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The structure of dairy products at different length scales drives the bioavailability of nutrients

Didier Dupont

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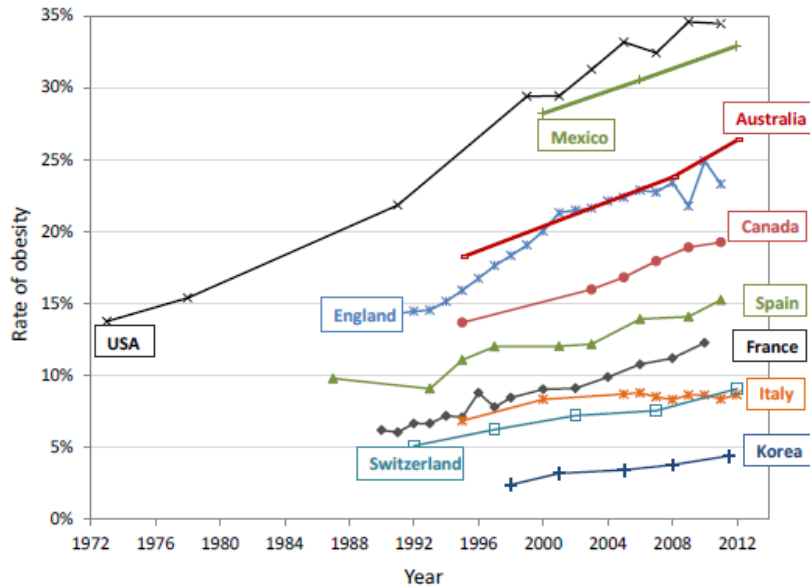
The structure of the food matrix at different length scales affects the mechanisms of digestion and the nutrient bioavailability



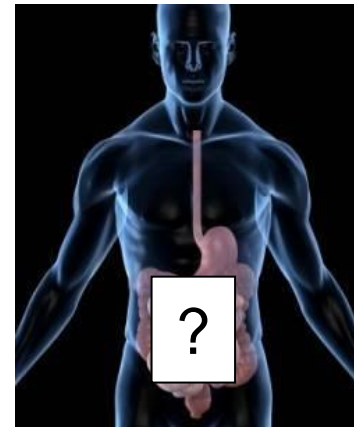
Dr Didier DUPONT, INRAE, STLO, Rennes, France



Food and human health: the key role of digestion



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



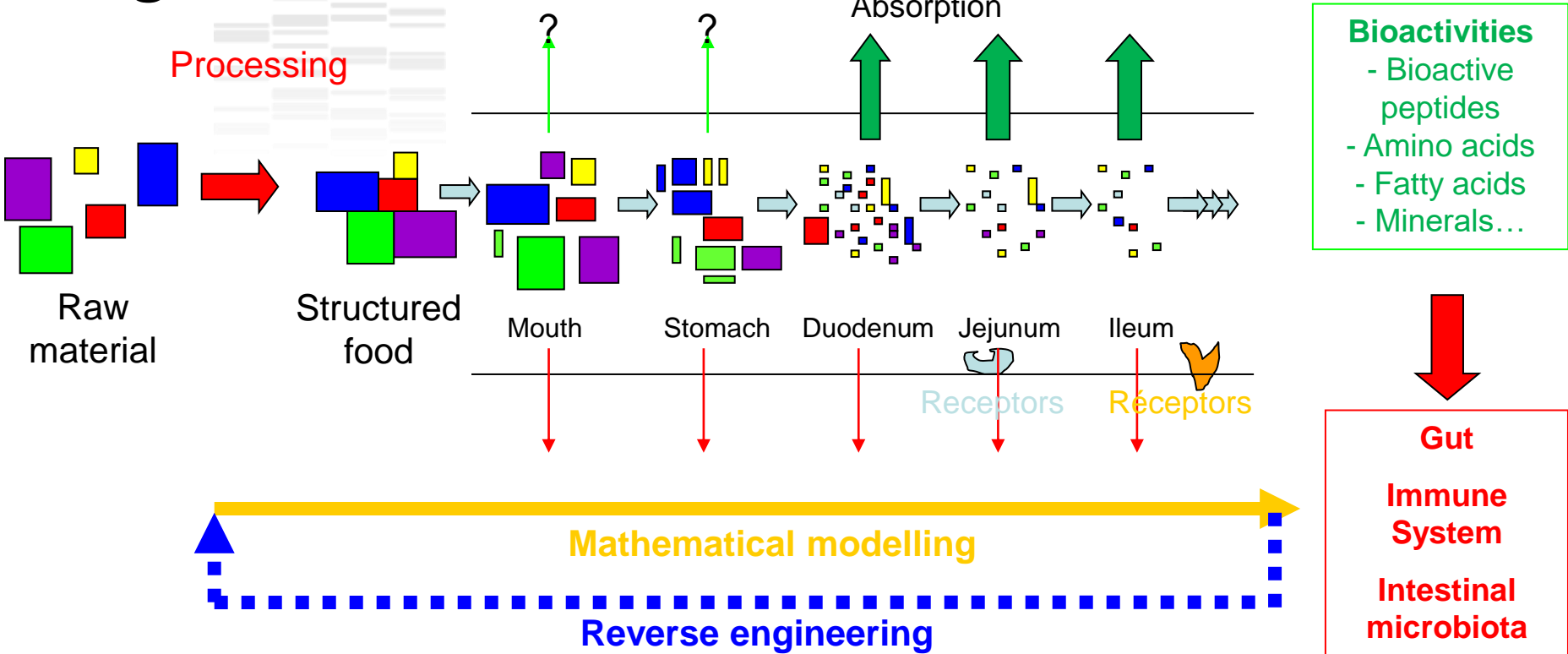
GI tract = interface between food and human body

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

... but the mechanisms of food disintegration in the gastrointestinal tract remain unclear and the digestive process has been considered as a black box so far

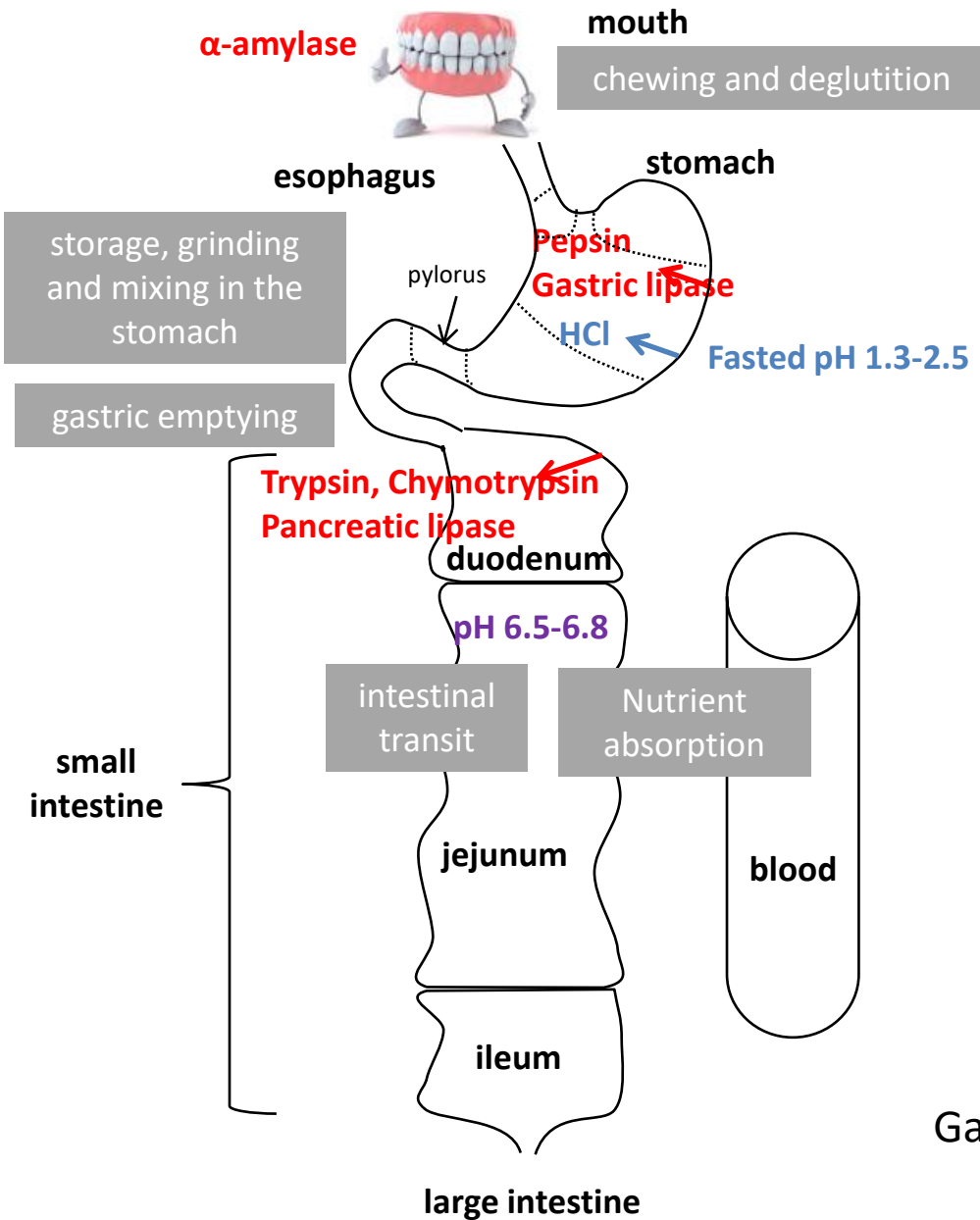
By increasing our knowledge on food digestion, we will increase our knowledge on the effect of food on human health

Our goals

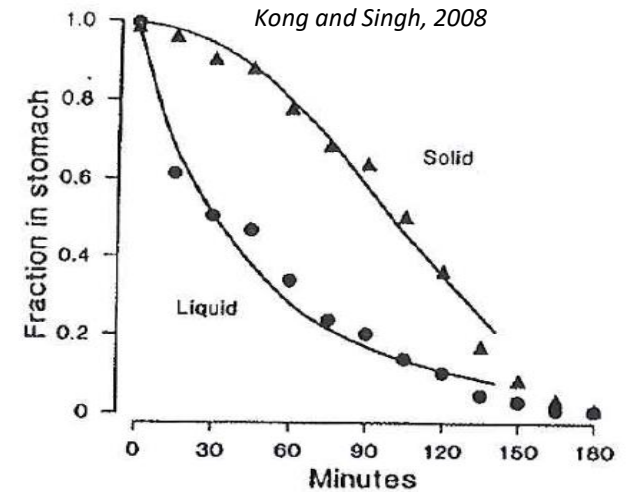


- ☞ To understand the mechanisms of breakdown of food matrices and their constituents in the gut and identify the beneficial/deleterious food components released during digestion
- ☞ To determine the impact of the structure of food matrices on nutrient bioavailability
- ☞ To model these phenomena in order to develop a reverse engineering approach

The digestive process



From Roger Lentle, Massey Univ. NZ



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

Models available at INRAE for simulating digestion

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

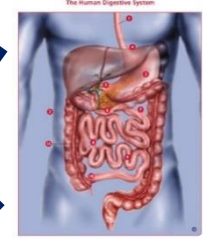
Menard et al. 2018, 2023
 Wang et al. 2022



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

Le Feunteun et al.
 2014, 2020

*In silico
 models*



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

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



*Human
 models*



Animal models



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



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

NERDT™ : the NEar Real Digestive Tract



Xiao Dong Pro-Health
Smart Digestion
Suzhou University



INRAE

L'INSTITUT
agro Rennes
Angers



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



Dupont D.

INRAE, Rennes, France



Comparison of 6 dairy products of identical composition but different structure

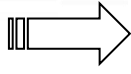


Macroscopic scale

Ultra Low Heat powder



rehydration in water 14.5%

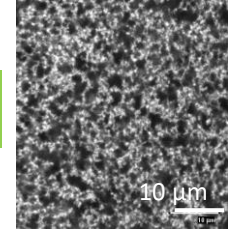


unheated milk ("raw" milk)

macrostructure

24h-20°C,
rennet 0.003 % v/w

rennet gel



pH 6.6



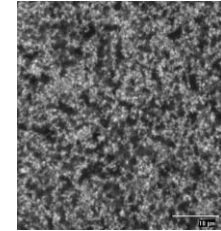
heat treatment
90°C-10 min

heated milk

microstructure

24h-20°C,
rennet 0.3 % v/w

rennet gel

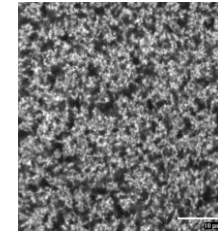


pH 6.6



24h-20°C,
GDL 3 % w/w

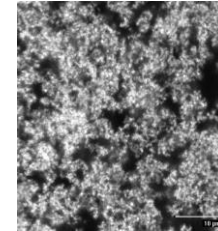
acid gel



pH 4

24h-20°C,
GDL 3 % w/w +
mixer 2 min

stirred acid gel



pH 4

Fat-free matrices:

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

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

The multi-cannulated mini-pigs

SOLID

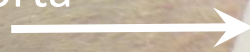
LIQUID



Macroscopic scale

6 minipigs (20 ± 1kg)

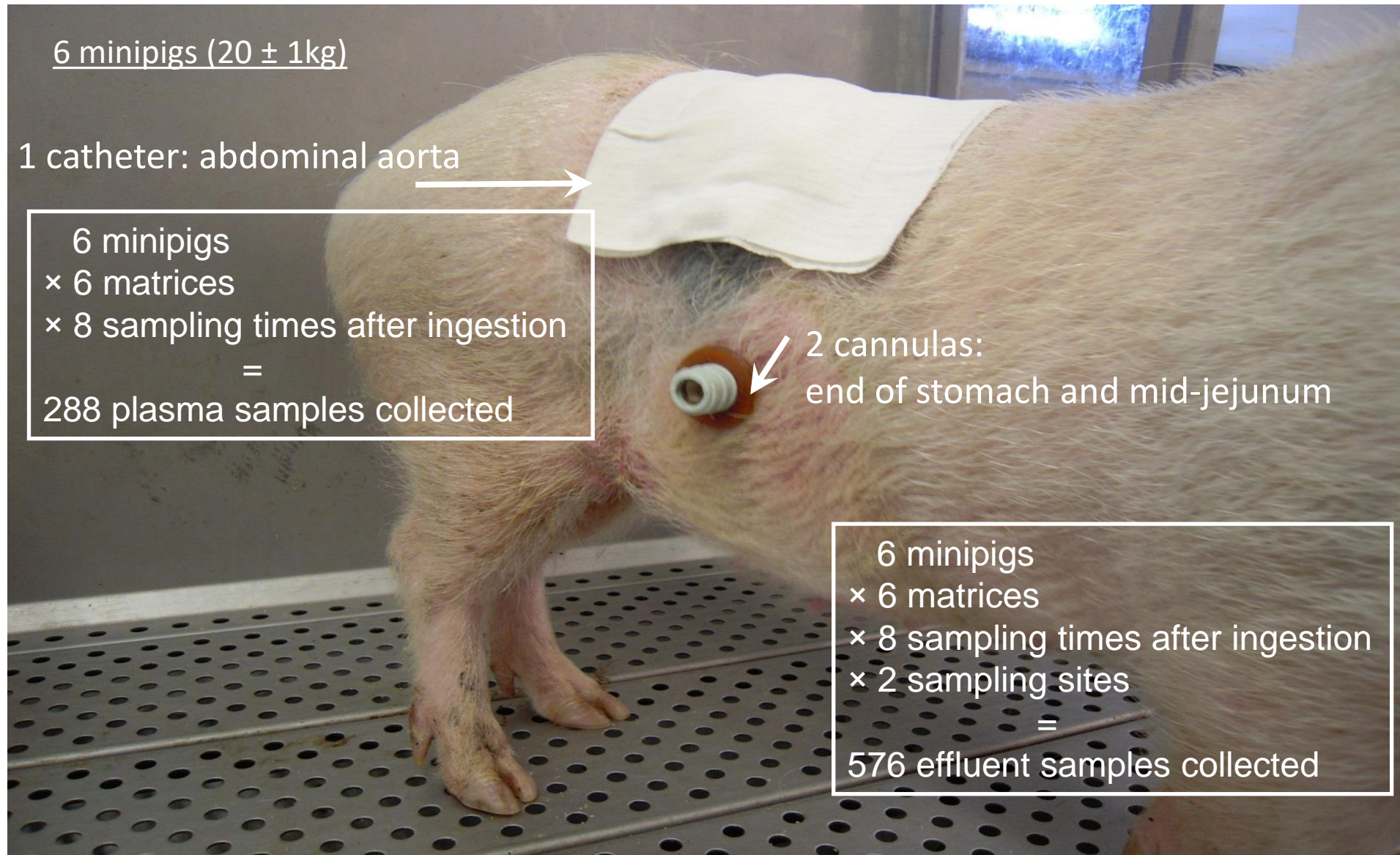
1 catheter: abdominal aorta



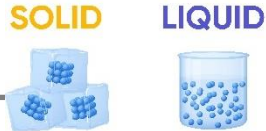
6 minipigs
× 6 matrices
× 8 sampling times after ingestion
=
288 plasma samples collected

2 cannulas:
end of stomach and mid-jejunum

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

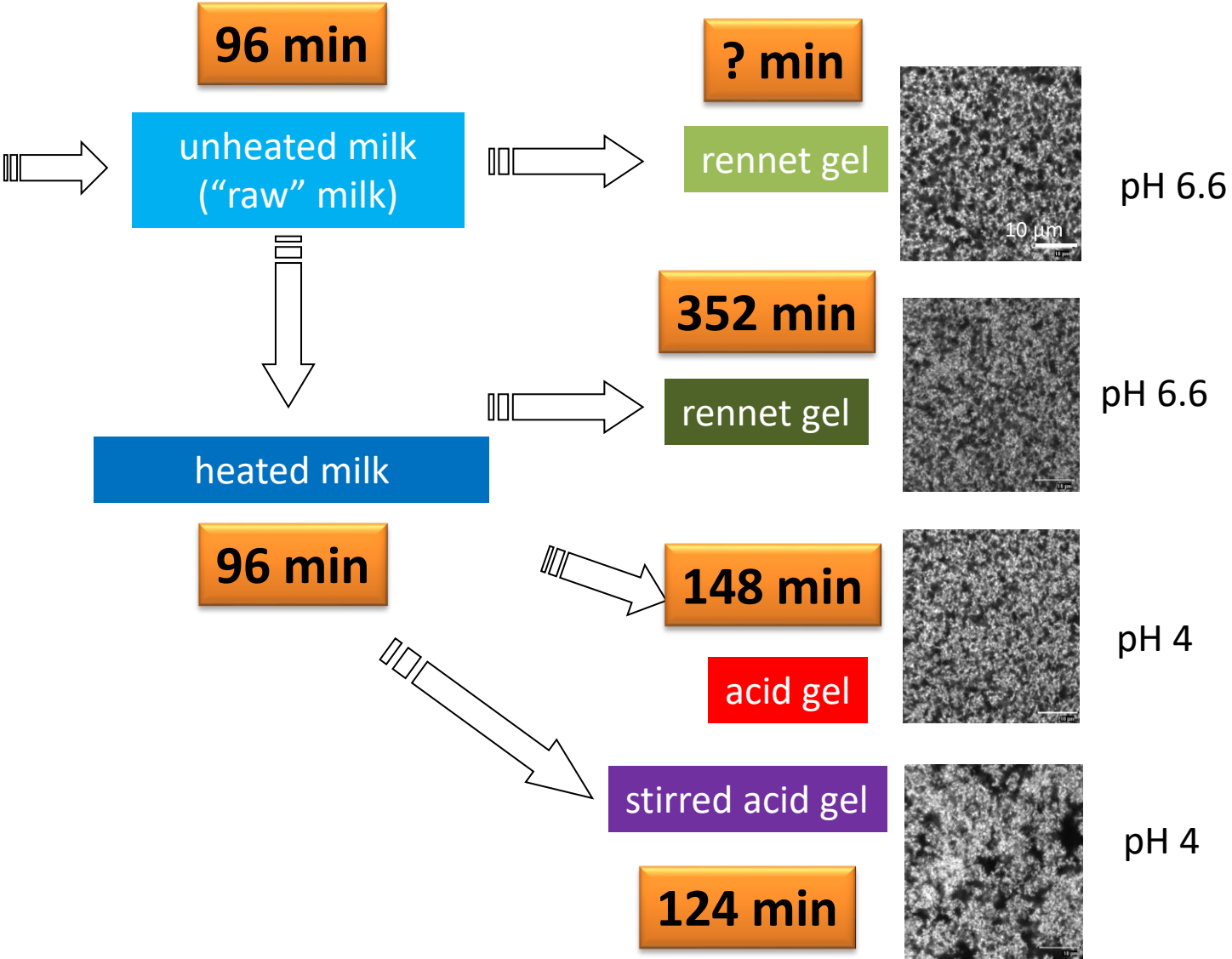


Gastric emptying half time

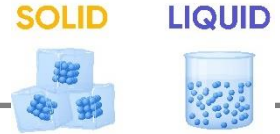


Macroscopic scale

Ultra Low Heat powder

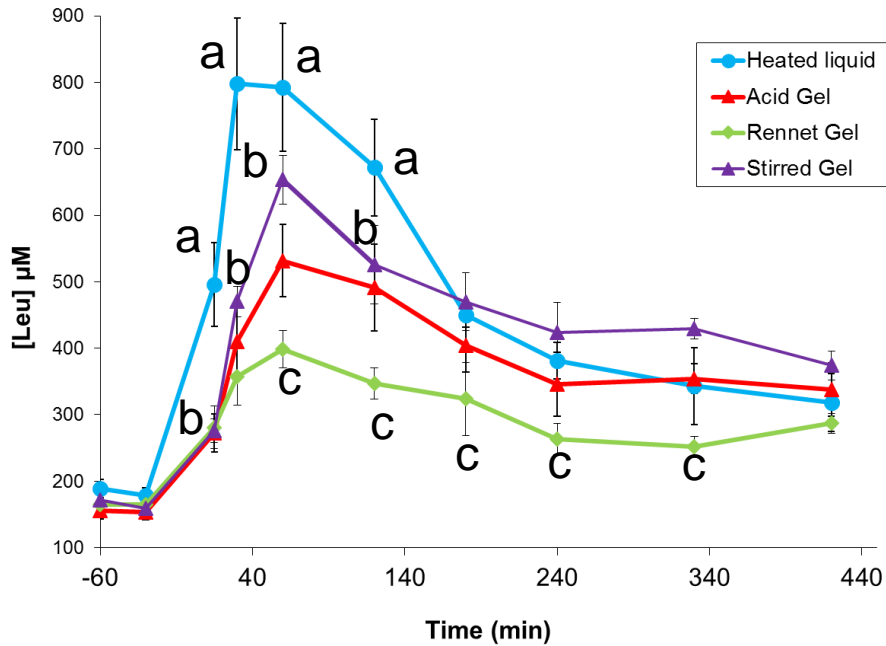


The liquid-gel transition



Macroscopic scale

Effect on absorption

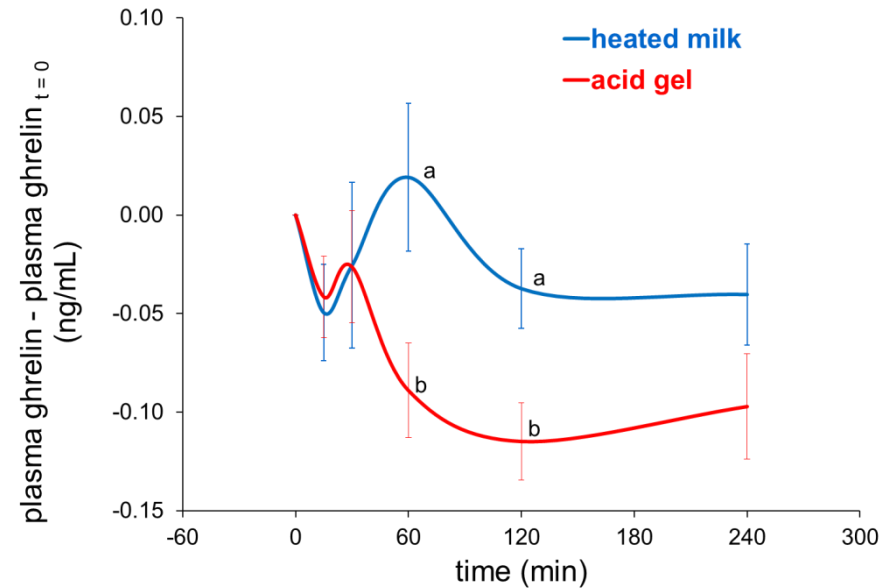


milk gelation:

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

Potential effect on satiety

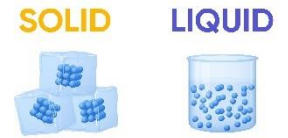
ghrelin (gastrointestinal hormone → appetite stimulation)



milk gelation:

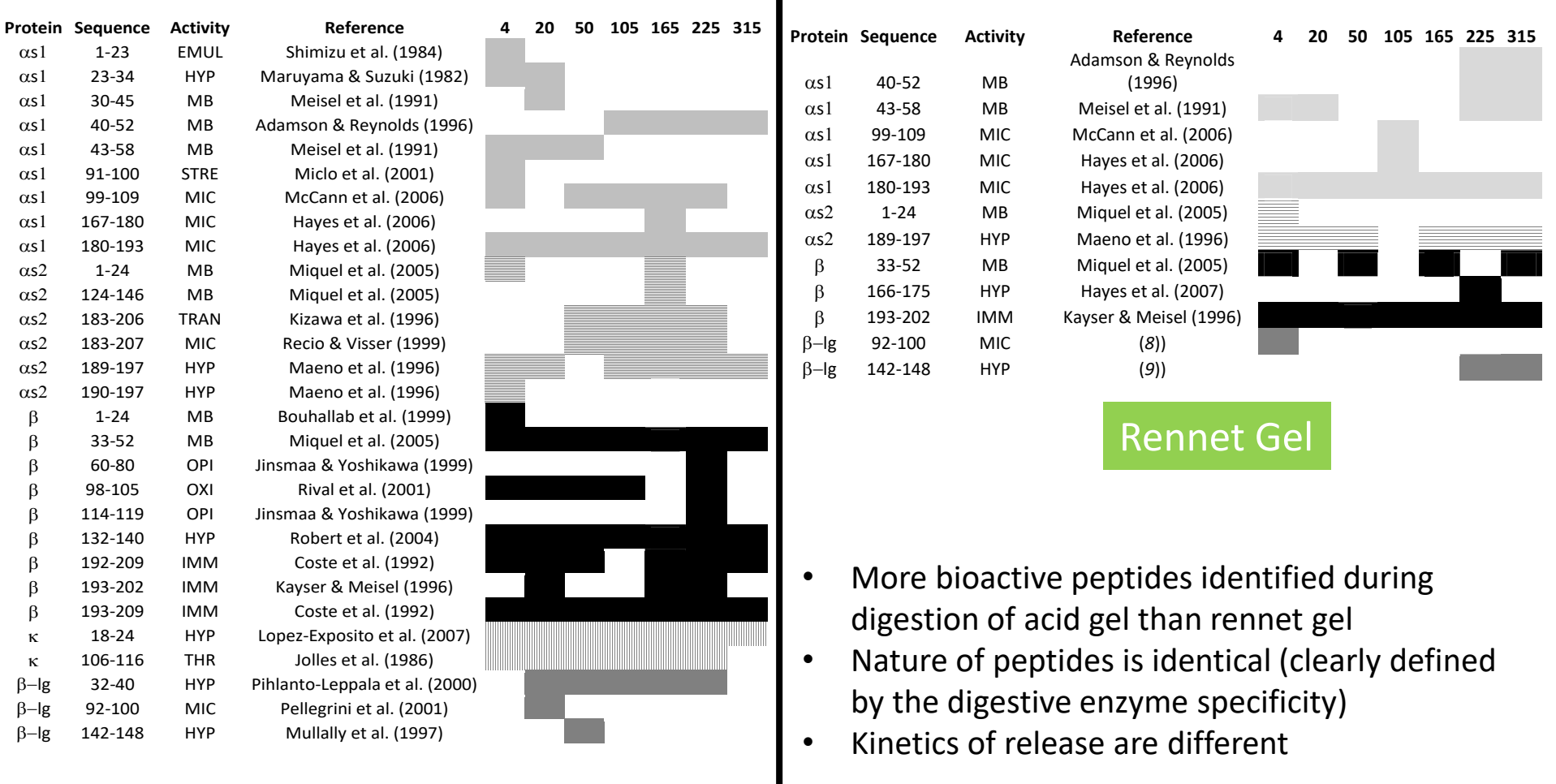
↘ postprandial ghrelin concentration =
↑ satiety ?

Bioactive peptides released during digestion differ from one matrix to another



Macroscopic scale

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



Acid Gel

Rennet Gel

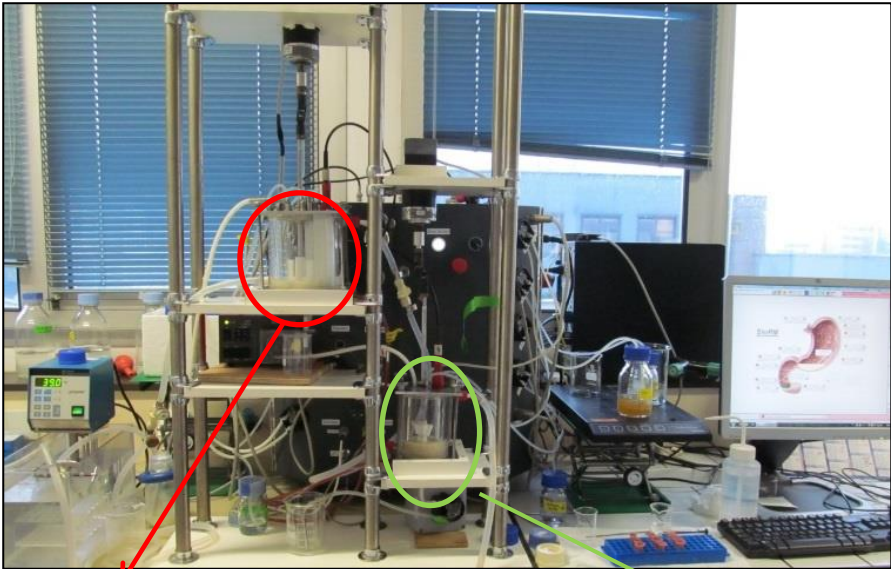
- More bioactive peptides identified during digestion of acid gel than rennet gel
- Nature of peptides is identical (clearly defined by the digestive enzyme specificity)
- Kinetics of release are different

Barbé et al. 2014
Food Res Int

Differential behaviour of acid/rennet gels in gastric conditions

- ☞ Acid/Rennet gel: identical composition, similar rheological properties and pore size
- ☞ ≠ Time of residence in the stomach (Acid 148 min /Rennet 352 min)
 - ☞ How can we explain this difference? Dynamic *in vitro* digestion of the 2 gels

**Ménard *et al.*
Food Chem 2014**



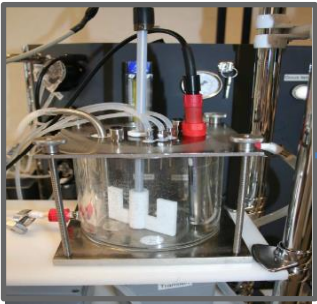
DIDGI®

StoRM® software

Stomach

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

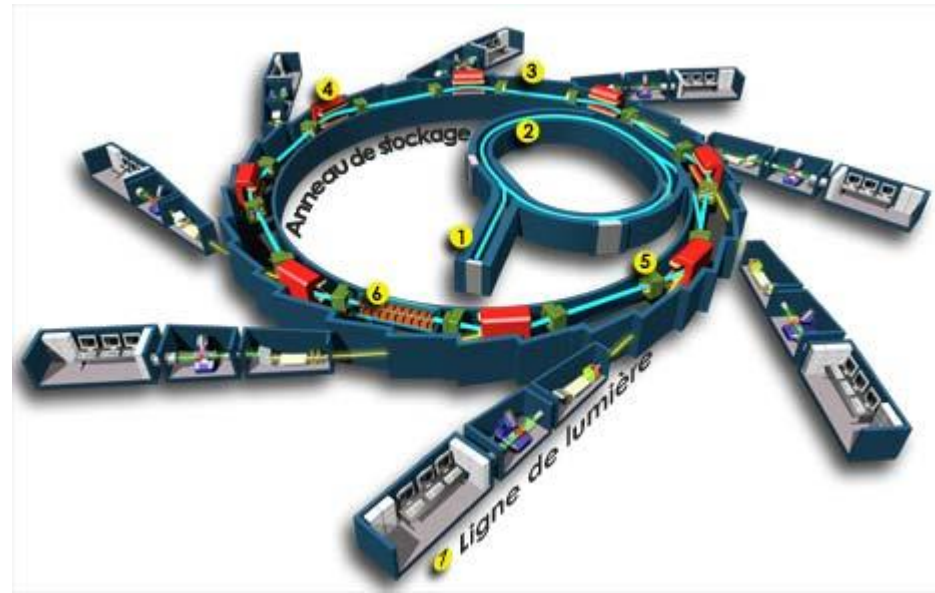


Soleil is a particle (electron) accelerator that produces the synchrotron radiation, an extremely powerful source of light that permits exploration of inert or living matter

DISCO

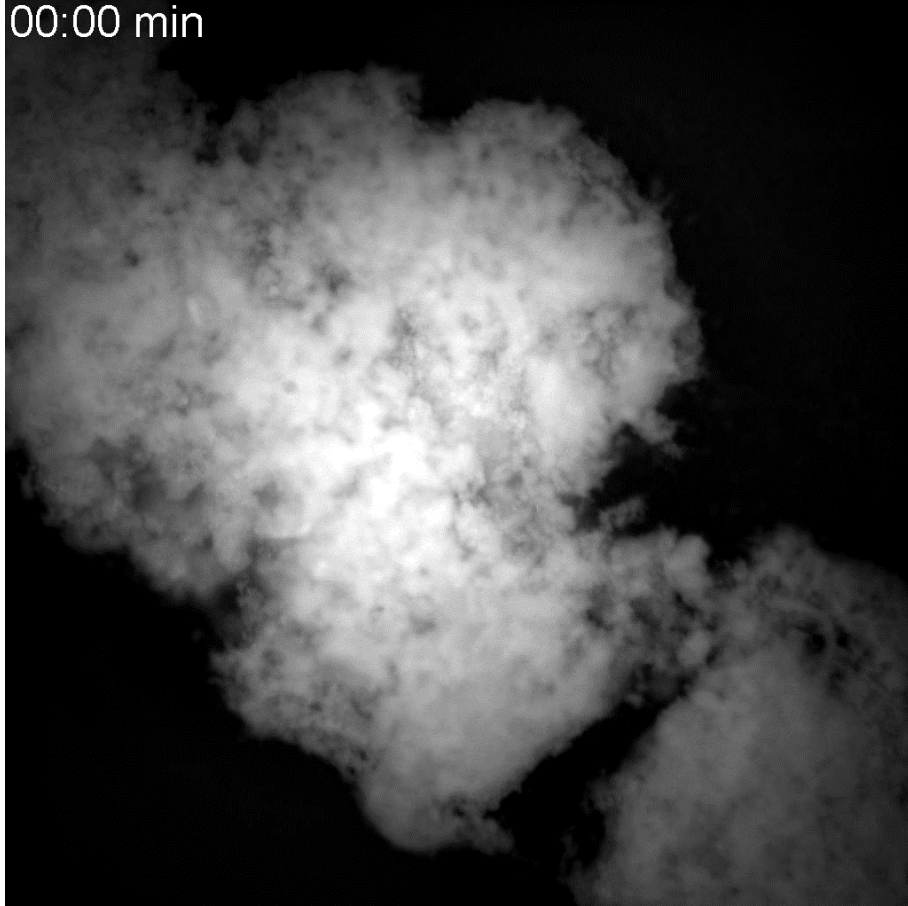
DISCO is a VUV to visible beamline dedicated to biochemistry, chemistry and cell biology. The spectral region is optimized between 60 and 700 nm with conservation of the natural polarization of the light

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



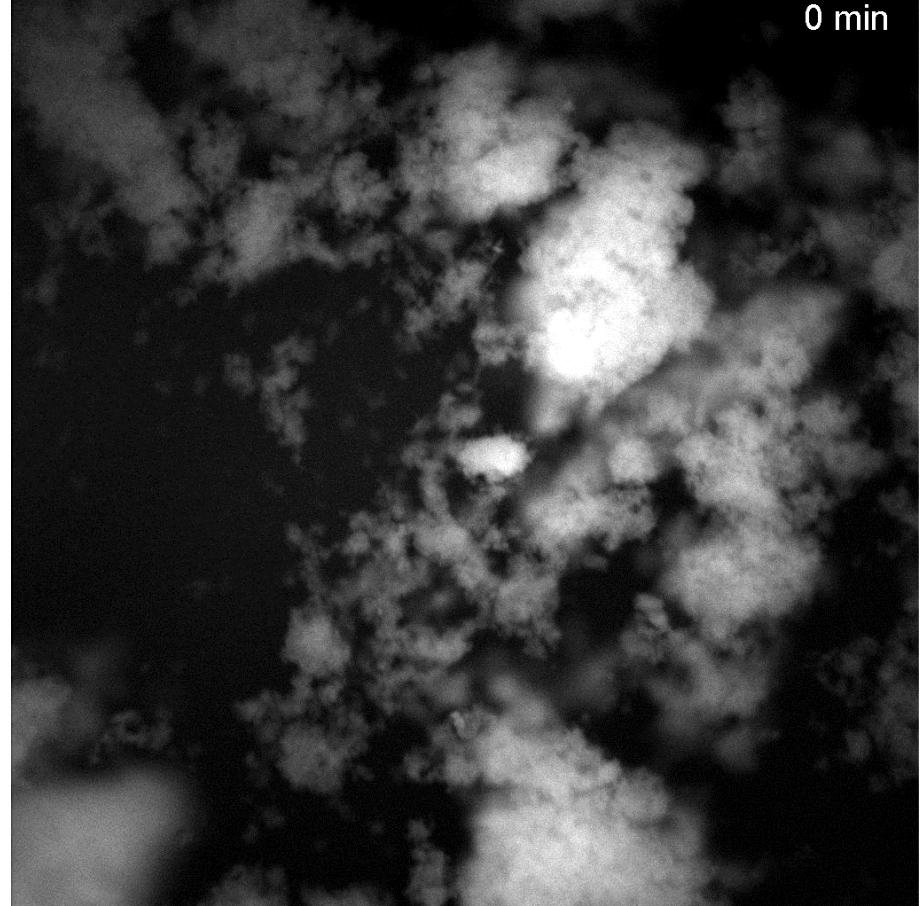
Kinetics of gel particles disintegration

00:00 min



Rennet Gel

0 min



Acid Gel

➤ Development of whey-based cheese for helping elderly people to fight against sarcopenia

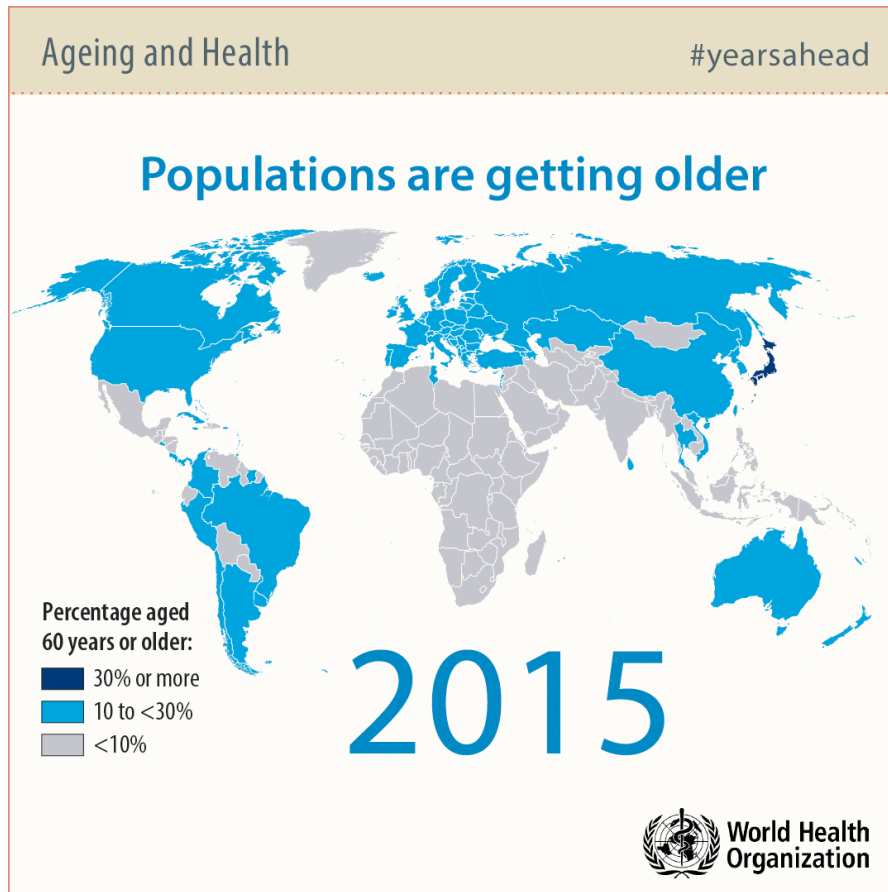
Anais Lavoisier, Olivia Menard, Stefano Nebbia,
Martine Morzel, Didier Dupont

Science & Technology of Milk & Eggs (STLO)
Rennes, FRANCE

AGEING = a world problem...



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



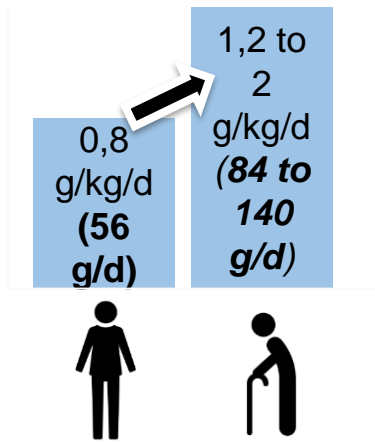
Challenge : healthy ageing

=

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

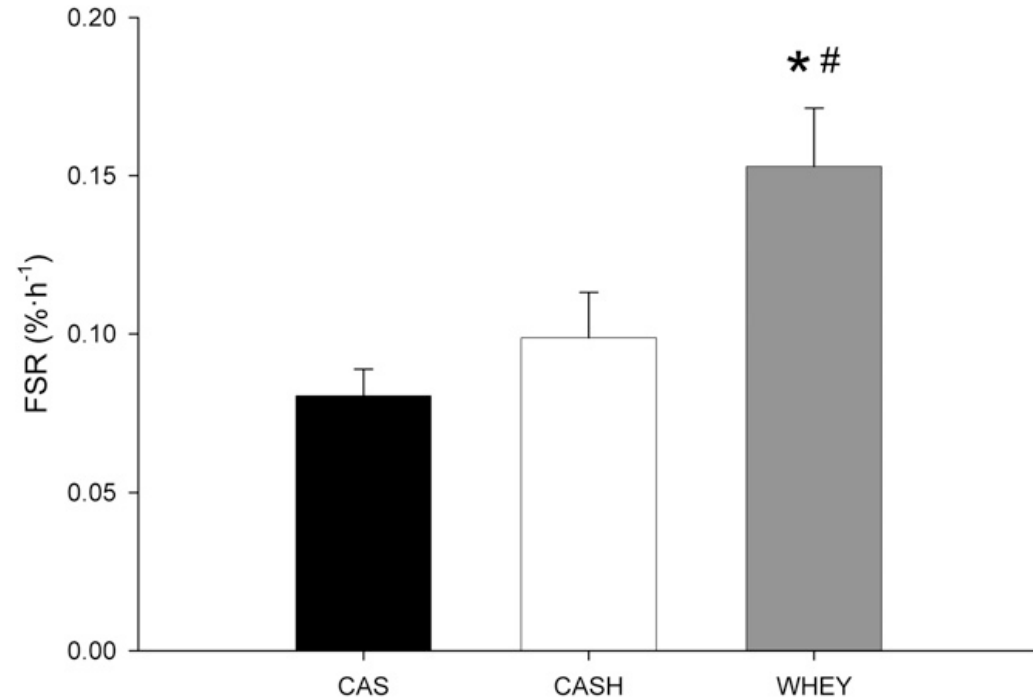
The nutritional needs of elderly people

Proteins



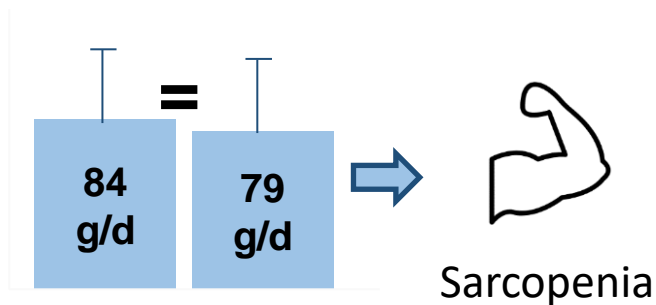
(Baum et al. 2017)

FSR= protein fractional synthesis rate



Pennings et al. 2013

Mean daily intake



(INCA 3, 2017)

Objectives of the EAT₄AGE project

Ageing leads to a decrease in the oro-gastrointestinal capacities (Rémond *et al.* 2015)

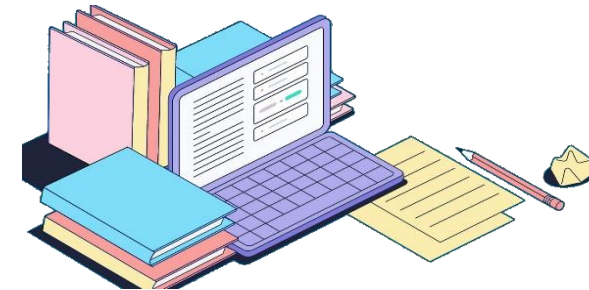
In vitro models simulating the altered conditions in the GI tract are needed to study food digestion in elderly

1. Exhaustive literature review

Oro-gastrointestinal tract physiological parameters

Healthy older adults ≤ 65 y.

In vivo data



2. Consensus static *in vitro* digestion model

Adaptation of the INFOGEST 2.0 digestion method

Brodkorb *et al.* *INFOGEST static in vitro simulation of gastrointestinal food digestion.* Nat Protoc 14, 991–1014 (2019)

EAT₄AGE consortium & International workshop

WP 3: *In vitro* digestibility and nutrient bioaccessibility of age-tailored food products

Overview of the *in vitro* digestion model for adults over 65

3-5 days
Preparations

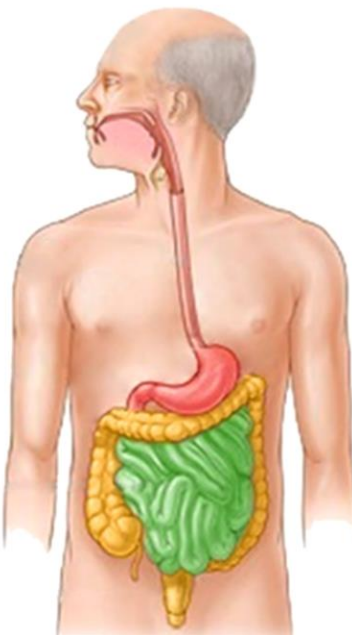
Prep-work

- Assay enzyme activity and bile salts concentration
- Prepare SSF, SGF and SIF, and CaCl₂ stock solution
- Perform pH adjustment pre-experiment

Parameters differing from the young adult model are in bold

1 Day digestion analysis

Elderly (>65y)



Oral

Dry food : SSF ratio (V/V)	1:1
Salivary amylase (U/mL)	75
Duration (min)	2
pH	7.0

Stomach

Oral bolus : SGF ratio (V/V)	1:1
Pepsin (U/mL)	1200
Gastric lipase (U/mL)	36
Duration (hour)	3
pH	3.7

Intestine

Gastric effluent : SIF ratio (V/V)	1:1
CaCl ₂ [mM]	1

Pancreatin & Bile OR Individual components

Pancreatin (U/mL)-	80
By trypsin	1600
By lipase	6.7
Bile salt [mM]	
Duration (hour)	2
pH	7.0

Trypsin (U/mL)	80
α-chymotrypsin(U/mL)	20
Pancreatic α-amylase(U/mL)	1600
Pancreatic lipase (U/mL)	1600
Sodium glycodeoxycholate [mM]	3.35
Taurocholic acid sodium salt hydrate[mM]	3.35

Sampling, inactivate enzymes and analyses



From the journal:
Food & Function

Menard et al. 2023, Food & Function, Highly Cited Paper

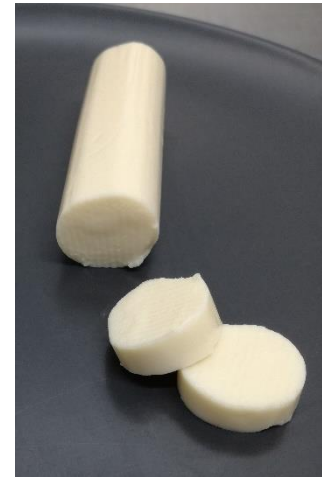
Cream cheese

Whey-based cheese

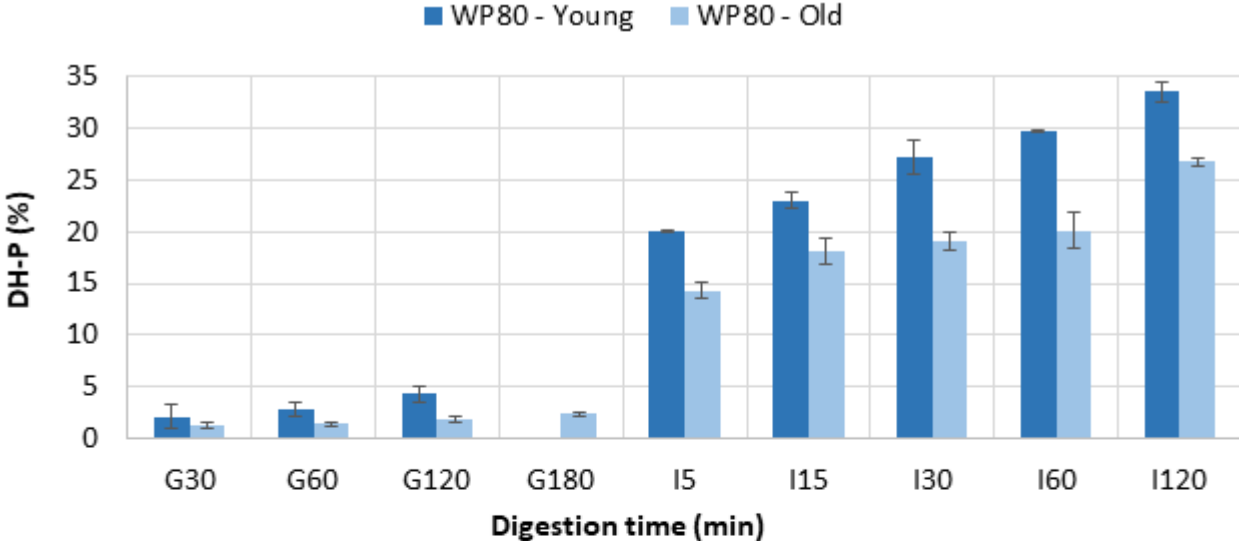
- Liquid emulsion: 24 % proteins, 20 % lipids, 0.6 % NaCl, 0.5% CaCO₃
- 80% whey proteins + 20% casein
- Heat treatment at 70°C for 35 min
- Emmental-cheese flavoured
- Texture: spreadable
- Corresponds to ≈ **0.93 g of leucine** /serving (= 30 g of cheese), ≈ 37 % of the recommendations /meal for the elderly

Control Casein-based cheese

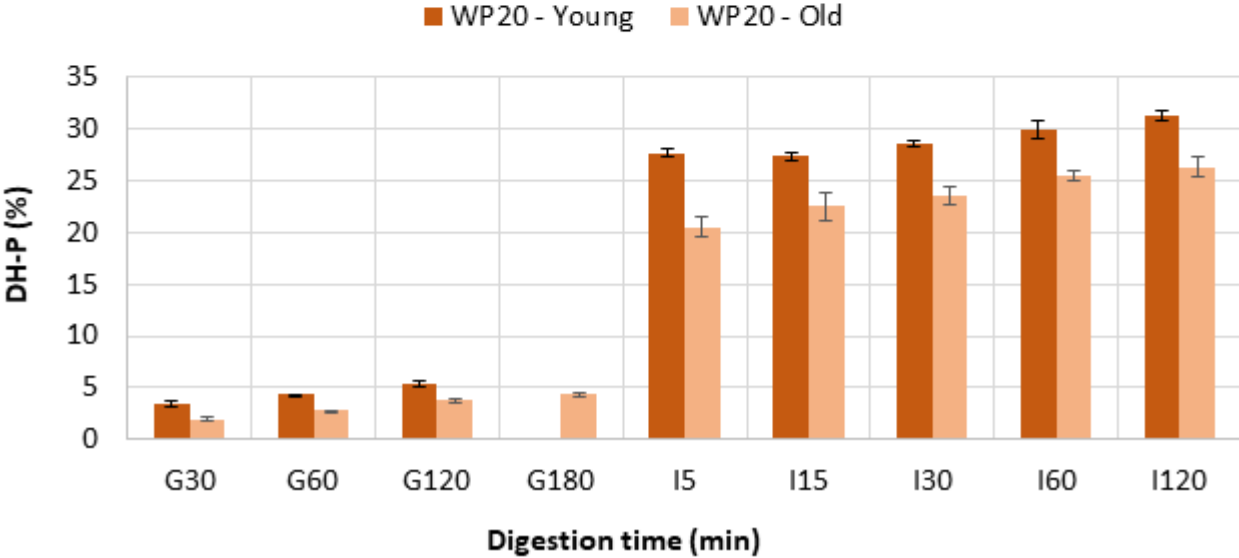
- Liquid emulsion: 24 % proteins, 20 % lipids, 0.6 % NaCl, emulsifying salts
- 80% casein + 20% whey proteins
- Emmental-cheese flavoured
- Texture: spreadable
- Corresponds to ≈ **0.70 g of leucine** /serving (= 30 g of cheese), ≈ 28 % of the recommendations /meal for the elderly



Ageing results in a slower digestion of cheese proteins



Lavoisier et al.
Food Res Int. 2014

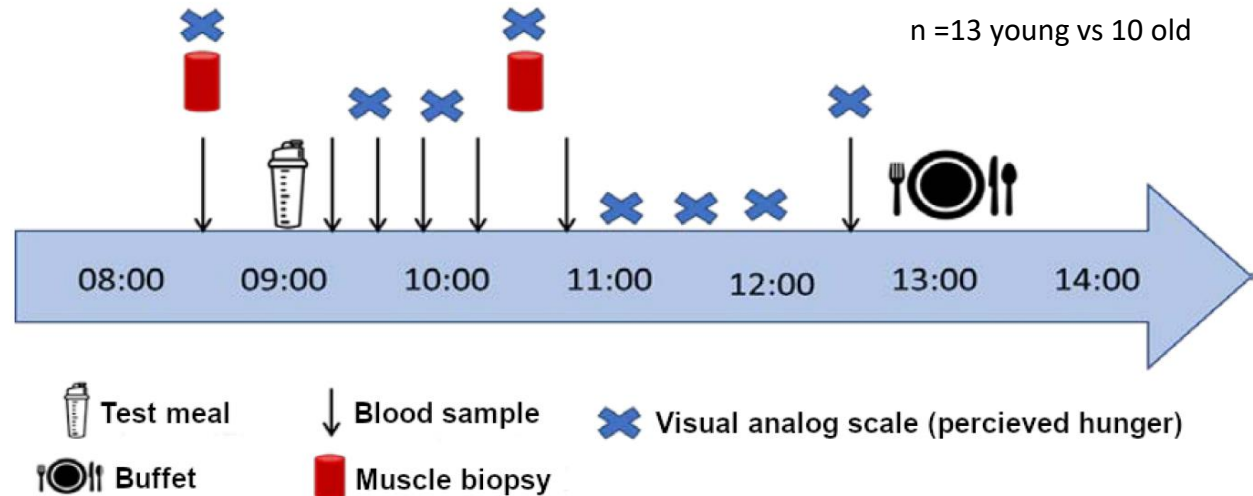


Clinical study to assess aminoacidemia and acute anabolic response in young and old adults after cheese consumption

- Control spreadable cheese: 80% casein + 20% whey proteins
- Modified spreadable cheese has switched the ratio of whey and casein (Whey 80%, casein 20%)
- Portion size: 45 g

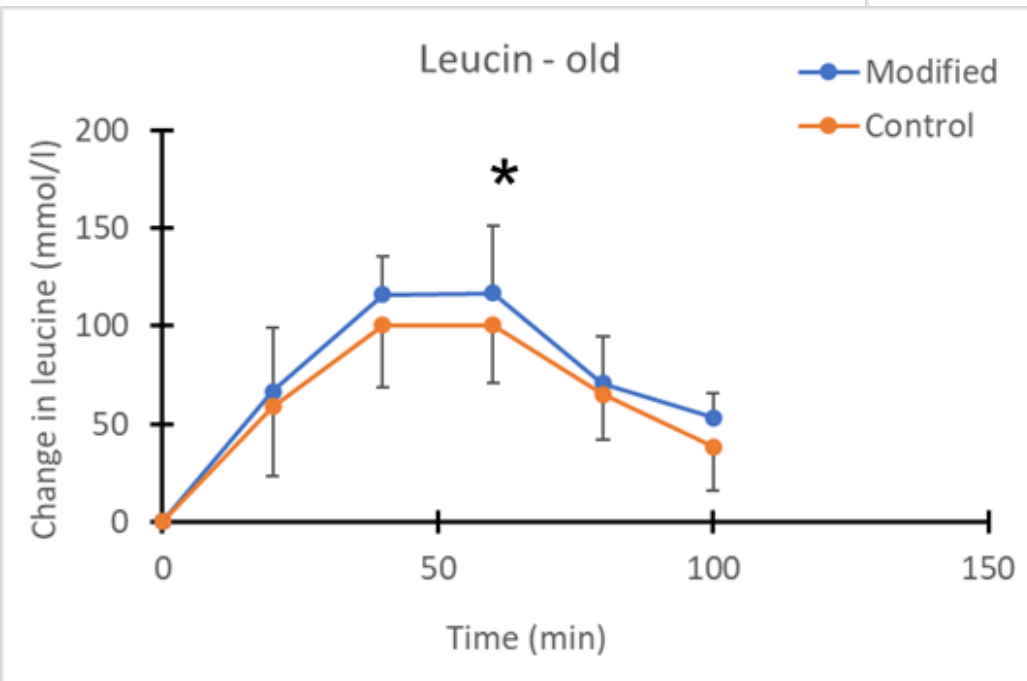
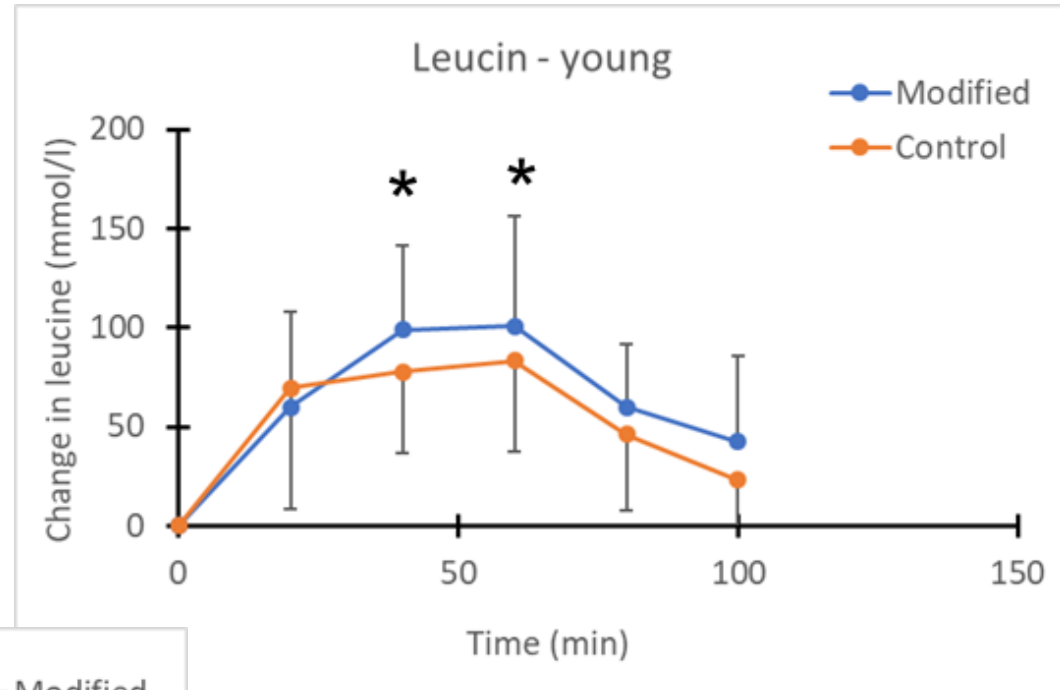
	Portion size	Protein	CHO	Fat	Energy	
Modified	45	10,8	2,7	9	135	kcal
Control	45	10,8	2,7	9	135	kcal

- Served on melba toast (23 g)



Whey-based vs. Casein-based cheese – Plasma [leucine]

By courtesy of
Pr Truls Raastad



* Limited effect on elderly people
* Data on the acute anabolic response released over the summer

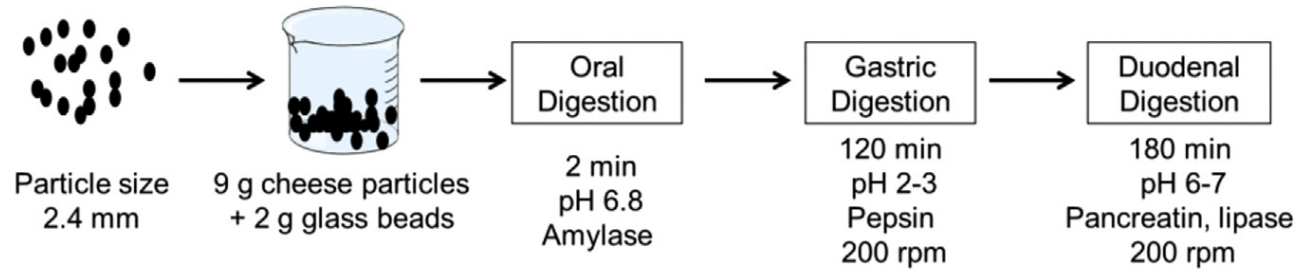
Does cheese texture affects its digestion?

Xixi Fang, Laurie-Eve Rioux, Steve Labrie, Sylvie L.
Turgeon

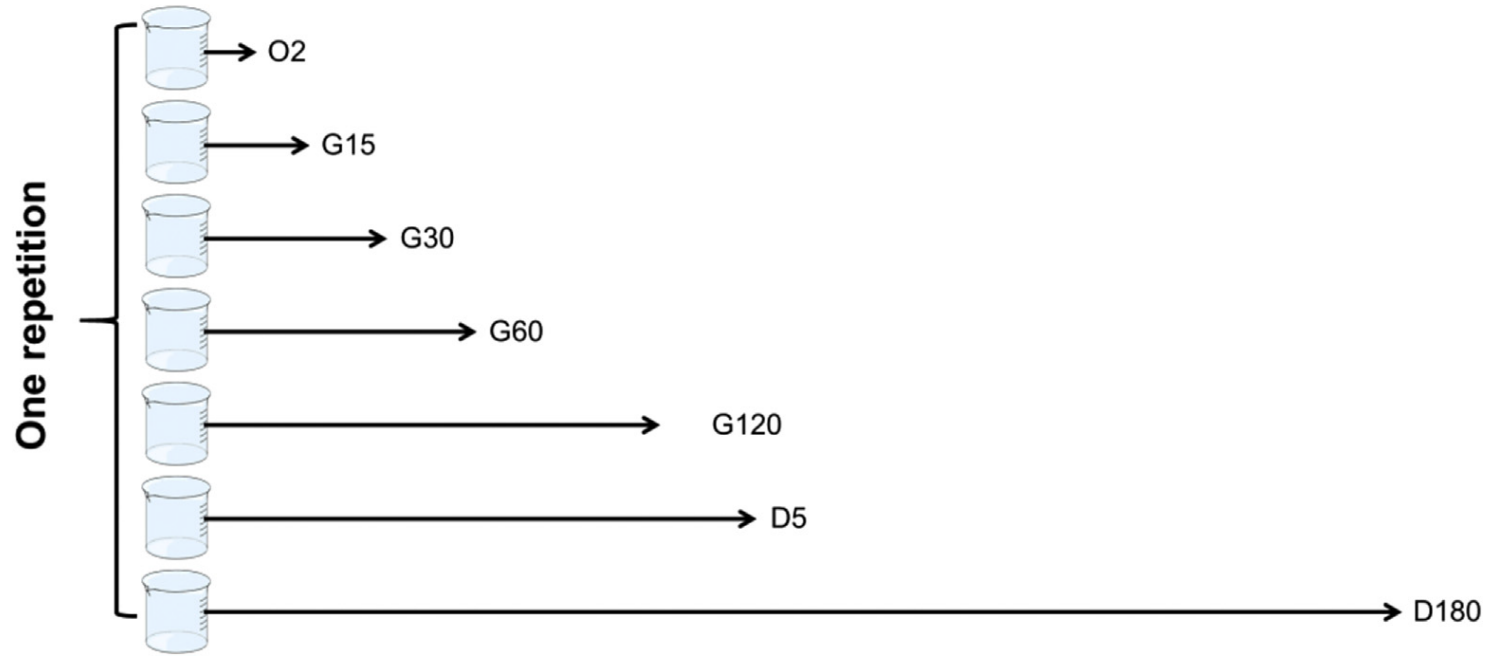
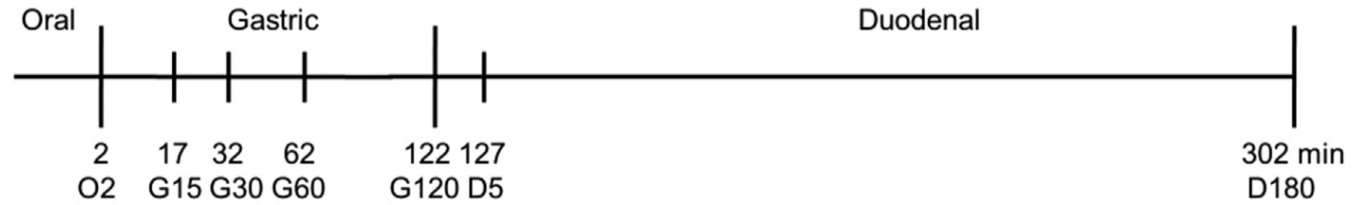
STELA Dairy Research Centre, Institute of Nutrition
and Functional Foods, Université Laval, Québec City,
Qc, G1V 0A6, Canada

In vitro digestion model

- Camembert
- Smear cheese
- Young Cheddar
- Aged Cheddar
- Mozzarella



Chyme sampling



Main findings of the study



Camembert showed a fast release of proteins/peptides in the gastric phase with a full hydrolysis in the stomach

Mozzarella exhibited a very slow digestion process with several proteins remaining intact at the end of the gastric phase


But all the cheese proteins were quickly hydrolyzed when entering the small intestine showing that cheese is a highly digestible food

Cheese disintegration at the end of gastric phase was higher when initial cheese hardness, cohesiveness, and chewiness were lower.

We have demonstrated that solid and elastic foods can limit the diffusion of acidic secretions and digestive enzymes in food particles (Nau et al. 2019, 2022).

Texture and composition of cheese are key parameters that drive the digestion process





Does cheese microbiota has an impact on protein hydrolysis during digestion?

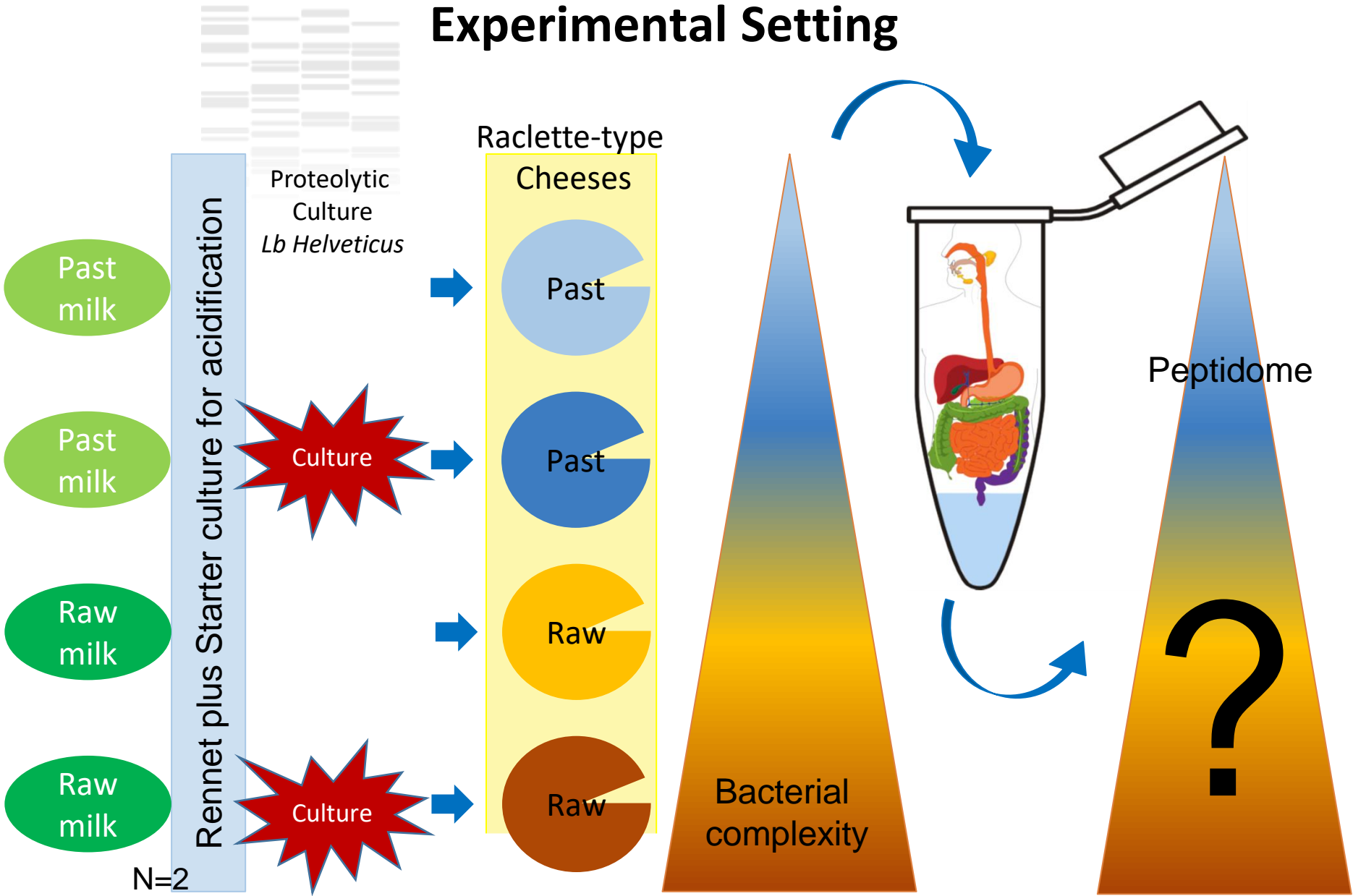


Egger L., Menard O., Portman R. & Dupont D.

Agroscope, Bern, Switzerland
INRAE, Rennes, France

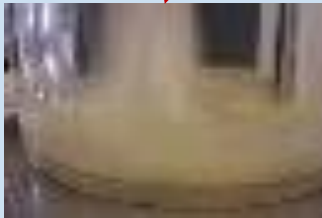


Experimental Setting



In vitro digestion methods

Dynamic (DIDGI®)



Gastric :

- Pepsin (2000U/ml) 0.5ml/min
- HCL 1M to achieve $\text{pH}=1.68+3.52^{(-t/42)}$
- Emptying $t_{1/2}=85\text{min}$ $\beta=1.8$
- duration: 150min

Intestinal digestion:

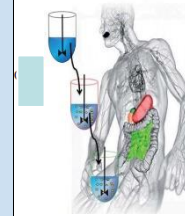
- Bile (2%) 0.5ml/min
- Pancreatin (7%) 0.25ml/min
- NaHCO_3 1M to achieve $\text{pH}=6.8$
- Emptying $t_{1/2}=250\text{min}$ $\beta=2.5$
- duration: 240min

Kinetic digestions n=2, cheeses: 4 X 2

Static (INFOGEST)

PAPER

INFOGEST



A standardised static *in vitro* digestion method suitable for food – an international consensus†

M. Minekus,^{†a} M. Alminger,^{†b} P. Alvito,^{†c} S. Ballance,^{†d} T. Bohn,^{†e} C. Bourlieu,^{†fn} F. Carrière,^{†g} R. Boutrou,^{†fn} M. Corredig,^{†i} D. Dupont,^{†fn} C. Dufour,^{†j} L. Egger,^{†k} M. Golding,^{†l} S. Karakaya,^{†m} B. Kirkhus,^{†n} S. Le Feunteun,^{†o} U. Lesmes,^{†p} A. Macierzanka,^{†q} A. Mackie,^{†r} S. Marze,^{†s} D. J. McClements,^{†t} O. Ménard,^{†fn} I. Recio,^{†u} C. N. Santos,^{†vw} R. P. Singh,^{†x} G. E. Vegarud,^{†y} M. S. J. Wickham,^{†z} W. Weitschies^{†aa} and A. Brodkorb^{†*ab}

Gastric:

- Pepsin (2000U/ml)
- $\text{pH}=3$
- duration: 120min

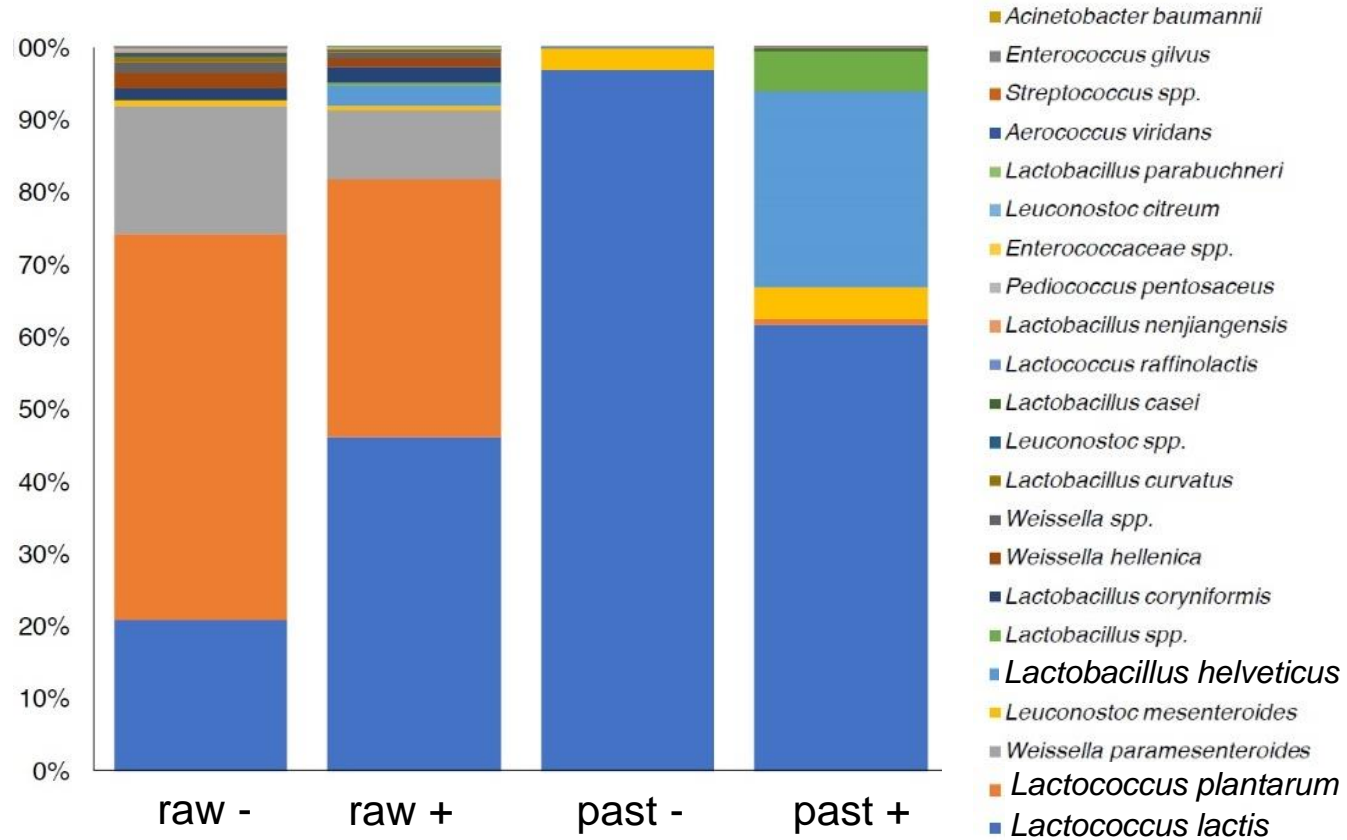
Intestinal digestion:

- Bile salts 10mM
- Pancreatin: to reach 100 U of trypsin/ml digesta
- $\text{pH}=7$
- duration: 120min

Kinetic digestion n=1, Endpoints n=3, cheeses: 4 X 2

Characterization of Cheeses

Microbial Diversity
Bacterial community based on 16S rRNA sequencing (v1v2) after 120 days of ripening:

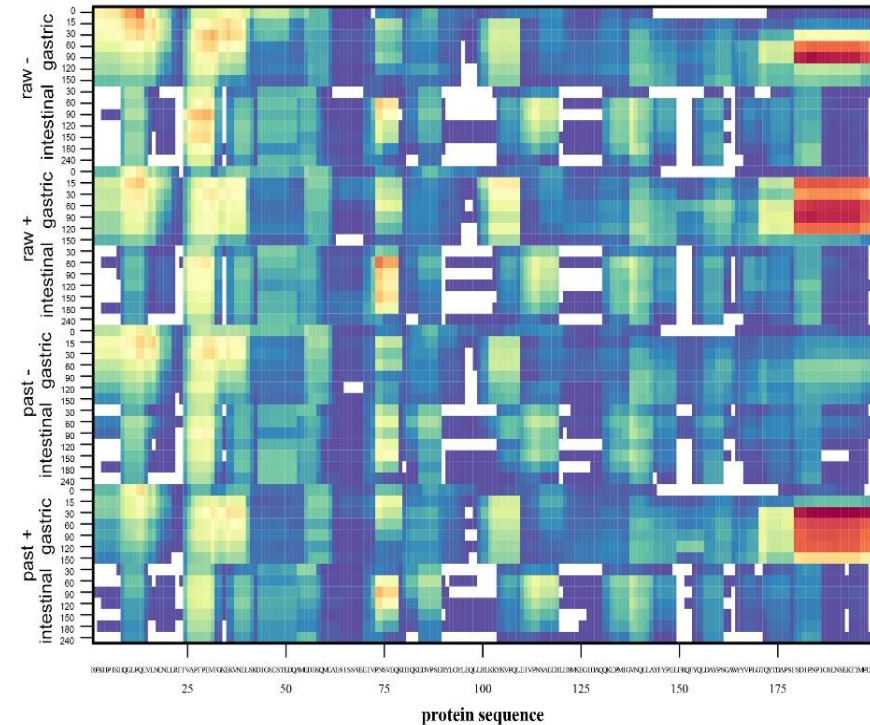


Composition
of four different (n=2)
Swiss Raclette cheeses
After 120 days of ripening:

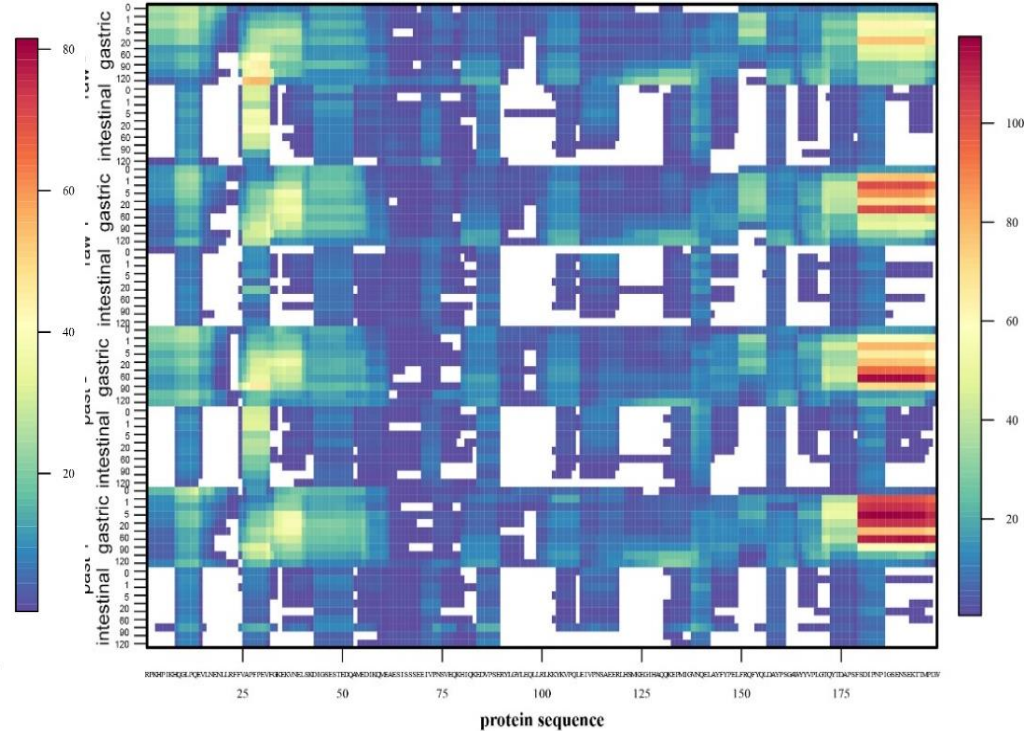
Cheese		sample	fat	NaCl	true protein	NPN	pH	Free NH ₂
milk	LH		g/kg	g/kg	g/kg	g/kg		mmol/kg
raw	no	raw -	290.3	18.9	244.37	5.7	5.1	151.3
	yes	raw +	295.8	17.9	250.26	5.6	5.1	151.5
past	no	past -	284.8	21.0	247.41	5.7	5.1	143.2
	yes	past +	288.3	18.9	238.26	5.8	5.1	145.2

Peptide generation during IVD

α_{s1} -casein



Dynamic N=4



Static N=2

Gastric digestion:

- Higher number of peptides at the C-terminus of the cheese samples with the proteolytic strain (observed in all caseins)

Intestinal digestion:

- Differences have been wiped off by the action of intestinal proteases

Opioid¹

Hypertension²

DPP-IV
inhibitory³

Peptide	Cheese								raw / past
	1	3	2	4	5	7	6	8	
EELNVPGEI	2	2	1	0	0	0	0	0	0.04
LNVPGEI	1	1	0	0	0	0	0	0	0.13
NVPGEIVES	0	0	0	0	2	1	1	0	0.05
NVPGEIVESL	10	10	10	6	5	7	8	0	0.10
FQSEEQQT	0	0	0	0	1	1	0	0	0.13
QTEDEL	1	1	0	3	4	6	3	1	0.11
TEDEL	18	18	20	13	11	15	11	11	0.03
TEDELQD	2	2	5	7	11	8	7	5	0.08
PFAQTQSLV	1	1	0	0	0	0	0	0	0.13
YVPPGPIP	0	0	0	2	3	3	2	0	0.13
PVPPGPIP	5	5	5	4	1	0	3	1	0.00
PQNIPPLTQTP	1	1	0	0	0	0	0	0	0.13
PQNIPPL	1	1	0	0	0	0	0	0	0.13
NIPPL	11	11	8	5	7	7	6	3	0.13
NIPPLT	8	8	3	0	0	2	1	0	0.10
PPIQT	0	0	0	0	0	1	0	1	0.13
PVVVPP	7	6	5	3	2	2	4	3	0.04
VVVVPPVQPEV	1	1	1	2	0	0	0	0	0.00
PPFLQPE	7	7	9	13	14	16	14	18	0.01
PFLQPEV	0	0	0	2	2	4	3	4	0.01
SLTLTDVENLHLPLPLLQSWMHQPHQPLP	1	1	1	0	0	0	0	0	0.02
HLPLP	0	0	0	0	2	0	0	3	0.15
LLQSWMHQPHQPLPPT	2	2	1	0	1	0	0	0	0.11
MHQPHQPLPPT	30	30	31	18	22	15	9	1	0.03
QPHQPLPPT	0	0	0	1	3	2	3	0	0.06
FPPQSV	14	14	13	21	12	10	7	13	0.07
SVLSLQSKVLPVPQKAVPY	1	1	0	0	0	0	0	0	0.13
LPVPQ	7	7	8	5	5	4	6	6	0.11
MEPPQ	2	2	3	2	1	2	1	1	0.03
MFPQVS	4	4	2	2	1	0	0	0	0.00
AVPYPP	5	5	5	5	0	0	0	5	0.02

Peptide	Cheese								culture +/-
	minus culture				plus culture				
YPVEP	3	3	2	4	0	1	3	2	0.10
TLTDVEN	4	4	4	3	4	1	3	1	0.11
LHLPLP	0	0	1	0	1	2	0	3	0.12
LLQSWMHQPHQPLPPT	2	2	1	0	1	0	0	0	0.11
SVLSLQSKVLPVPQKAVPY	1	1	0	0	0	0	0	0	0.13
RDMPI	0	0	0	0	1	0	0	1	0.13
QEPVLPV	0	0	3	2	4	2	3	3	0.09
LNVPGEIVESL	3	3	2	2	2	0	1	0	0.02
LNVPGEI	1	1	0	0	0	0	0	0	0.13
PVLGPV	3	3	5	4	8	5	3	6	0.18
FQSEEQQT	0	0	1	1	0	0	0	0	0.13
DELQDKI	1	1	0	2	0	0	0	0	0.05
PFAQTQSLV	1	1	0	0	0	0	0	0	0.13
VYVPPGPIP	11	11	10	10	8	9	8	0	0.09
VYVPPGPI	9	9	9	7	9	11	16	27	0.19
PPVPP	16	16	12	14	13	10	10	11	0.03
LPQNIPPL	9	9	6	5	2	2	7	2	0.05
PQNIPPLTQTP	1	1	0	0	0	0	0	0	0.13
PQNIPPL	1	1	0	0	0	0	0	0	0.13
NIPPL	11	11	7	7	8	5	6	3	0.07
NIPPLT	8	8	0	2	3	0	1	0	0.16
TQTPVVVPPFLQPEV	0	0	1	0	1	3	0	1	0.19
TQTPV	3	3	2	1	1	2	2	0	0.19
TPVVVPP	2	2	2	0	0	0	0	0	0.02

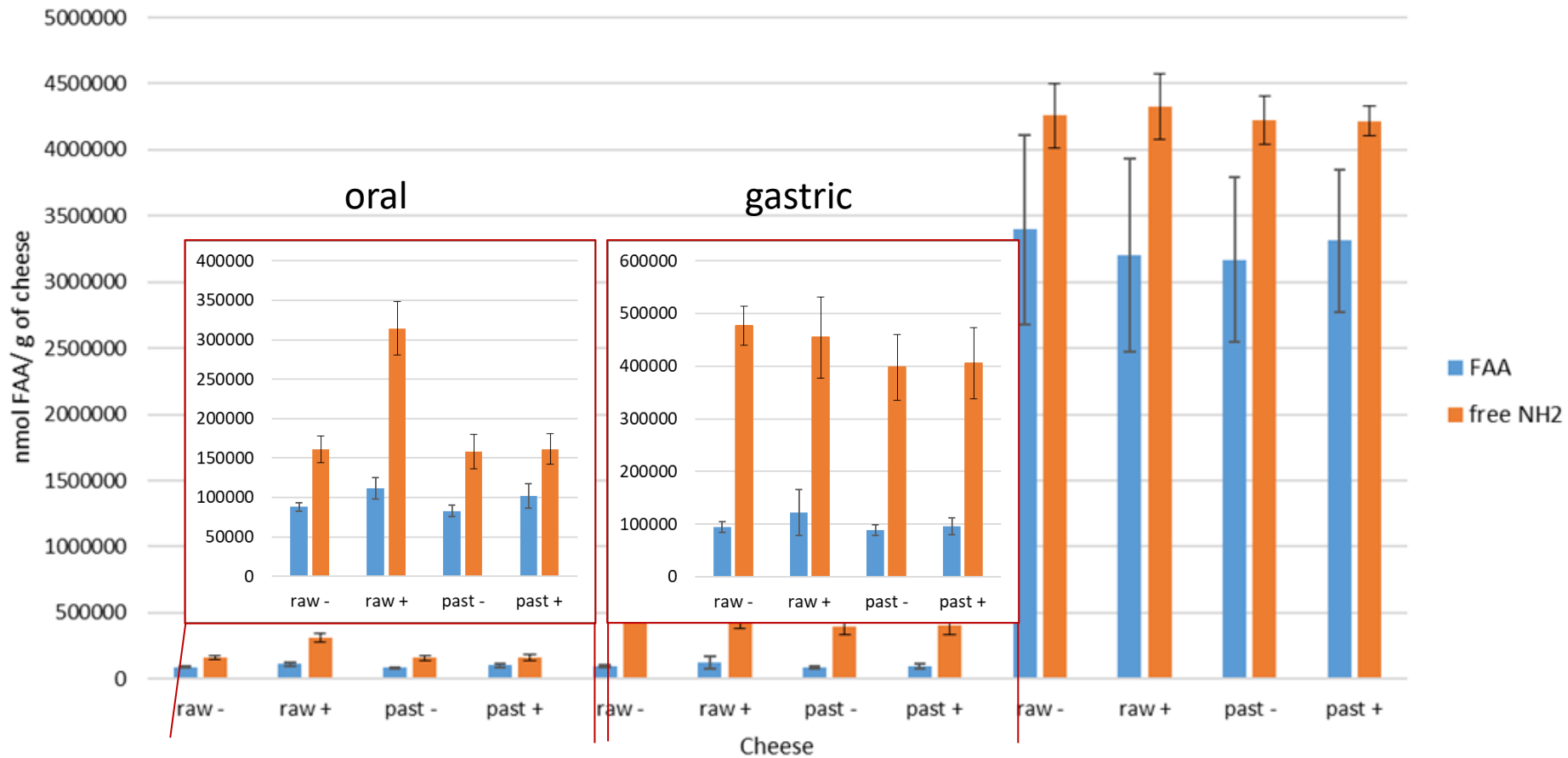
Tendency: ↗ Bioactive Peptides

- raw milk
- without proteolytic culture

¹Nongonierma, 2015; ²Aluko, 2015, ³Nongonierma, 2016

Release of free amino acids

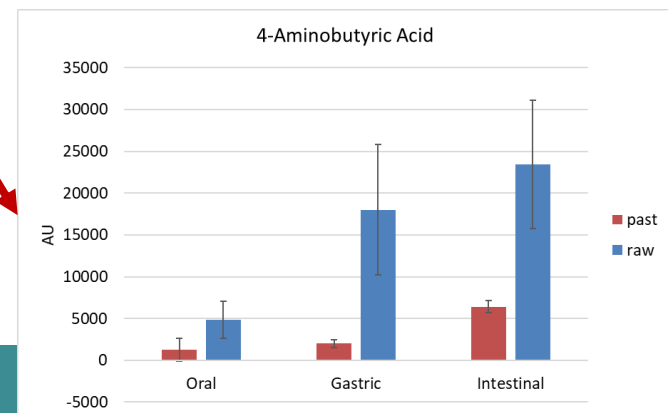
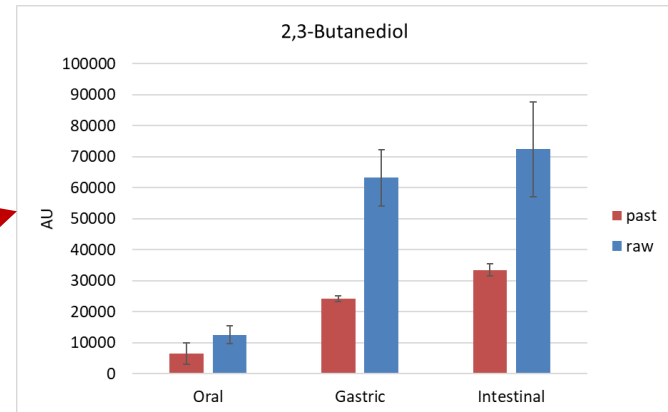
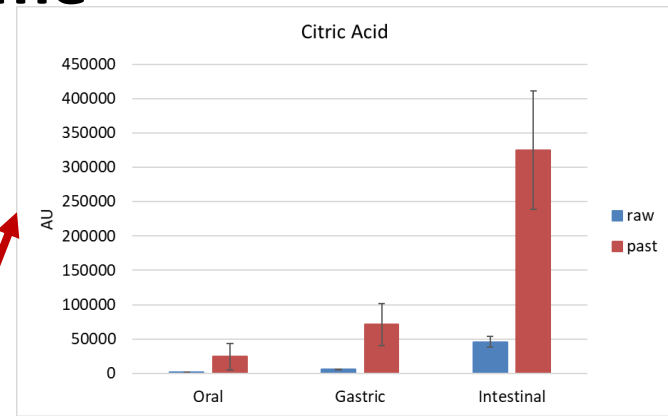
FAA in 1 g cheese gastric digest



→ Some differences between cheeses are visible in the oral or gastric phase but are wiped off after the intestinal phase

Metabolome

	Name	Gastric		Intestinal	
		t-Test	Kruskal-Wallis	t-Test	Kruskal-Wallis
Increased in Past milk Cheese	L-Serine	0.007	0.003	0.748	0.753
	Glyceric acid	0.000	0.001	0.077	0.115
	L-Serine	0.000	0.001	0.021	0.016
	Malic acid	0.000	0.001	0.158	0.172
	L-Serine	0.000	0.001	0.085	0.093
	L-Arabitol	0.000	0.001	0.472	0.016
	L-Glutamine	0.001	0.003	0.749	0.753
	Citric acid	0.001	0.001	0.000	0.001
	D-Tagatose	0.000	0.001	0.000	0.002
	D-Tagatose	0.000	0.001	0.829	0.401
Increased in Raw milk Cheese	2,3-Butanediol	0.000	0.001	0.000	0.001
	2,3-Butanediol	0.000	0.001	0.000	0.001
	2-Hydroxybutyric acid	0.000	0.001	0.022	0.016
	L-Isoleucine	0.017	0.027	0.065	0.036
	Succinic acid	0.000	0.001	0.000	0.001
	L-Aspartic acid	0.012	0.006	0.010	0.012
	4-Aminobutyric acid	0.001	0.001	0.000	0.001
	Tyramine	0.017	0.001	0.034	0.027
	3-Phenyllactic acid	0.000	0.001	0.012	0.016
	L-Ornithine	0.000	0.001	0.029	0.046
	L-Glutamic acid	0.000	0.001	0.548	0.753
	5-Aminovaleric acid	0.055	0.401	0.122	0.753
	Cadaverine	0.000	0.001	0.000	0.001
	L-Tryptophan	0.000	0.003	0.058	0.046
2-Monomyristin			0.078	0.115	





Conclusions

- Raw milk cheeses have a higher microbial diversity after 4 months of ripening, resulting in a higher content of free amino acids and differences in specific metabolites
- After gastric digestion, cheeses with higher microbial complexity have more complex peptide patterns and a slightly higher proteolysis
- All cheese digesta have only minor differences after intestinal IVD under dynamic and static conditions and these differences originate from non digestible metabolites present in the cheeses

Take home messages

The structure of dairy products regulate the kinetics of protein (and lipids as well!) digestion and the release of amino acids in the bloodstream

Gastric emptying rate and the structure that the product adopts in the stomach cavity regulates the whole digestion process.

Cheeses are food of very high nutritional quality providing all of the indispensable amino acids needed.

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



The Bioactivity & Nutrition team at INRA Rennes



Head

Didier DUPONT - Senior Scientist

Scientists

Amélie DEGLAIRE – Lecturer

Juliane FLOURY – Lecturer

Catherine GUERIN - Lecturer

Steven LE FEUNTEUN – Senior Scientist

Joëlle LEONIL – Senior Scientist

Martine MORZEL – Senior Scientist

Françoise NAU – Professor

Frédérique PEDRONO – Lecturer

Xiaoxi YU – Post-doc

Guilherme FURTADO – Post-doc

PhD students

Yohan REYNAUD (2016-2019)

Amira HALABI (2017-2020)

Jun WANG (2018-2021)

Lea SALLELES (2018-2021)

Elise CHARTON (2019-2022)

Lucile CHAUVET (2019-2022)

Ousmane SUWAREH (2019-2022)

Technicians

Gwenaële HENRY

Yann LE GOUAR

Nathalie MONTHEAN

Engineers

Julien JARDIN

Olivia MENARD

Jordane OSSEMOND

Masters students

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

International Network

Dr. Didier DUPONT, Senior Scientist, INRAE, France

●
INFOGEST
●



Main objective: understanding the mechanisms of food digestion

- Develop new *in vitro*, *in vivo* and *in silico* digestion models including some for specific populations (infant, elderly)
- Harmonize the methodologies and propose guidelines for performing experiments
- Validate *in vitro* models towards *in vivo* data (animal and/or human)
- Identify the beneficial/deleterious components that are released in the gut during food digestion
- Determine the effect of the matrix structure on the bioavailability of food nutrients and bioactive molecules

Industry involvement

~ 60 private companies are following INFOGEST





INFOGEST



Chair
Didier Dupont - France
didier.dupont@inrae.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

Imaging Technologies applied to digestion
WG7



Isidra Recio



Pasquale Ferranti



Linda Giblin



Frederic Carriere



Daniela Freitas



Steven Le Feunteun



Paul Smeets



Andre Brodkorb



Lotti Egger



Uri Lesmes



Brigitte Graf



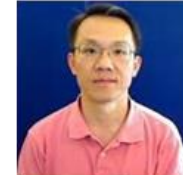
Marion Letisse



Leslie Couedelo



Marilisa Alongi



Choi-Hong Lan



Luca Marciani

Some outputs

In vitro gastrointestinal digestion Consensus INFOGEST protocol

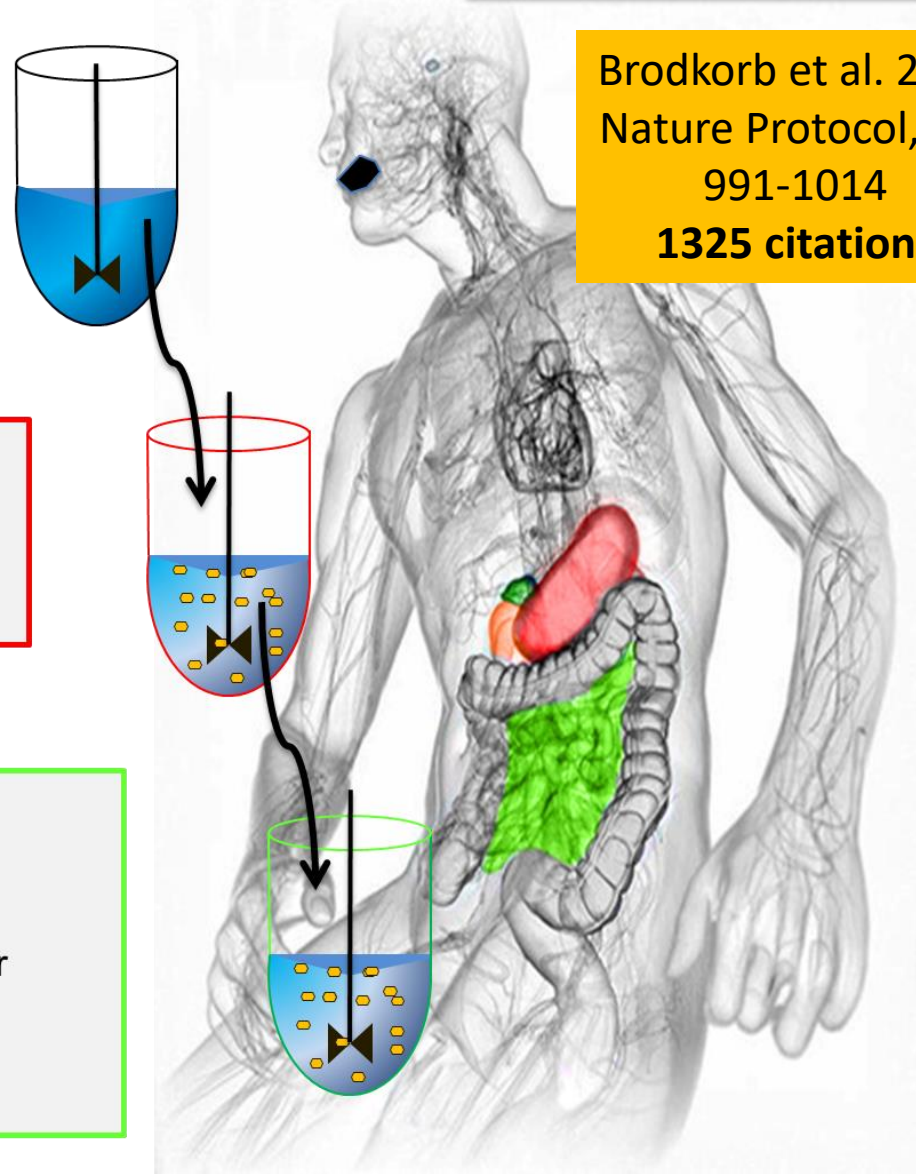
Minekus et al. 2014
Food & Function, 5, 1113-1124
3125 citations

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

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





We are pleased to announce the next
9th International Conference on Food Digestion



in Gdansk, Poland, 19-21 May 2026