

Influence of raw milk microflora and starter cultures in cheese on protein hydrolysis and bioactive peptide generation during digestion

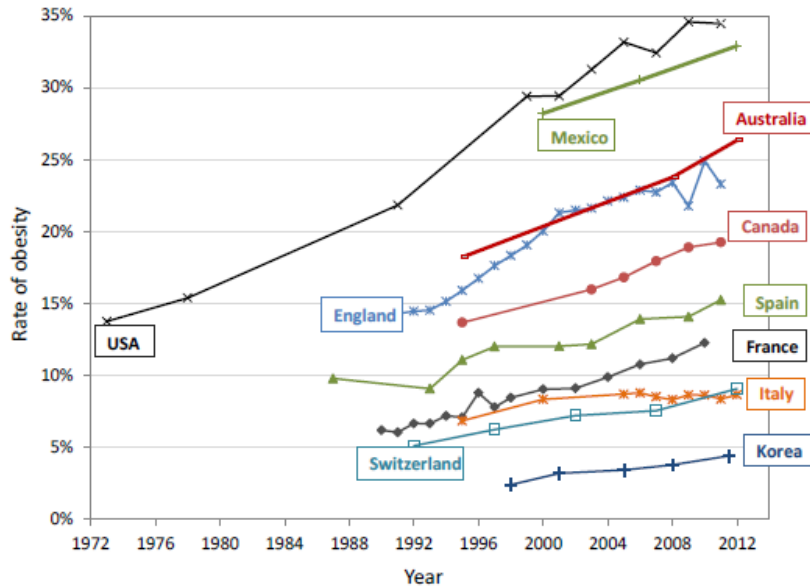


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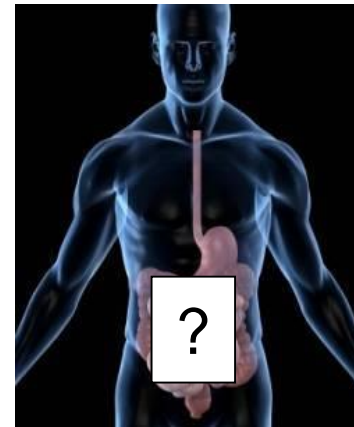
¹Agroscope, CH-3003 Berne, www.agroscope.ch; ²STLO, UMR 1253, INRA, Agrocampus Ouest, 35000 Rennes, France



Food and human health: the key role of digestion



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



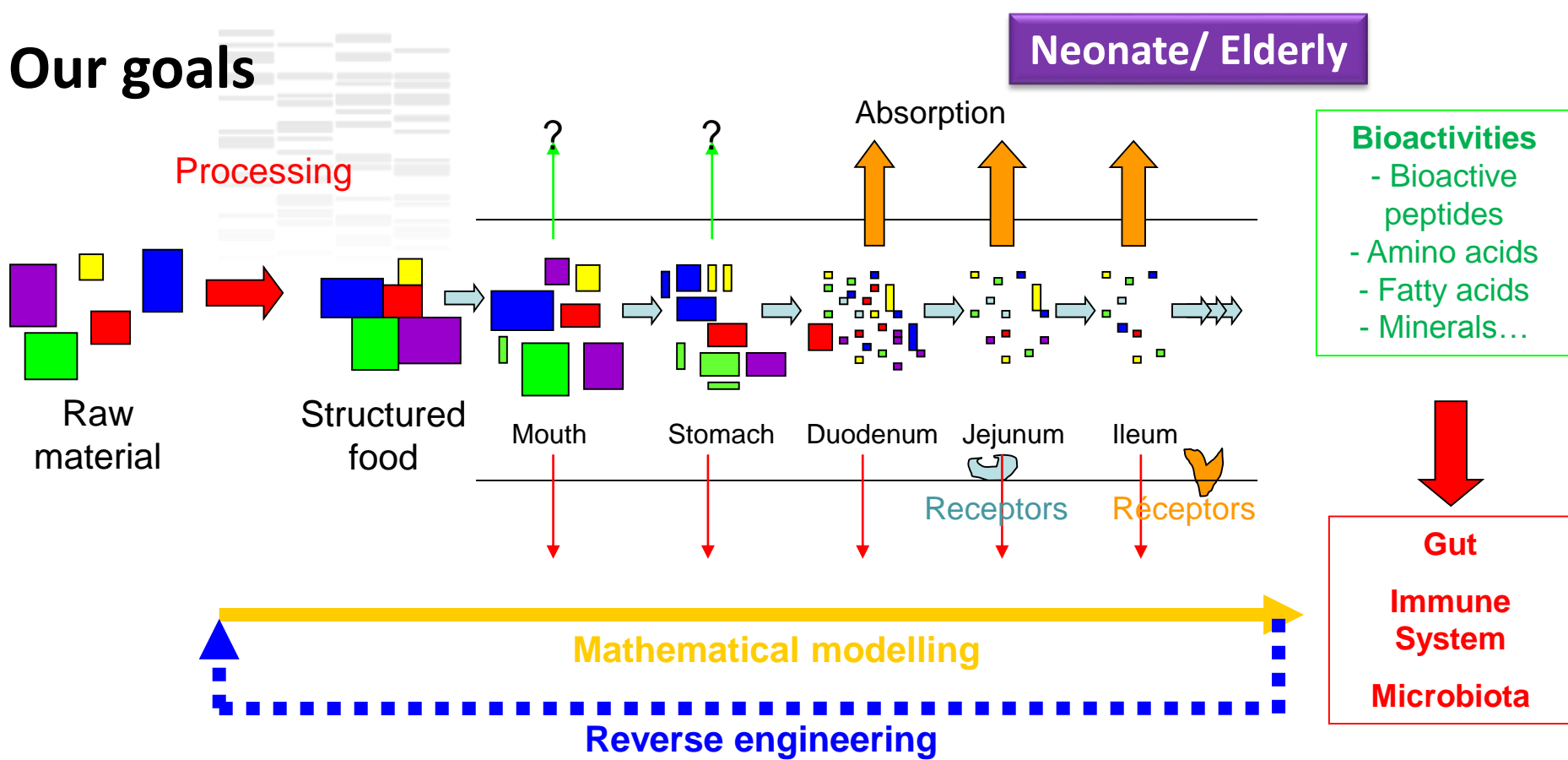
Gut = interface between food and human body

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

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

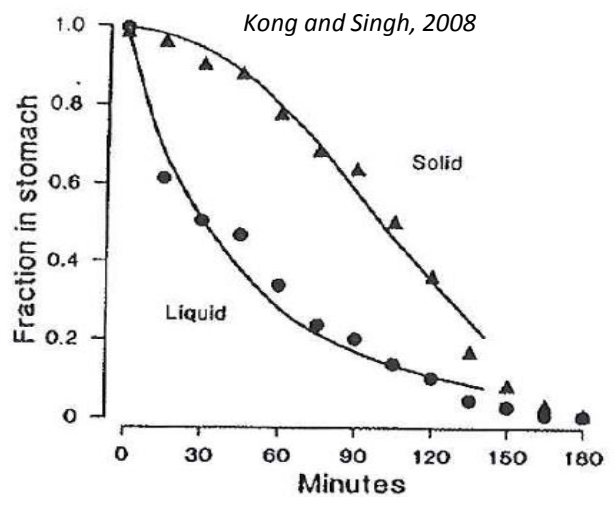
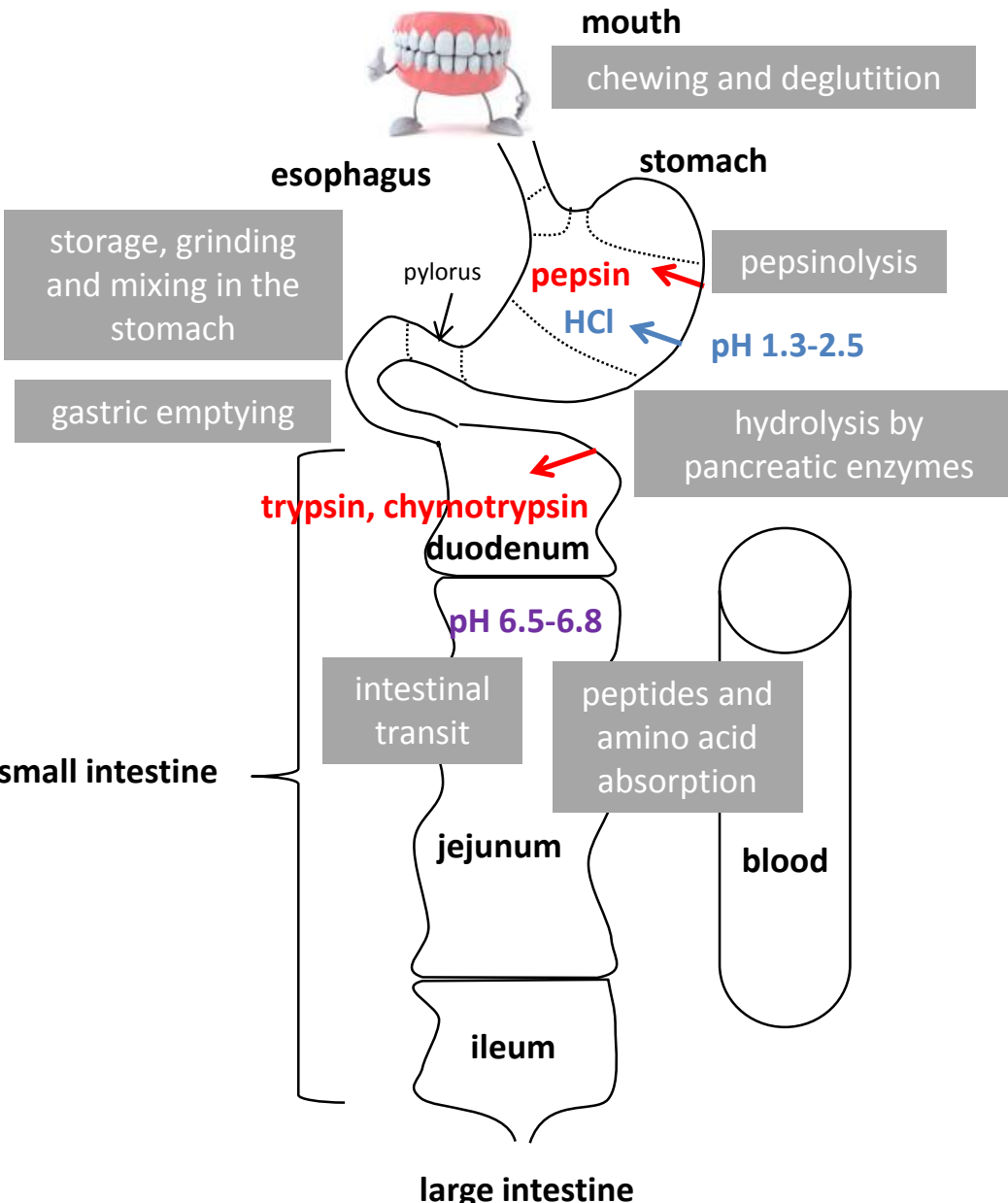
By increasing our knowledge on food digestion, we will increase our knowledge on the effect of food on human health

Our goals



- ☞ 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 digestive process



→ Gastric phase = key 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)*



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

Le Feunteun et al.
2014
Food Bioprocess
Tech

*In silico
models*



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



*Human
models*



Animal models



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



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

Context of the study

Cow milk cheeses are highly-consumed products all over Europe

They provide highly digestible proteins and lipids, bioavailable calcium as well as a complex microbial ecosystem to the body

However, cheese digestion has been scarcely studied so far and their digestion products have never been exhaustively characterized

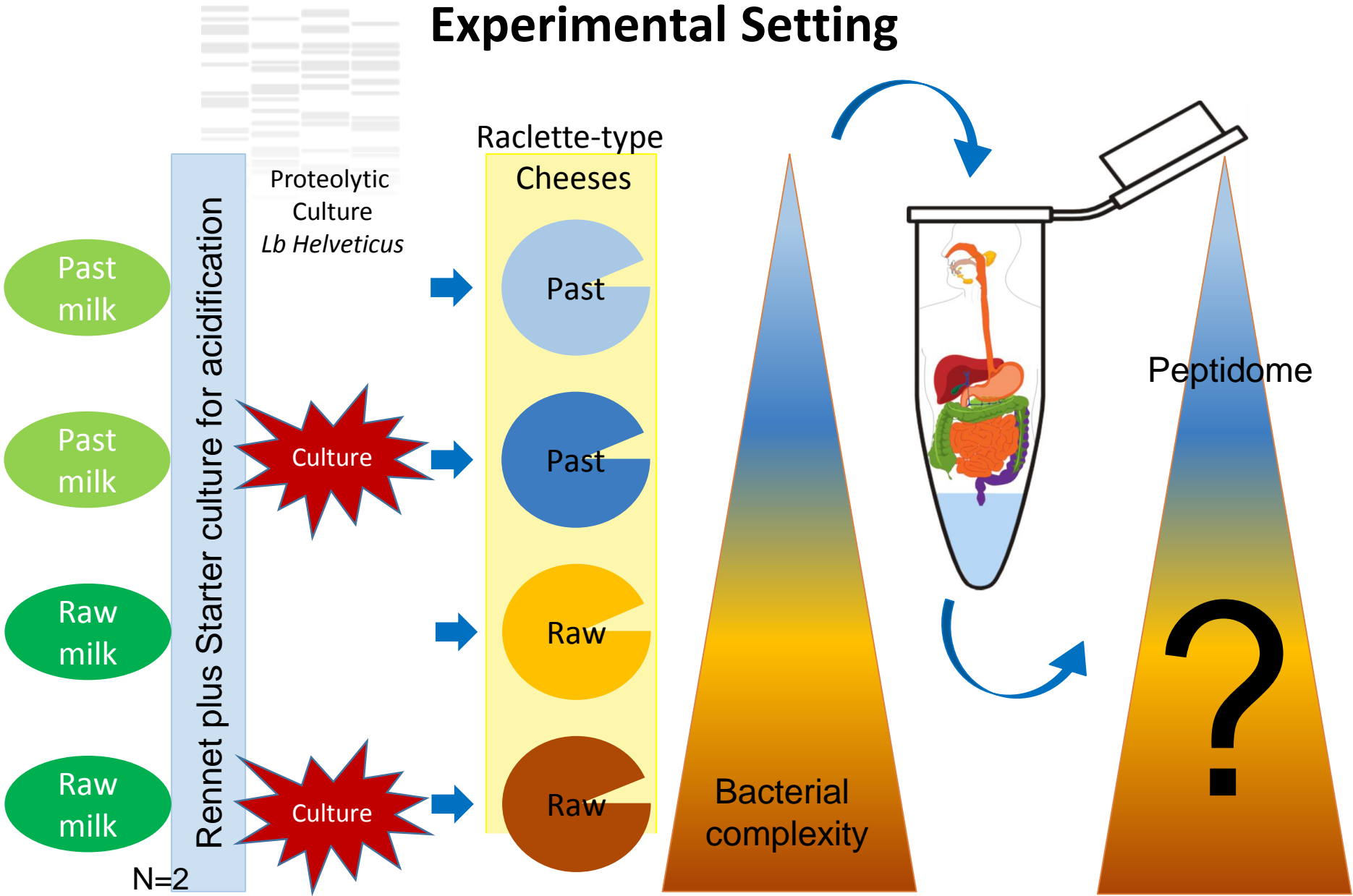
Research Questions

Do bacterial strains in cheese have an impact on protein hydrolysis during human digestion?

Does a higher microbial diversity lead to the generation of a higher number and diversity of peptides after digestion?

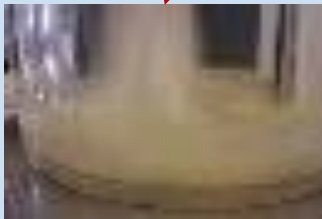
Are there differences at a molecular level after digestion of cheeses with a different bacterial background?

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:

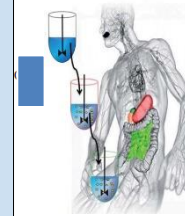
- Bile (2%) 0.5ml/min
- Pancreatin (7%) 0.25ml/min
- NaHCO_3 1M to achieve $\text{pH}=6.8$
- Emptying $t_{1/2}=250\text{min}$ $\beta=2.5$
- duration: 240min

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†

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Gastric:

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

Intestinal digestion:

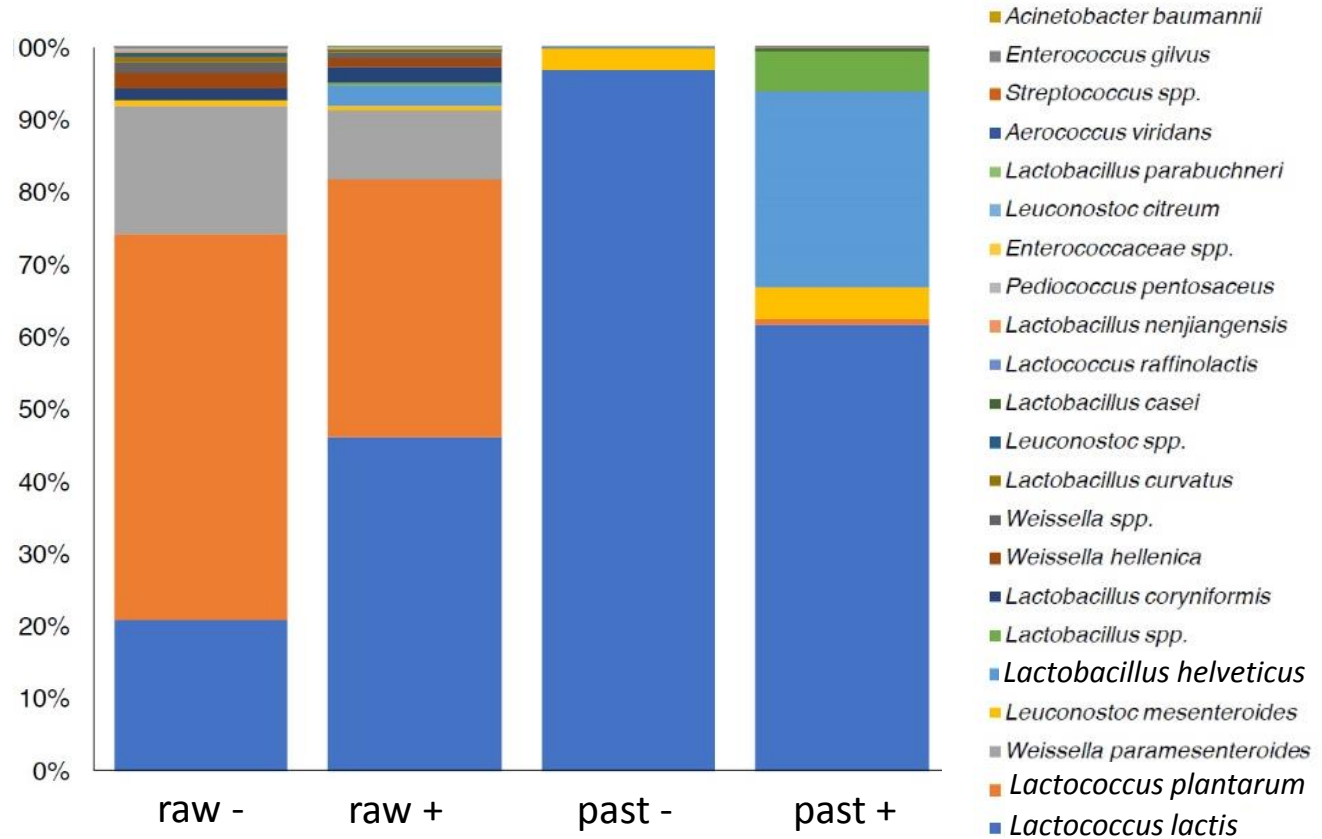
- Bile salts 10mM
- Pancreatin: to reach 100 U of trypsin/ml digesta
- $\text{pH}=7$
- duration: 120min

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:

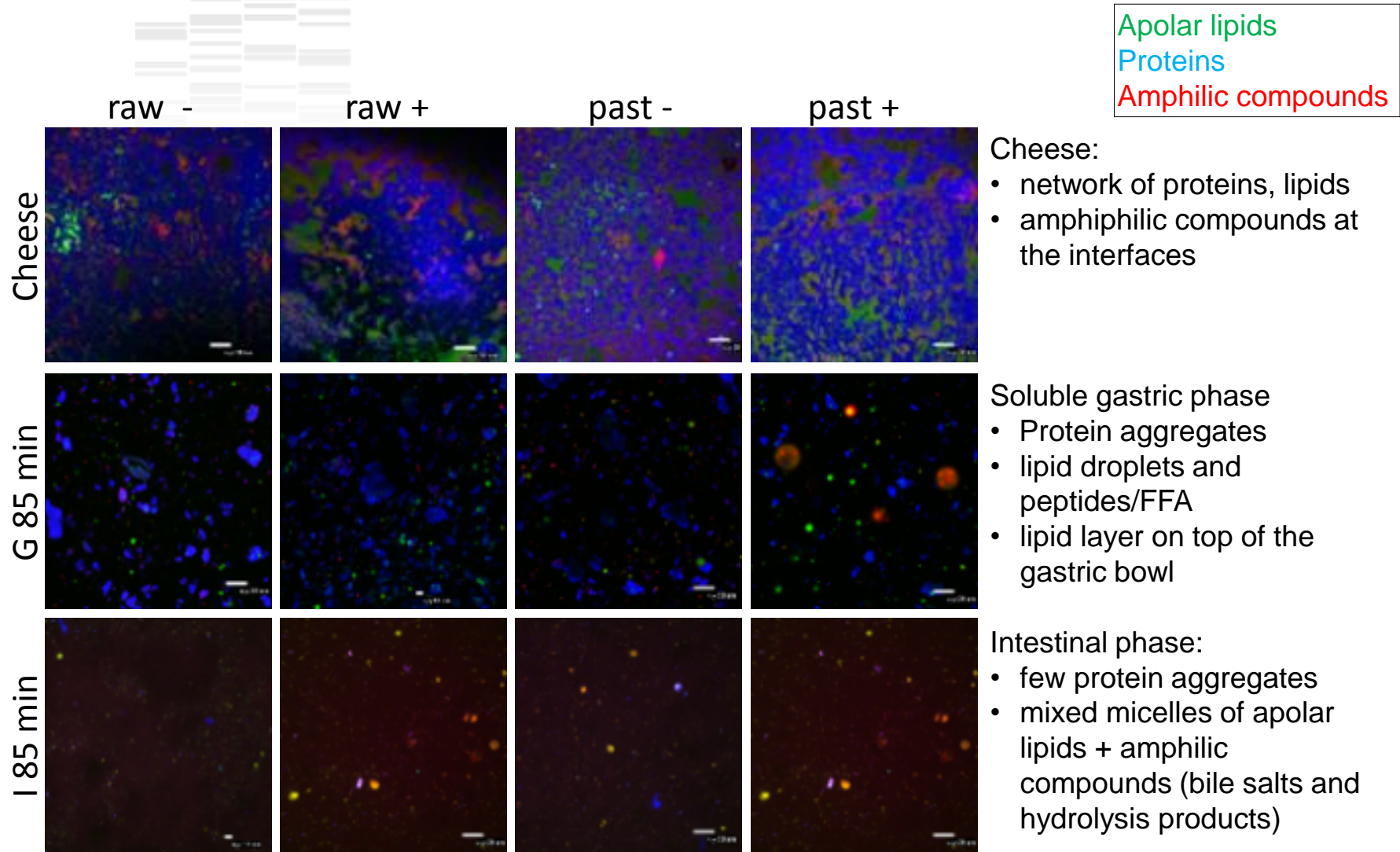


Composition

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

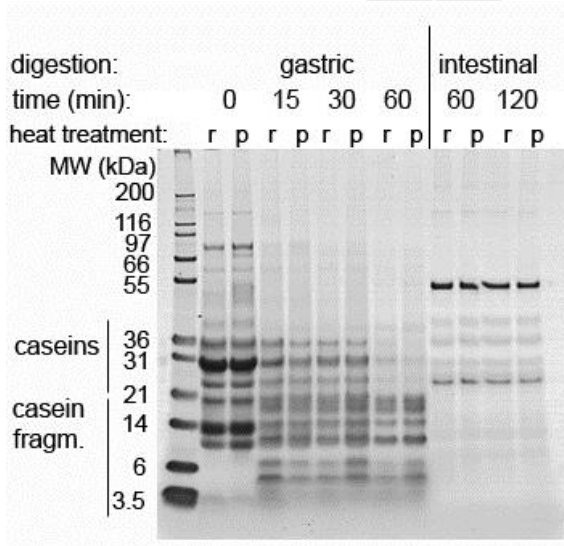
Structural evolution of the cheeses during dynamic IVD



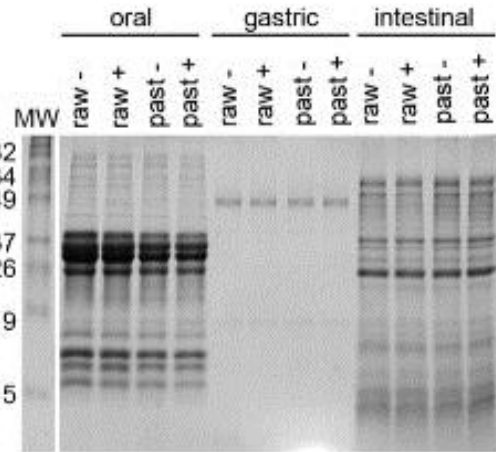
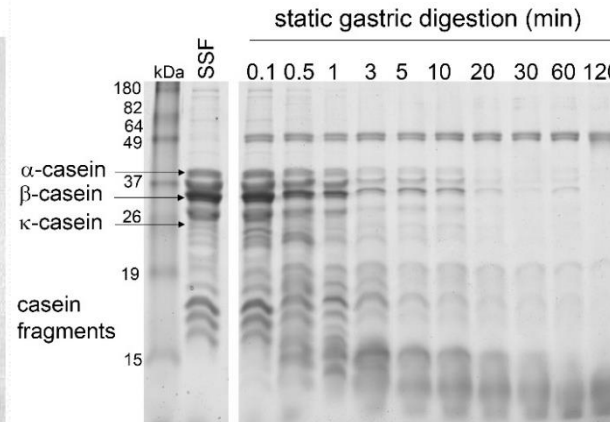
→ No structural differences visible between the four cheeses

Protein Hydrolysis

Dynamic



Static



Similar proteolysis pattern in dynamic and static *in vitro* digestion

Gastric digestion:

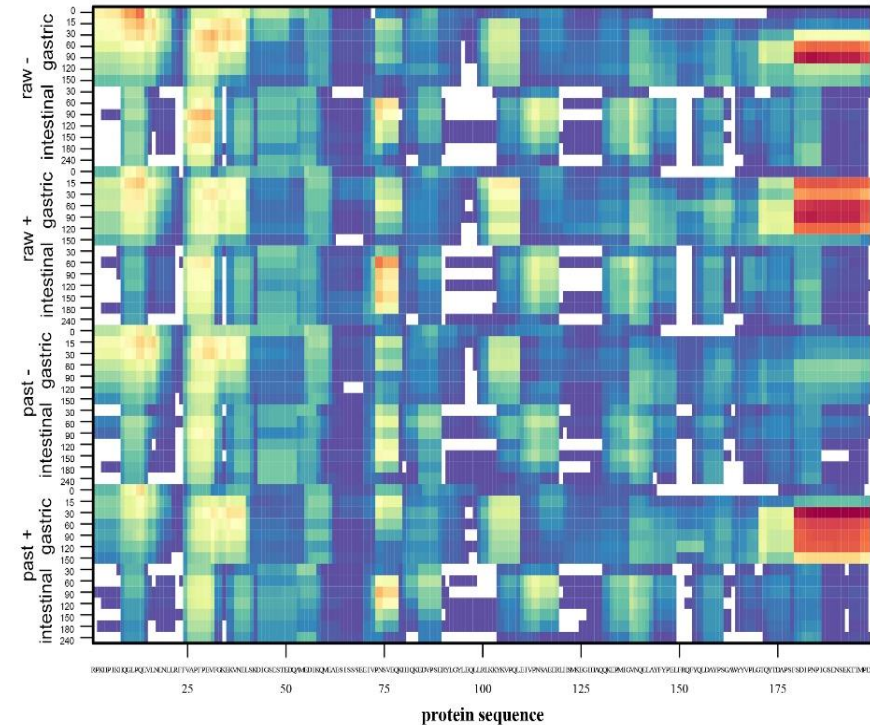
- Fast hydrolysis of intact caseins in all cheeses
- At 60 min of digestion no intact caseins remain but large fragments present

Intestinal digestion:

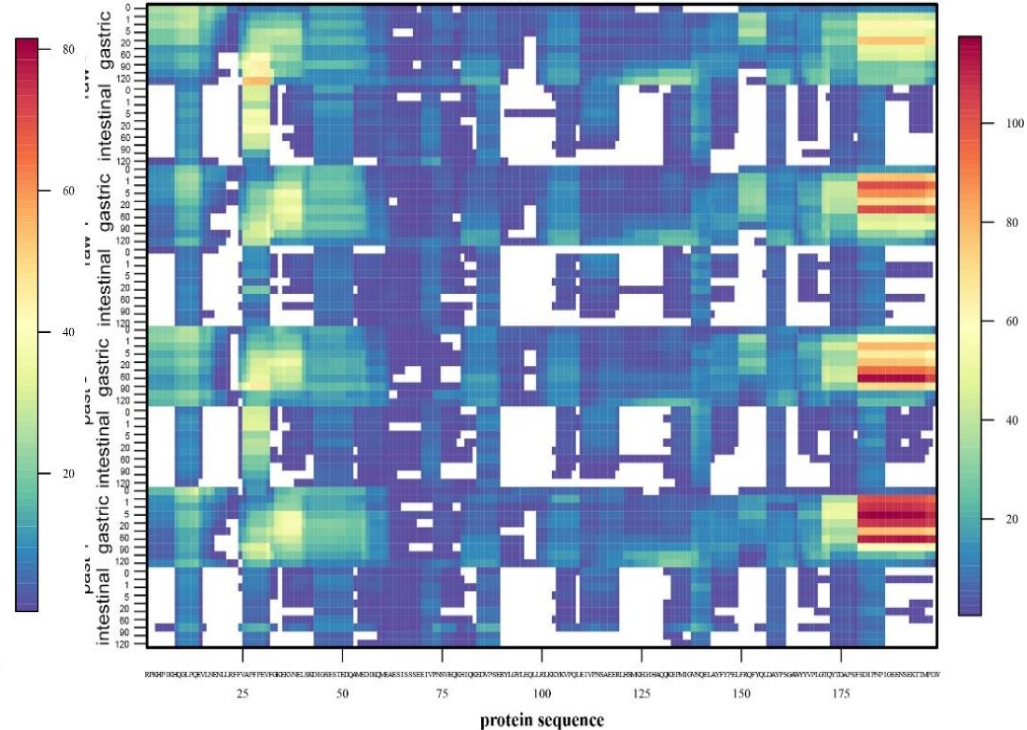
- Only proteins from pancreatin visible

Peptide generation during IVD

α_{s1} -casein



Dynamic N=4



Static N=2

Gastric digestion:

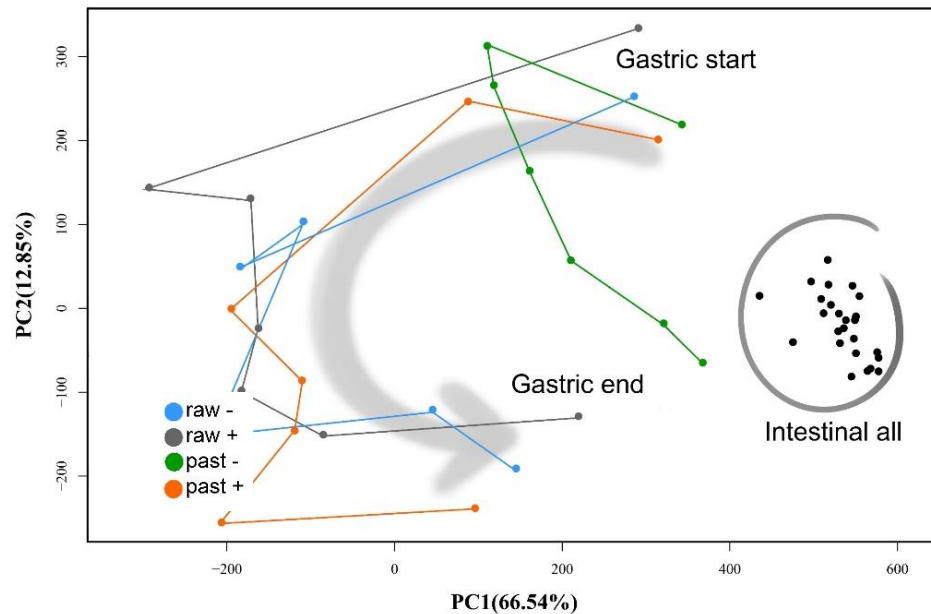
- Higher number of peptides at the C-terminus of the cheese samples with the proteolytic strain (observed in all caseins)

Intestinal digestion:

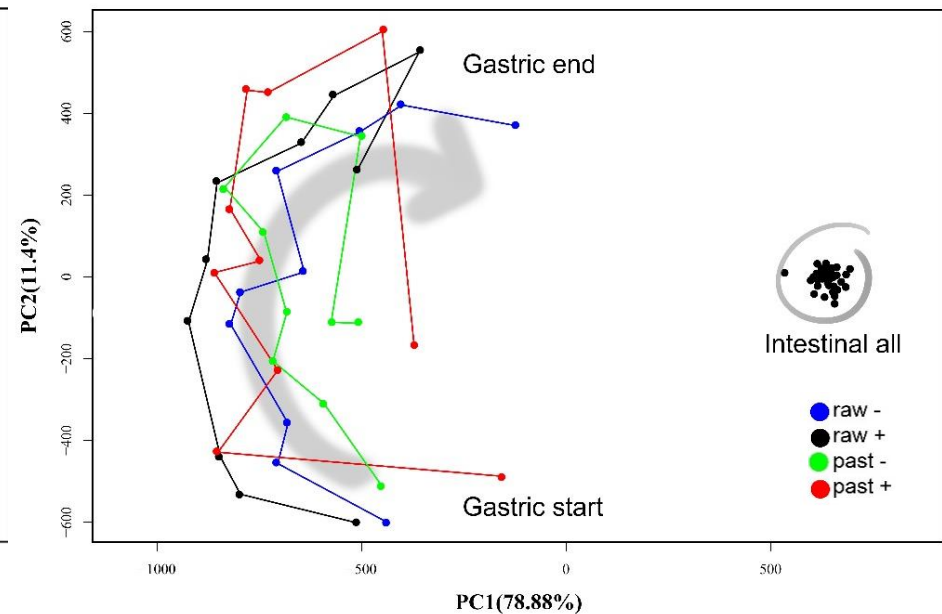
- Differences have been wiped off by the action of intestinal proteases

Peptide generation during IVD

Dynamic



Static



Gastric digestion:

- Peptidomes of cheese samples show an evolution in both digestion models

Intestinal digestion:

- No differences observed in the intestinal phase for the four different cheeses in both digestion models

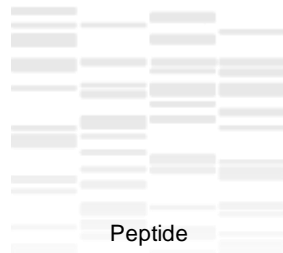
Bioactive Peptides

β-casein, dynamic digestion

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
YPPFGPIP	0	0	0	2	3	3	2	0	0.13
PFPFGPIP	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
VVVVPP	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
SLTLTDVENLHLPLPLLSQSWMHQPHQPLP	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
SVLSLSQSKVLPVPQKAVPY	1	1	0	0	0	0	0	0	0.13
LPVPQ	7	7	8	5	5	4	6	6	0.11
MFPPQ	2	2	3	2	1	2	1	1	0.03
MFPPQS	4	4	2	2	1	0	0	0	0.00
AVPYPPQ	5	5	5	5	0	0	0	5	0.02

Peptide	Cheese								culture +/-
	minus culture				plus culture				
	1	3	5	7	2	4	6	8	
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
SVLSLSQSKVLPVPQKAVPY	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
VYPPFGPIP	11	11	10	10	8	9	8	0	0.09
VYPPFGPI	9	9	9	7	9	11	16	27	0.19
PFPPI	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

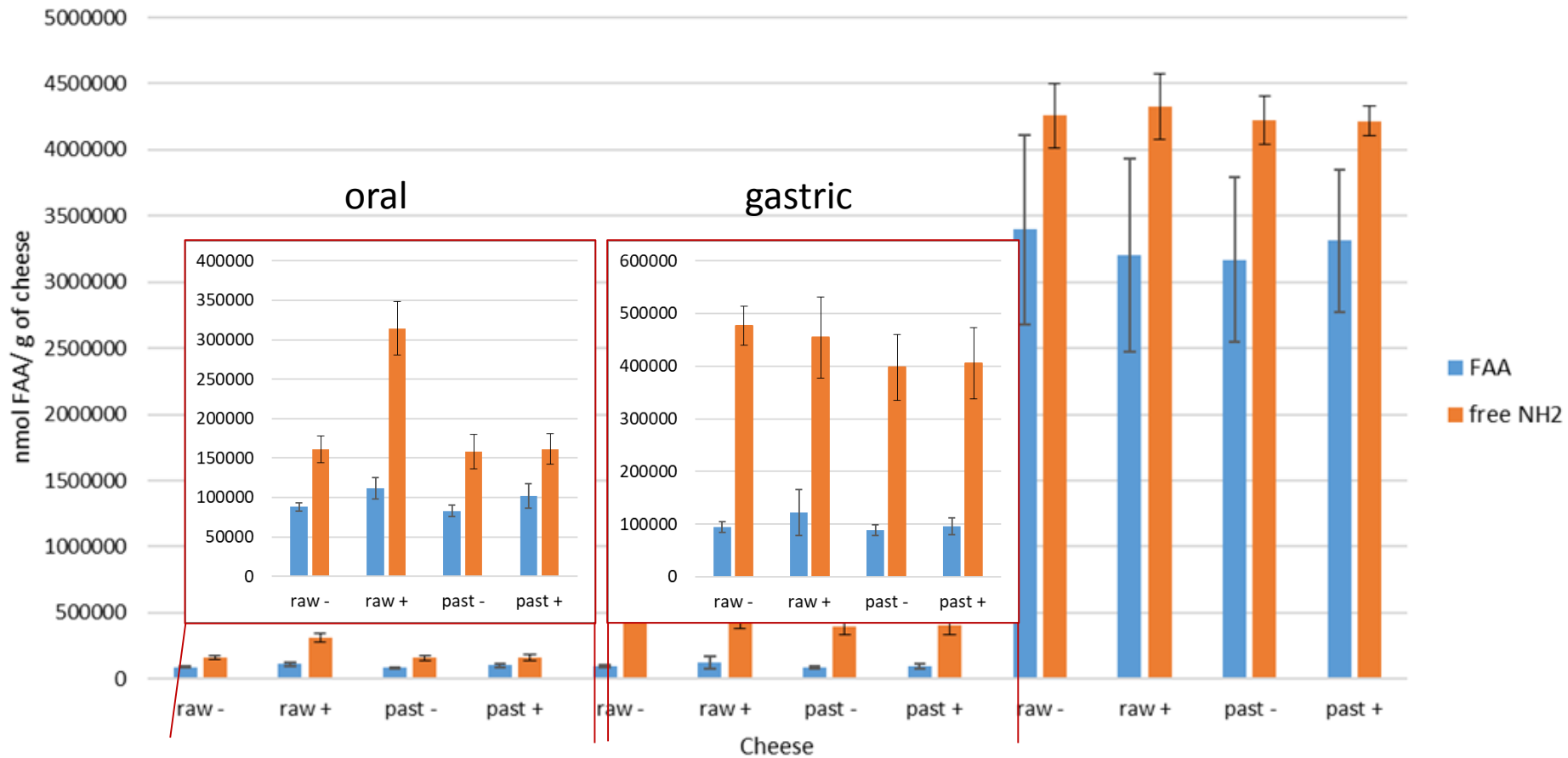
Tendency: ↗ Bioactive Peptides

- raw milk
- without proteolytic culture

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

Release of free amino acids

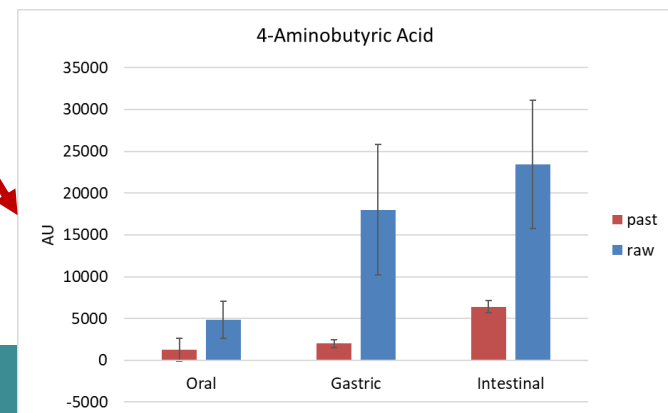
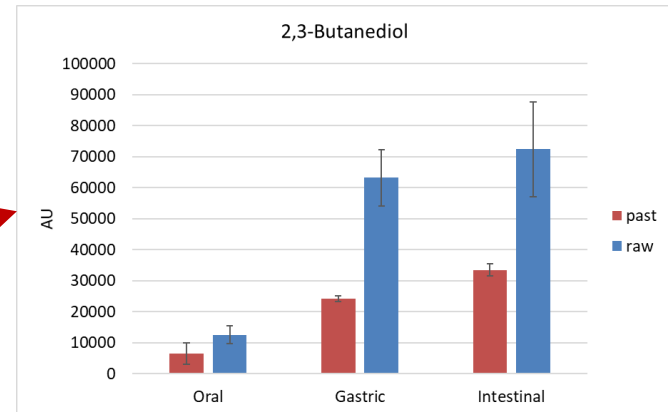
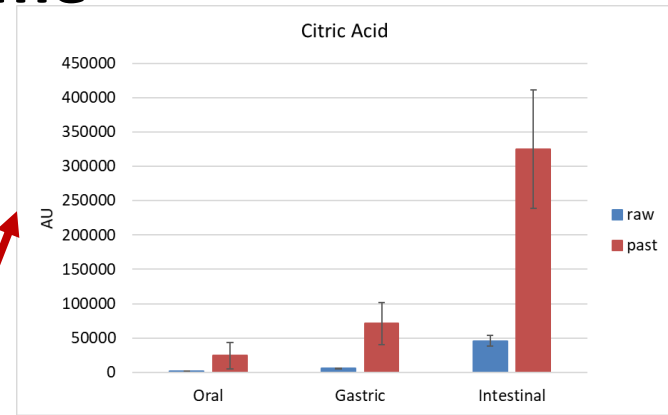
FAA in 1 g cheese gastric digest



→ 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 digests have only minor differences after intestinal IVD under dynamic and static conditions and these differences originate from non digestible metabolites present in the cheeses



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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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New Zealand

Riddett Inst
 Plant Food Res



Japan

Nagoya Univ

400 scientists - 140 institutes – 41 countries

Industry involvement

☞ ~ 50 private companies are following INFOGEST





Chair
Didier Dupont - France

INFOGEST



Vice-chair
Alan Mackie - UK



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In vitro/in vivo correlations
WG1

In vitro semi-dynamic model of digestion
WG2

Models for specific populations
WG3

Digestive lipases and lipid digestion
WG4

Digestive amylases and starch digestion
WG5

In silico models of digestion
WG6



Didier Dupont



Alan Mackie



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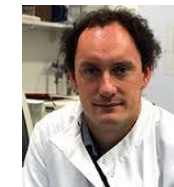


Guy Vergeres

The "Mind-the-Gap" group



Frederic Carriere



Fred Warren



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, 2-4 April 2019