

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

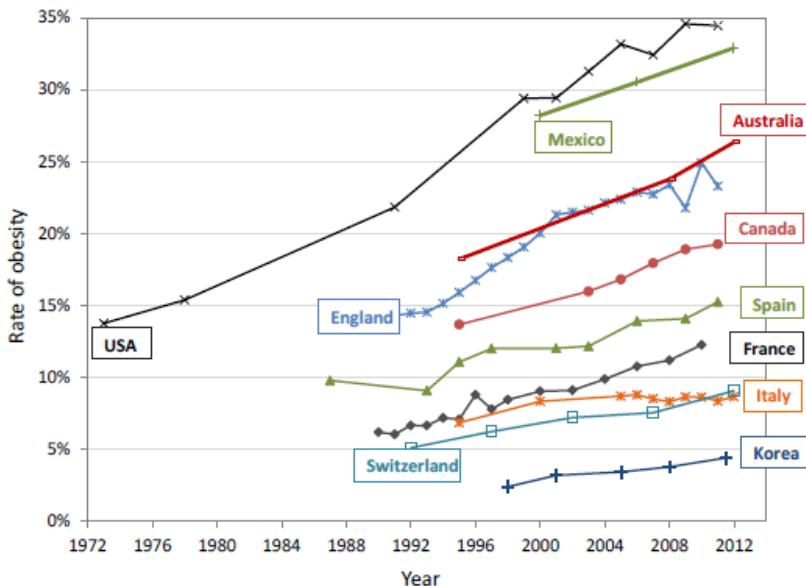


Dr Didier DUPONT

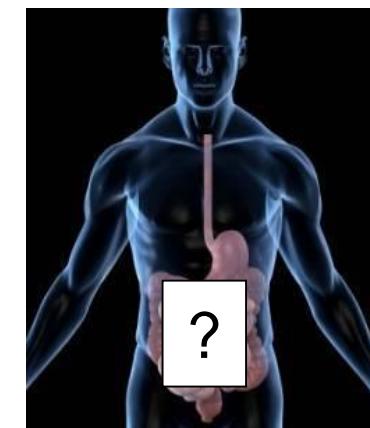
INRA, Rennes, France



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

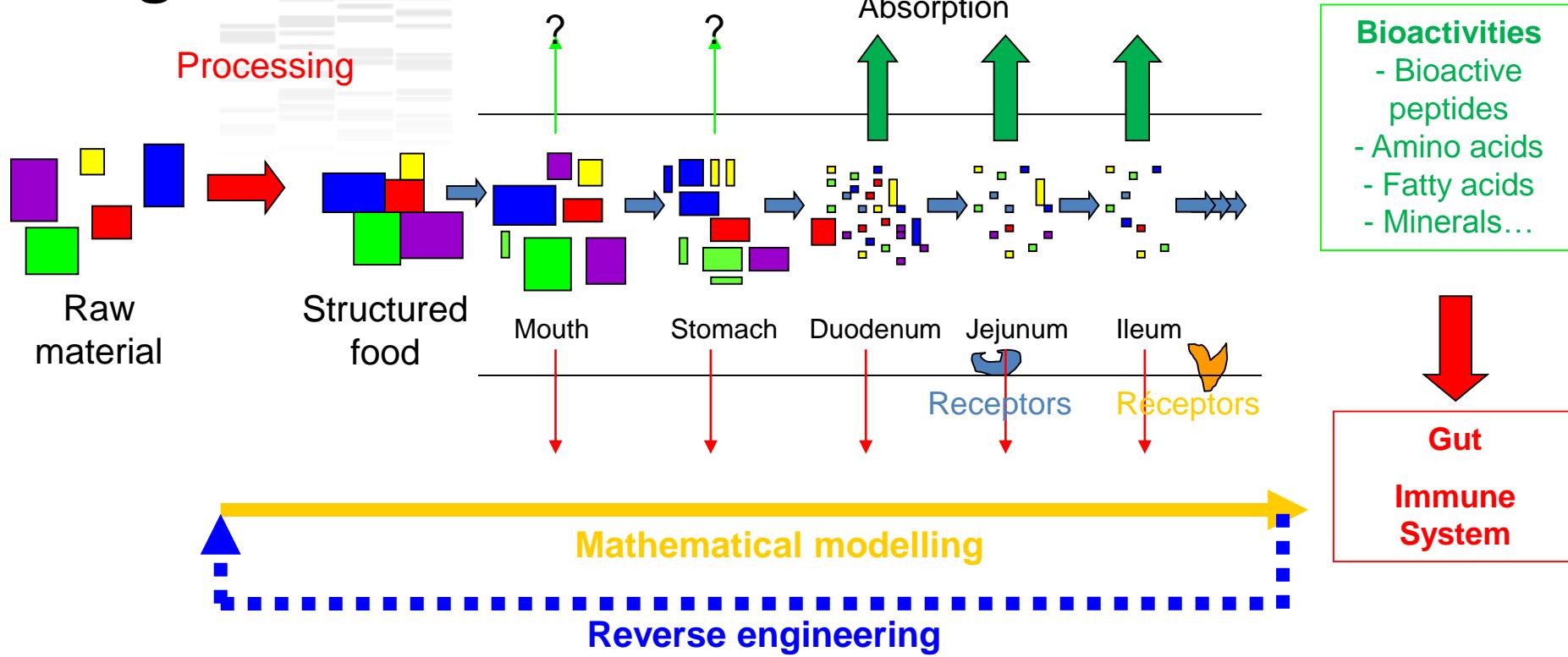
Digestion releases food components that can have a beneficial or a deleterious effect on human health

... 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



- 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



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⁴ INRA GMPA Grignon, France

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

Ultra Low Heat powder

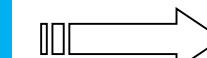


unheated milk
("raw" milk)

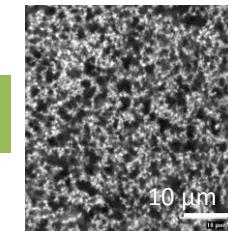
rehydration in
water 14.5%



macrostructure



rennet gel



pH 6.6

microstructure

heated milk

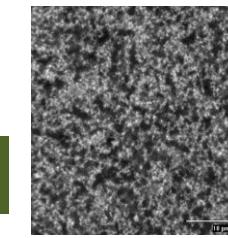


heat treatment
90°C-10 min



24h-20°C,
rennet 0.3 % v/w

rennet gel

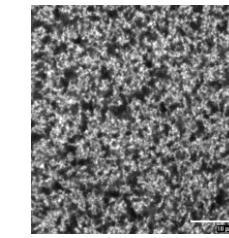


pH 6.6



24h-20°C,
GDL 3 % w/w

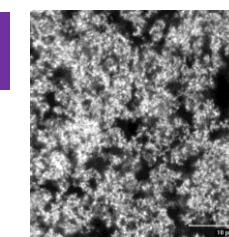
acid gel



pH 4

24h-20°C,
GDL 3 % w/w +
mixer 2 min

stirred acid gel

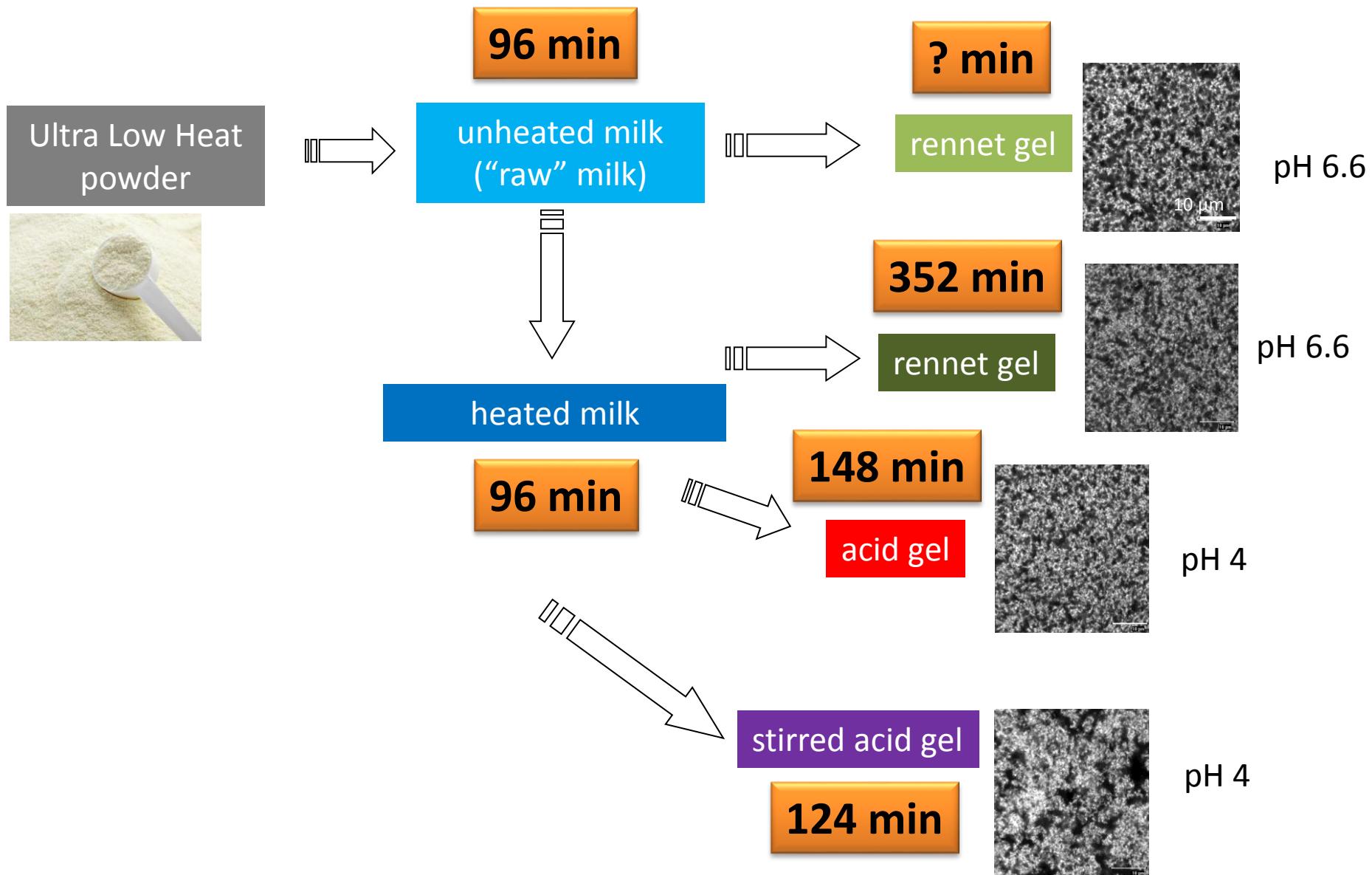


pH 4

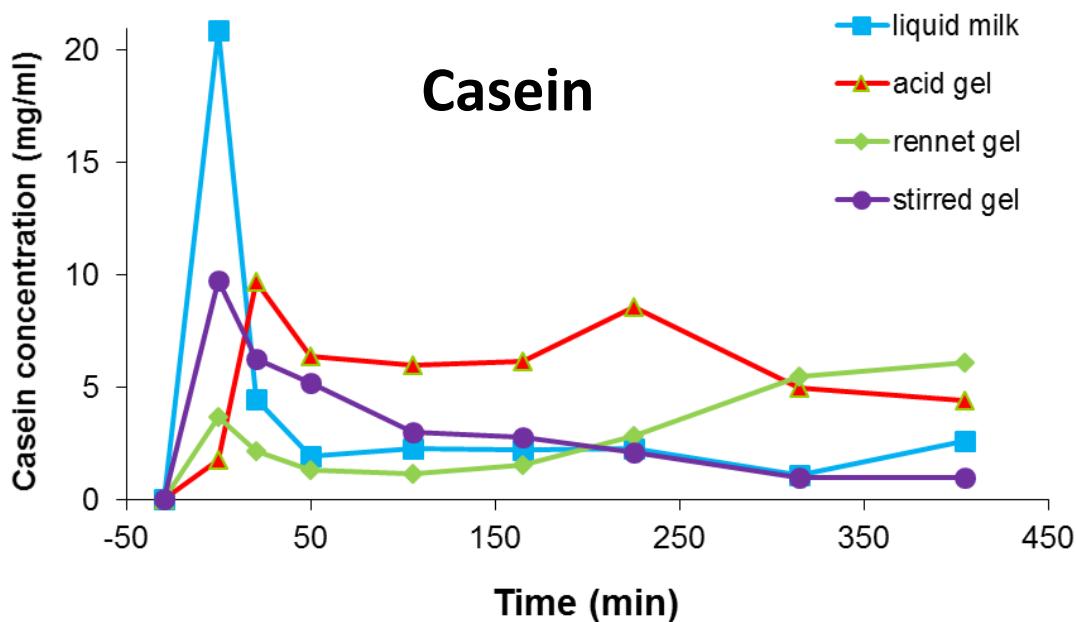
Fat-free matrices: 40 g/L caseins, 10 g/L whey proteins,
95 g/L lactose and minerals

+ marker of the meal transit (Cr^{2+} -EDTA) → Mean Retention Time in the stomach

Time of residence in the stomach

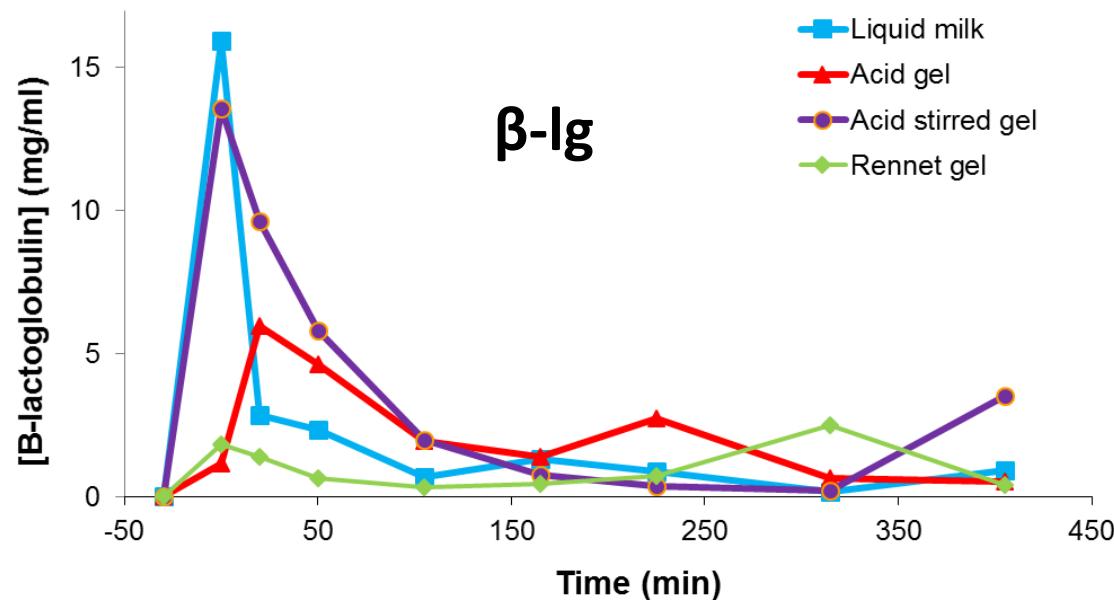


Milk proteins in the duodenum (ELISA)

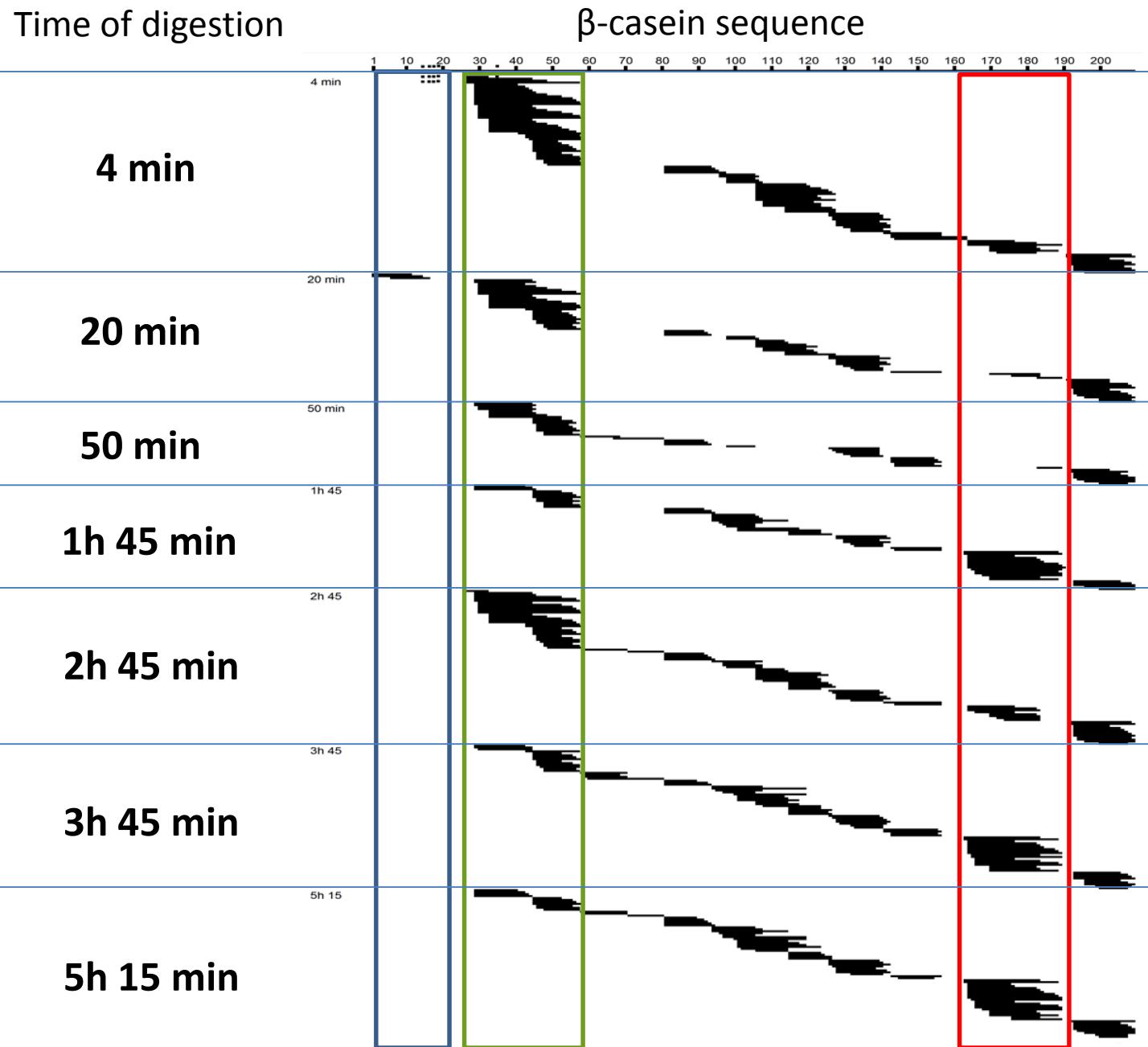


- Intense and early peak with milk
- Lower and delayed with gels
- Intermediate behaviour with stirred gel
- Low concentrations with rennet gel but casein release tends to increase over time

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



β -cn peptides released in the duodenum during digestion of acid gels



Bioactive peptides released during digestion differ from one matrix to another

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

Acid Gel

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

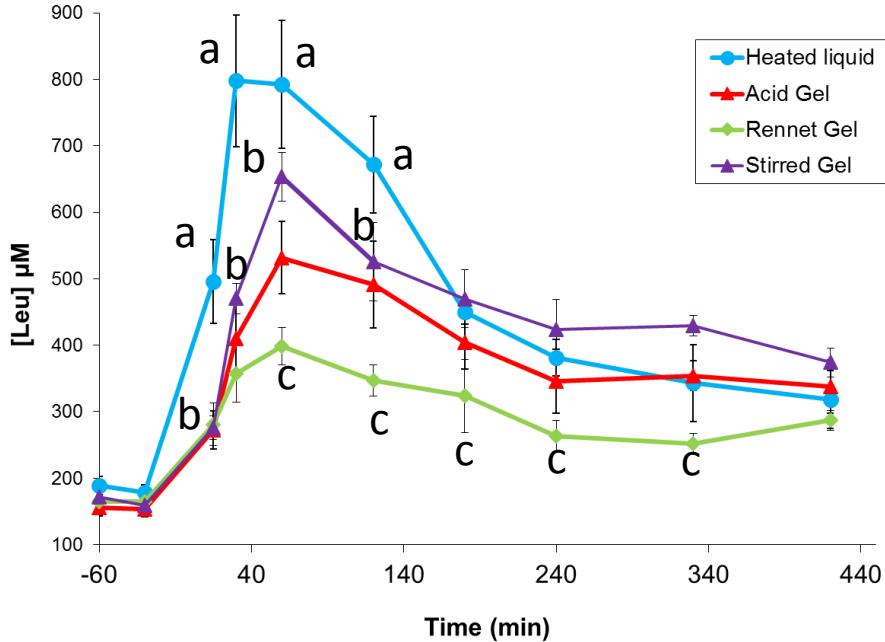
Rennet Gel

- 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

2) effect on absorption

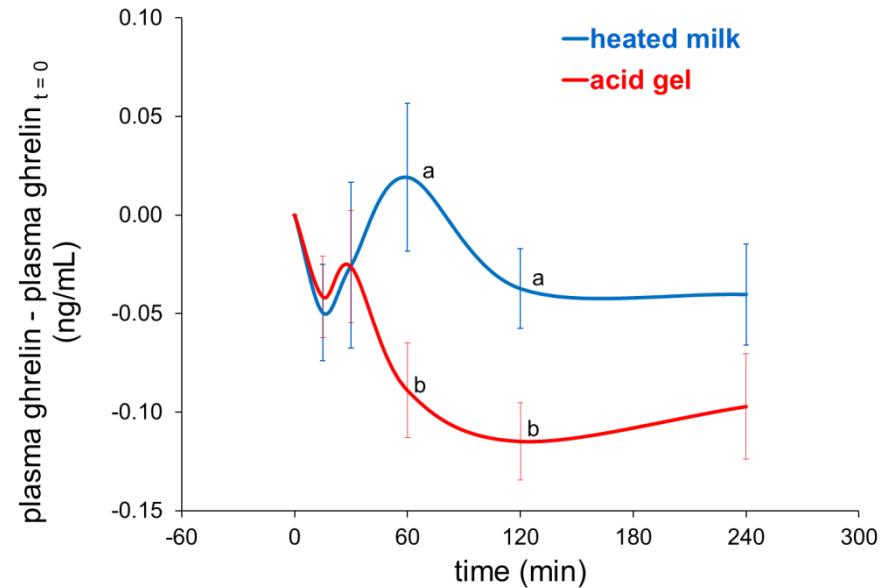


milk gelation:

→ delayed proteins transit → delayed AA absorption
→ maximal AA concentration in the plasma

3) potential effect on satiety

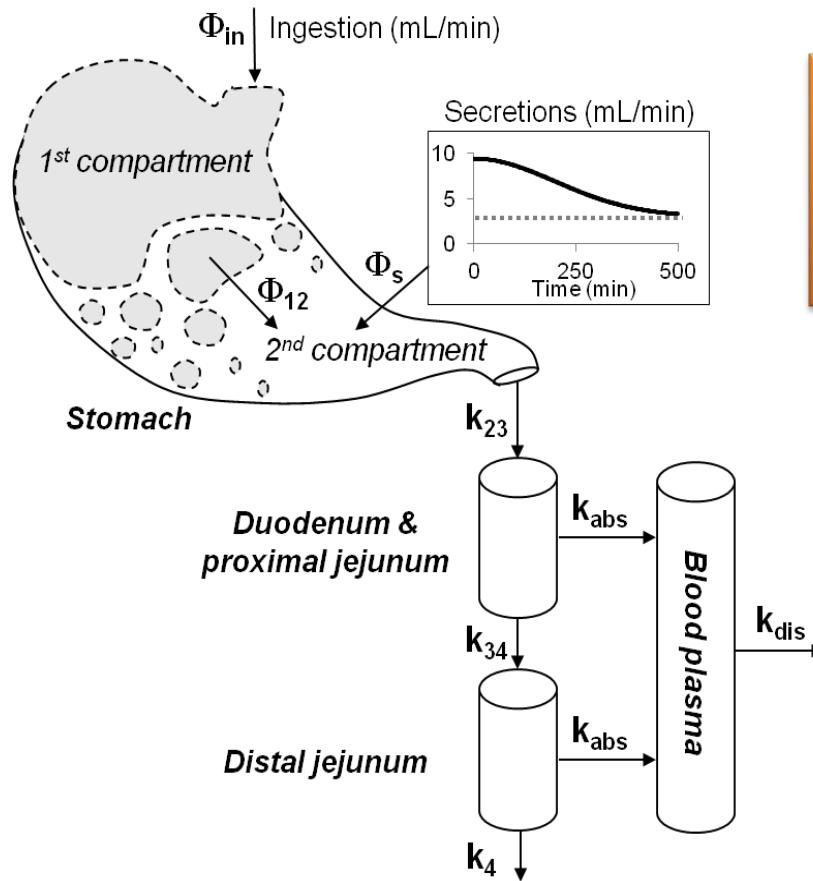
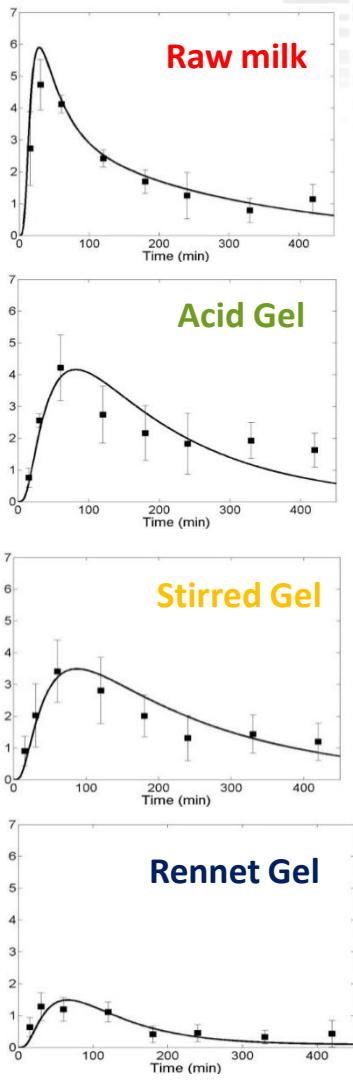
ghrelin (gastrointestinal hormone \rightarrow appetite stimulation)



milk gelation:

postprandial ghrelin concentration =
↑ satiety ?

In silico model of transit and absorption



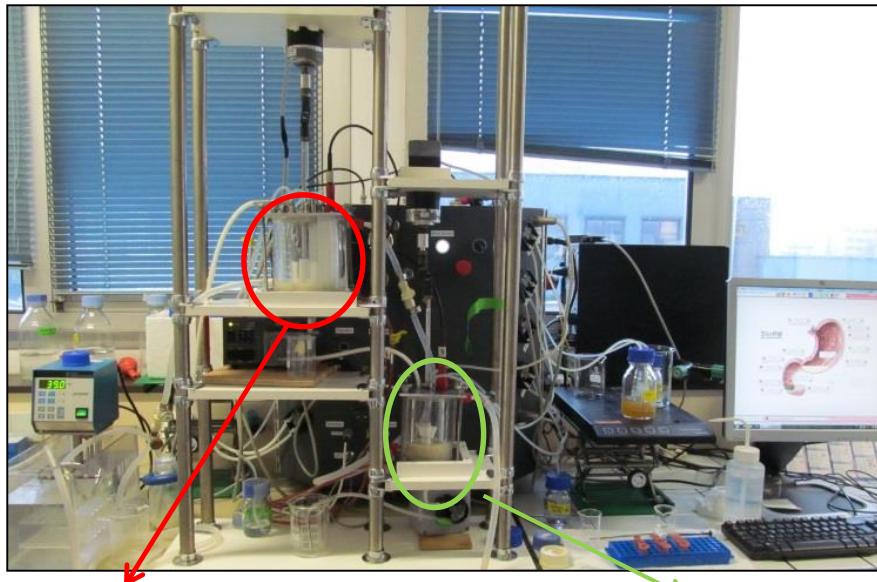
Le Feunteun
et al. Food
Bioprocess
Tech 2014

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

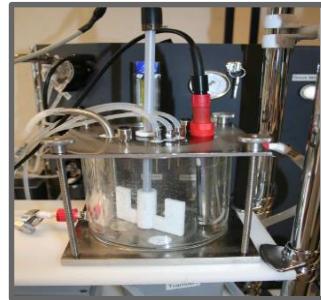
Ménard *et al.*
Food Chem 2014



DIDGI®

StoRM® software

- Pepsine
- Gastric lipase
- Simulated gastric fluid
- HCl



Stomach

Emptying :
Elashoff's model

Small intestine



- 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

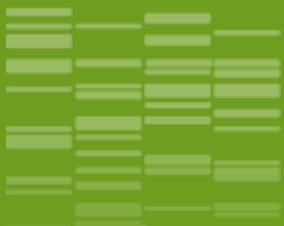


Rennet Gel



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



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

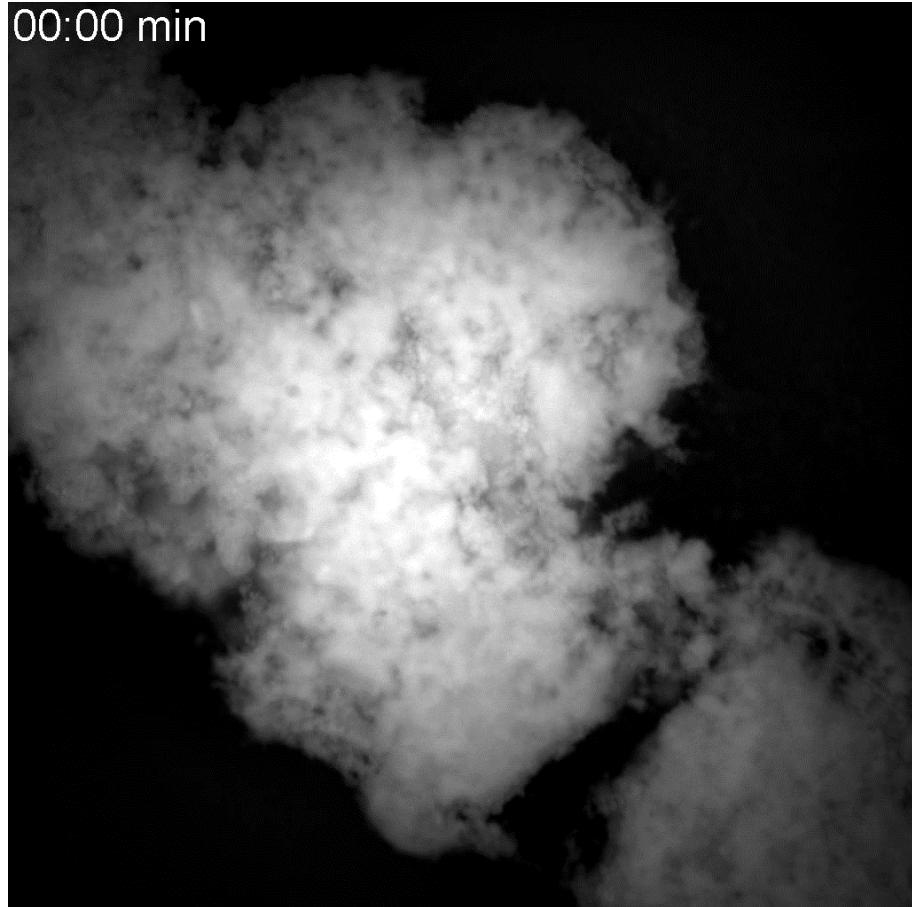
- Allow the imaging of protein intrinsic fluorescence with a UV microscope



SOLEIL is the French national synchrotron facility, a multi-disciplinary instrument and research laboratory.

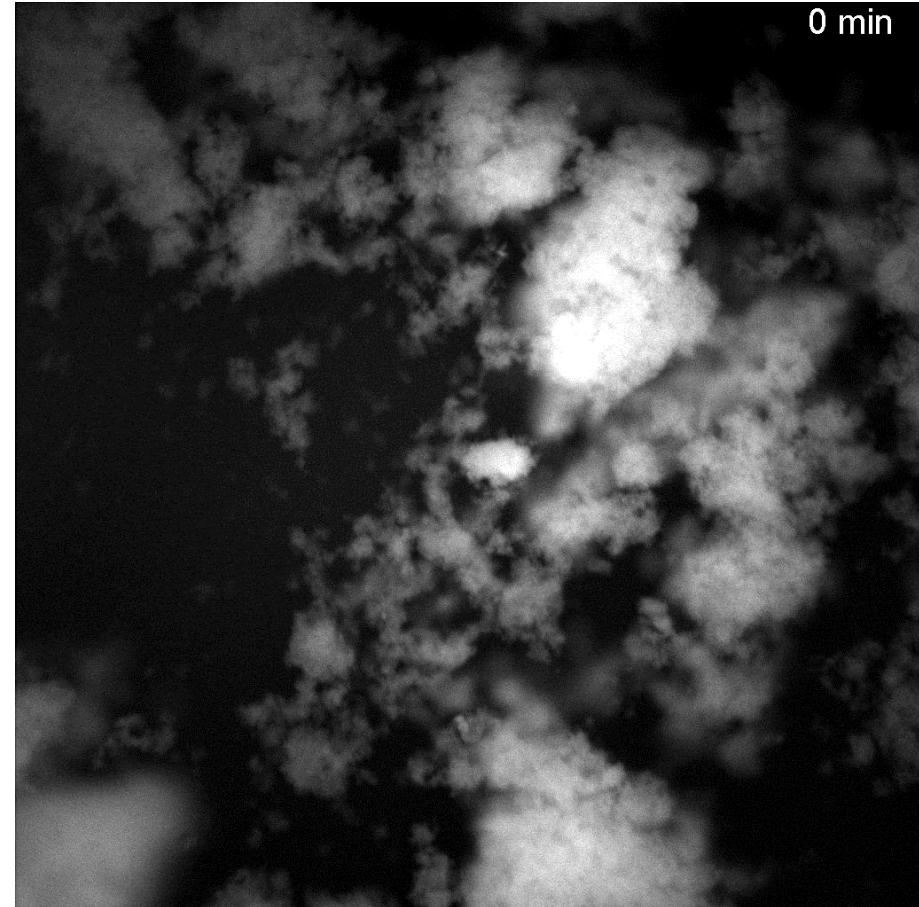
Kinetics of gel particles disintegration: comparison of rennet/acid gel

00:00 min



Rennet Gel

0 min



Acid Gel



Effect of processing on the digestion of milk macronutrients – The case of homogenization



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

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

2 model infant formulas standardized in fat and proteins

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

Effect of homogenization

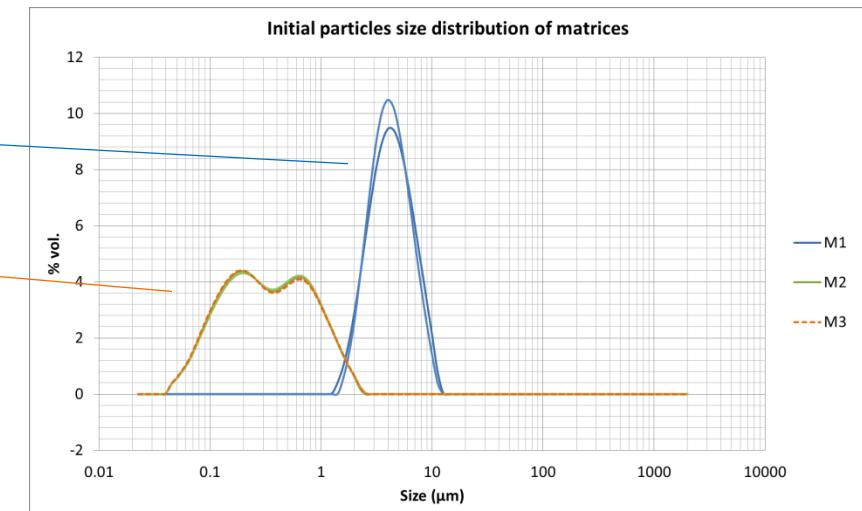
Raw formula
(M1)

Homogenized formula
(M2)

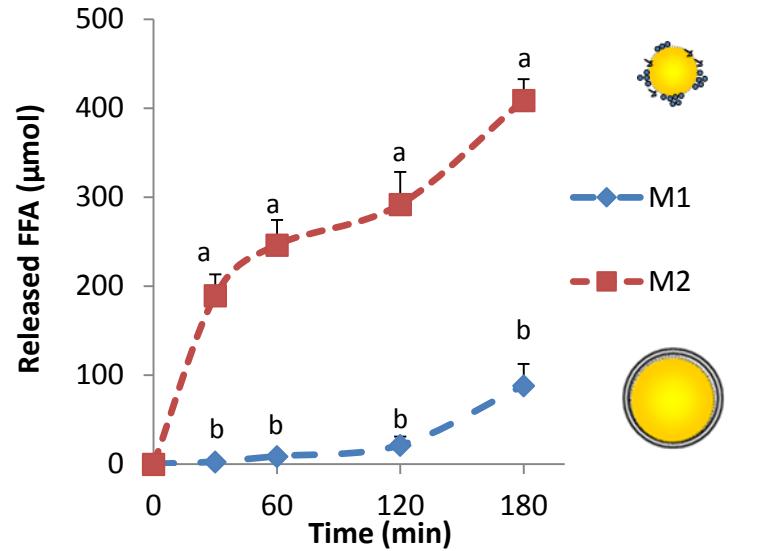
Raw (M1)



Homogenized (M2)



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



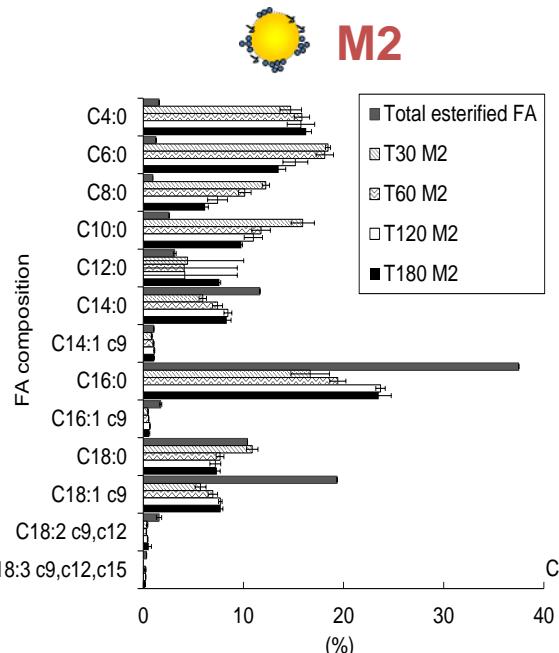
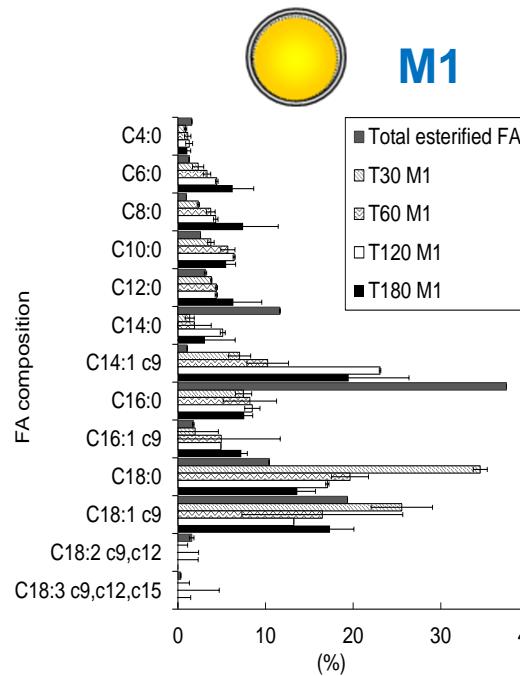
Interface

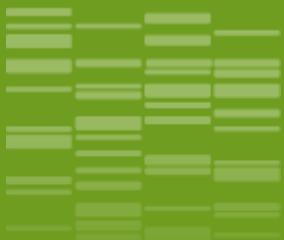
Surface spécifique [m²/g de lipide]

M1
1.81

M2
31.90

Bourlieu *et al.* Food Chem. 2015





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

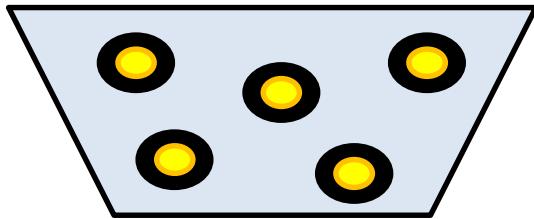
² INRA STLO Rennes, France

³ BIOFORTIS, Saint-Herblain, France

⁴ LACTALIS, Retiers, France

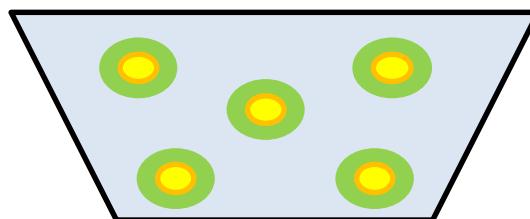
Infant formulas: can we create lipid structures biomimetic on the native fat globule?

Formula T1



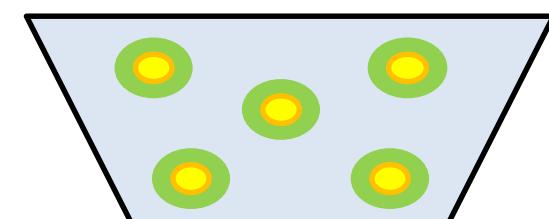
Interface 100 % Proteins
100% vegetable oil

Formula T2

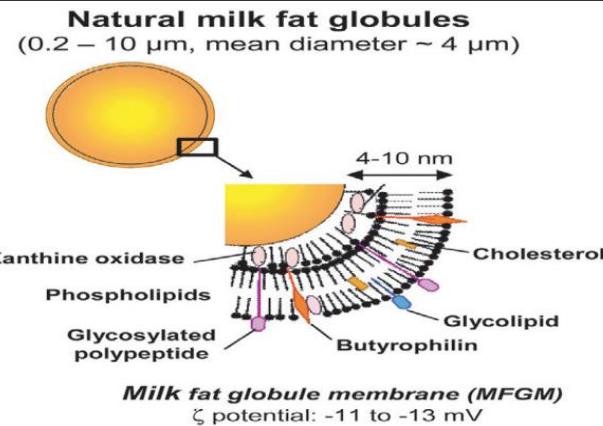


Interface 100 % phospholipids
100% vegetable oil

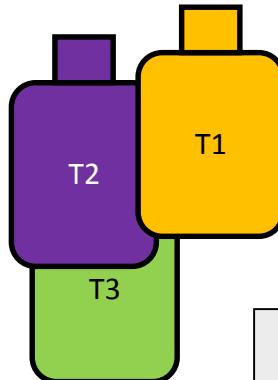
Formula T3



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)

+
Mother-fed piglets
(MF = + control)



Slaughtering
after

7 days

28 days

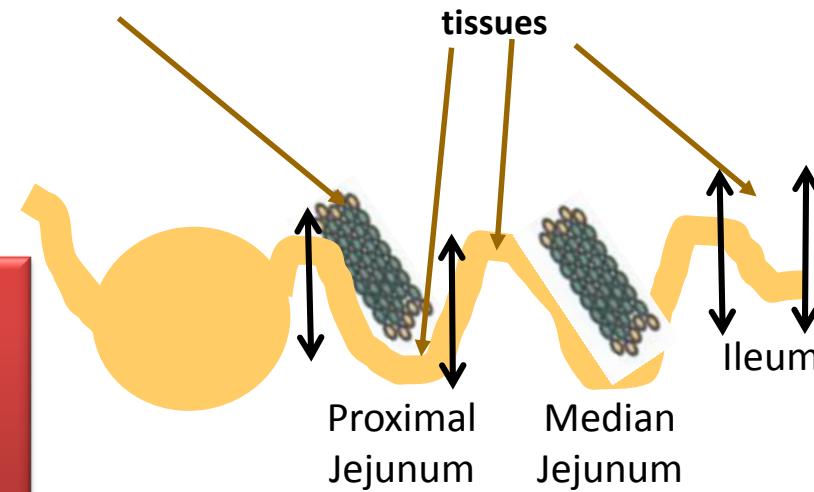
(90 min postprandial)

Mesenteric Lymph Nodes (MLN)

Collect of effluents and
tissues

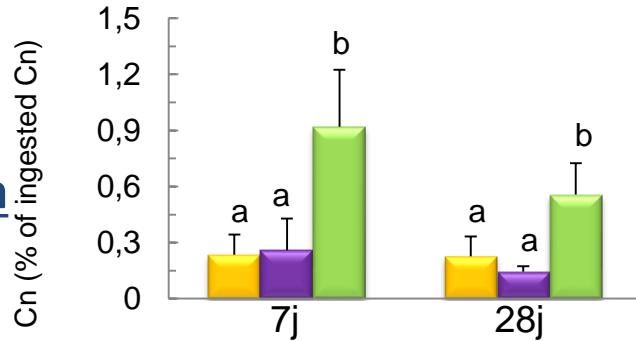
Effluents:
-SDS-PAGE
-Elisa

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

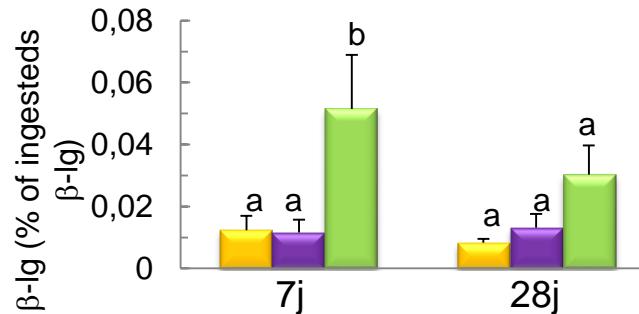
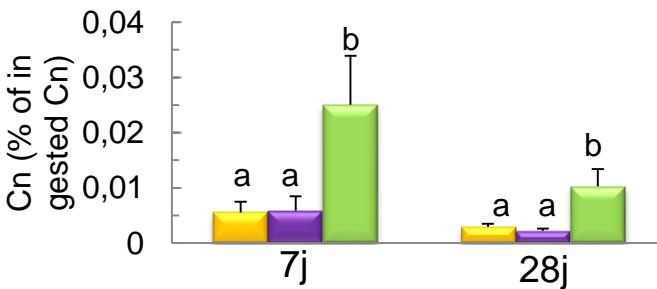
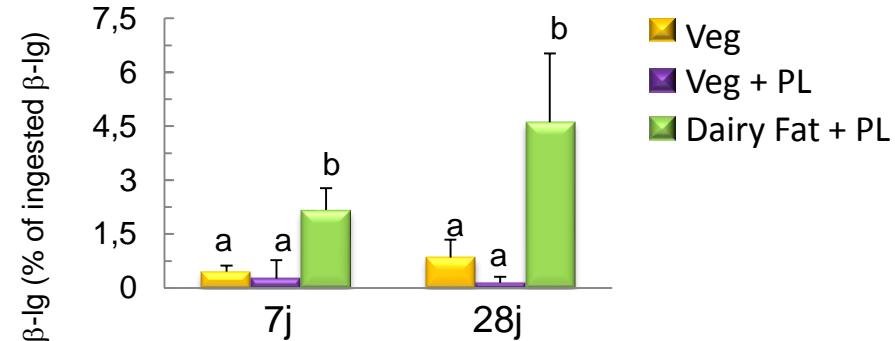


Protein Digestion

Casein



β -lactoglobulin



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

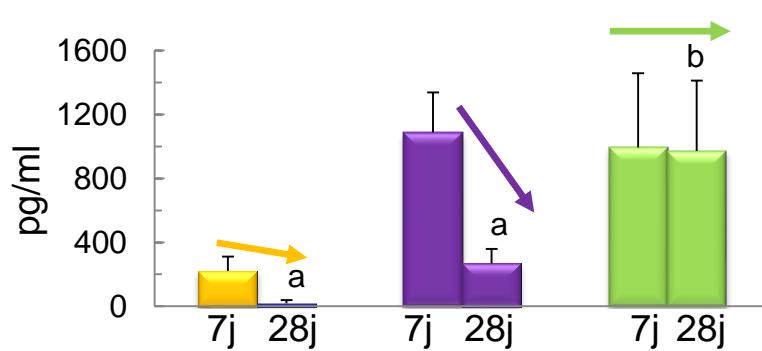
→ Modification of the interface

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

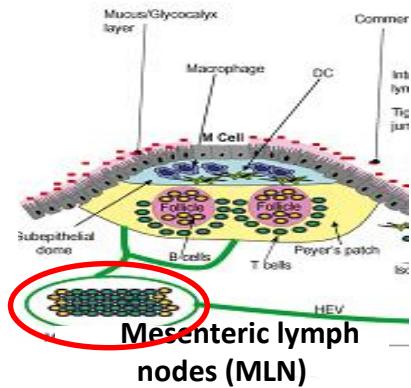
Secretory activity of MLN



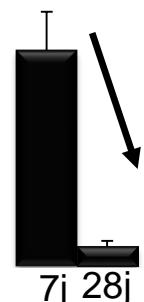
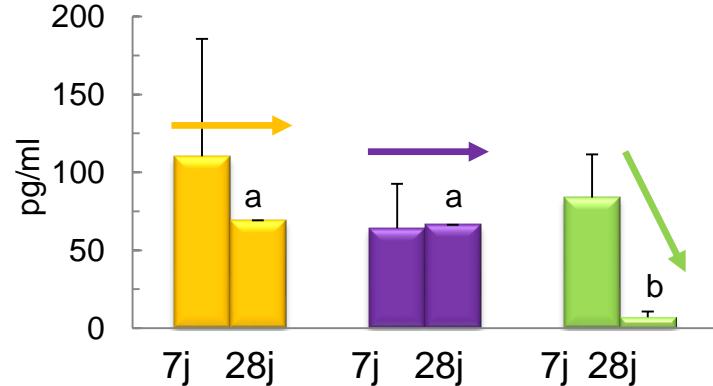
Interferon- γ (Th1 pro-inflammatory)



- Veg
- Veg + PL
- Dairy Fat + PL
- Porcelets SM

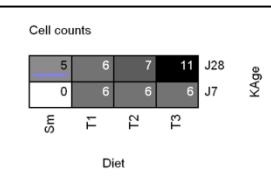


Interleukine-10 (Th2 anti-inflammatory)

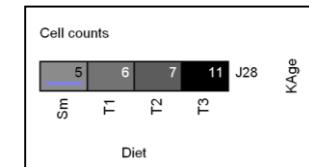
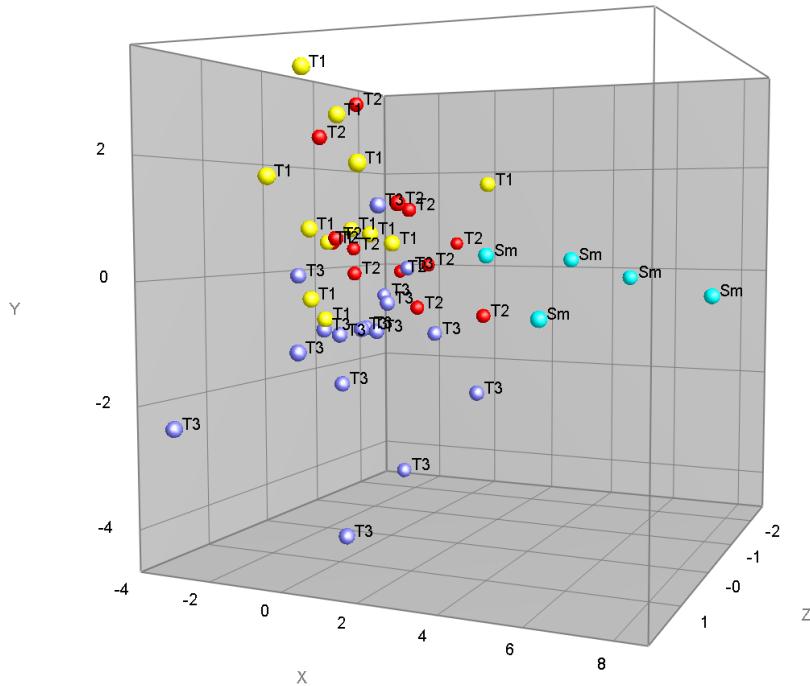


Milk lipids → maturation of the piglet's immune system more similar than with sow's milk

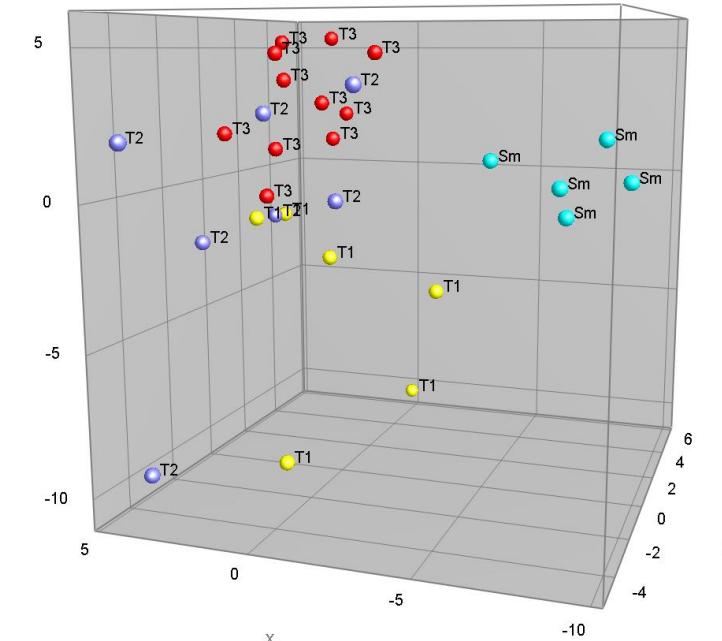
Microbiota by DHPLC



D7 & D28

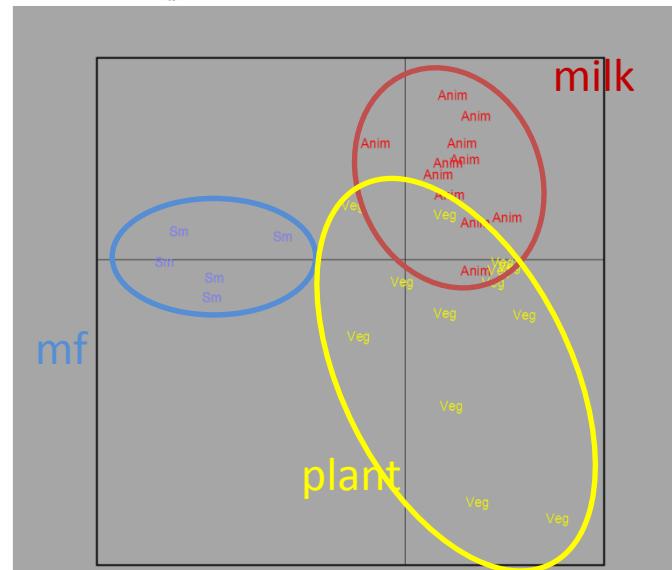


D28



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

More Proteobacteria with milk fat /
More Firmicutes with plant oil



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



The Bioactivity & Nutrition team



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Rachel BOUTROU – Junior Scientist

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Catherine GUERIN - Lecturer

Joëlle LEONIL – Senior Scientist

Françoise NAU – Professor

Jonathan THEVENOT – Post-doc



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Linda LEROUX (2016-2019)

Manon HIOLLE (2016-2019)

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Gwenaële HENRY

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Julien JARDIN

Olivia MENARD

Jordane OSSEMOND

Masters students

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

COST Action FA1005

Dr. Didier DUPONT, Senior Scientist, INRA, France



June 2011 – May 2015



INFOGEST
International Conference
Rennes, France
April 2017

We are pleased to announce the next

5th International Conference on Food Digestion



in Rennes, France, 4-6 April 2017