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Investigating in vitro digestion of food models to explain in vivo micronutrient bioavailability

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6th INTERNATIONAL
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GRANADA 2019
Granada Conference Centre

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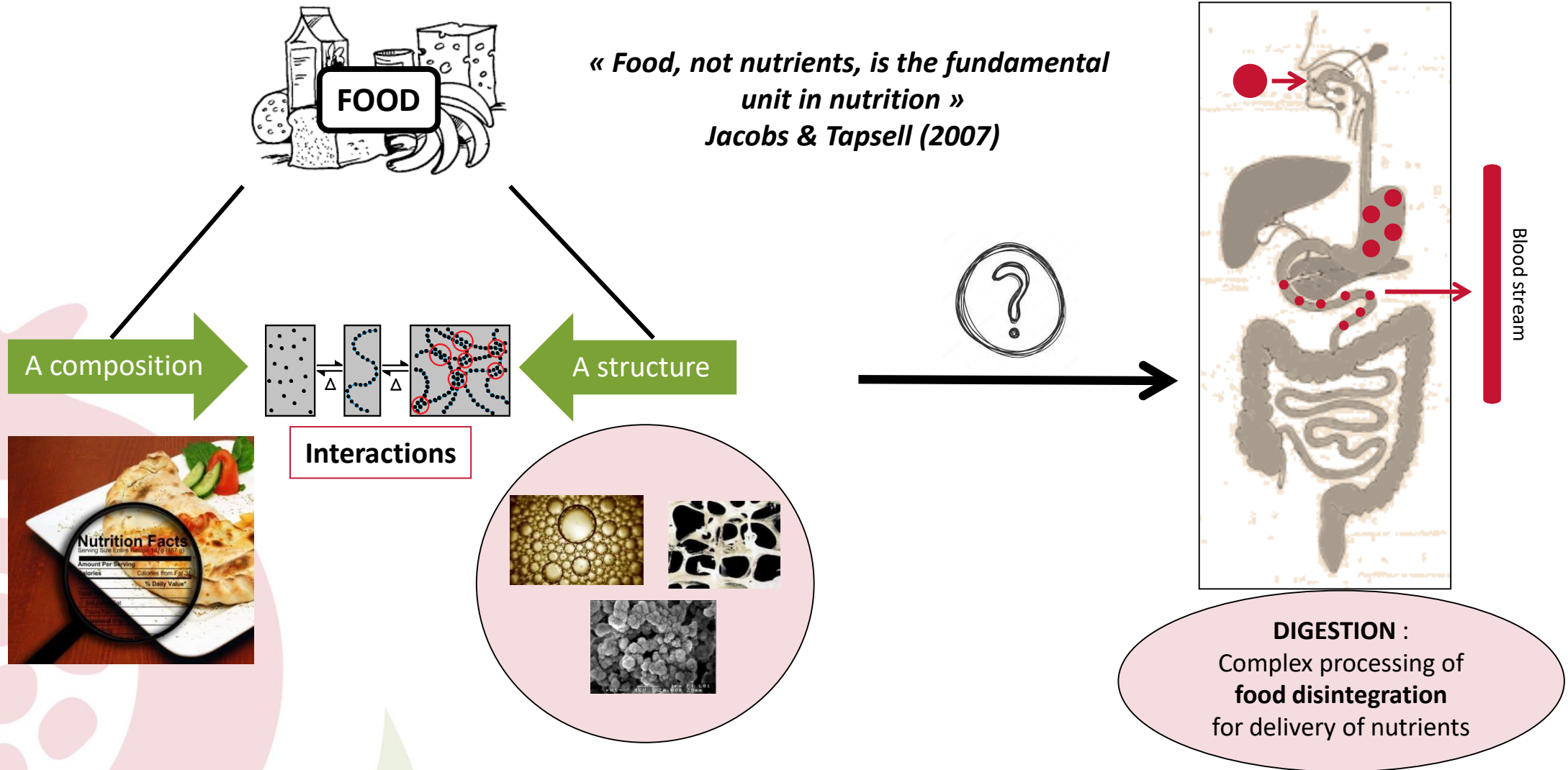
GRANADA
CIENCIA E INNOVACIÓN

INRA
SCIENCE & IMPACT

Investigating *in vitro* digestion of food models
to explain *in vivo* micronutrient bioavailability

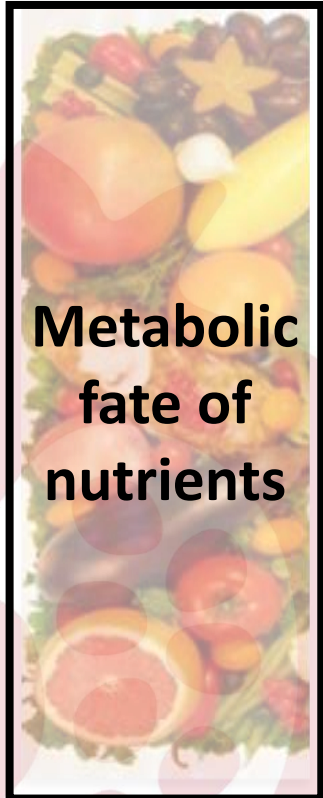
Speaker : Manon Hiolle

Nutritional quality : beyond food composition



Nutritional quality : beyond food composition

Food structure



Metabolic fate of nutrients

Bioaccessibility

Bioavailability

Kinetics

Metabolic responses

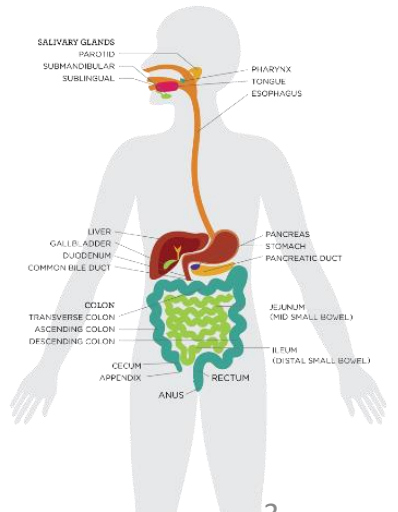
Macronutrients :

Micronutrients : composition effect versus structure effect ?

Models micronutrients :

- vitamins B9 and B12
- vitamin D and Lutein

THE DIGESTIVE TRACT



Strategy : clinical study conduct

Four products with identical composition

Enriched with the four micronutrients studied



Products characterization

Clinical study

- 12 healthy male subjects
- 20 to 30 years old
- Cross-over experimental design
- 8 hours postprandial follow-up
- Hourly blood samples and plasma separation by centrifugation

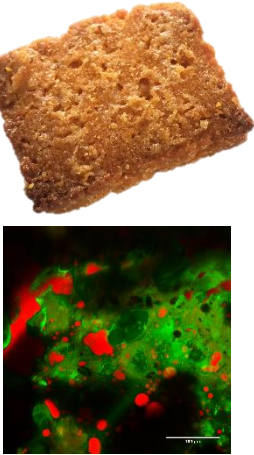
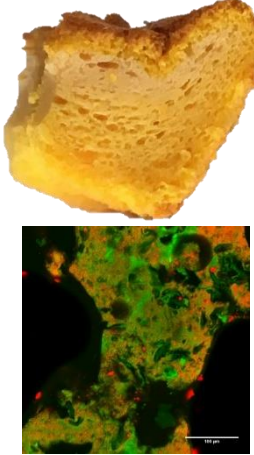

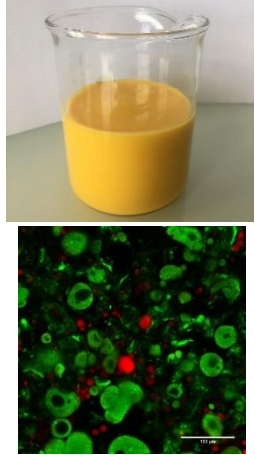
Bioavailability

- Absorption kinetics: quantitative analysis of micronutrients in plasma or chylomicrons

Results for B9 : poster n°378/214, session 2

- Microstructure : confocal microscopy
- Macrostructure : texture analysis

Strategy : development of food models

Matrices	Biscuit	Sponge cake	Pudding	Custard
Photo				
Water content	6 %	38 %	48 %	68 %
Heating treatment	180 °C/18 min	180 °C/30 min	180 °C/20 min	110 °C/20 min 80 °C/7 min
Portion size	135 g	206 g	242 g	512 g

✓ Same composition on dry matter :

- 17 % proteins
- 52 % carbohydrates
- 30 % lipids

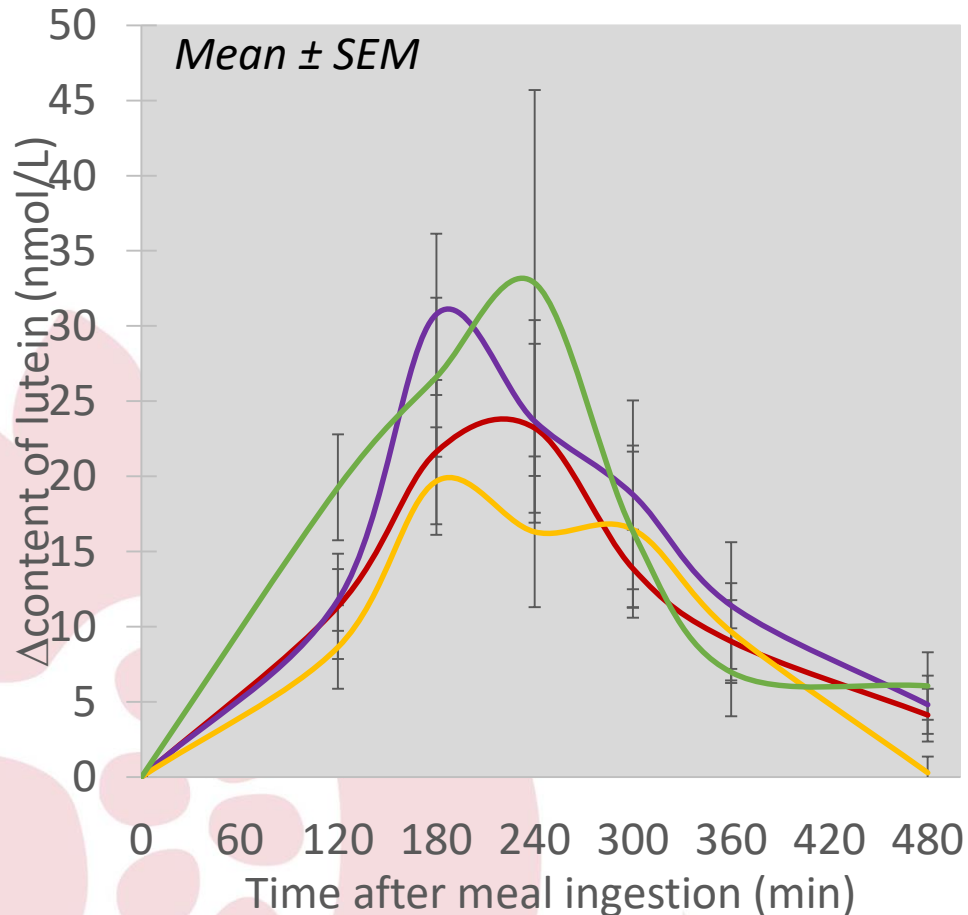
✓ Enriched in (per portion) :

- Lutein : 20 mg
- Vitamin D : 1,25 mg
- B9 : 1 mg
- B12 : 12 µg

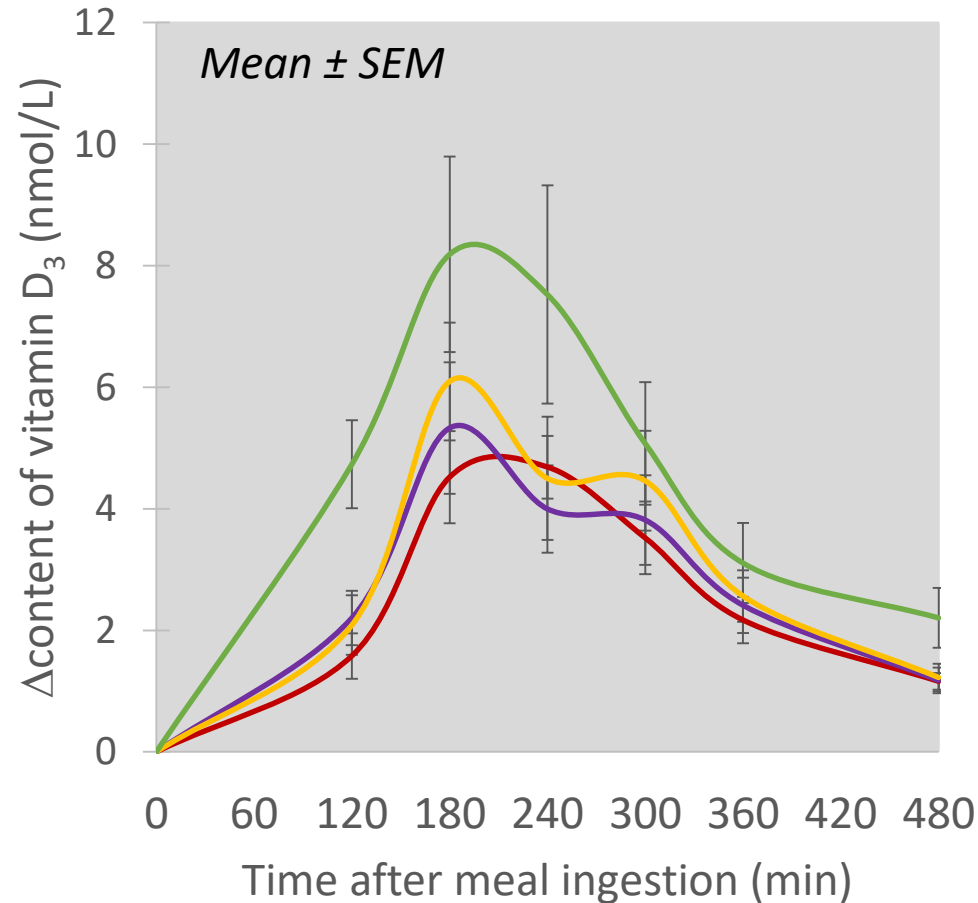
■ : lipids (Red Nile)
■ : proteins (Fast Green)

Results - clinical study

Lutein



Vitamin D

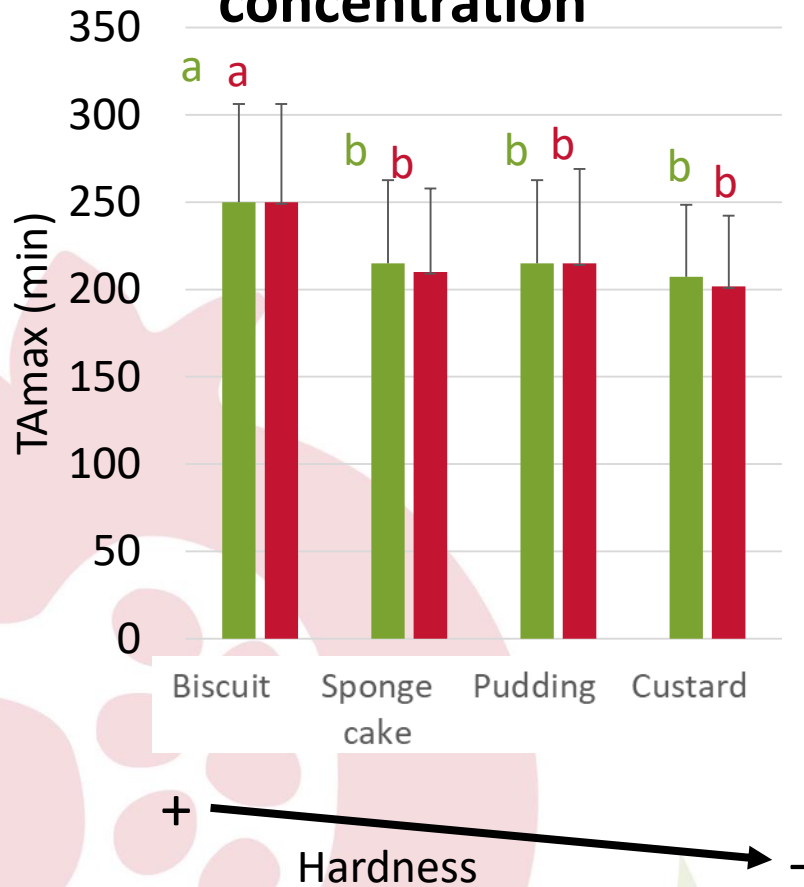


- Extraction of 2 parameters from the curves :
- T_{Am} : Time of maximal absorption
 - AUC : Area under the curve

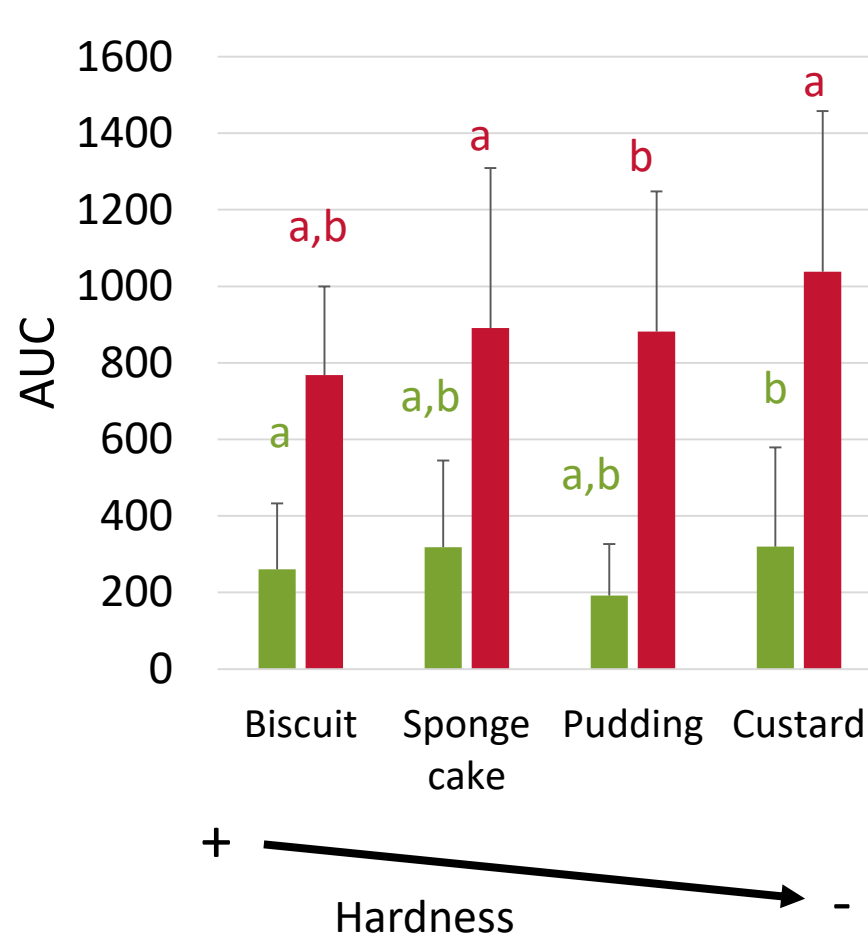
— Biscuit — Sponge cake — Pudding — Custard

Results - clinical study

Time of maximal concentration



Area under the curve



■ Vitamin D
■ Lutein

- ✓ Later T_{max} for harder texture
- ✓ Tendency to higher total bioavailability for softer matrices

Research question and strategy

HYPOTHESIS



Food structure impacts kinetics of matrix disintegration during digestion

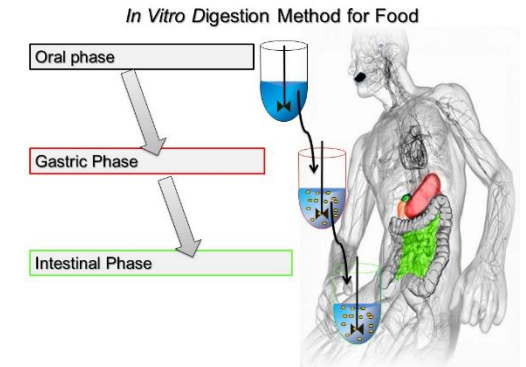
Can we explain *in vivo* digestion mechanisms by using an *in vitro* digestion model ?

Following INFOGEST *in vitro* protocol with special conditions :

- ✓ Use of human saliva in oral phase (mix 1:1)
- ✓ Constant water content between matrices (difference adjustment with water)

Focus point :

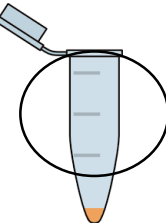
- ✓ Matrix disintegration
 - Proteolysis
 - Lipolysis
 - Amylolysis
- ✓ Release of micronutrients (lutein and vitamin B9)



(Minekus, 2014)

Digestion kinetics

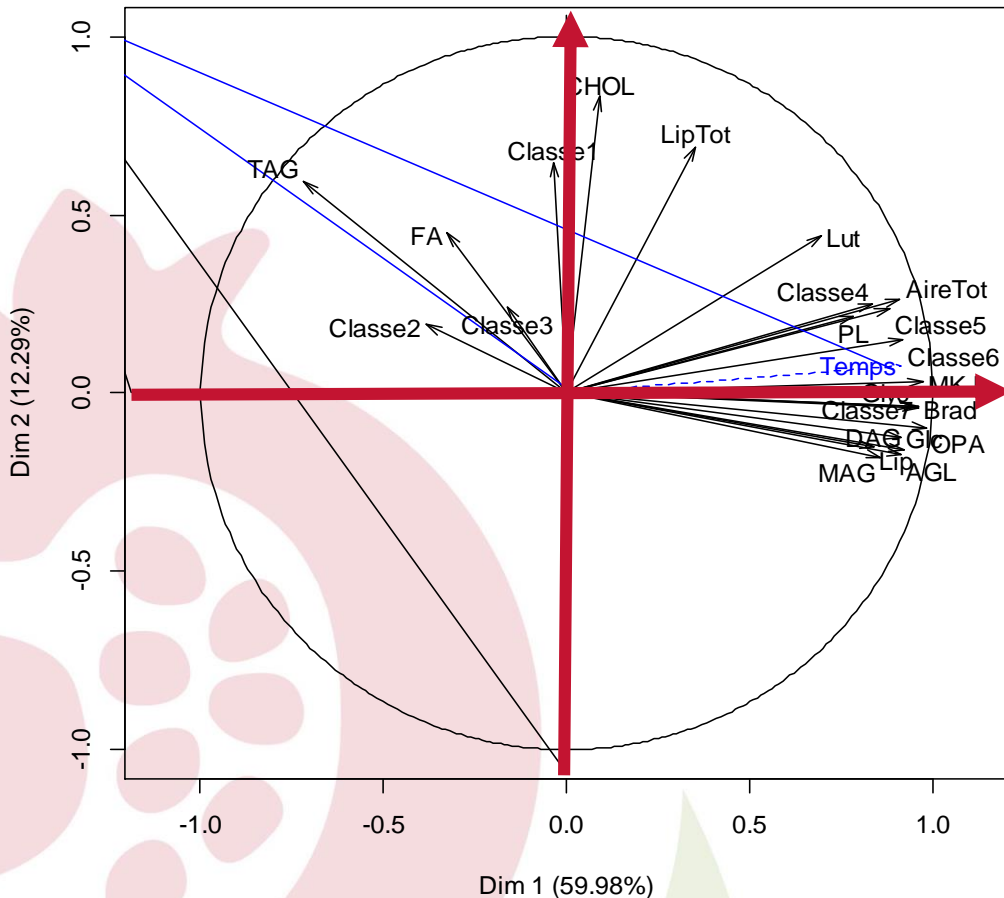
(S0, G0, G30, G120, I0, I30, I120)



Principal component analysis – graph of variables

Axis n°2 :

Solubilization of macronutrients
in the supernatant



Axis n°1 :
Hydrolysis of
macronutrients

Axis n°1 :

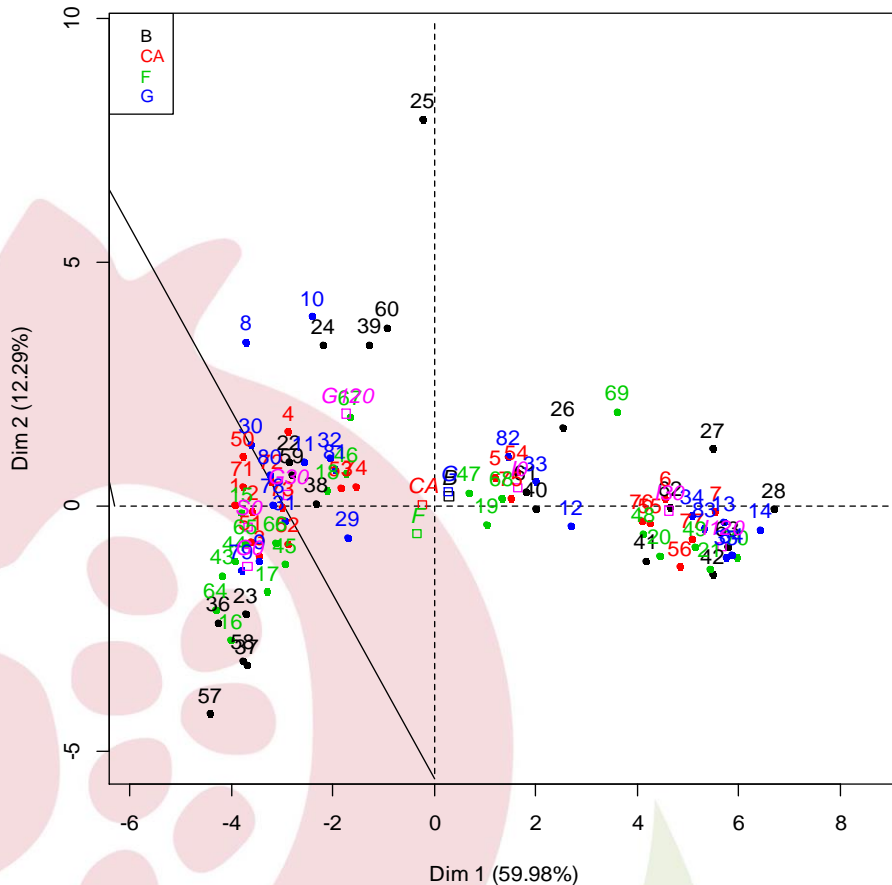
- Low molecular weight peptides (< 25 kDa) and free amino group content
- FFA, MAG and DAG
- Carbohydrates less than 10 glucose units equivalent
- **Hydrolysis of macronutrients**

Axis n°2 :

- High molecular weight peptides (> 100 kDa)
- Triglycerides and total lipids
- **Solubilization of macronutrients in the supernatant**

Principal component analysis – map of individuals

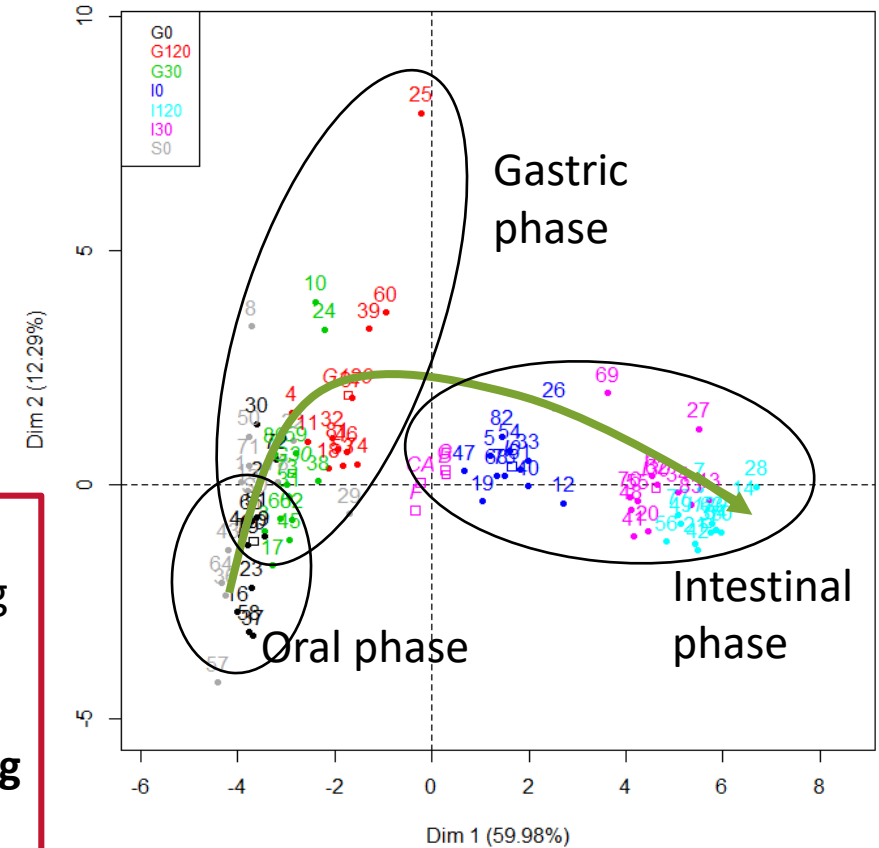
Axis n°2 :
Solubilization of macronutrients
in the supernatant



1°) Grouped by matrix : no matrix effect highlighted

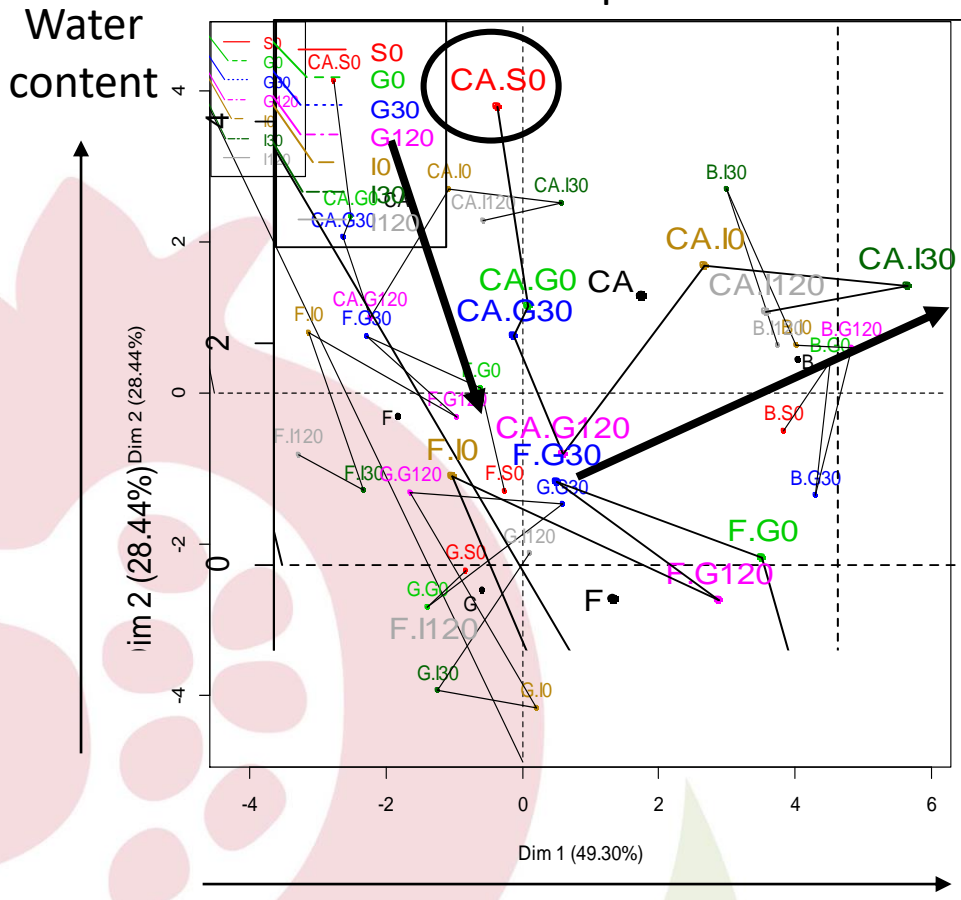
Axis n°1 :
Hydrolysis of macronutrients

2°) Grouped by digestion time : evidence of a strong "digestion time" effect on individuals
→ Samples split according to digestion time



Multiple factor analysis – map of individuals

Axis n°2 :
Solubilization of macronutrients
in the supernatant



Axis n°1 :
Hydrolysis of
macronutrients

Matrix effect highlighted by this analysis

- Axis n°1 isolates **biscuit**
- The **3 softer matrices** are separated along the **axis n°2**

Focus on **custard** behavior during *in vitro* digestion :

- Oral phase : macronutrients solubilized in the supernatant
- Gastric phase : drop in pH leads to macronutrients aggregation
- Intestinal phase : re-solubilization and hydrolysis of macronutrients

Matrix of Pearson correlations – p-values

	AGL	> 100 kDa	50 kDa	25 kDa	10 kDa	5 kDa	1 kDa	< 1 kDa	DAG	B9	Glc	Glyc	Lip	Lut	MAG	MKJ	OPA	TAG
AGL		0.1752	0.0505	0.6405	<.0001	<.0001	<.0001	<.0001	<.0001	0.0131	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
> 100 kDa	0.1752		0.7055	0.4193	0.2575	0.1624	0.2762	0.5820	0.1630	0.0032	0.0627	0.4111	0.3951	0.0758	0.2104	0.5493	0.1655	0.0103
50 kDa	0.0505	0.7055		<.0001	0.2164	0.6387	<.0001	<.0001	0.0923	0.6511	0.1283	0.0338	0.0200	0.1880	0.1097	0.0260	0.0166	0.0162
25 kDa	0.6405	0.4193	<.0001		0.4850	0.0662	0.0197	0.0117	0.9289	0.5243	0.9186	0.7120	0.2819	0.6995	0.8595	0.6308	0.4731	0.4055
10 kDa	<.0001	0.2575	0.2164	0.4850		<.0001	<.0001	<.0001	<.0001	0.1846	<.0001	<.0001	0.0002	<.0001	0.0007	<.0001	<.0001	0.0057
5 kDa	<.0001	0.1624	0.6387	0.0662	<.0001		<.0001	<.0001	<.0001	0.2743	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0002
1 kDa	<.0001	0.2762	<.0001	0.0197	<.0001	<.0001		<.0001	<.0001	0.3762	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
< 1 kDa	<.0001	0.5820	<.0001	0.0117	<.0001	<.0001	<.0001		<.0001	0.0172	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
DAG	<.0001	0.1630	0.0923	0.9289	<.0001	<.0001	<.0001	<.0001		0.0074	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
B9	0.0131	0.0032	0.6511	0.5243	0.1846	0.2743	0.3762	0.0172	0.0074		0.0278	0.0240	0.0325	0.1914	0.0385	0.0308	0.0034	0.0017
Glc	<.0001	0.0627	0.1283	0.9186	<.0001	<.0001	<.0001	<.0001	<.0001	0.0278		<.0001	<.0001	0.0001	<.0001	<.0001	<.0001	<.0001
Glyc	<.0001	0.4111	0.0338	0.7120	<.0001	<.0001	<.0001	<.0001	<.0001	0.0240	<.0001		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Lip	<.0001	0.3951	0.0200	0.2819	0.0002	<.0001	<.0001	<.0001	<.0001	0.0325	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001	<.0001
Lut	<.0001	0.0758	0.1880	0.6995	<.0001	<.0001	<.0001	<.0001	<.0001	0.1914	0.0001	<.0001	<.0001		0.0005	<.0001	<.0001	0.1704
MAG	<.0001	0.2104	0.1097	0.8595	0.0007	<.0001	<.0001	<.0001	<.0001	0.0385	<.0001	<.0001	<.0001	0.0005		<.0001	<.0001	<.0001
MKJ	<.0001	0.5493	0.0260	0.6308	<.0001	<.0001	<.0001	<.0001	<.0001	0.0308	<.0001	<.0001	<.0001	<.0001	<.0001		<.0001	<.0001
OPA	<.0001	0.1655	0.0166	0.4731	<.0001	<.0001	<.0001	<.0001	<.0001	0.0034	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		<.0001
TAG	<.0001	0.0103	0.0162	0.4055	0.0057	0.0002	<.0001	<.0001	<.0001	0.0017	<.0001	<.0001	<.0001	0.1704	<.0001	<.0001	<.0001	<.0001

Matrix of Pearson correlations – p-values

	Lipids in supernatant		Lipolysis				Proteins in supernatant		Proteolysis				Amylolysis					
	TAG	Total lipids	FFA	DAG	MAG	FFA/tot lip	Total nitrogen	> 100 kDa	50 kDa	25 kDa	10 kDa	5 kDa	1 kDa	< 1 kDa	Free amino	Glc	B9	Lut
B9	0.0017	0.0240	0.0131	0.0074	0.0385	0.0325	0.0308	0.0032	0.6511	0.5243	0.1846	0.2743	0.3762	0.0172	0.0034	0.0278		0.1914
Lut	0.1704	<.0001	<.0001	<.0001	0.0005	<.0001	<.0001	0.0758	0.1880	0.6995	<.0001	<.0001	<.0001	<.0001	<.0001	0.0001	0.1914	

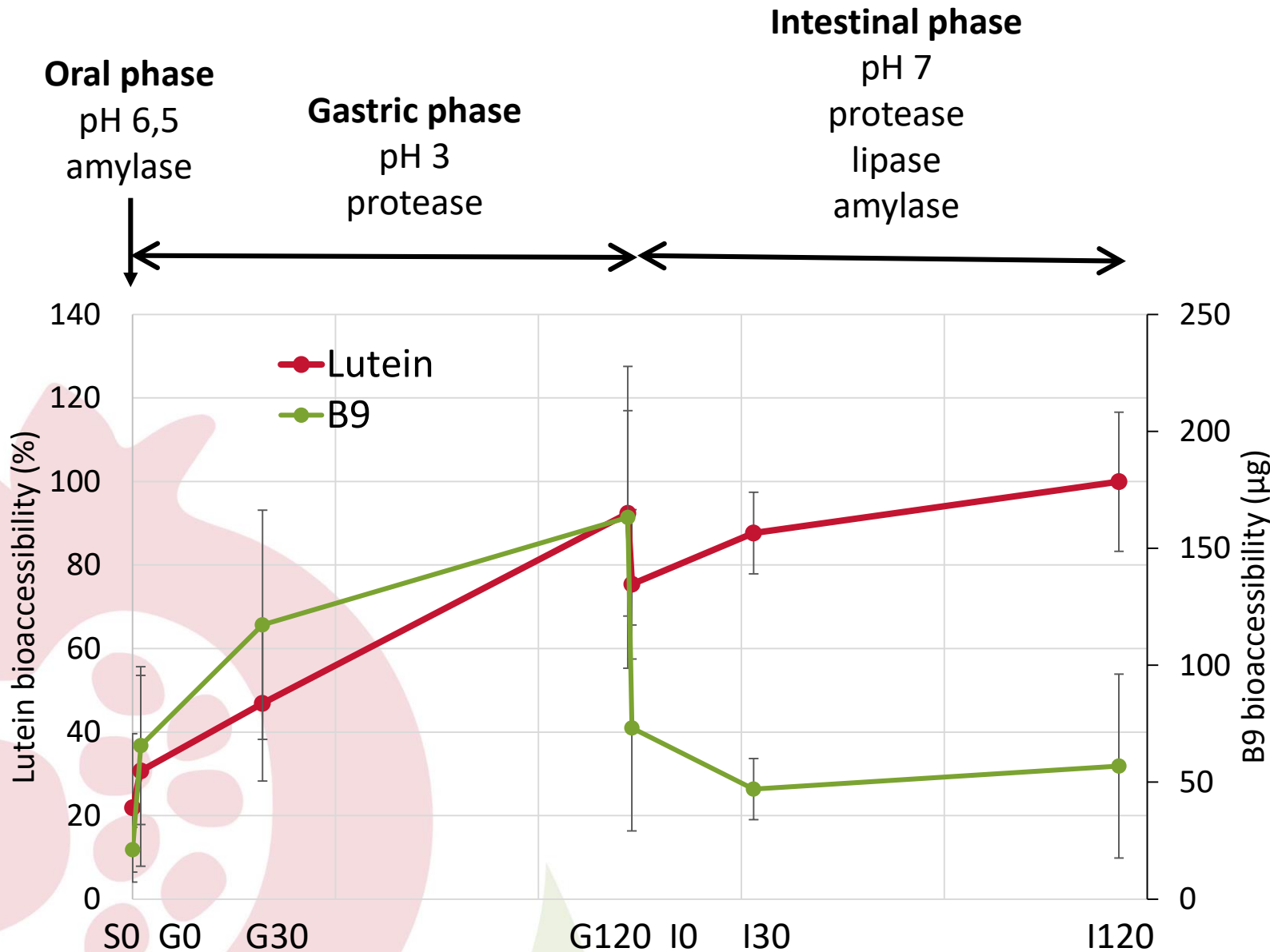
Vitamin B9 correlates with :

- Solubilization of macronutrients (TAG, total lipids, high weight class of proteins)
- Markers of matrix disintegration (lipolysis, proteolysis, amylolysis)

Lutein correlates with :

- Markers of matrix disintegration (lipolysis, proteolysis, amylolysis)

Hydrolysis of all macronutrient classes has an impact on micronutrients release from the matrix
→ The key factor of **micronutrients bioaccessibility** seems to be **matrix disintegration**



- ✓ Lutein is released continuously during digestion
→ driven by proteolysis ?
- ✓ Release of B9 mainly during gastric step: impacted by solubilization of macronutrient
- ✓ Drop in B9 bioaccessibility in intestinal phase : low solubility at neutral pH

Conclusion and perspectives

HYPOTHESIS



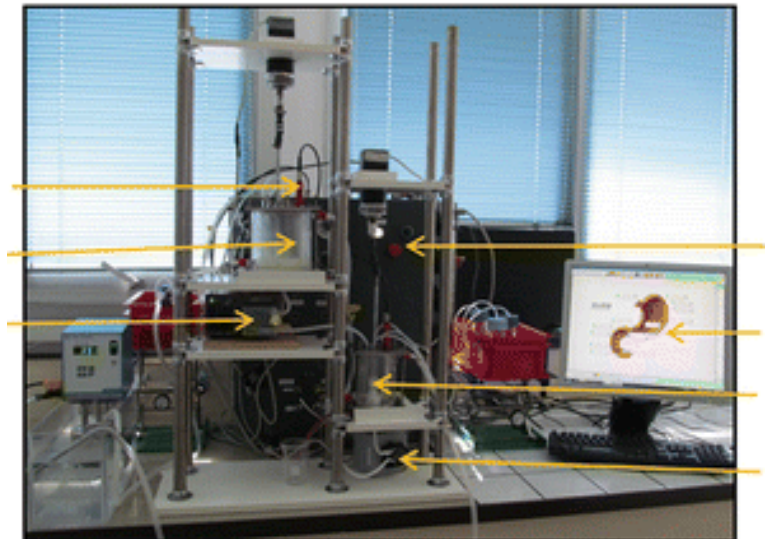
Food structure impacts kinetics of matrix disintegration during digestion



Food structure has an impact on matrix behaviour during *in vitro* digestion

✓ Is the structure solely involved in bioavailability differences observed *in vivo*?

→ ***In vitro* dynamic study** using the **DIDGI** to better mimic physiological responses during digestion (gastric emptying, progressive addition of enzymes, etc.)





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**Thank you
for your
attention**



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Poster n°378/214, session 2