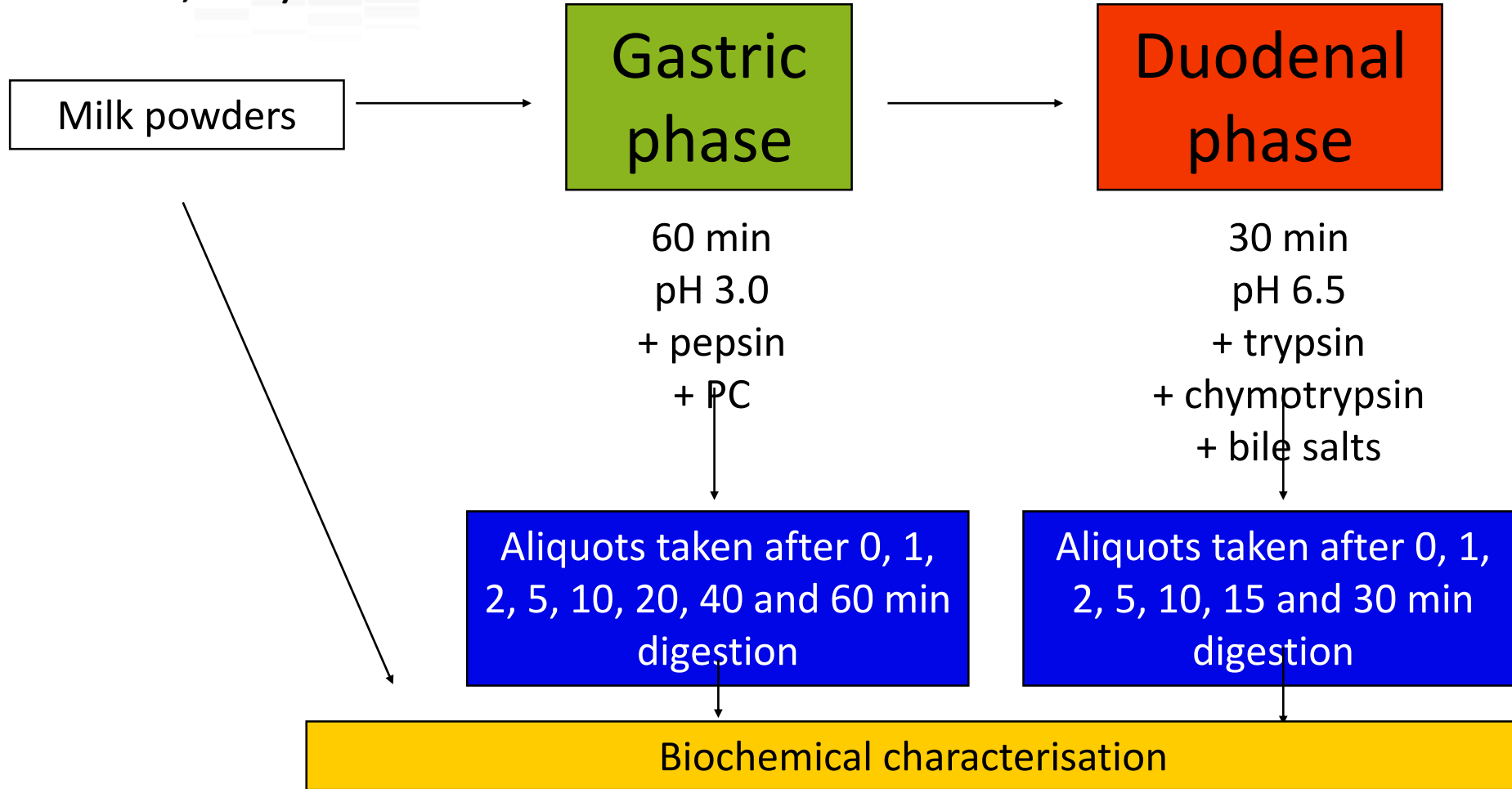
In vitro digestion



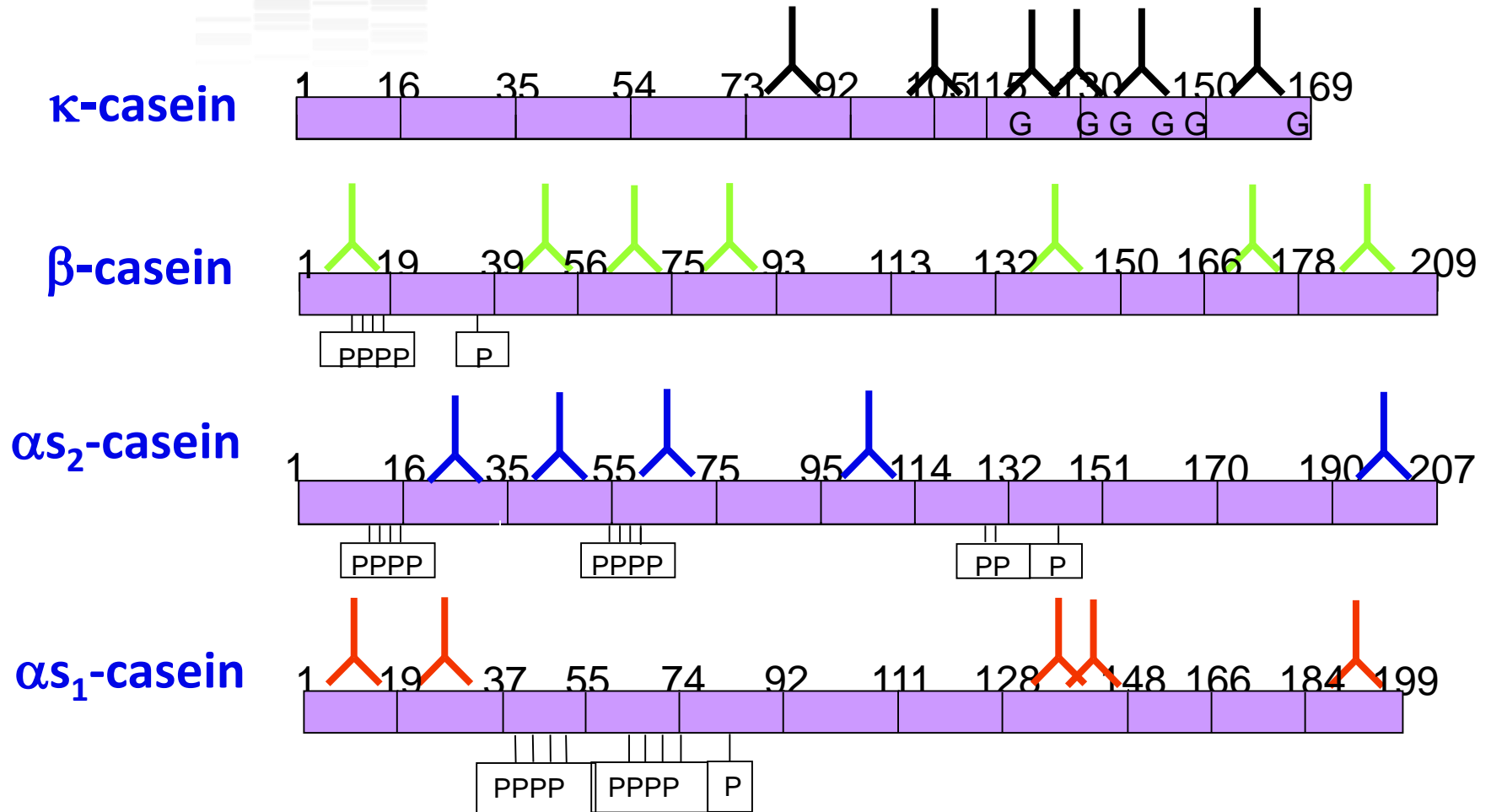
Infant Gut Model



Cooperation with the Institute of Food Research in Norwich UK (Dupont *et al.* Mol. Nutr. Food Res., 2010)



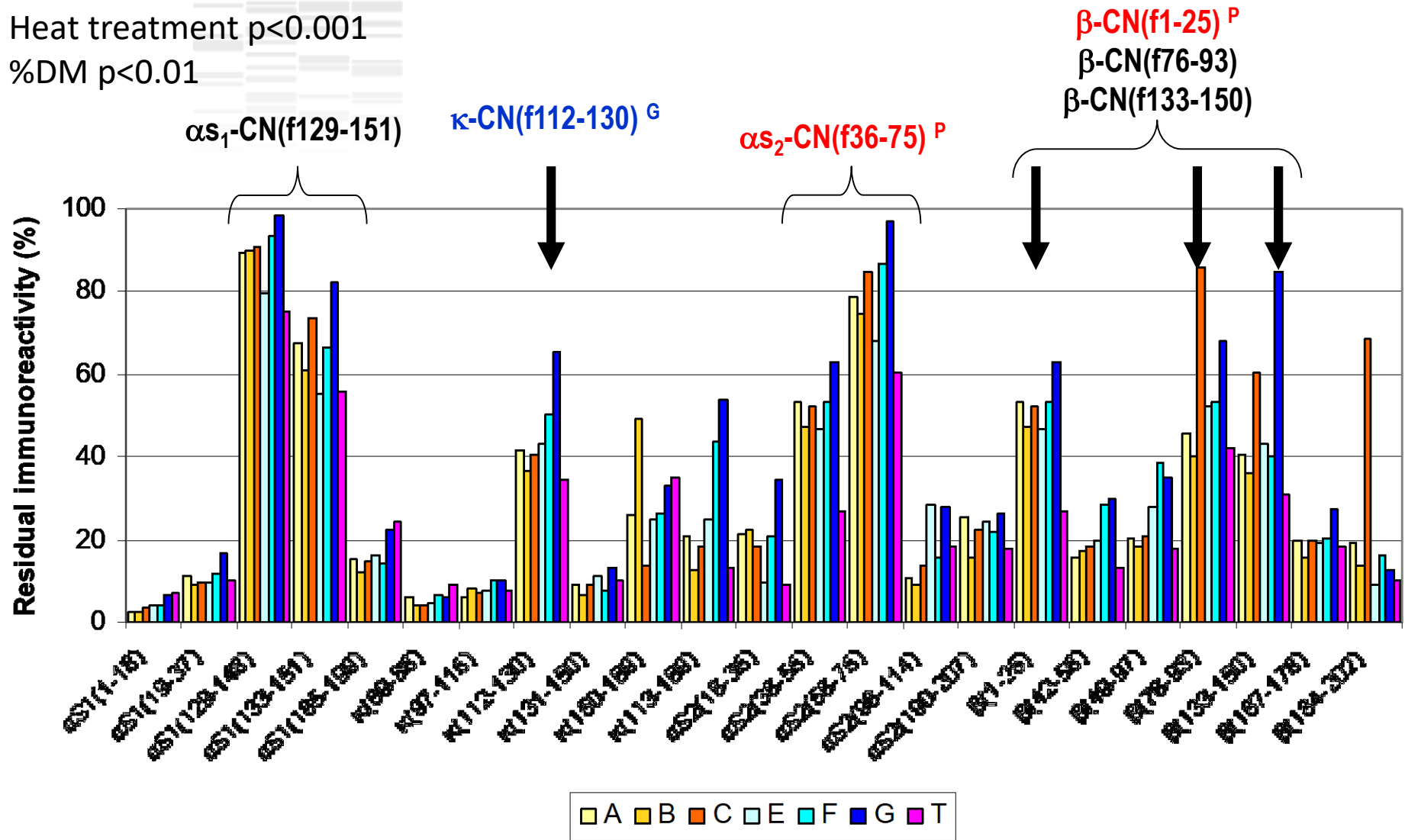
Identification of epitopes resistant to digestion by ELISA using specific Mabs



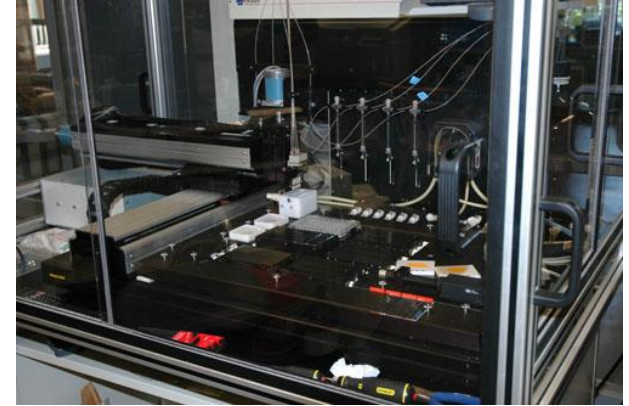
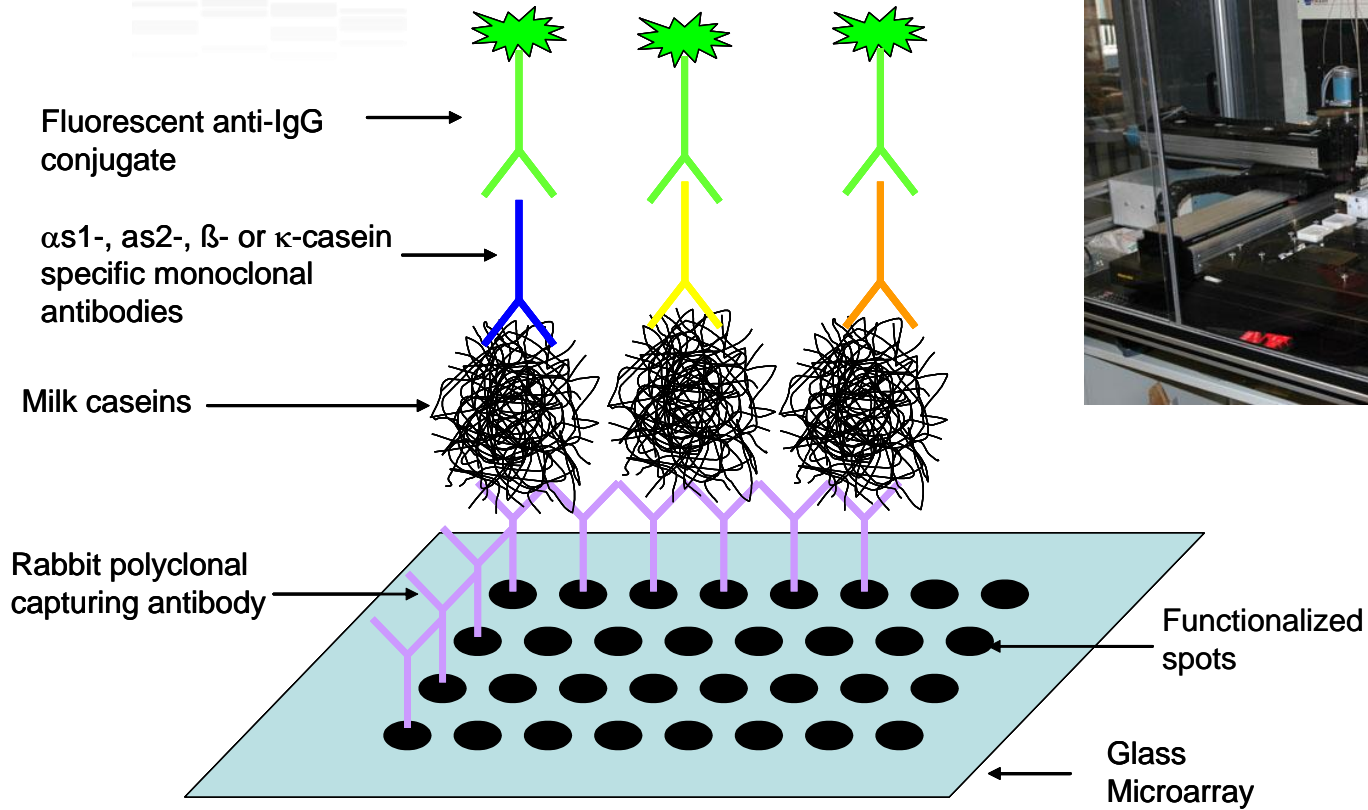
Residual immunoreactivity of casein fragments

Heat treatment $p < 0.001$

%DM $p < 0.01$



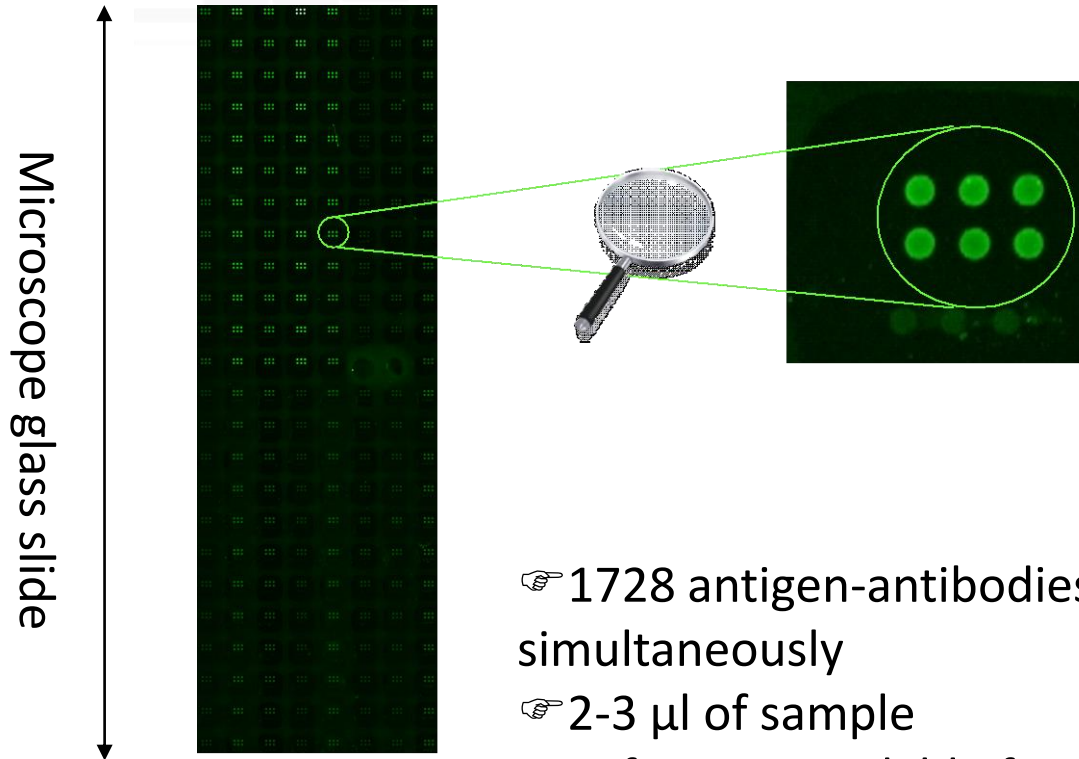
Development of antibody array technologies



Cooperation with the Van Andel Research Institute

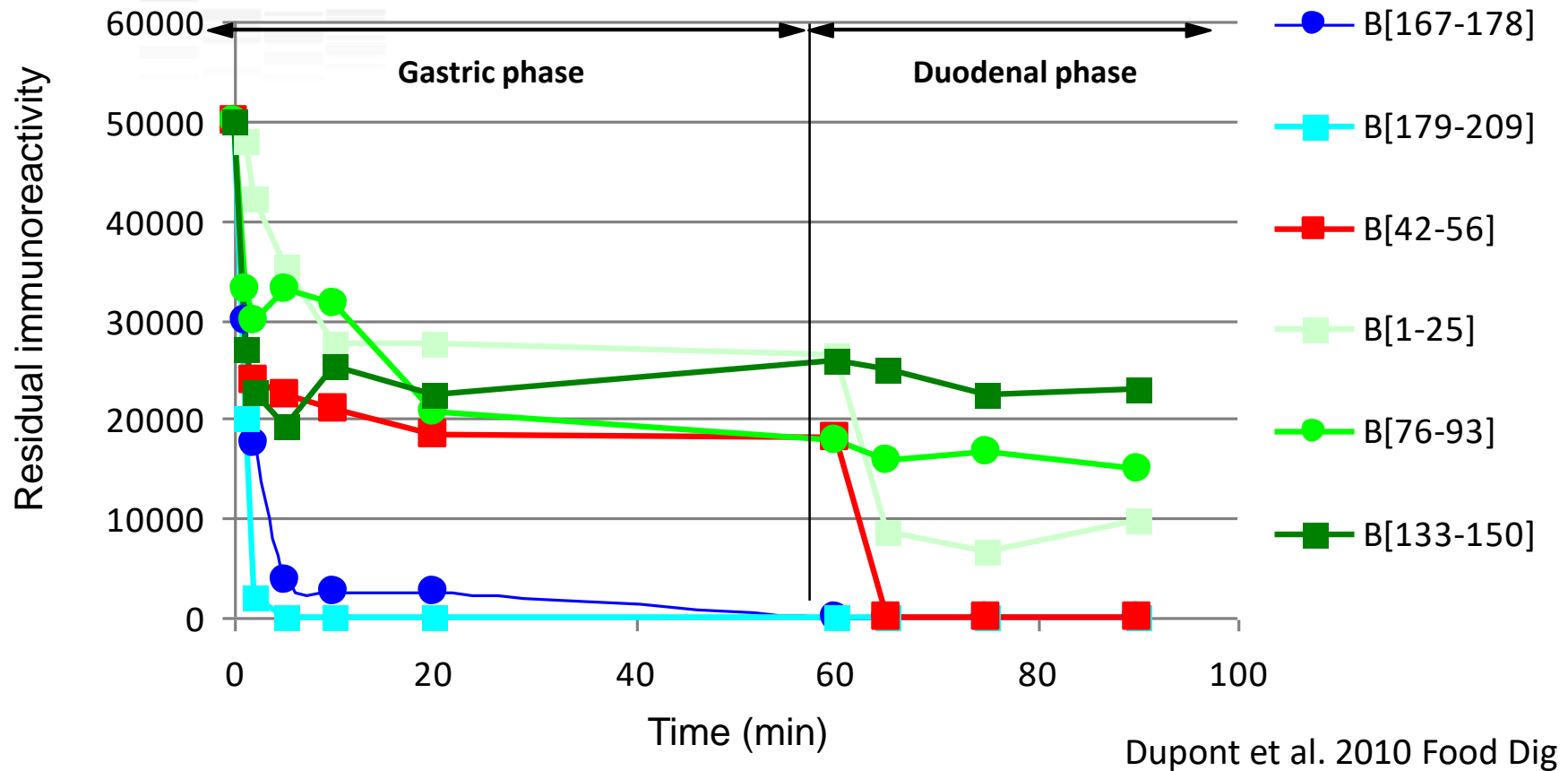


Antibody arrays – an example of results

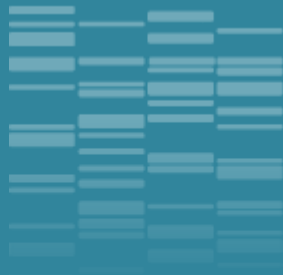


- 👉 1728 antigen-antibodies interactions studied simultaneously
- 👉 2-3 μl of sample
- 👉 Softwares available for data integration

Kinetics of β -casein digestion



👉 Kinetics of β -casein digestion differ according to the area studied

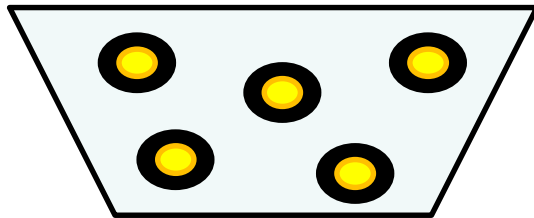


Can we design new generation of infant formula that would be biomimetic of human milk?



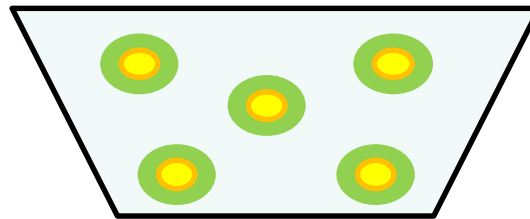
Infant formulas: can we create lipid structures biomimetic on the native fat globule?

Formula
T1



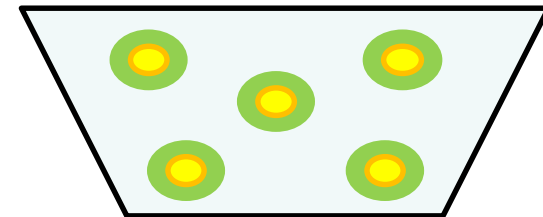
Interface 100 % Proteins
100% vegetable oil

Formula
T2

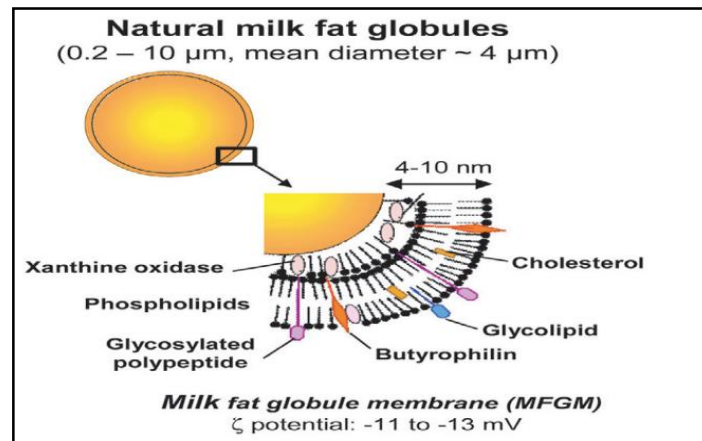


Interface 100 % phospholipids
100% vegetable oil

Formula
T3

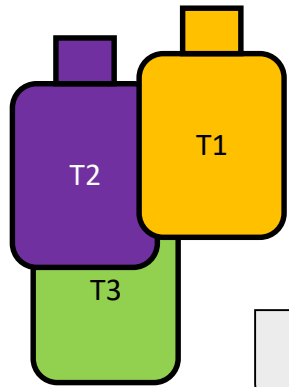


Interface 100 % phospholipids
40% vegetable oil + 60% milk fat



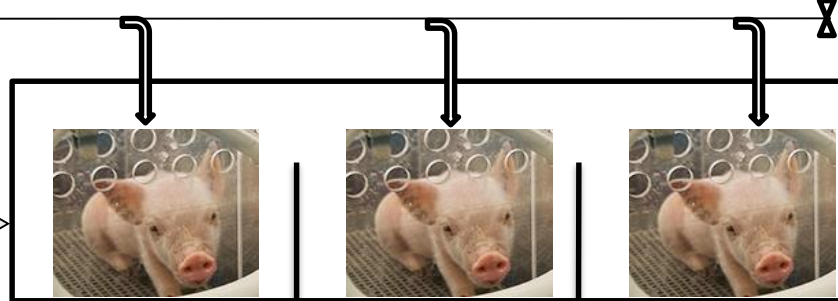
Lopez, (2007)

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



- Veg
- Veg + PL
- Dairy Fat + PL

Automatic meal delivery (10 meals/ day)



+
Mother-fed piglets
(MF = + control)



Slaughtering after

7 days

28 days

(90 min postprandial)

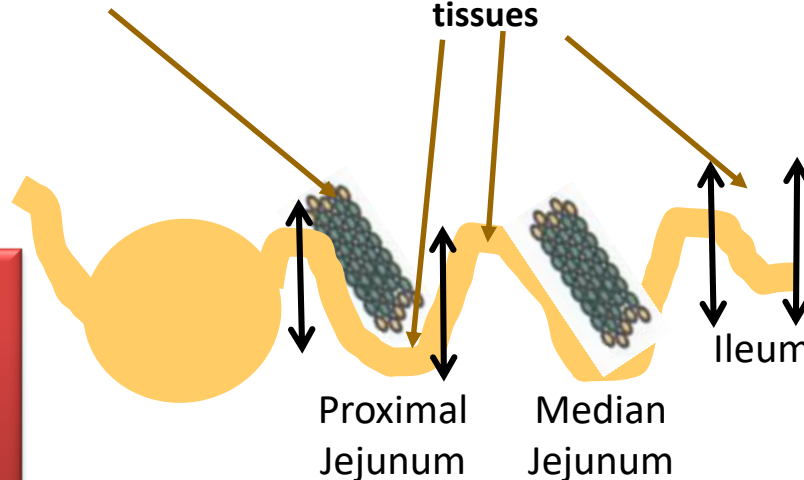
Mesenteric Lymph Nodes (MLN)

Collect of effluents and tissues

Effluents:
-SDS-PAGE
-Elisa

Tissues:

- Morphometry
- Enzyme Activities
- Intestinal Permeability
- Local immune response
- Microbiota

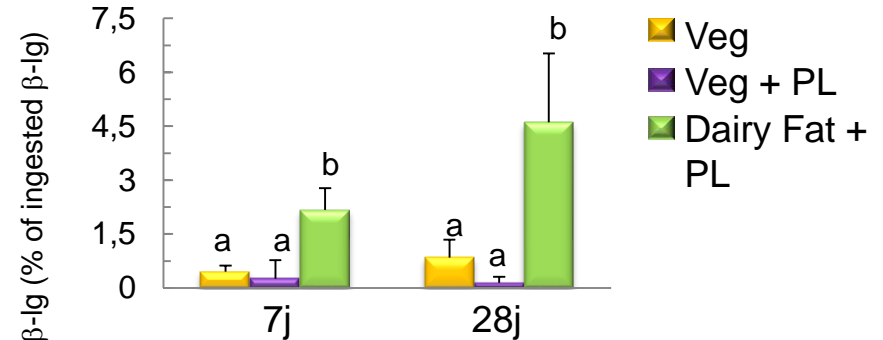
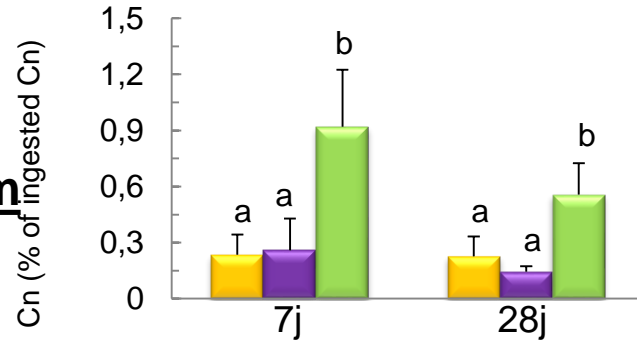


Protein Digestion

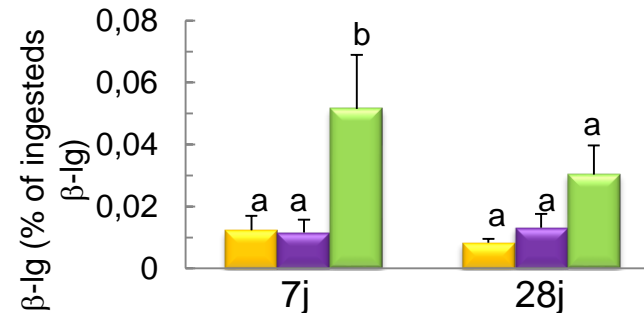
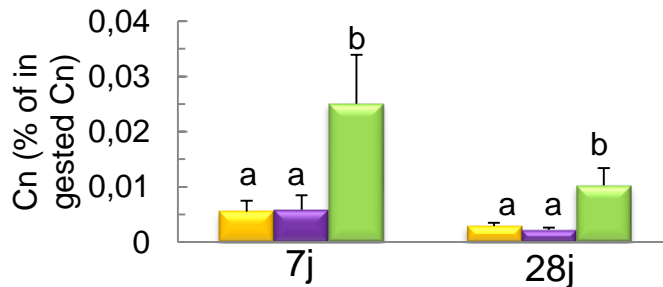
Casein

β -lactoglobulin

Jejunum



Ileum

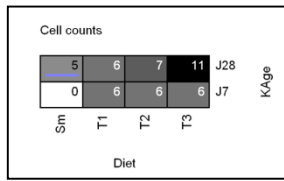


Milk Proteins better resist to intestinal digestion in the presence of dairy fat

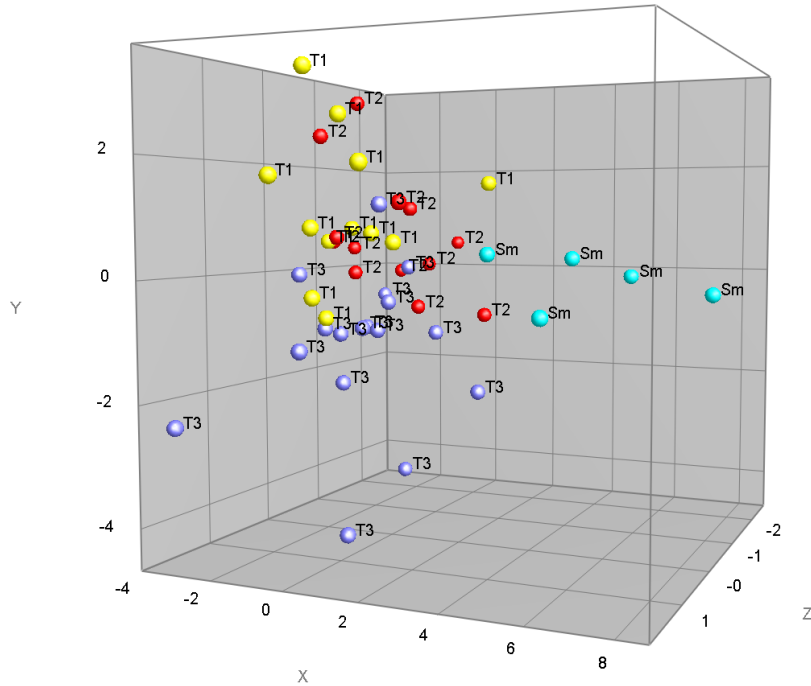
➔ Modification of the interface

(Granger *et al* 2005; Davies *et al*, 2001)

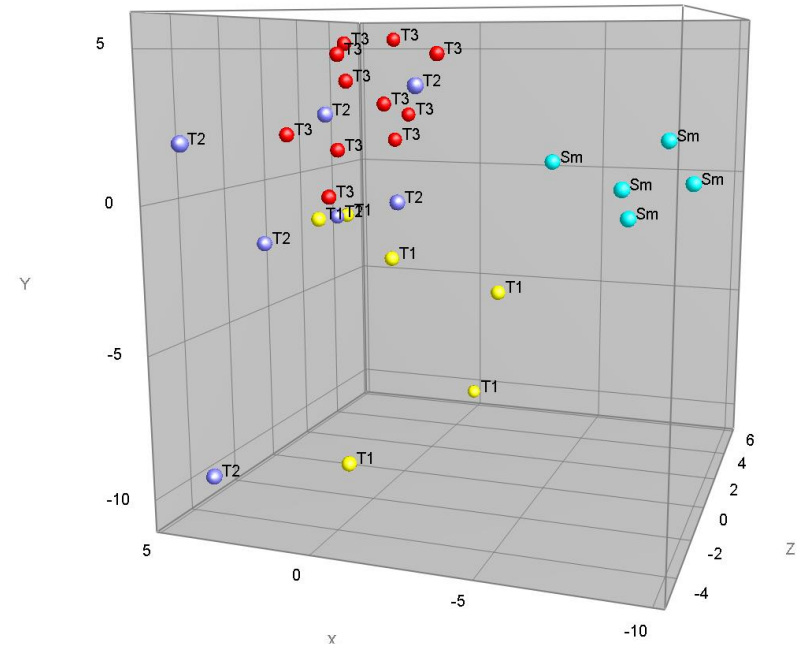
Microbiota by DHPLC



D7 & D28

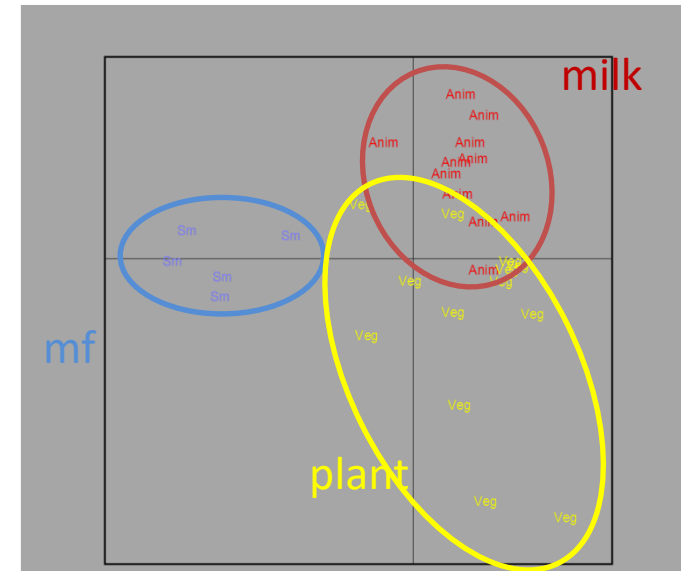


D28

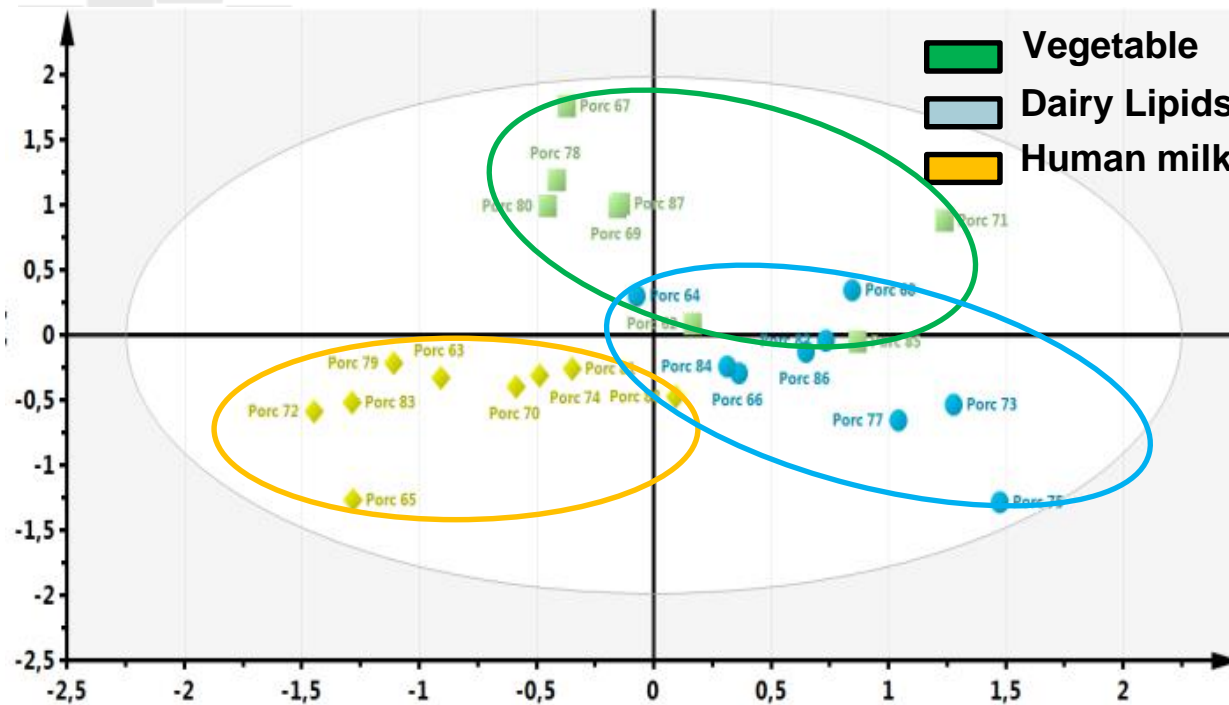


The composition/structure of the infant formula « orientates » the microbiota

More Proteobacteria with milk fat /
More Firmicutes with plant oil



What happens when they become older (140 d)?



Some differences remain in:

- * the microbiota composition
- * the metabolome with 5 discriminating metabolites (including propionate)
- * the immune system with a reduced susceptibility to inflammation with milk lipids (lower TNF α and IL8 with dairy lipids)



Design of a « native » infant formula using filtration technologies : nutritional, physiological and sensory impacts

Coordinator : Amélie Deglaire



Involved partners : PNCA (AM Davila), CSGA (S Nicklaus)

Laboratoire de Physiologie de la Nutrition et du Comportement Alimentaire



Duration of the project : 36 months (January 2018-December 2020)



Main objective :

To assess the nutritional, physiological and sensory potential of infant formula as native as possible but microbiologically safe using a cascade of filtration technologies compares to one submitted to several heat treatments



NativIF - phase 1 : results



Formula produced	T- : No heat treatment	T+ : One final heat treatment (75°C, 2 min)	T+++ : Multiple heat treatments (Max: 85°C, 2 min)
Microbiological safety/ Nutritional Composition	validated	validated	validated
% of protein denaturation	4,1 ± 0,7 %	7,2 ± 1,1 %	57,6 ± 0,2%
Furosine (mg / 100 g Total Nitrogen)	576 ± 14	516 ± 48	544 ± 29
Carboxymethyl-lysine (mg / 100 g NT)	8,2 ± 0,3	8,2 ± 0,3	10,3 ± 2,1
Amount conditioned in boxes	35 kg	35 kg	35 kg

Bacteriological analysis of the formulas

	T-	T+	T+++
	ufc / g poudre		
Flore totale à 30°C	500	340	100
Bacillus cereus	<10	<10	<10
Coliformes totaux	<10	<10	<10
Cronobacter spp	non détecté	non détecté	non détecté
Entérobactéries	non détecté	non détecté	non détecté
Listeria monocytogenes	absence	absence	absence
Salmonella	absence	absence	absence
Staphylocoque à coagulase positive	absence	absence	absence
Levures	<10	<10	<10
Moisissures	<10	<10	<10

→ In agreement with the legislation

Nutritional composition

		T-	T+	T+++
	Unité	/ 100 g poudre		
Extrait Sec Total	g	97.9	97.8	97.7
Energie	kcal	526	532	531
Proteines	g	10.6	11.2	10.2
ratio massique CN : PS		55:45	55:45	57:43
Lipides	g	29	30	30
Lactose	g	56	54	55
Cendres	g	2.7	2.5	2.7

Minerals and vitamins added

→ Composition in agreement with the legislation



Phase 2

Task 1 : Impact of the infant formulas T- and T+++ on the homeostasis of the intestinal microbiota on a rat model (12 months)

Laboratoire de Physiologie de la Nutrition et du
Comportement Alimentaire

T1.1 : Kinetics of digestion and protein and amino acid digestibility

Nitrogen and amino acid quantification in intestinal effluents and in the bloodstream

- After weaning, adaptation to the formulas during 2 weeks
- At 1h, 2h, 3h and 6h after the meal (n = 6)



T1.2 : Impact on the differentiation and the energy metabolism of the intestinal epithelium

Rats adapted to the formulas between 14 and 21d, then fed the formulas during 2 weeks (n = 8)

- Assessment of the growth, food intake and bone density
- Euthanasia at 35d : intestinal epithelium (**differentiation, permeability**), colon cryptes (**energy metabolism**)

T1.3 : Impact on the structuration of the intestinal microbiota

Microbiota characterization and quantification of amino acid-derived bacterial metabolites

**Task 3: Data
analysis
(6 months)**

Task 2 : Sensory properties of infant formulas and acceptability by the neonate (12 months)

T2.1 : Sensory characterization

Global Difference between T-, T+, T+++ and a control commercial infant formula → triangular test, adult panel

T2.2 : Analytical characterization

Aroma characterization of T-, T+, T+++ by GC-MS

Olfactometry coupled to gas chromatography if important differences

T2.3 : Acceptability by the neonate (T- vs T+++)

Consumer behavior by comparing formula T- vs. T+++

- 25 infants between 4 and 6 months
- oro-facial behavior, liking, amount consumed, digestive confort
- 3 sequences in laboratory + 1 day trial at home



ChemoSens



Amino acid bioavailability, particularly tryptophane, in human milk and infant formula

Supervisor of the thesis : Didier Dupont (INRA, STLO)

Co-supervisors :

- **Amélie Deglaire** (Agrocampus Ouest, STLO)
- **Isabelle Le Huërou-Luron** (INRA Saint Gilles, Numecan - NGB)
- **Paul Moughan** (Riddet Institute, Nouvelle-Zélande)

Funding

Student salary:

½ AO + ½ Brittany Council

Consumables :

22 k€/year (Riddet Institute) for part 1



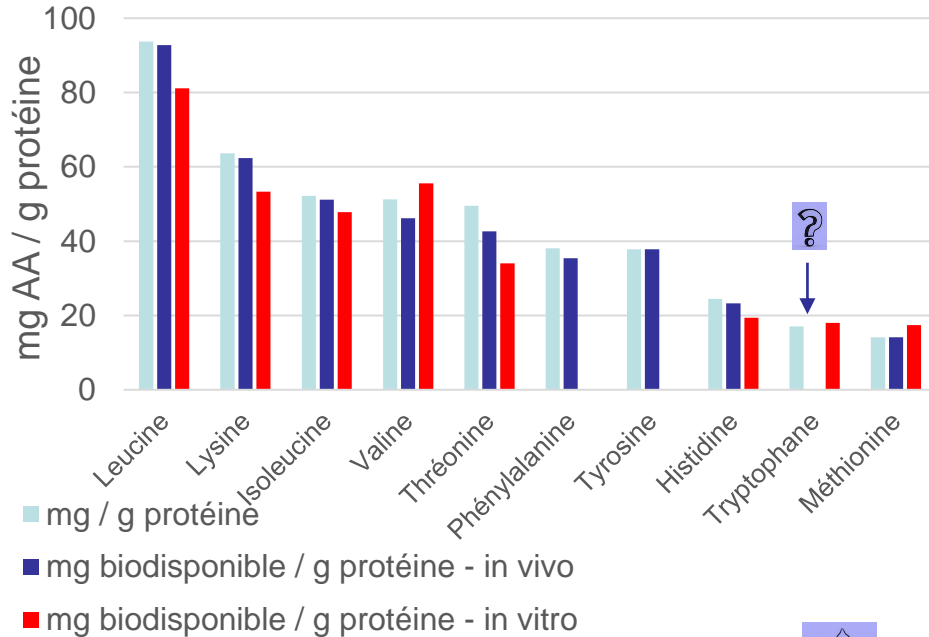
Research Questions :

- What is the amino acid bioavailability, especially that of tryptophane, in human milk and infant formula?
- What is the effect of the protein type and amount of the infant formula on :
 - the tryptophane bioavailability?
 - the production of tryptophane metabolites (e.g. sérotonine, mélatonine, indole) ?
 - The intestinal maturation and the brain development ?



Scientific context

Essential amino acid bioavailability in human milk



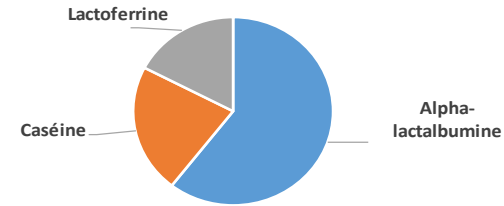
(Darragh & Moughan, 1998)
(Maathuis et al., 2017)

More research needed especially for Trp (FAO, 2013)



Tryptophane: an essential amino acid

Origin in human milk



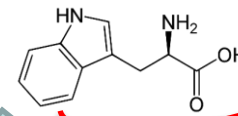
Digestion

Bacterial metabolism

Indol

inflammation
Intégrité barrière intestinale
(Bansal et al., 2010; Shimada et al., 2013)

Forte dose : Cpt anxieux et dépressif (Jaglin et al. 2018)



Liver

Niacin (Vit B3)

Absorption

1%

Intestine
Brain

Serotonin

Neuro-transmittor

Brain

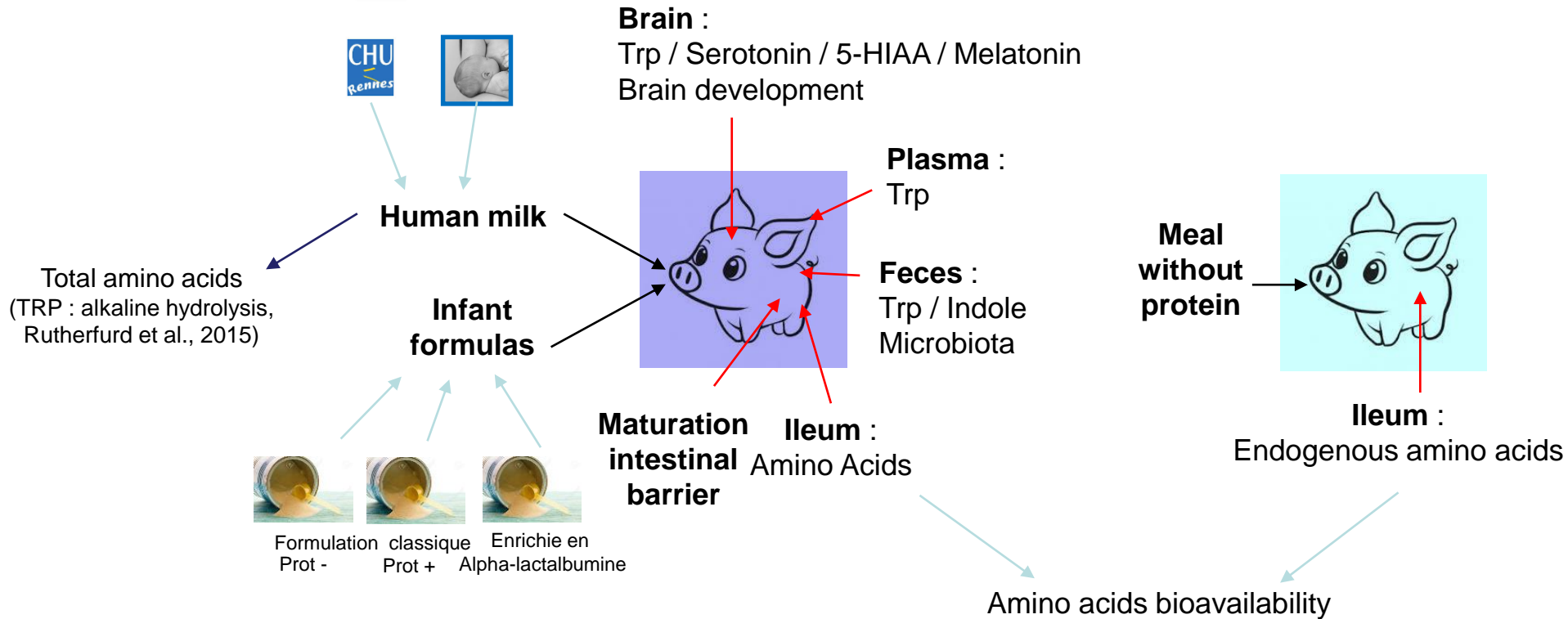
Melatonin

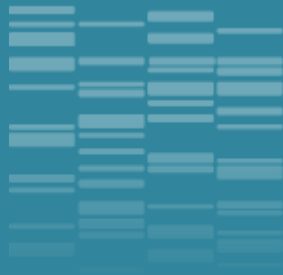
Hormone of the chronobiological regulation

Protein synthesis



Experimental Plan



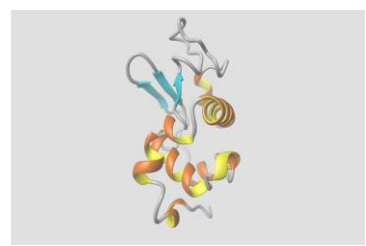


May bioactive peptides released
during digestion have an effect *in*
vivo?



In vivo digestion of milk proteins

- 16 subjects were fed with 30g of either:
 - Caseins
 - Whey proteins

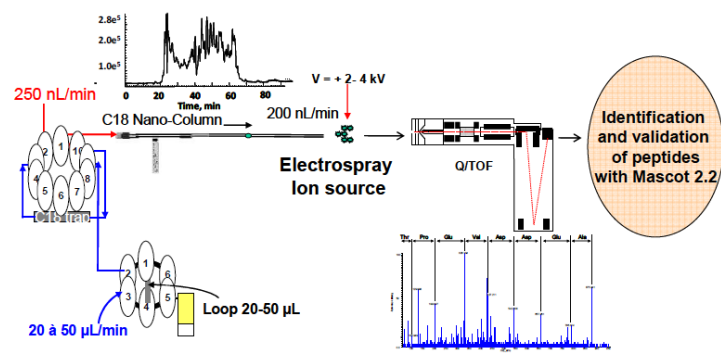


- Intestinal contents were collected through a double lumen nasogastric tube placed in their jejunum during 6 h after ingestion (location of the sampling site was controlled by radiography)



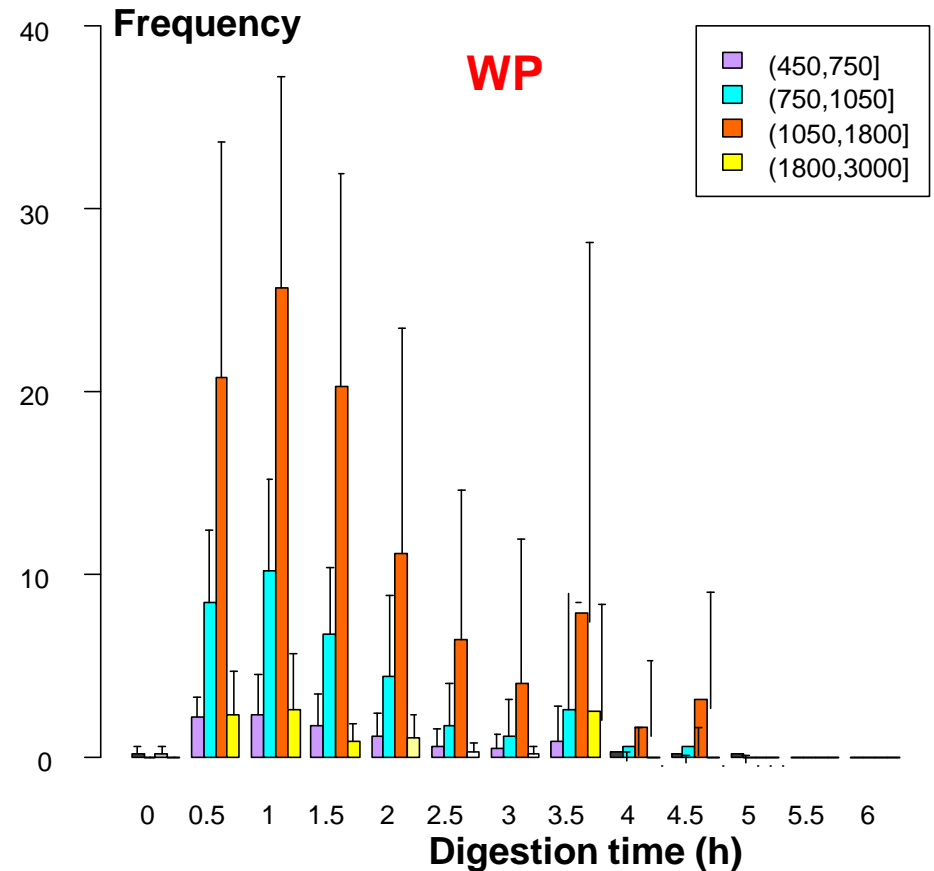
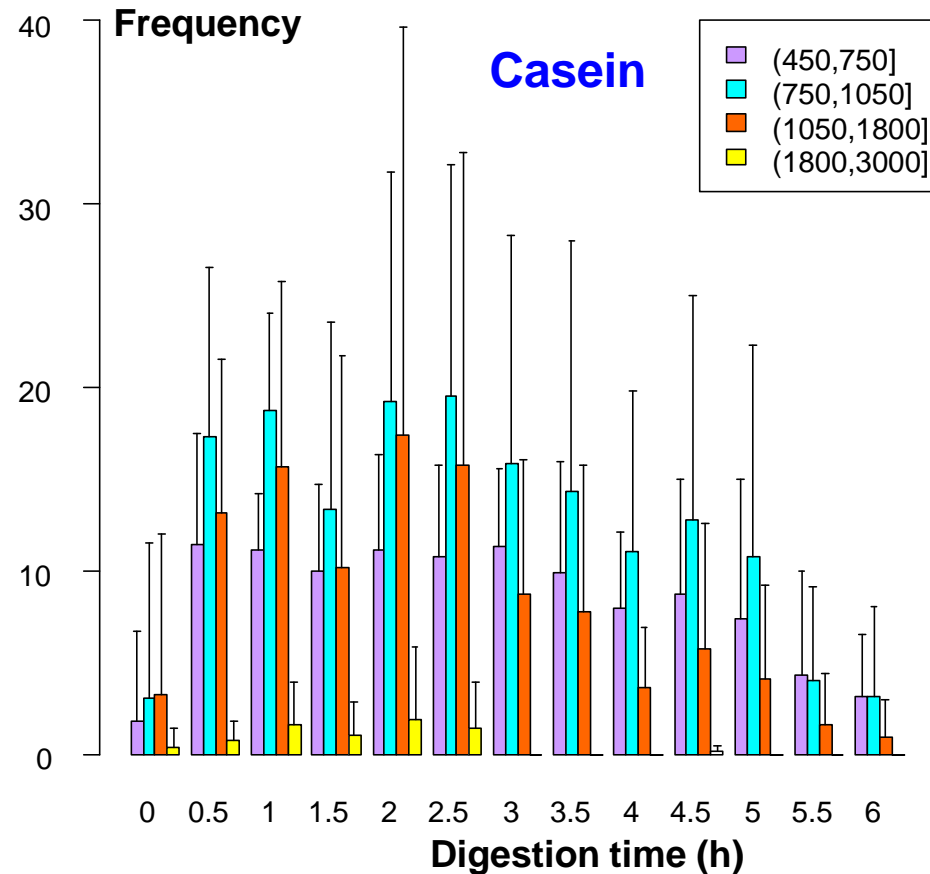
- Effluents were then freeze-dried until further use. Before analysis, effluents were rehydrated in 50 mM Tris HCl buffer pH 8.0 and 2M urea, centrifuged for 10 min a 2000 g

- Samples were characterized by ESI-MS-MS. Only peptides larger than 5 amino acids are considered



Kinetics of peptides release

↪ 4704 dietary peptides unambiguously identified



☞ Release of casein peptides throughout digestion

☞ Peptides generated from whey proteins digestion are larger

↪ Generation of a unique database of dietary peptides bioaccessible

A great variability in patterns but some peptides present in all subjects

31 peptides present throughout digestion

14 peptides found in all the subjects

1-6	RELEEL		
57-66	SLVYFPFGPI	57-66	SLVYFPFGPI
58-66	LVYFPFGPI		
58-68	LVYFPFGPIP		
59-66	VYFPFGPI	59-66	VYFPFGPI
59-67	VYFPFGPIP		
59-68	VYFPFGPIP	59-68	VYFPFGPIP
60-66	YFPFGPI	60-66	YFPFGPI
61-66	PFGPI	60-67	YFPFGPIP
73-79	NIPPLTQ		
73-80	NIPPLTQT		
73-82	NIPPLTQTPV		
80-91	TPVVVPFLQPE		
81-87	PVVVPPF		
81-91	PVVVPPFLQPE	81-91	PVVVPPFLQPE
81-92	PVVVPPFLQPEV	81-92	PVVVPPFLQPEV
83-91	VPPFLQPE		
84-91	VPPFLQPE		
85-091	PPFLQPE		
107-113	KEMPFK		
108-113	EMPFK	108-113	EMPFK
114-119	YPVEPF	114-119	YPVEPF
134-139	HLPLPL		
135-139	LPLPL		
156-160	MFPPQ		
168-173	VAPFPQ	168-173	VAPFPQ
170-175	VLPVPQ	170-175	VLPVPQ
171-175	LPVPQ		
194-201	QEPVLGPV	177-182	AVPYPQ
195-201	EPVLGPV	194-201	QEPVLGPV
196-201	PVLGPV	195-201	EPVLGPV

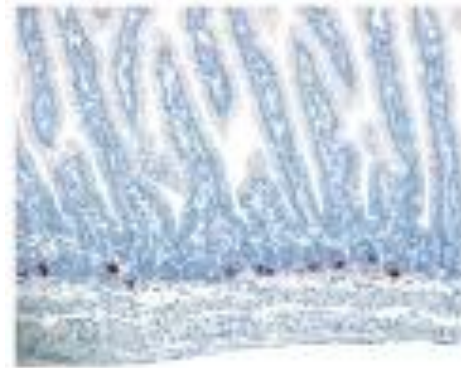
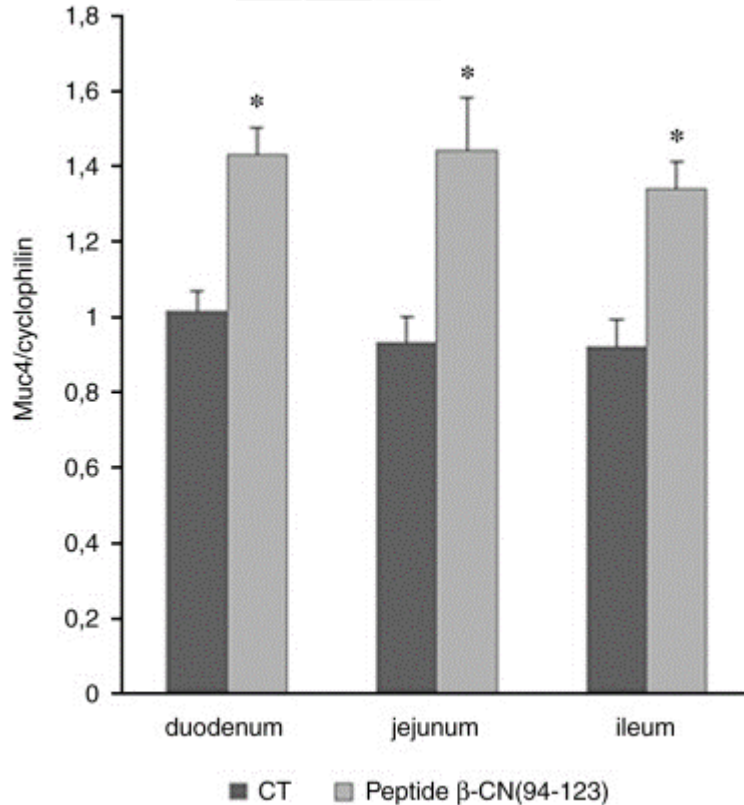
β-CN

Among those 5 bioactive peptides

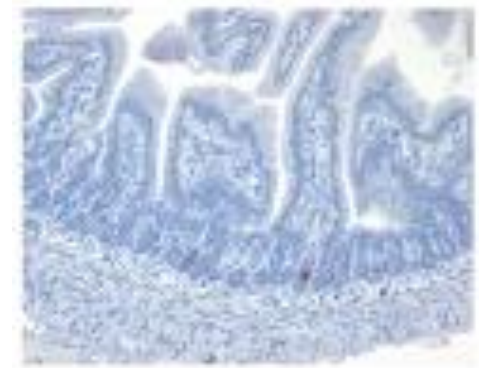
59-66	VYFPFGPI	} Anti-hypertensive Opioid
59-68	VYFPFGPIP	
60-66	YFPFGPI	
108-113	EMPFK	
114-119	YPVEPF	

Boutrou et al. 2013
Am J Clin Nutr

Bioactive peptides released during digestion can reinforce the defense of the intestinal epithelium



Before



After

Sections of duodenum after 10 d of administration of peptide β -CN (94-123)

β -CN(94-123) stimulates the mucus production *in vitro* (HT-29 MTX) and *in vivo* (rat)

Plaisancie et al. 2013, J Nutr Biochem
Plaisancie et al. 2015, J Dairy Res

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 group at INRA in Rennes



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

International Network

Dr. Didier DUPONT, Senior Scientist, INRA, France

●
INFOGEST
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 Plant Food Res

Nagoya Univ

Japan

410 scientists - 150 institutes – 45 countries

Industry involvement

☞ ~ 50 private companies are following INFOGEST





INFOGEST



Chair

Didier Dupont - France

didier.dupont@inra.fr



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**



Didier Dupont



Pasquale Ferranti



Milena
Corredig



Linda Giblin



Myriam Grundy



Nadja Siegert



Choi-Hong Lai



Alan Mackie



Uri Lesmes



Brigitte Graf



Frederic Carriere



Caroline Orfila



Steven Le Feunteun

Some outputs

In vitro gastrointestinal digestion
Consensus INFOGEST protocol

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

Oral phase

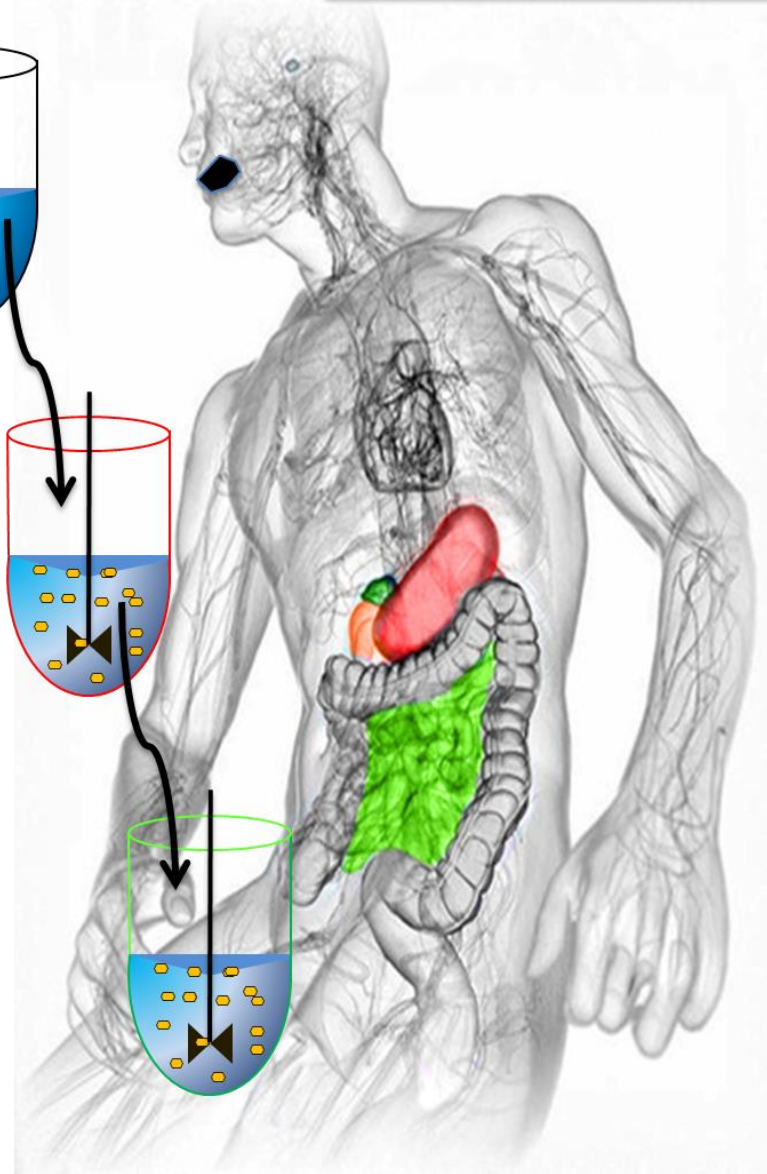
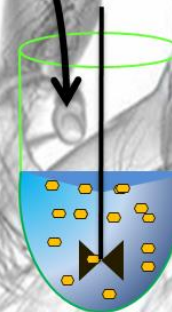
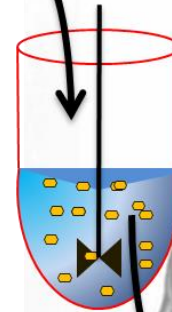
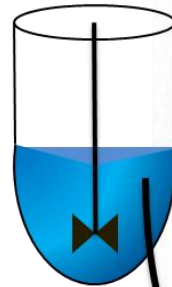
Mix 1:1 with Simulated Salivary Fluid (SSF)
salivary amylase (75 U/mL)
2 min, pH 7

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



An updated version of the protocol published last week!

nature
protocols

PROTOCOL

<https://doi.org/10.1038/s41596-018-0119-1>

INFOGEST static in vitro simulation of gastrointestinal food digestion

André Brodkorb^{1*}, Lotti Egger², Marie Alminger³, Paula Alvito⁴, Ricardo Assunção⁴, Simon Ballance⁵, Torsten Bohn⁶, Claire Bourlieu-Lacanal⁷, Rachel Boutrou⁸, Frédéric Carrière⁹, Alfonso Clemente¹⁰, Milena Corredig¹¹, Didier Dupont⁸, Claire Dufour¹², Cathrina Edwards¹³, Matt Golding¹⁴, Sibel Karakaya¹⁵, Bente Kirkhus⁵, Steven Le Feunteun⁸, Uri Lesmes¹⁶, Adam Macierzanka¹⁷, Alan R. Mackie¹⁸, Carla Martins⁴, Sébastien Marze¹⁹, David Julian McClements²⁰, Olivia Ménard⁸, Mans Minekus²¹, Reto Portmann², Cláudia N. Santos^{22,23}, Isabelle Souchon²⁴, R. Paul Singh²⁵, Gerd E. Vegarud²⁶, Martin S. J. Wickham²⁷, Werner Weitschies²⁸ and Isidra Recio²⁹

5-year Impact Factor=15,269

The logo for INFOGEST, featuring the word "INFOGEST" in a bold, black, sans-serif font. Above the letter "O" is a solid orange circle, and below the letter "T" is another solid orange circle.

The consensus model can be learned with videos on YouTube

The screenshot shows a web browser window displaying the YouTube channel page for 'cost infogest'. The browser's address bar shows the URL 'https://www.youtube.com/channel/UCdc-NP9kTDGyH_kZCgpQWg'. The page features a search bar with 'cost infogest' entered, a navigation menu on the left, and a main content area with a video player and a list of recent uploads. The video player shows a video titled 'In vitro food digestion - COST action INFOGEST'. Below the video player, there are two video thumbnails with titles: 'Alpha Amylase Activity Assay for In Vitro Food Digestion' and 'Static In Vitro Digestion Method for Food'. The right sidebar contains a list of popular channels on YouTube, including 'Seb la Frite', 'MissCreatives', 'LanguedePub / M...', 'JunkFoodTasterD...', 'Madame Patachou', and 'halawiyat khadija...'. The bottom of the page shows a Windows taskbar with various application icons and the system clock.

Fichier Édition Affichage Historique Marque-pages Outils ?

Intranet UMR STLO - Accueil x GRR (Gestion et Réservatio... x YaZiba: 30/3 - 3/4 x In vitro food digestion - C... x +

https://www.youtube.com/channel/UCdc-NP9kTDGyH_kZCgpQWg

youtube

YouTube FR

cost infogest

Mettre en ligne Connexion

Vidéos à regarder

LE MEILLEUR DE YOUTUBE

- Populaire sur YouTube
- Musique
- Sport
- Jeux vidéo et autres
- Films
- Émissions TV
- Actualité

Parcourir les chaînes

Connectez-vous maintenant pour consulter vos chaînes et les recommandations.

Se connecter

Toutes les activités

In vitro food digestion - COST action INFOGEST a mis en ligne une vidéo. il y a 10 mois

Oral phase
Gastric Phase
Intestinal Phase

Alpha Amylase Activity Assay for In Vitro Food Digestion
de In vitro food digestion - COST action INFOGEST
il y a 10 mois • 846 vues
A step-by-step description of a static α -amylase activity assay for an in vitro digestion method presented suitable for food; recently published and available online (open access...)

5:12

In vitro food digestion - COST action INFOGEST a mis en ligne une vidéo. il y a 10 mois

Oral phase
Gastric Phase
Intestinal Phase

Static In Vitro Digestion Method for Food
de In vitro food digestion - COST action INFOGEST
il y a 10 mois • 723 vues
A step-by-step description of a static in vitro digestion method presented suitable for food (international consensus); recently published and available online (open access), see ...

8:55

Chaînes populaires sur YouTube

- Seb la Frite S'abonner
- MissCreatives S'abonner
- LanguedePub / M... S'abonner
- JFT S'abonner
- Madame Patachou S'abonner
- halawiyat khadija... S'abonner

FR

10:38

https://www.youtube.com

Kitty Verhoeckx · Paul Cotter
Iván López-Expósito
Charlotte Kleiveland · Tor Lea
Alan Mackie · Teresa Requena
Dominika Swiatecka
Harry Wichers *Editors*

The Impact of Food Bioactives on Health

In Vitro and Ex Vivo Models

 COST

 Springer Open

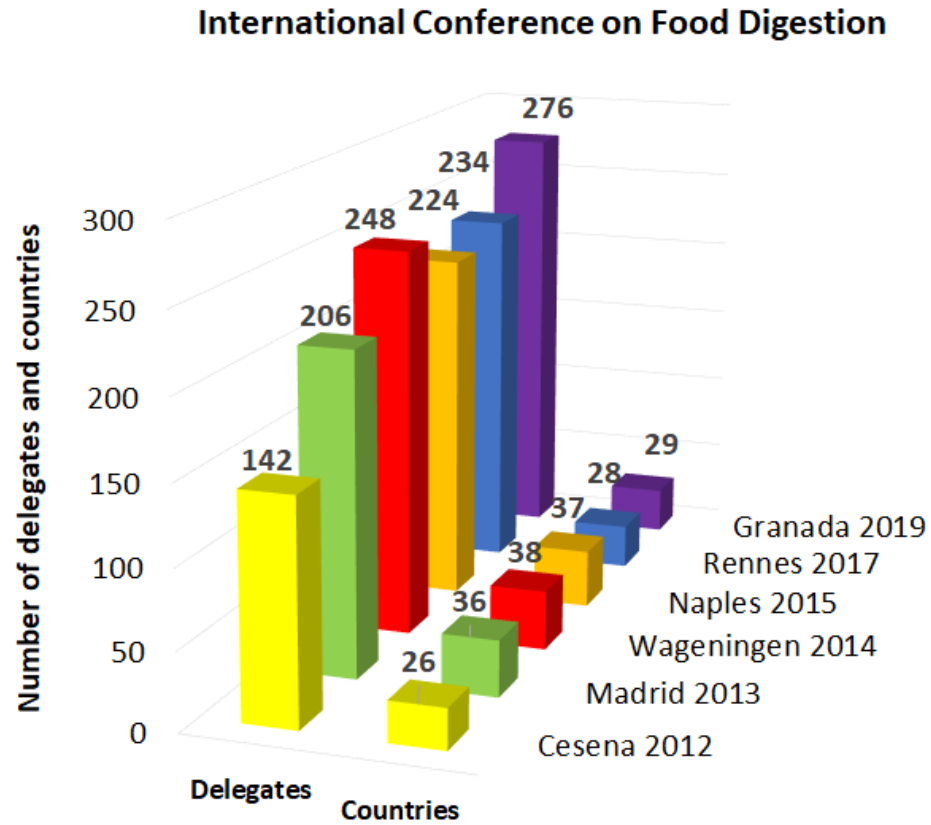
Some outputs

Release of a 341-page book on all the in vitro and ex vivo models to study the impact of food bioactives on health

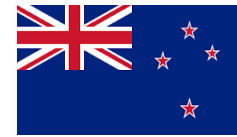
OPEN ACCESS

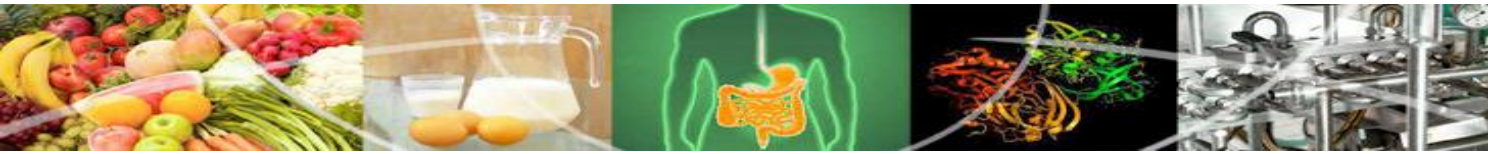
- * More than 620,000 chapter downloads
- * top 25% most downloaded eBooks in 2017

The International Conference on Food Digestion



The Conference has been created by INFOGEST and is now an event regularly followed by 200 scientists





We are pleased to announce the next
7th International Conference on Food Digestion



in Cork, Ireland, 2021