Structuring foods to improve the bioavailability of bioactives and nutrients

Didier Dupont

The structure of dairy products drives the kinetics of proteolysis and lipolysis in the GI tract and the bioavailability of nutrients

Dr Didier DUPONT

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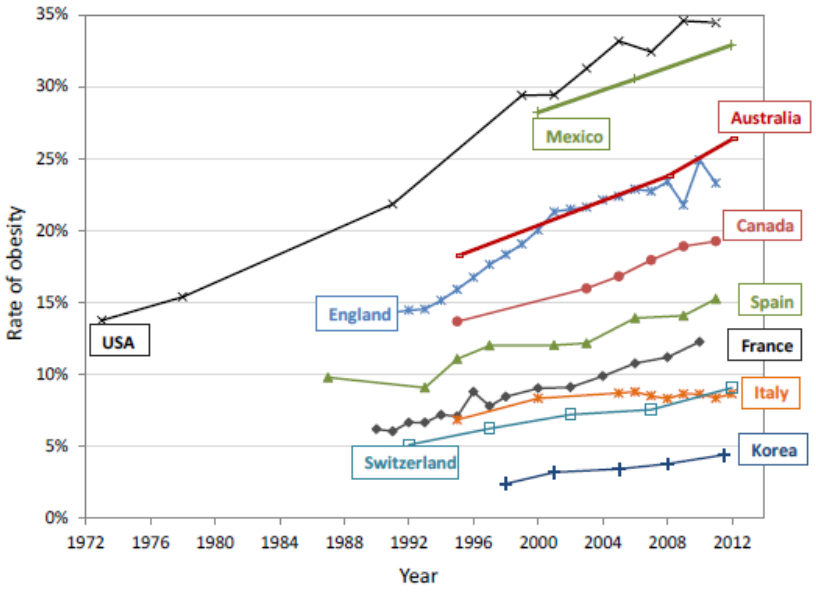
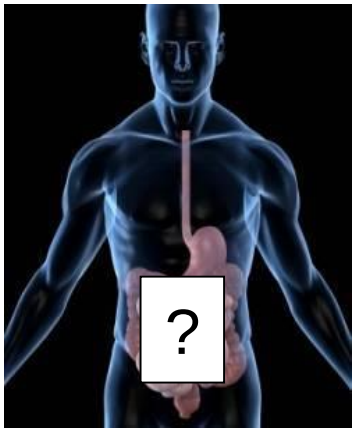
Why are we interested in understanding food digestion?



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



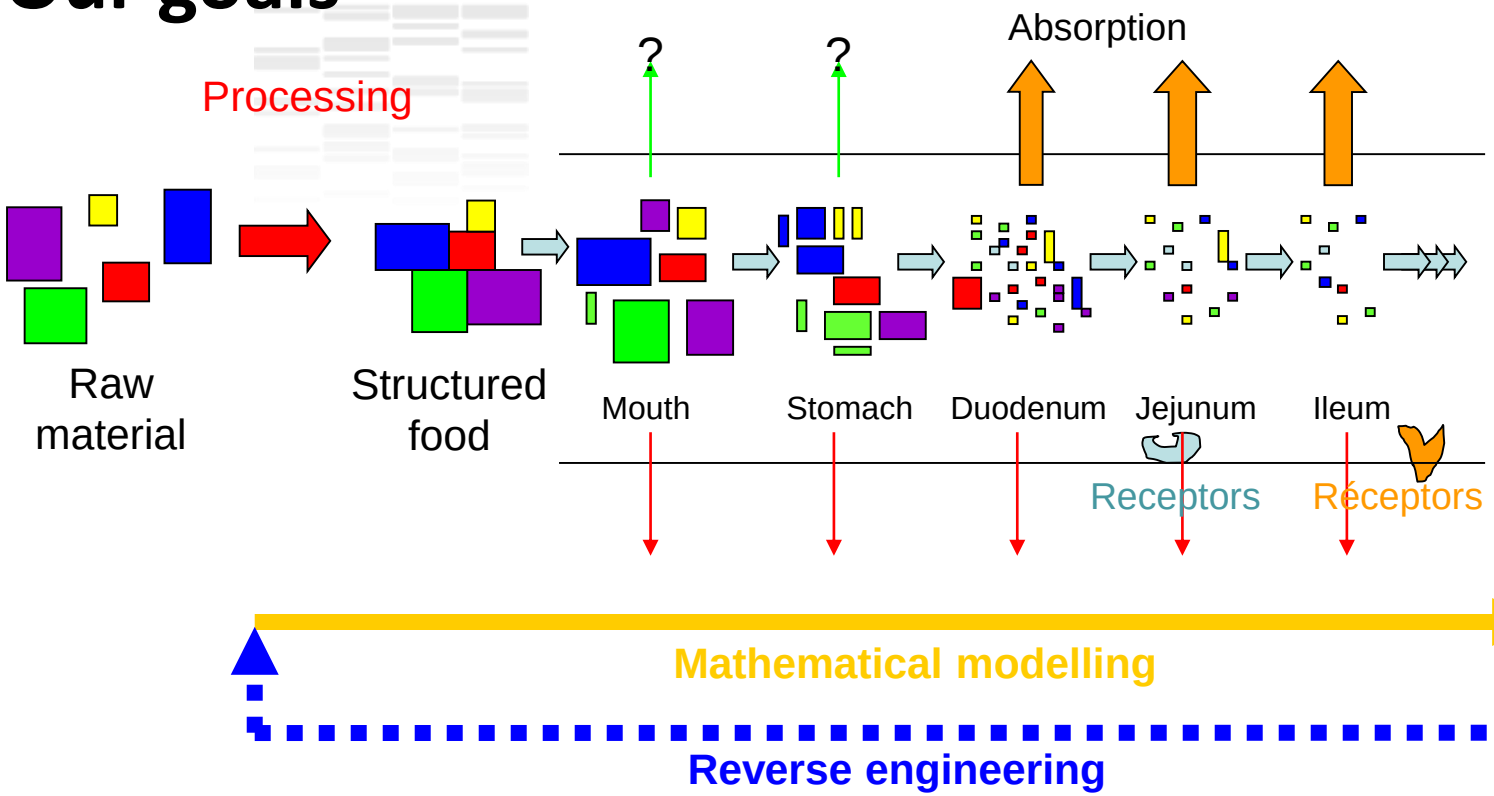
Gut = interface between food and human body
Digestion releases food components that can have a beneficial or a deleterious effect on human health

… 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/ Neonate/ Elderly



Bioactivities
- Bioactive peptides
- Amino acids
- Fatty acids
- Minerals…

Gut

Immune System

Microbiota

☞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 these mechanisms

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

The structure of dairy products modulate their kinetics of digestion

Barbé F.[1], Ménard O.[1], Le Gouar Y.[1], Buffière C.[2], Famelart M.-H.[1], Laroche B.[3], Le Feunteun S.[4], Rémond D.[2] and **Dupont D.[1]**

[1] INRA STLO Rennes, France
[2] INRA UNH Clermont-Ferrand, France
[3] INRA MIA Jouy-en-Josas, France
[4] INRA GMPA Grignon, France

Objective: to compare kinetics of digestion of dairy products of identical composition but different structure

Ultra Low Heat powder

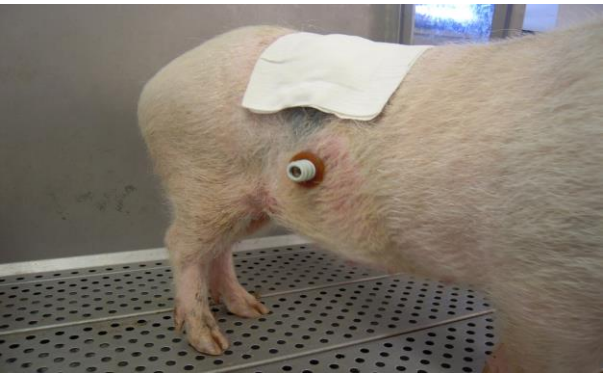
rehydration in water 14.5%

unheated milk ("raw" milk)

heat treatment 90°C-10 min

microstructure

heated milk



macrostructure

24h-20°C, rennet 0.003 % v/w

rennet gel

24h-20°C, rennet 0.3 % v/w

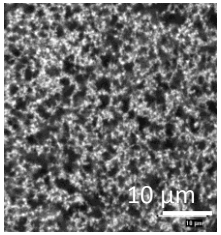
rennet gel

24h-20°C, GDL 3 % w/w

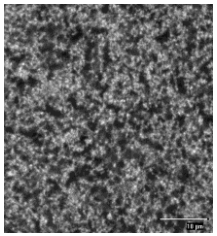
acid gel

24h-20°C, GDL 3 % w/w + mixer 2 min

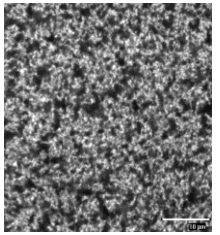
stirred acid gel



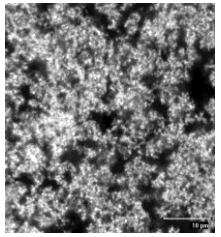
pH 6.6



pH 6.6



pH 4

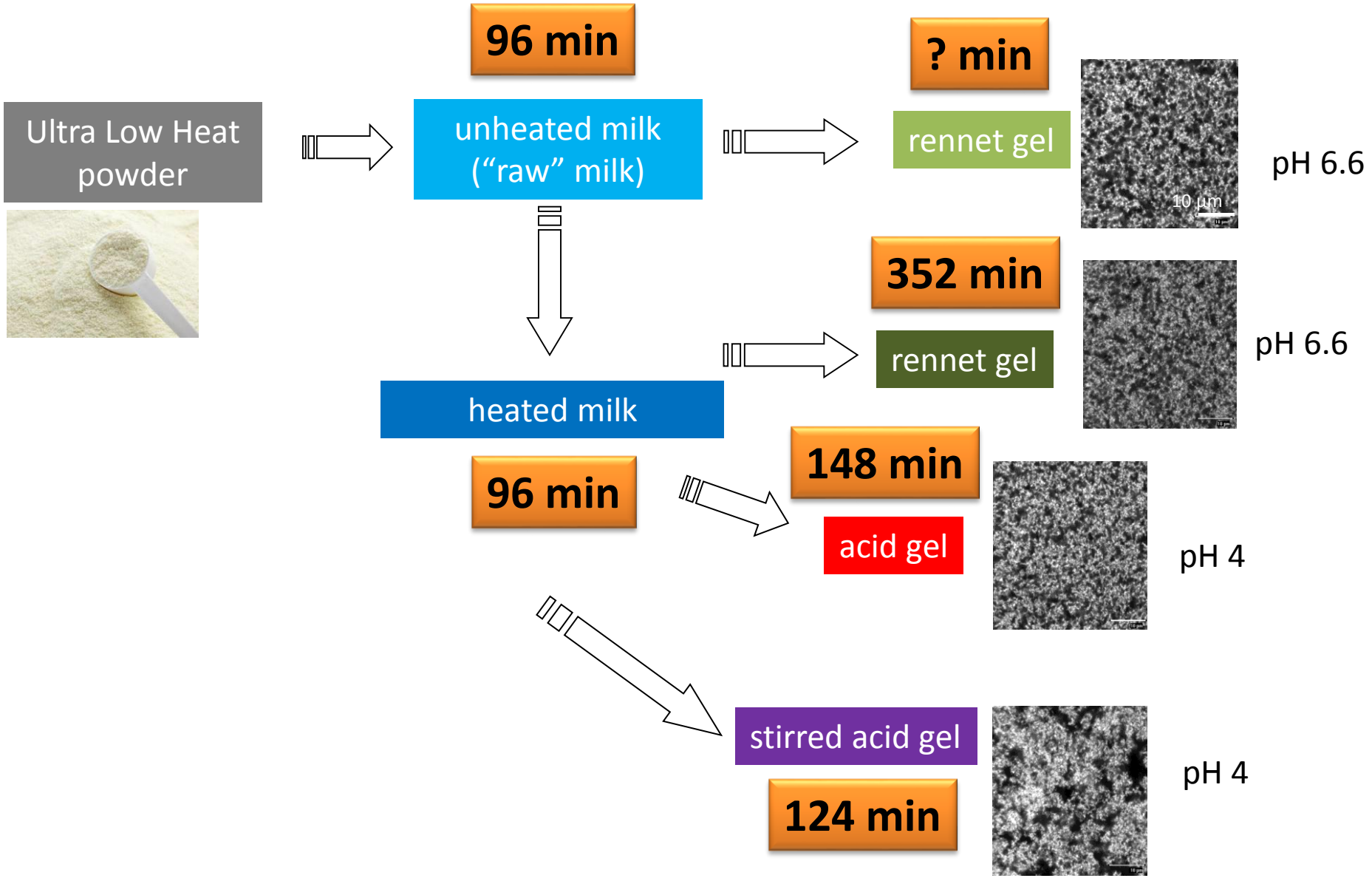


pH 4

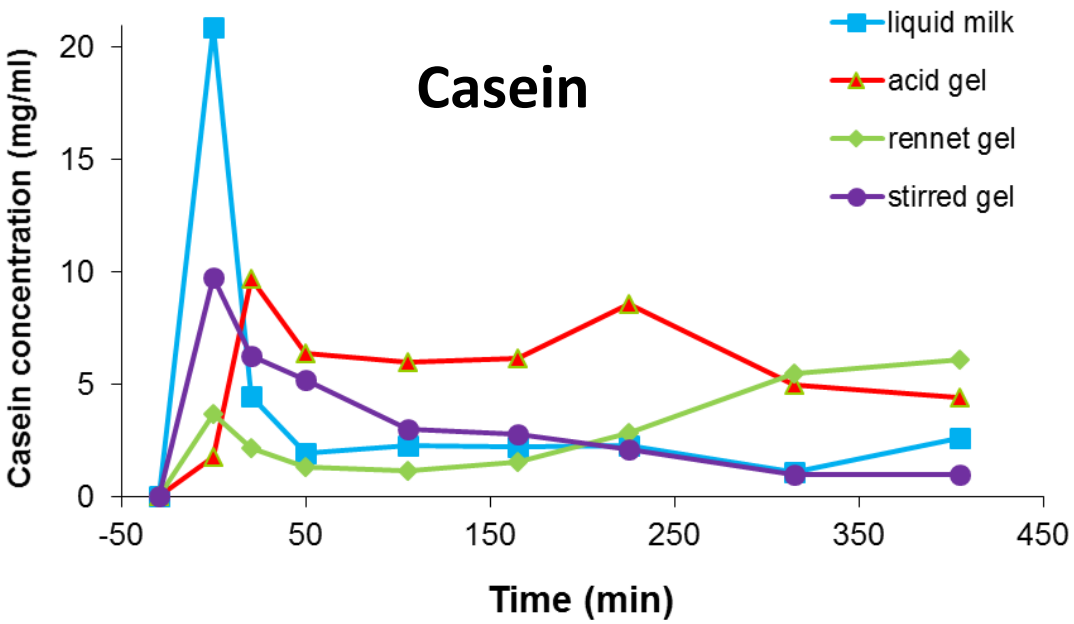
Fat-free matrices: 40 g/L caseins, 10 g/L whey proteins, 95 g/L lactose and minerals

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

Gastric emptying half-time

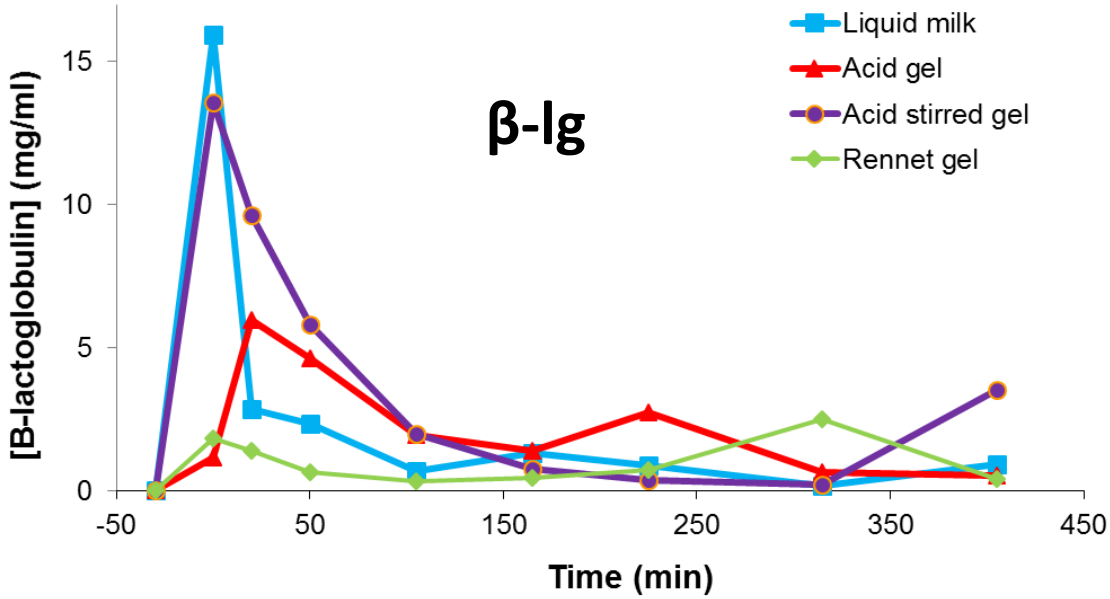


Milk proteins in the duodenum (ELISA)



- 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

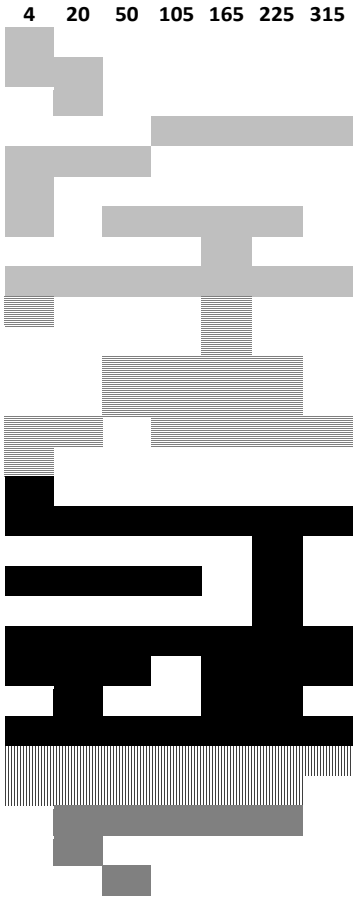
- Only traces of milk proteins found in the jejunum
- Dairy products remain highly digestible



Bioactive peptides released during digestion differ from one matrix to another

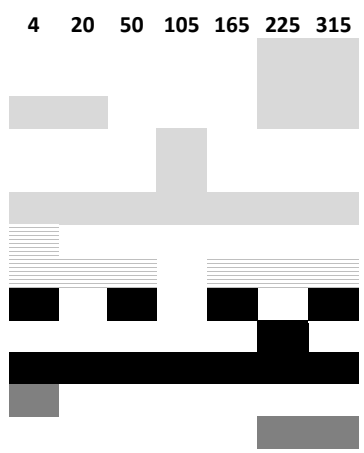
More than 4000 peptides were identified in the gut lumen!!!

Protein	Sequence	Activity	Reference
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
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

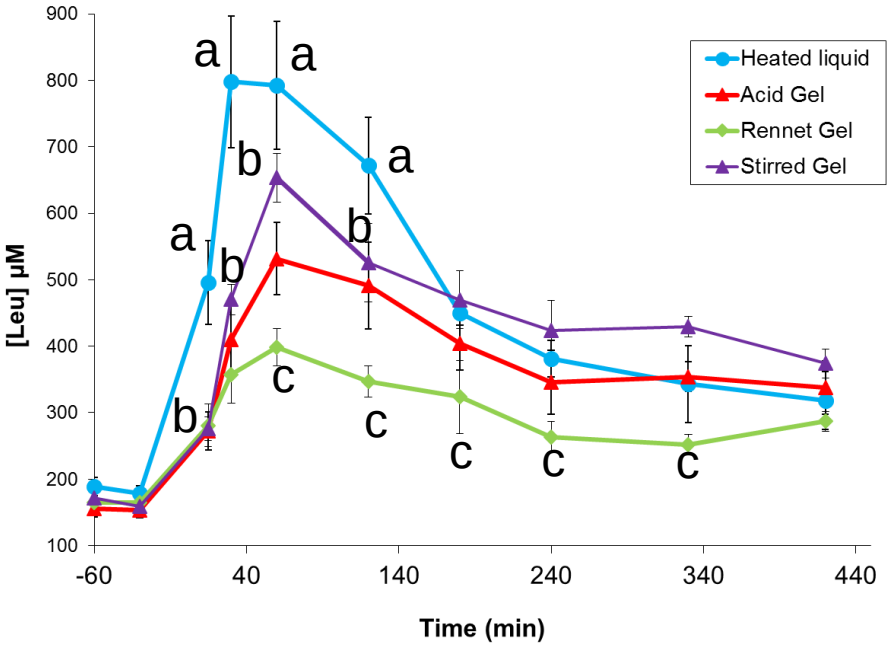
- More bioactive peptides identified during digestion of acid gel than rennet gel
- Nature of peptides is identical (clearly defined by the digestive enzyme specificity)
- Kinetics of release are different

Barbé et al. 2014 Food Res Int

The liquid-gel transition

Barbé *et al*. Food Chem 2013
Highly cited paper

2) effect on absorption



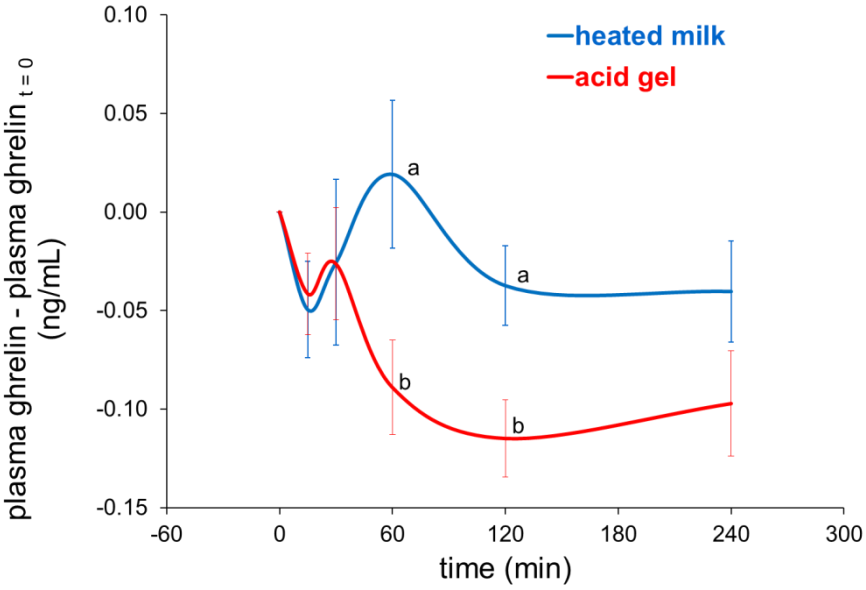
milk gelation:

→ delayed proteins transit → delayed AA absorption

↘ maximal AA concentration in the plasma

3) potential effect on satiety

ghrelin (gastrointestinal hormone → appetite stimulation)

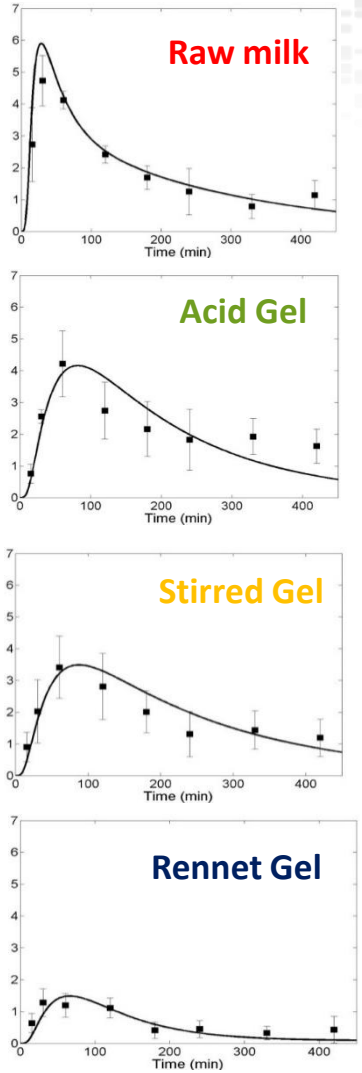


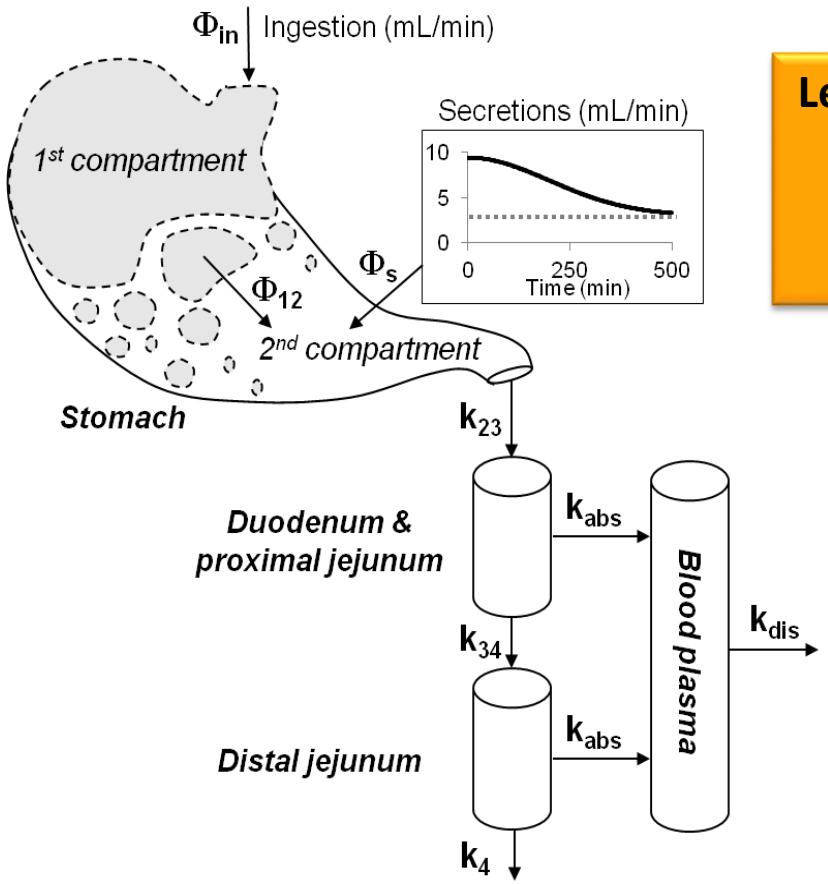
milk gelation:

↘ postprandial ghrelin concentration =

↗ satiety ?

In silico model of transit and absorption





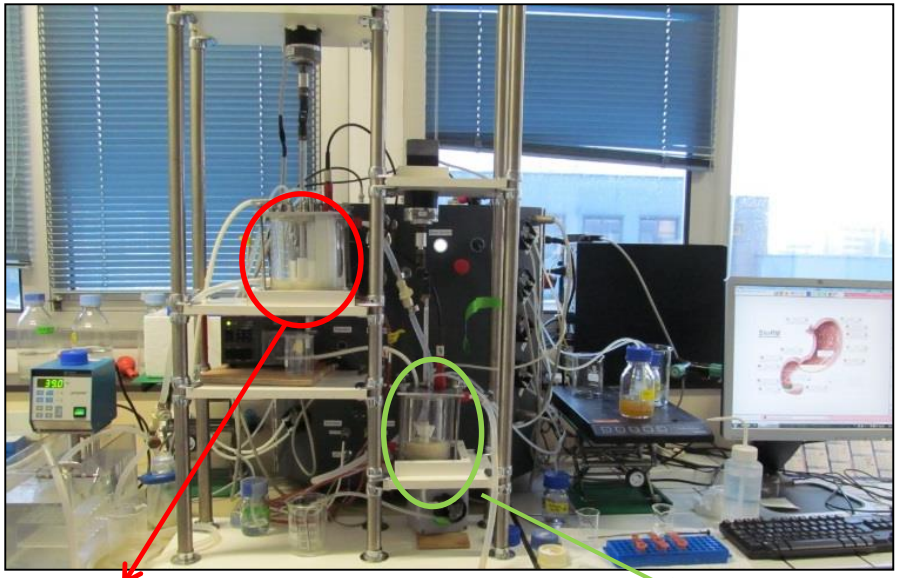
Le Feunteun et al. Food Bioprocess Tech 2014

Better understanding of the food behaviour in the stomach
Predictive model???

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

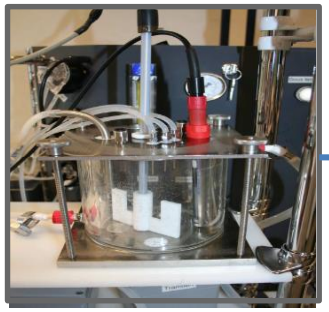
Ménard *et al*.
Food Chem 2014



DIDGI®

StoRM® software

Stomach



- Pepsine
- Gastric lipase
- Simulated gastric fluid
- HCl

Emptying : Elashoff's model

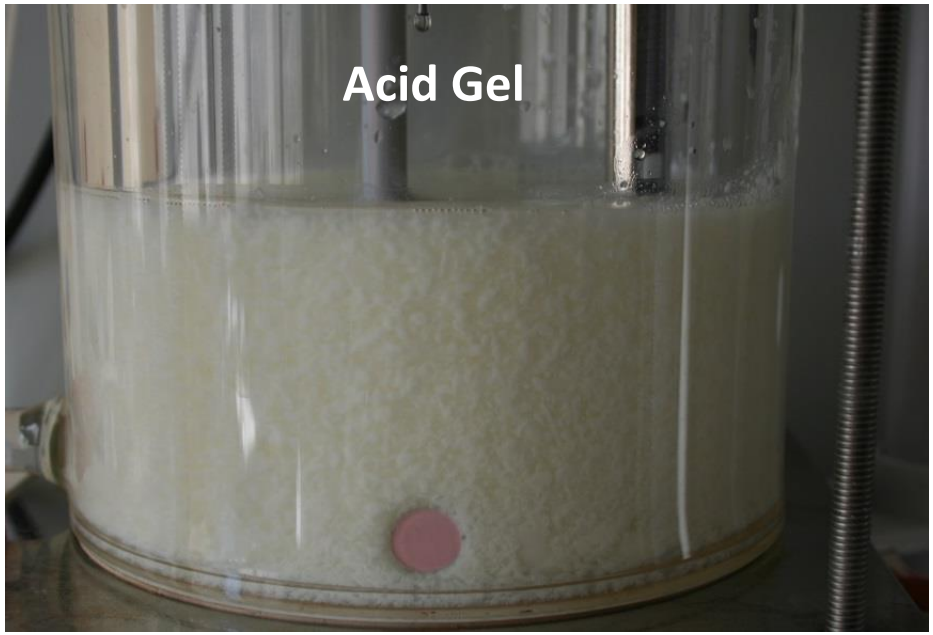
Small intestine

- 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

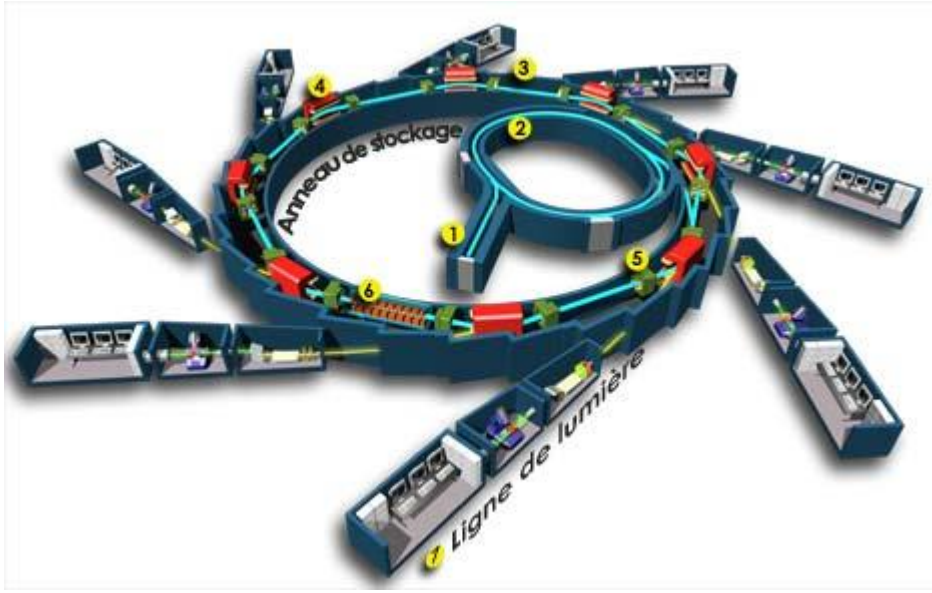
The structure that a food adopts in the stomach is essential to understand its digestion

Understanding the mechanisms of dairy gel particles degradation in the stomach

Floury J.[1], Cardoso Bianchi T.L.[1], Thévenot J.[1], Dupont D.[1], Jamme F.[2], Lutton E.[3], Panouillé M.[3], Boué F.[3], Le Feunteun S.[4]

[1] INRA STLO Rennes, France
[2] SOLEIL Synchrotron Gif-sur-Yvette, France
[3] INRA GMPA Grignon, France

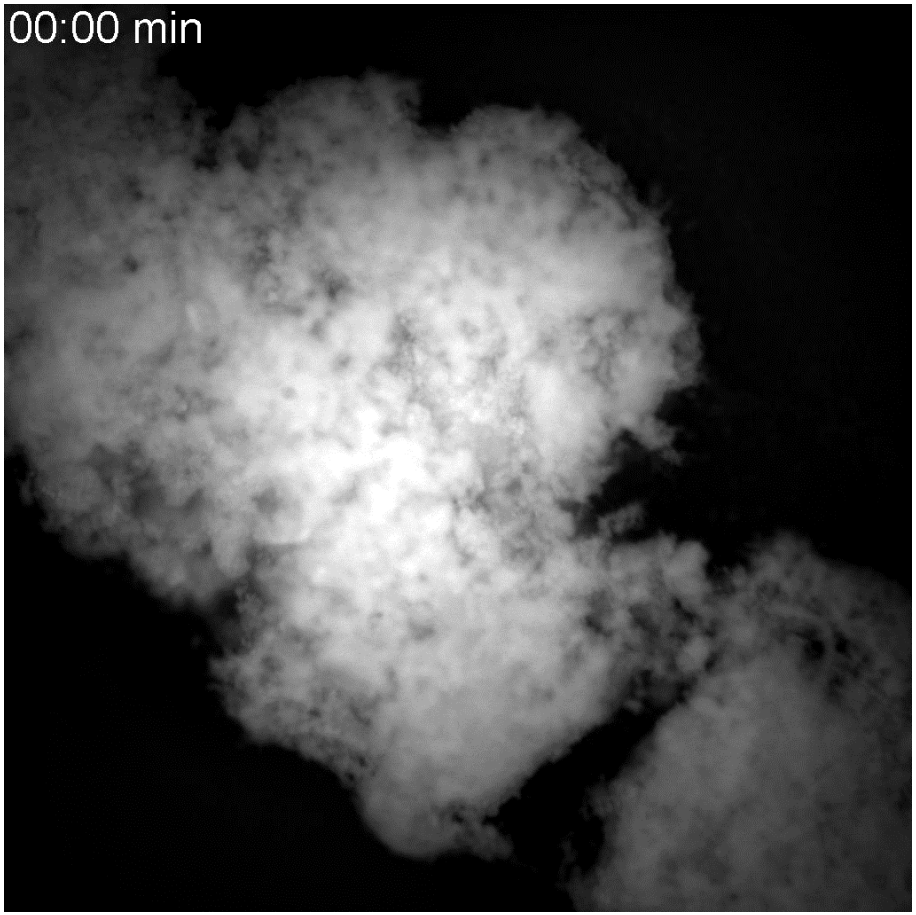
Soleil is a particle (electron) accelerator that produces the synchrotron radiation, an extremely powerful source of light that permits exploration of inert or living matter

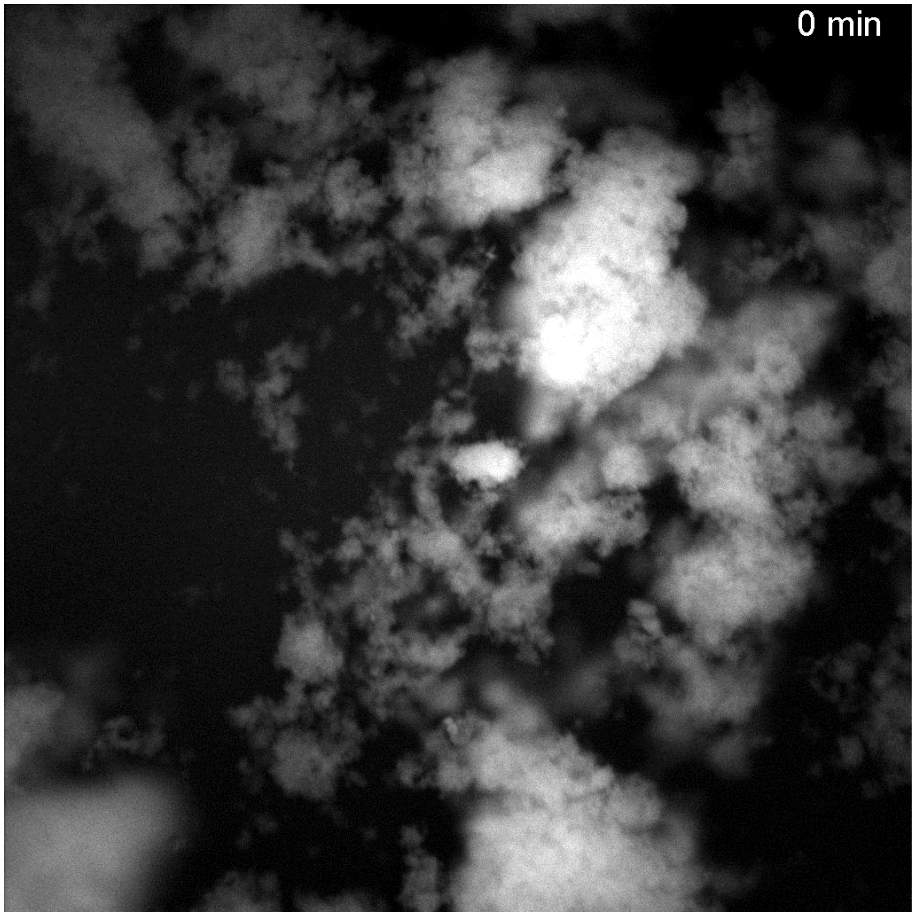


DISCO is a VUV to visible beamline dedicated to biochemistry, chemistry and cell biology. The spectral region is optimized between 60 and 700 nm with conservation of the natural polarization of the light

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

Kinetics of gel particles disintegration: comparison of rennet/acid gel





Rennet Gel

Acid Gel

Nutrition of the neonate - Effect of the fat globule homogenization on the digestion of milk macronutrients

Bourlieu C.[1], Ménard O.[1], De Langle A.[1], Rousseau F.[1], Madec M.-N.[1], Deglaire A.[1], Pezennec S.[1], Robert B.[1], Bouhallab S.[1], Carrière F.[2], Dupont D.[1]

[1] INRA STLO Rennes, France
[2] CNRS EIPL Marseille, France

Human/ bovine milk / Infant Formula

Protein structures

Human milk

Bovine milk

Infant Formula

(Turck *et al*, 2010)

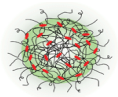
Ø = 64 nm

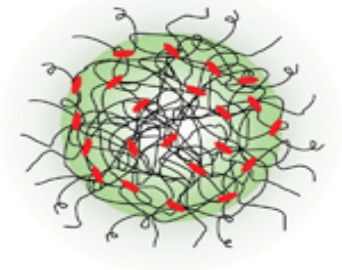
(β, κ–casein)

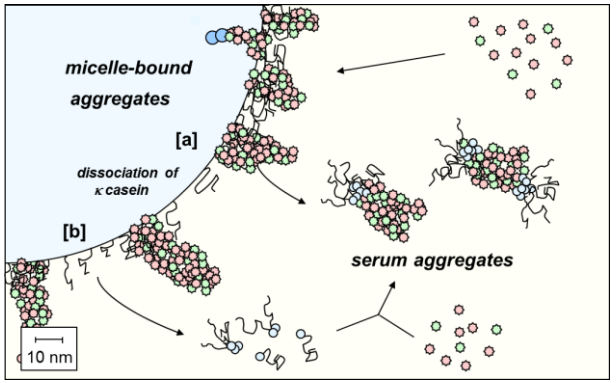
ø = 182 nm

(αs1, β–casein)

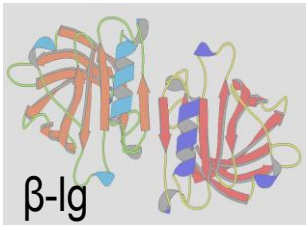
Casein micelle







Whey Proteins



Human/ bovine milk / Infant Formula

Lipid globule structure

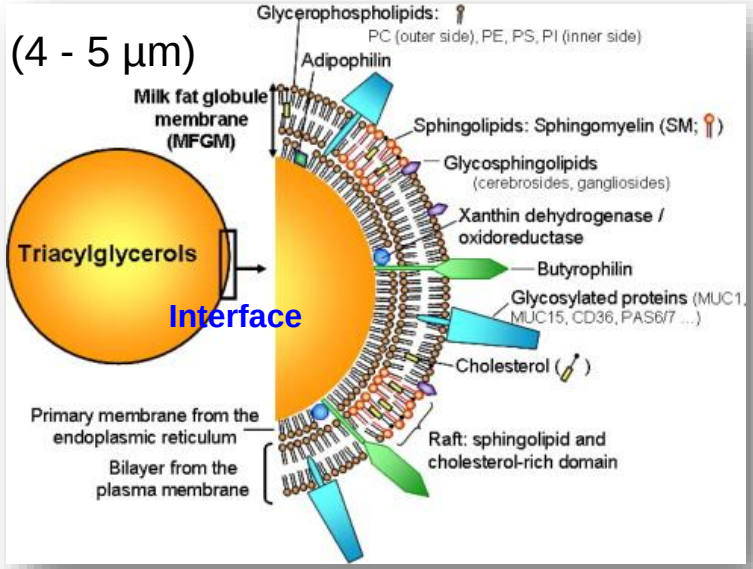
Human milk

Bovine milk

Infant Formula

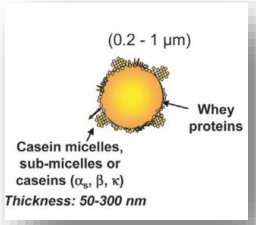
Native milk fat globule

(4 - 5 µm)



(Lopez, 2010)

Lipid droplets



(0,2 - 1 µm)

(Lopez and Briard-Bion, 2007)

Do technological processes have an impact on the kinetics of lipolysis in the stomach?

Effect of homogenization

2 model infant formulas standardized in fat and proteins

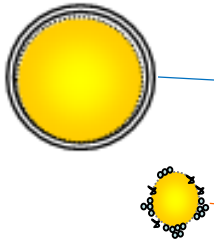
(1.8 % proteins 40:60 caseins/whey proteins, 3.2 % fate with either native or homogenized globules)

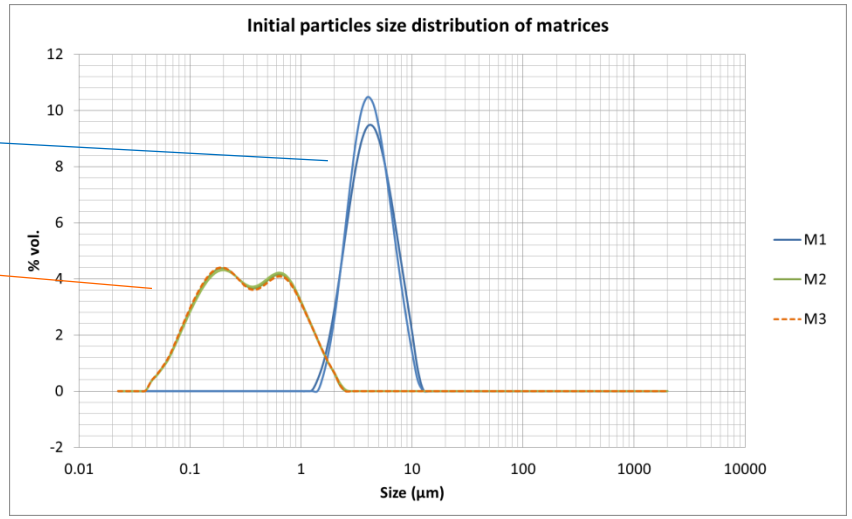
Raw formula (M1)

Homogenized formula (M2)

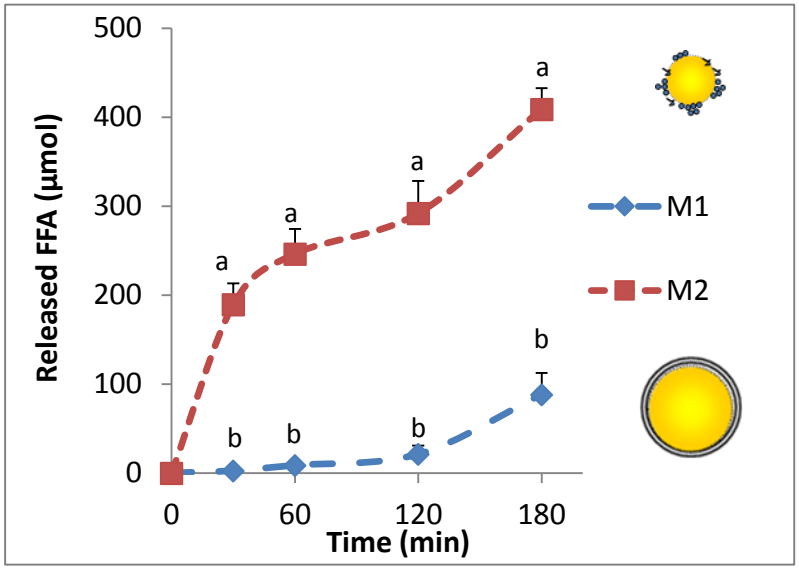
Raw (M1)

Homogenized (M2)



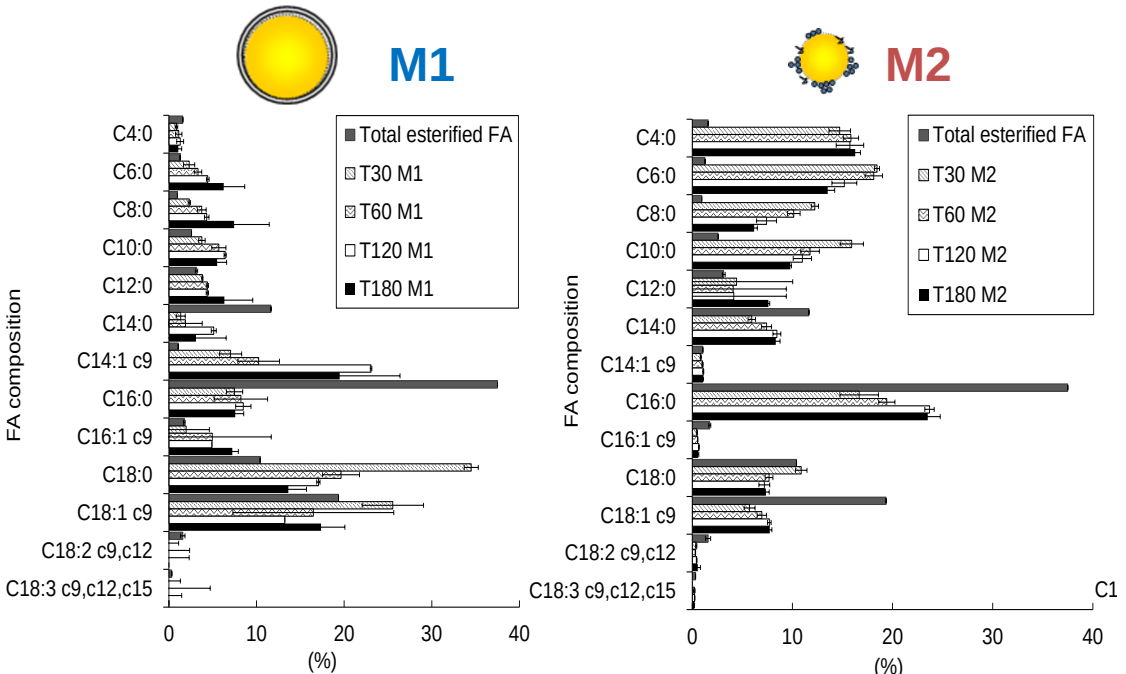


The increased lipolysis of the homogenized formula can be explained by the increase in specific surface of the o/w interface



Specific surface [m²/g of lipid]	M1 1.81	M2 31.90

Bourlieu *et al*. Food Chem. 2015



In vivo study in the preterm infant

NCT02112331 (ClinicalTrials.gov)

- Preterm hospitalized infants (GA < 32 wk)
- Nasogastric tube feeding
- Enteral feeding ≥ 120 mL/kg/day at 3 h intervals

GROUP A

HM from their **own mother**

Raw HM → collected < 24h before feeding

Past HM → 1 pool aliquoted in 6 bottles

GROUP B

HM from **anonymous donor**

Past HM

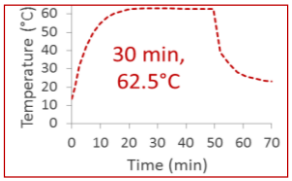
P+Homog HM

→ The same pool from one donor was used for the two types of milk

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

HM bank

Holder pasteurization



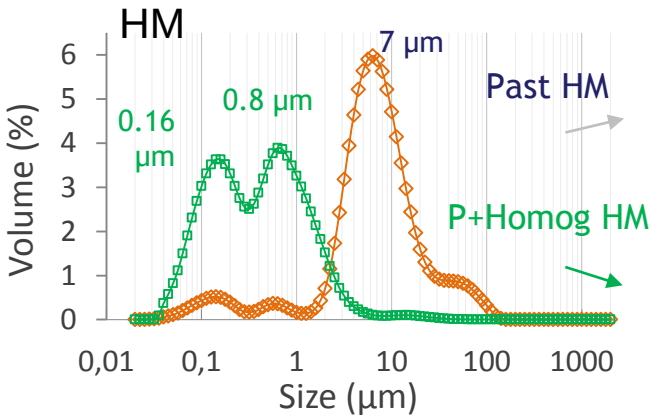
Indirect homogenization by ultrasonication

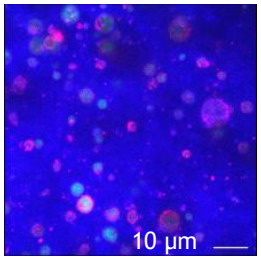
→ 595 W, 3 periods of 5 min interrupted by 30s of pause

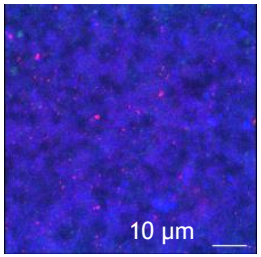
Homogenization affected the initial structure and the emulsion disintegration of HM

(n = 5 infants)

Initial structure







Past HM: **4.1** ± 1.2 m^2/g of fat

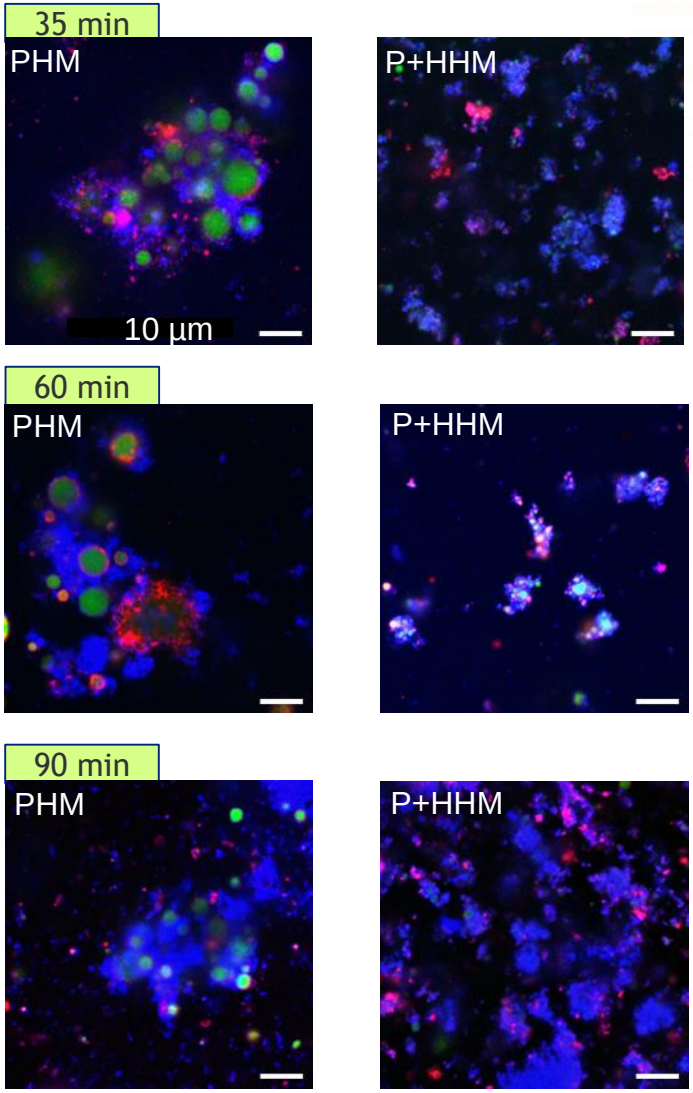
P+Homog HM: **25.5** ± 3.8 m^2/g of fat

Disintegration of the emulsion structure after 35 min of gastric digestion

Differences in terms of aggregates morphology

Persistance of native fat globules throughout gastric digestion

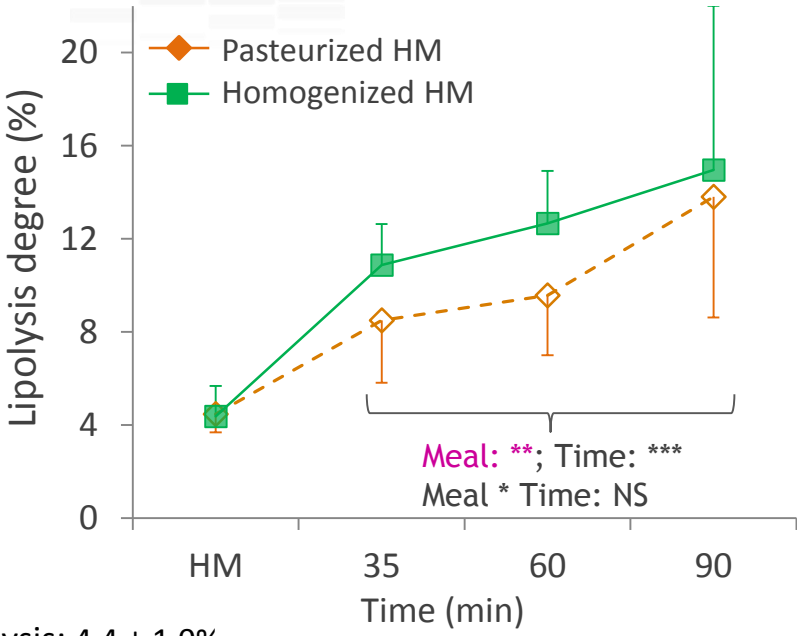
Gastric disintegration



Apolar lipids | Amphiphiles | Proteins

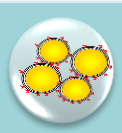
Homogenization impacted gastric lipolysis

Instantaneous lipolysis level

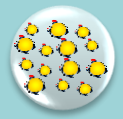


Pre-lipolysis: 4.4 ± 1.0%

De Oliveira *et al.* Clin Nutr. 2017



Past HM



P+Homog HM

- Same milk composition, initial pre-lipolysis degree and inactivation of BSSL
- **Different structure**

↗ Increase of specific surface of droplets facilitating HGL adsorption

Human milk homogenization accelerates gastric lipolysis: what are the consequences on the growth and health of pre-term neonates?

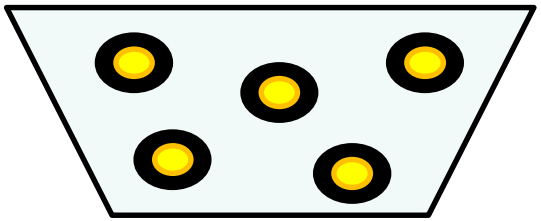
Infant formulas
Can we create lipid structures biomimetic of the native fat globule?

Le Huërou-Luron I.[1], Bouzerzour K.[2], Ferret-Bernard S.[1], Ménard O.[2], Le Normand L.[1], Perrier C.[1], Le Bourgot C.[1], Jardin J.[2], Bourlieu C.[2], Carton T.[3], Le Ruyet P.[4], Cuinet I.[4], Bonhomme C.[4], Dupont D.[2]

[1] INRA ADNC Rennes, France
[2] INRA STLO Rennes, France
[3] BIOFORTIS, Saint-Herblain, France
[4] LACTALIS, Retiers, France

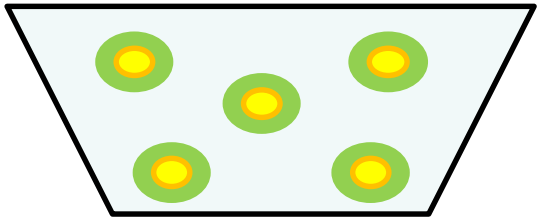
Infant formulas: can we create lipid structures biomimetic on the native fat globule?

Formula T1



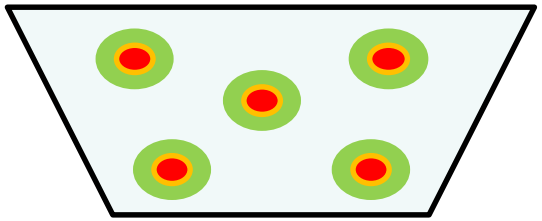
Interface 100 % Proteins
100% vegetable oil

Formula T2

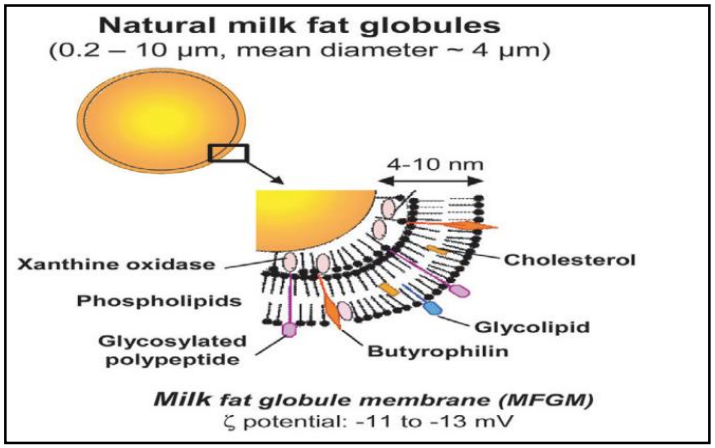


Interface 100 % phospholipids
100% vegetable oil

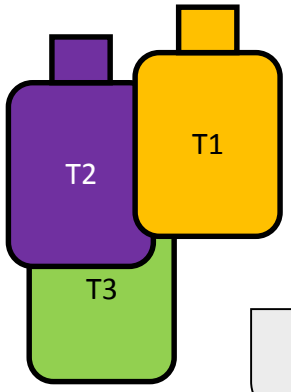
Formula T3



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



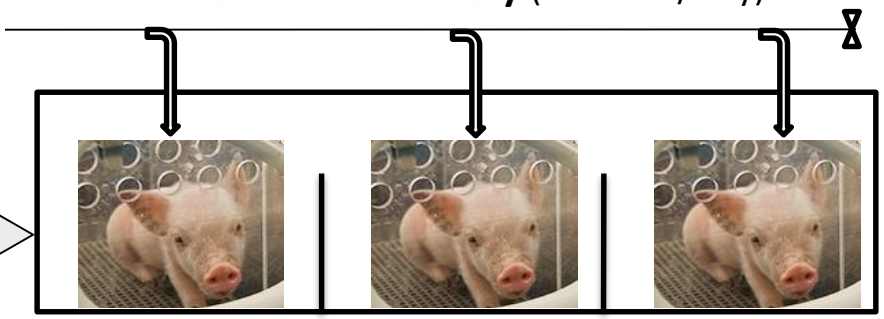
Can the composition of Infant Formula modulate the physiological response of the neonate?



Veg
Veg + PL
Dairy Fat + PL

Automatic meal delivery (10 meals/ day)

Rehydration at 20%



\+

Mother-fed piglets (MF = + control)

Slaughtering after

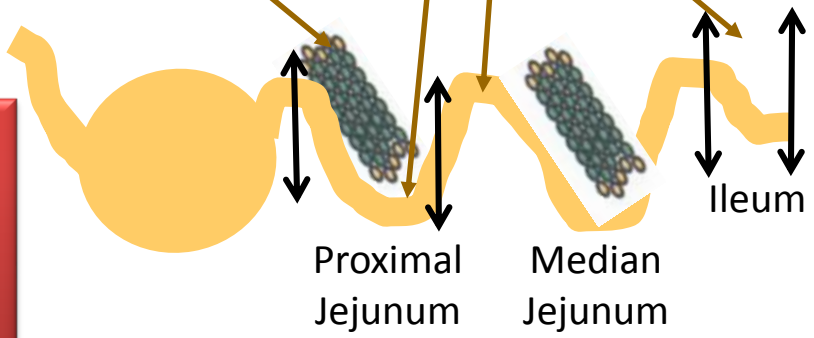
7 days

28 days

(90 min postprandial)

Mesenteric Lymph Nodes (MLN)

Collect of effluents and tissues



Effluents:
-SDS-PAGE
-Elisa

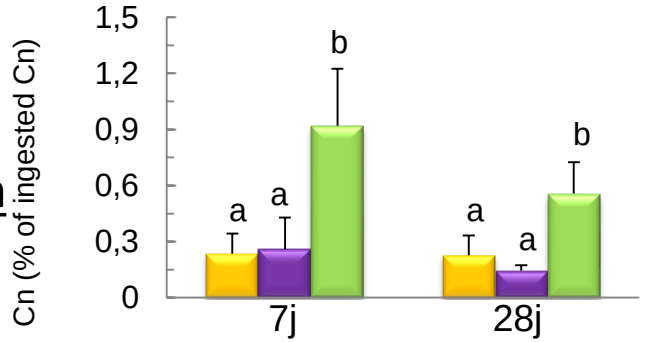
Tissues:
-Morphometry
-Enzyme Activities
-Intestinal Permeability
-Local immune response
-Microbiota

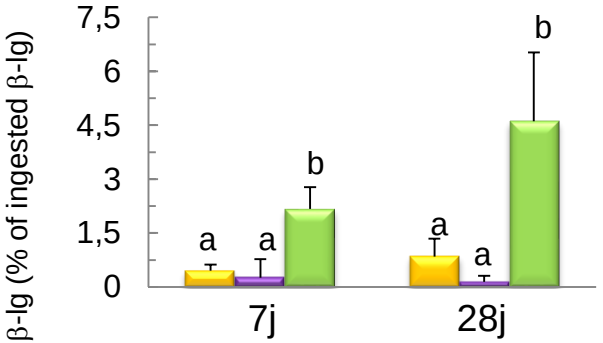
Protein Digestion

Casein

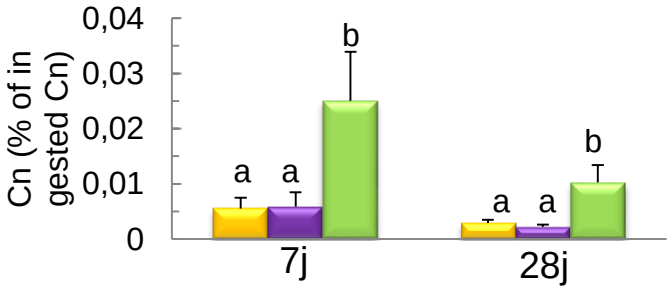
β-lactoglobulin

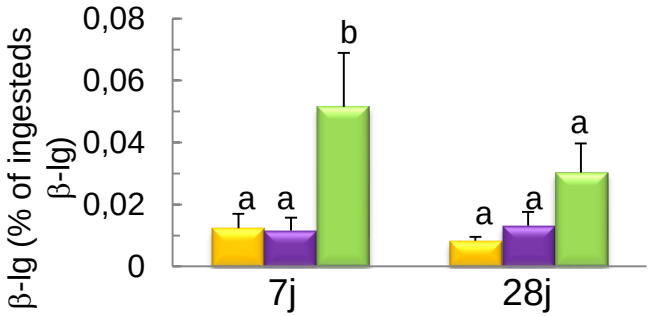
Jejunum





Ileum





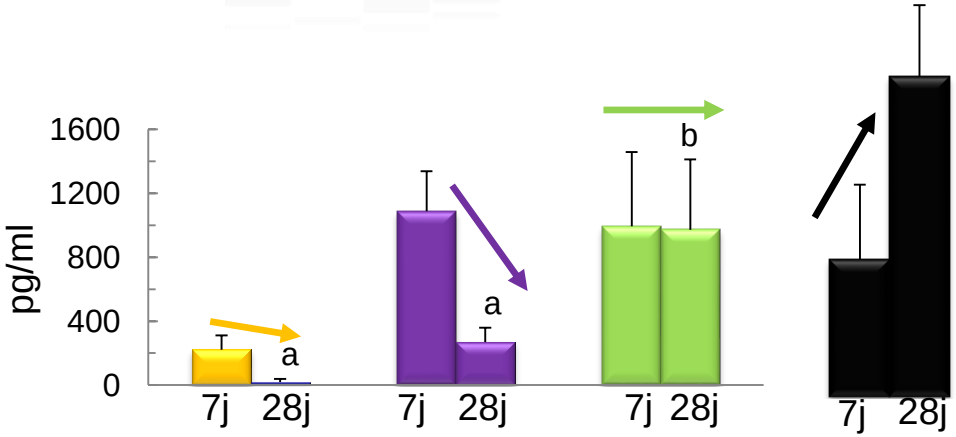
Milk Proteins better resist to intestinal digestion in the presence of dairy fat

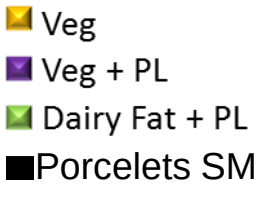
➔ Modification of the interface

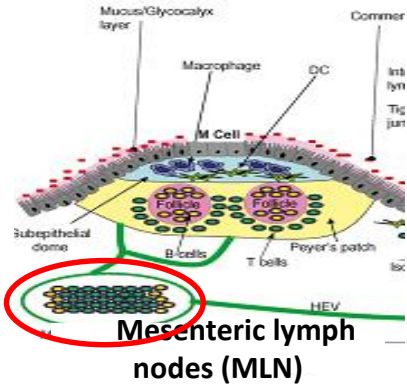
(Granger *et al* 2005; Davies *et al*, 2001)

Secretory activity of MLN

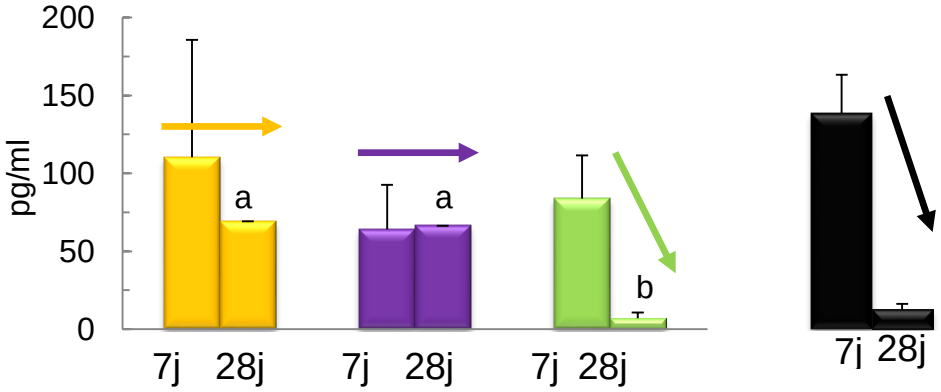
Interferon-g (Th1 pro-inflammatory)







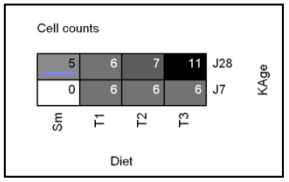
Interleukine-10 (Th2 anti-inflammatory)



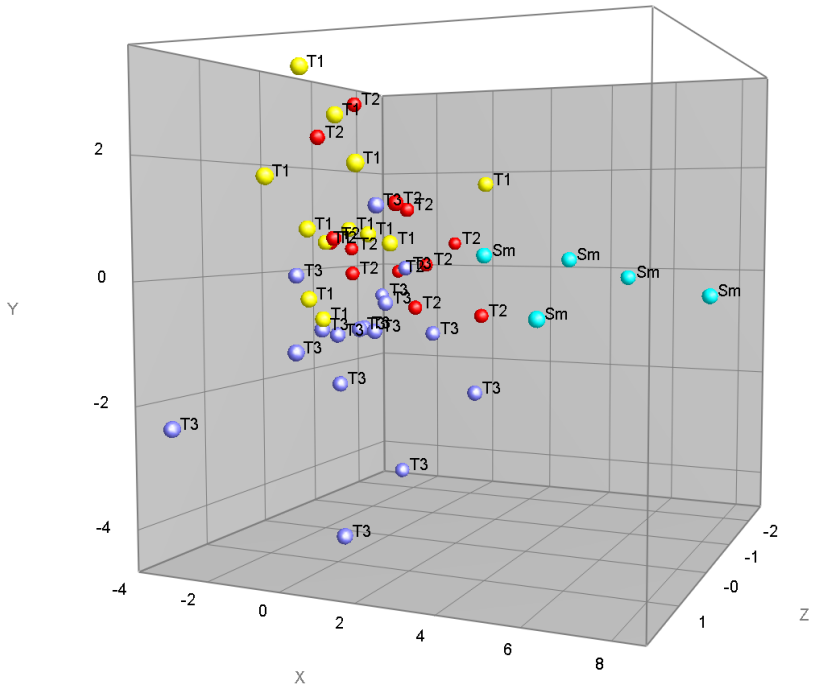
Milk lipids ➔ maturation of the piglet's immune system more similar than with sow's milk

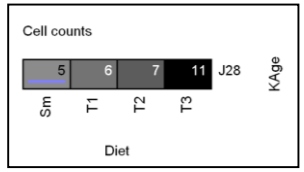
Le Huerou-Luron *et al.*
Eur J Nutr. 2016

Microbiota by DHPLC

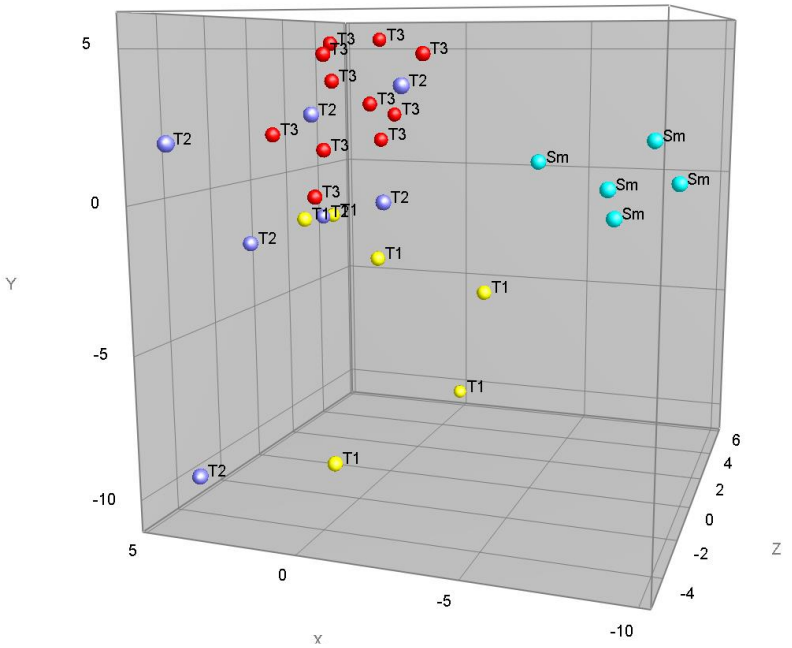


D7 & D28



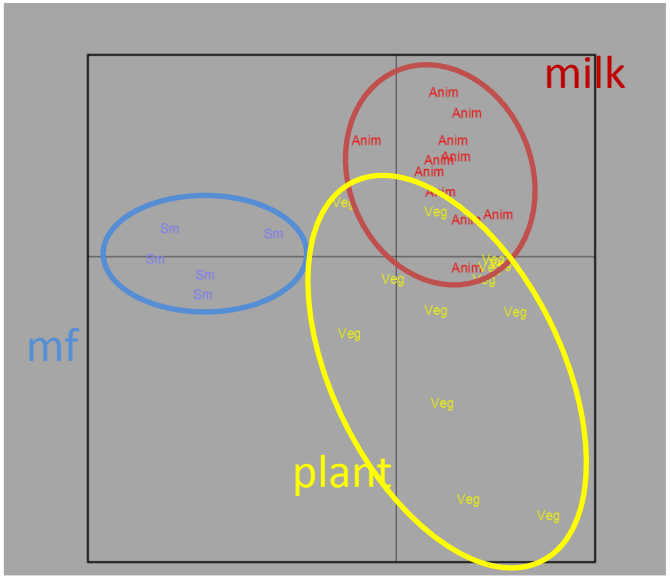


D28



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

More Proteobacteria with milk fat /
More Firmicutes with plant oil



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Conclusion

The structure/composition of dairy products 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

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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

350 scientists - 130 institutes – 38 countries

Industry involvement

☞ ~ 40 European companies are involved in INFOGEST

Chair
Didier Dupont - France

INFOGEST

Vice-chair
Alan Mackie - UK

www.cost-infogest.eu

In vitro/in vivo correlations WG1

Didier Dupont

In vitro semi-dynamic model of digestion WG2

Alan Mackie

Guy Vergeres

Models for specific populations WG3

Uri Lesmes

The "Mind-the-Gap" group

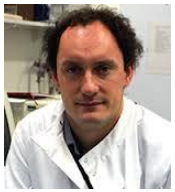
Digestive lipases and lipid digestion WG4

Myriam Grundy

Frederic Carriere

Digestive amylases and starch digestion WG5

Nadja Siegert



Fred Warren

In silico models of digestion WG6

Choi-Hong Lai

Steven Le Feunteun

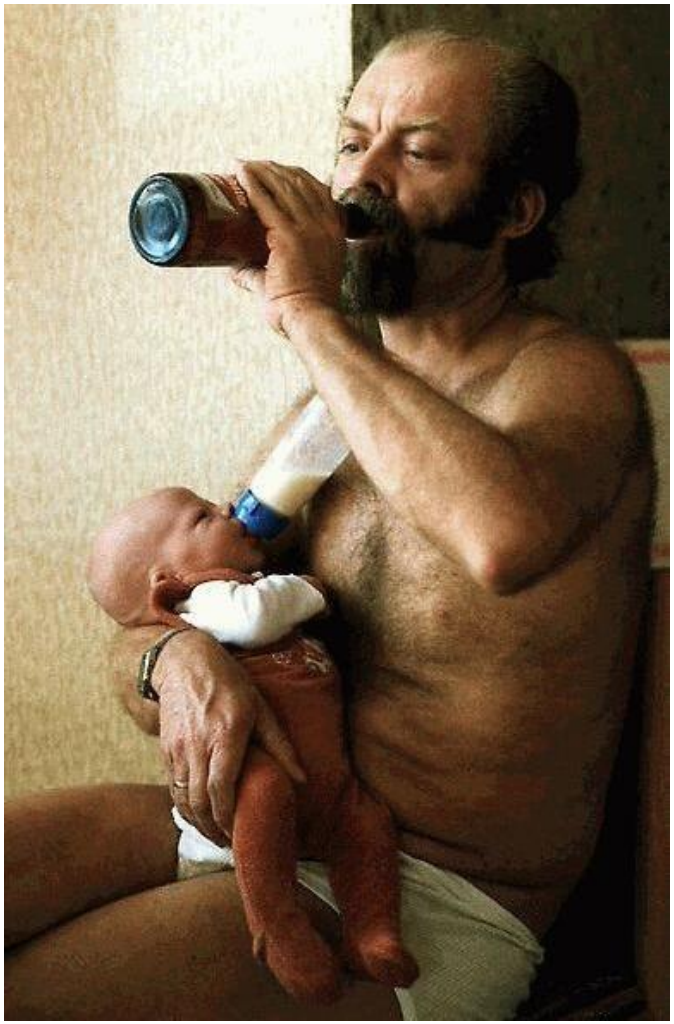
INFOGEST
International Conference
Granada, Spain
April 2019

We are pleased to announce the next

6th International Conference on Food Digestion

in Granada, Spain, April 2019

Improving infant formula for improving human life…



Thanks for your kind attention !!!