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

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6<sup>th</sup> INTERNATIONAL  
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GRANADA 2019  
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DE GRANADA



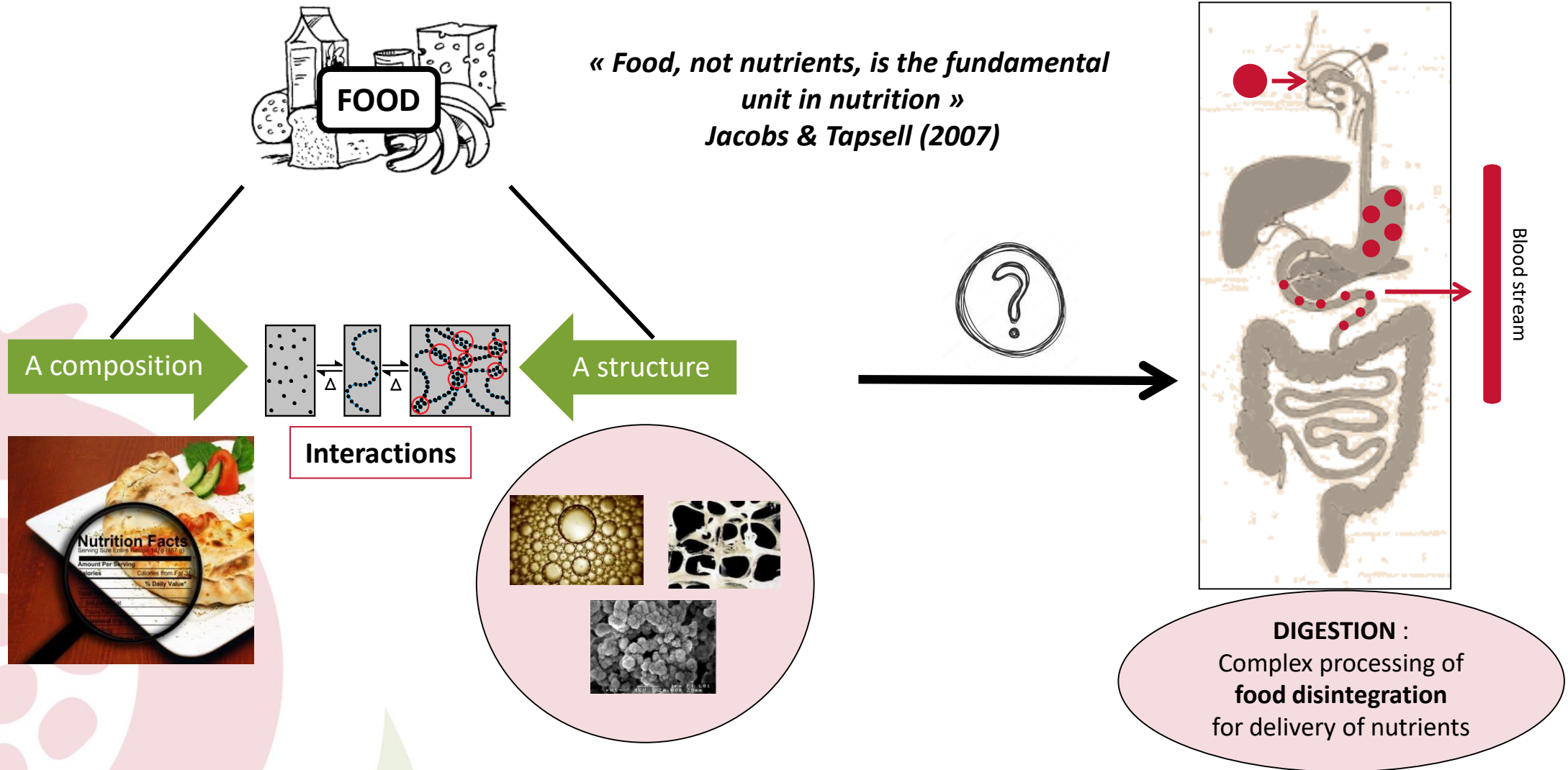
GRANADA  
CIENCIA E INNOVACIÓN

INRA  
SCIENCE & IMPACT

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

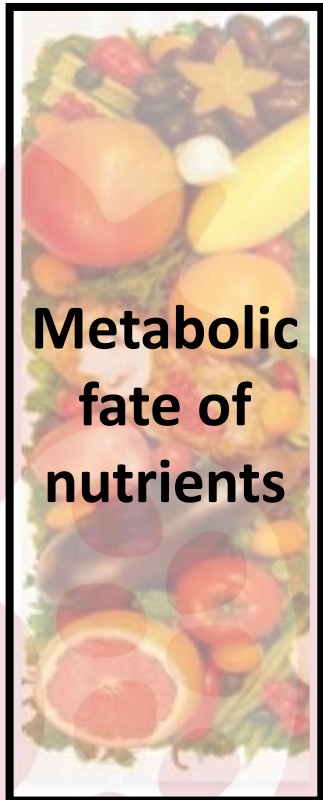
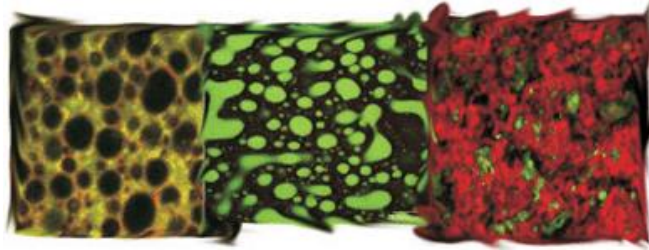
Speaker : Manon Hiolle

# Nutritional quality : beyond food composition



# Nutritional quality : beyond food composition

Food structure



**Metabolic fate of nutrients**

Bioaccessibility

Bioavailability

Kinetics

Metabolic responses

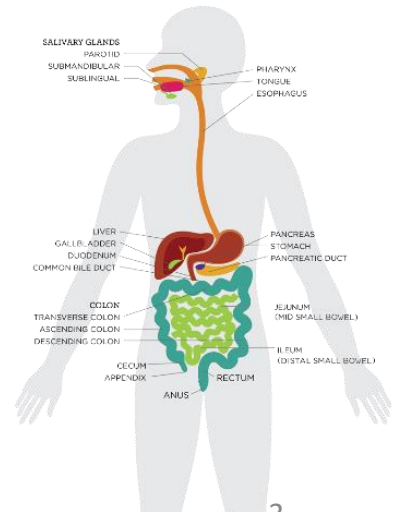
Macronutrients :

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



## 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

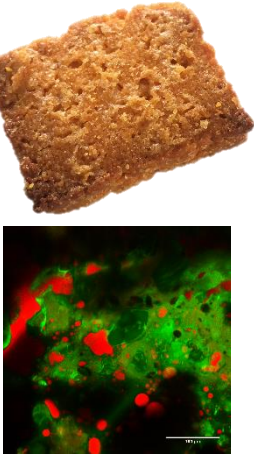
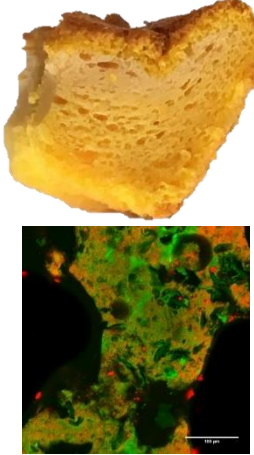

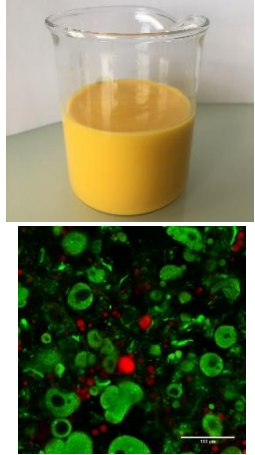
Results for B9 : poster n°378/214, session 2

## Products characterization

- 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

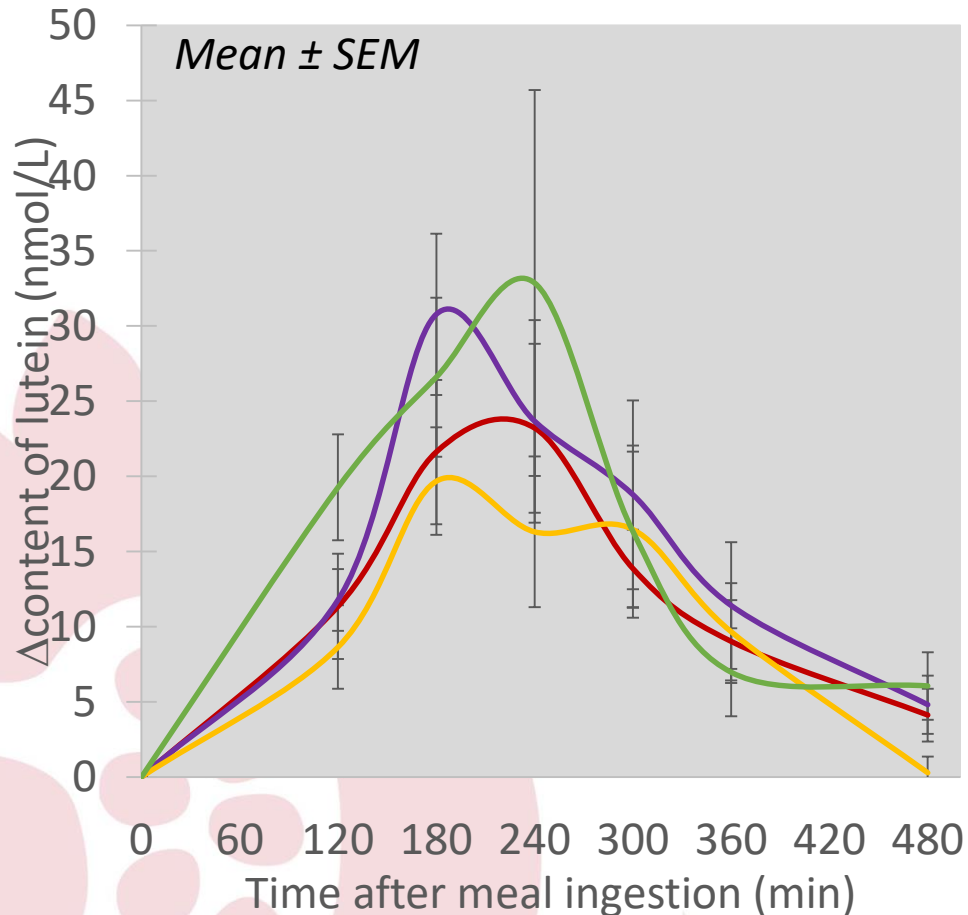
✓ Enriched in (per portion) :

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

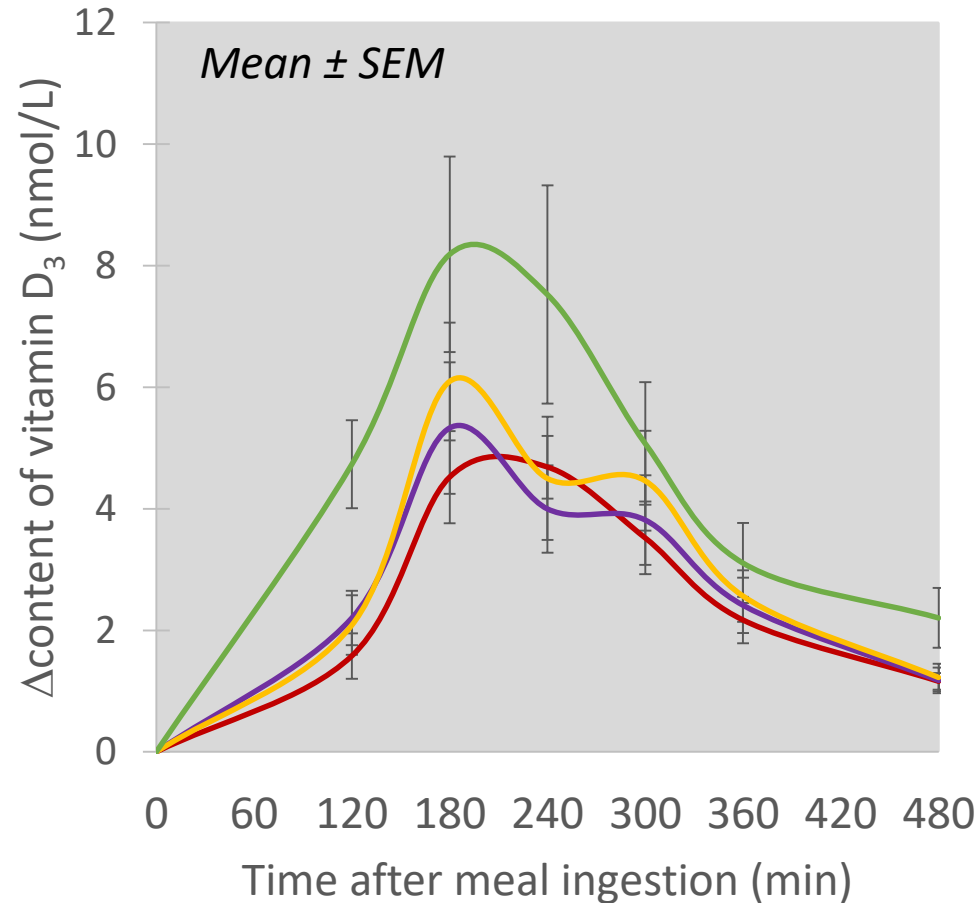
■ : lipids (Red Nile)  
■ : proteins (Fast Green)

# Results - clinical study

## Lutein



## Vitamin D

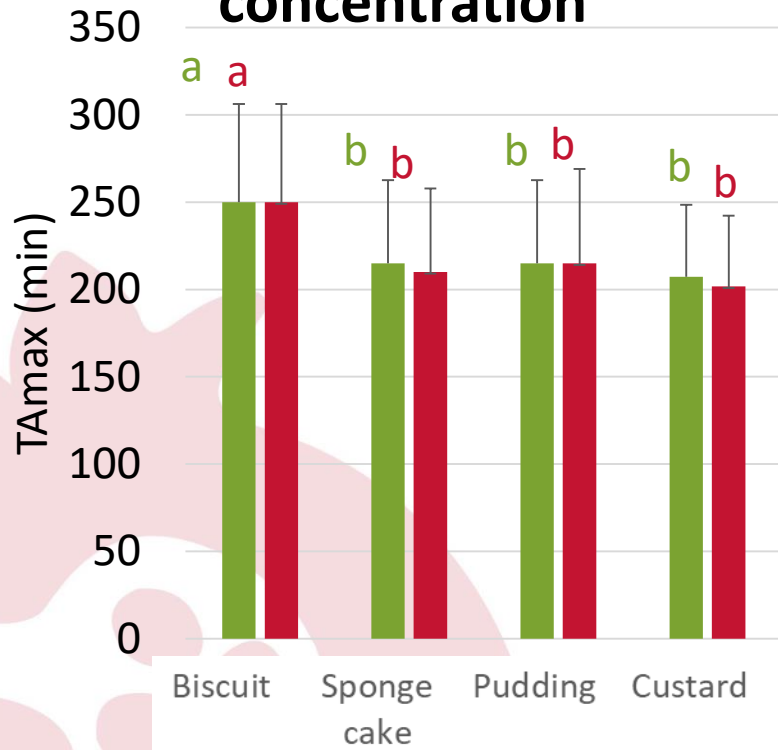


- Extraction of 2 parameters from the curves :
- T<sub>Am</sub> : Time of maximal absorption
  - AUC : Area under the curve

— Biscuit — Sponge cake — Pudding — Custard

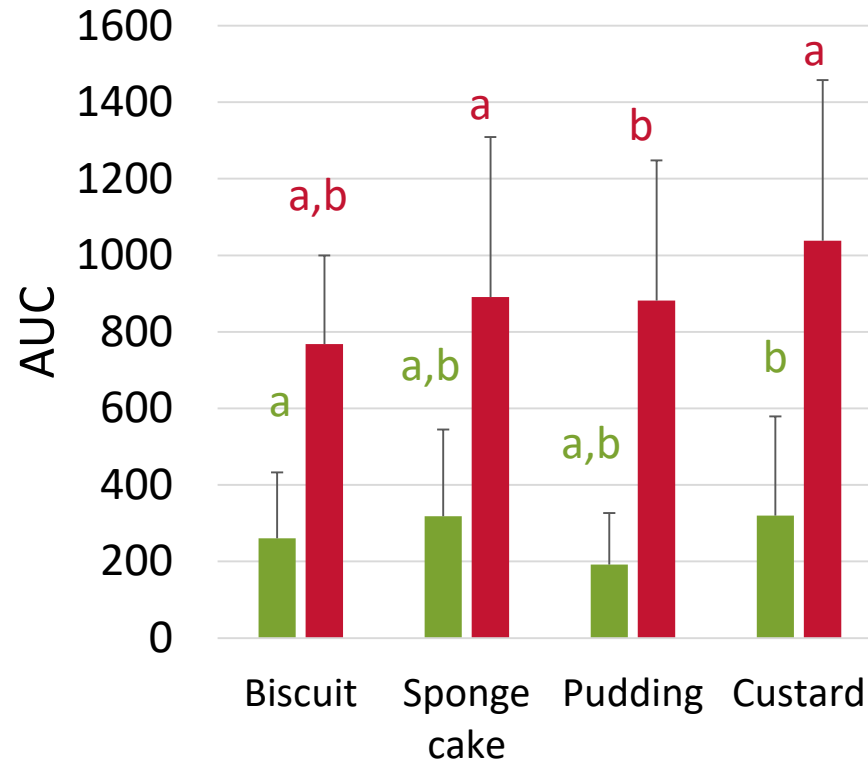
# Results - clinical study

## Time of maximal concentration



+ -  
Hardness

## Area under the curve



+ -  
Hardness

■ Vitamin D  
■ Lutein

- ✓ Later T<sub>max</sub> 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

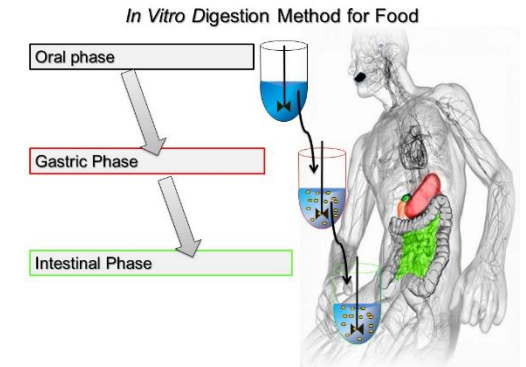
**Can we explain *in vivo* digestion mechanisms by using an *in vitro* digestion model ?**

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 :

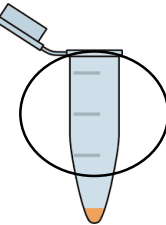
- ✓ Matrix disintegration
  - Proteolysis
  - Lipolysis
  - Amylolysis
- ✓ Release of micronutrients (lutein and vitamin B9)



(Minekus, 2014)

Digestion kinetics

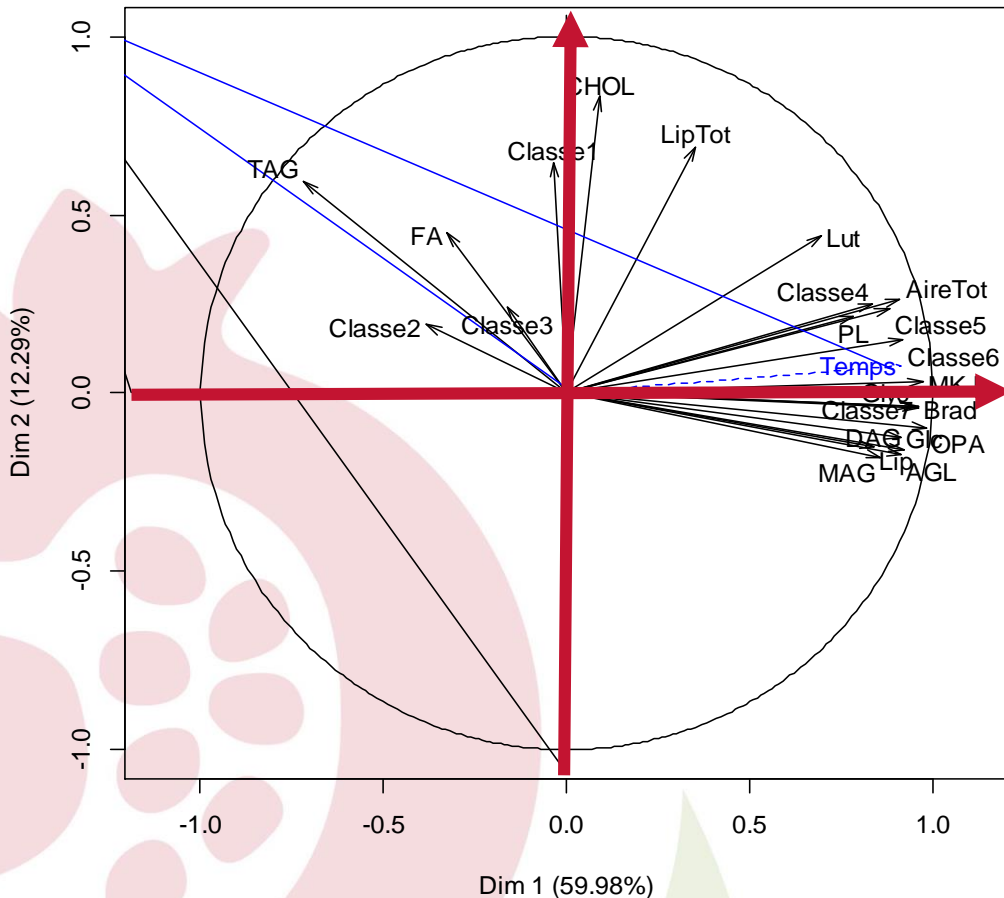
(S0, G0, G30, G120, I0, I30, I120)



# Principal component analysis – graph of variables

Axis n°2 :

Solubilization of macronutrients  
in the supernatant



Axis n°1 :  
Hydrolysis of  
macronutrients

Axis n°1 :

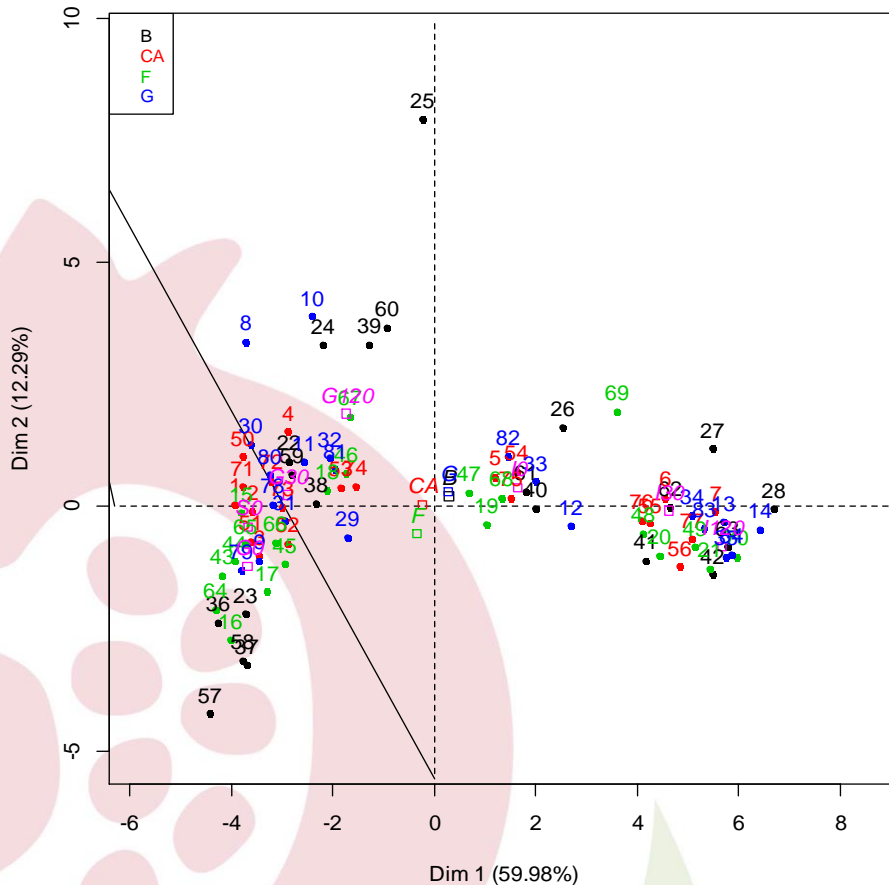
- Low molecular weight peptides (< 25 kDa) and free amino group content
- FFA, MAG and DAG
- Carbohydrates less than 10 glucose units equivalent
- **Hydrolysis of macronutrients**

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

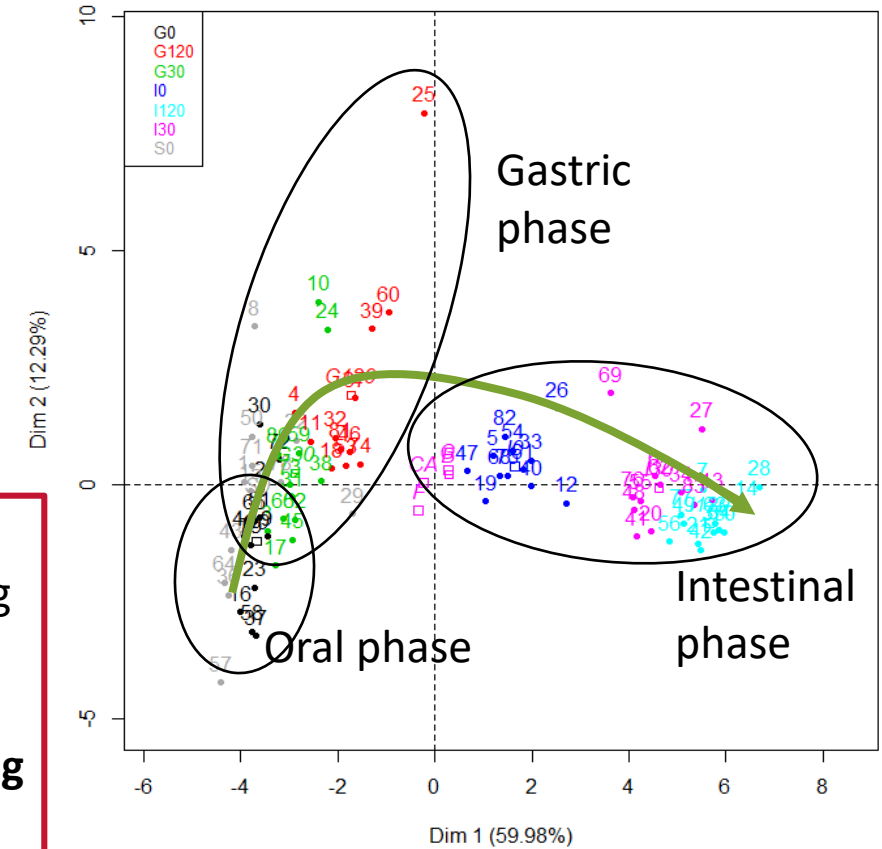
Axis n°2 :  
Solubilization of macronutrients  
in the supernatant



1°) Grouped by matrix : no matrix effect highlighted

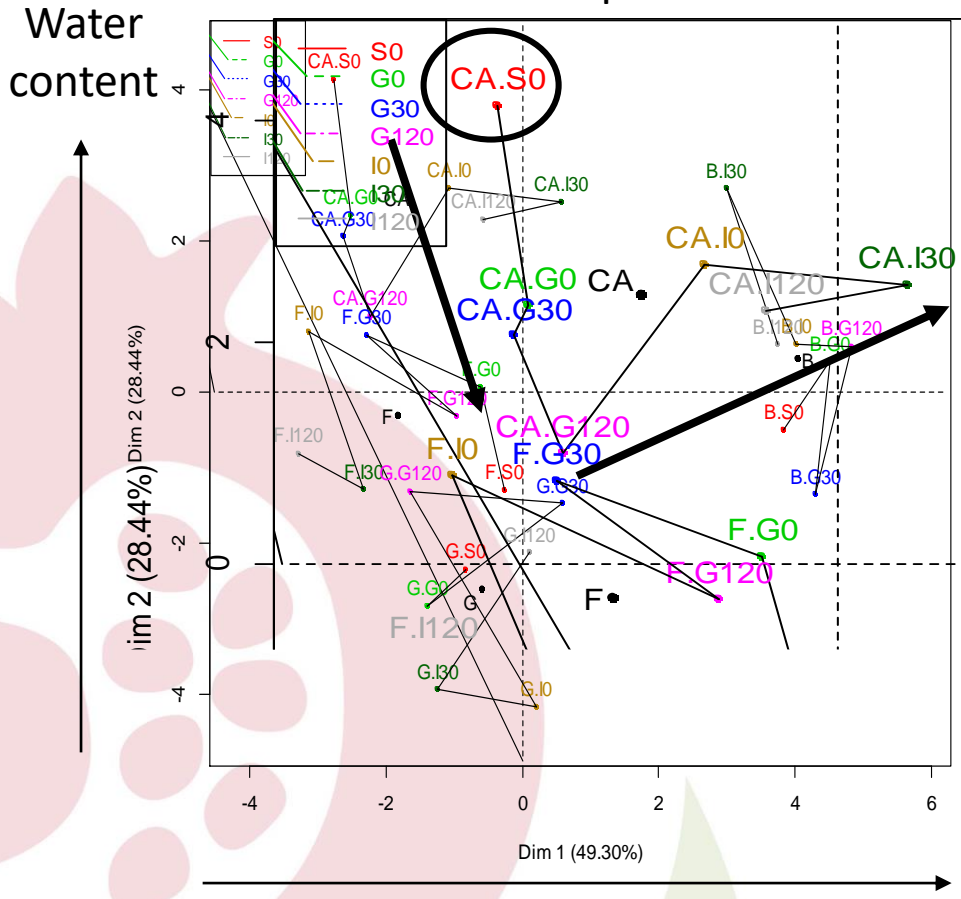
Axis n°1 :  
Hydrolysis of macronutrients

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
<b>B9</b>	<b>0.0131</b>	<b>0.0032</b>	<b>0.6511</b>	<b>0.5243</b>	<b>0.1846</b>	<b>0.2743</b>	<b>0.3762</b>	<b>0.0172</b>	<b>0.0074</b>		<b>0.0278</b>	<b>0.0240</b>	<b>0.0325</b>	<b>0.1914</b>	<b>0.0385</b>	<b>0.0308</b>	<b>0.0034</b>	<b>0.0017</b>
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
<b>Lut</b>	<b>&lt;.0001</b>	<b>0.0758</b>	<b>0.1880</b>	<b>0.6995</b>	<b>&lt;.0001</b>	<b>&lt;.0001</b>	<b>&lt;.0001</b>	<b>&lt;.0001</b>	<b>&lt;.0001</b>	<b>0.1914</b>	<b>0.0001</b>	<b>&lt;.0001</b>	<b>&lt;.0001</b>		<b>0.0005</b>	<b>&lt;.0001</b>	<b>&lt;.0001</b>	<b>0.1704</b>
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	

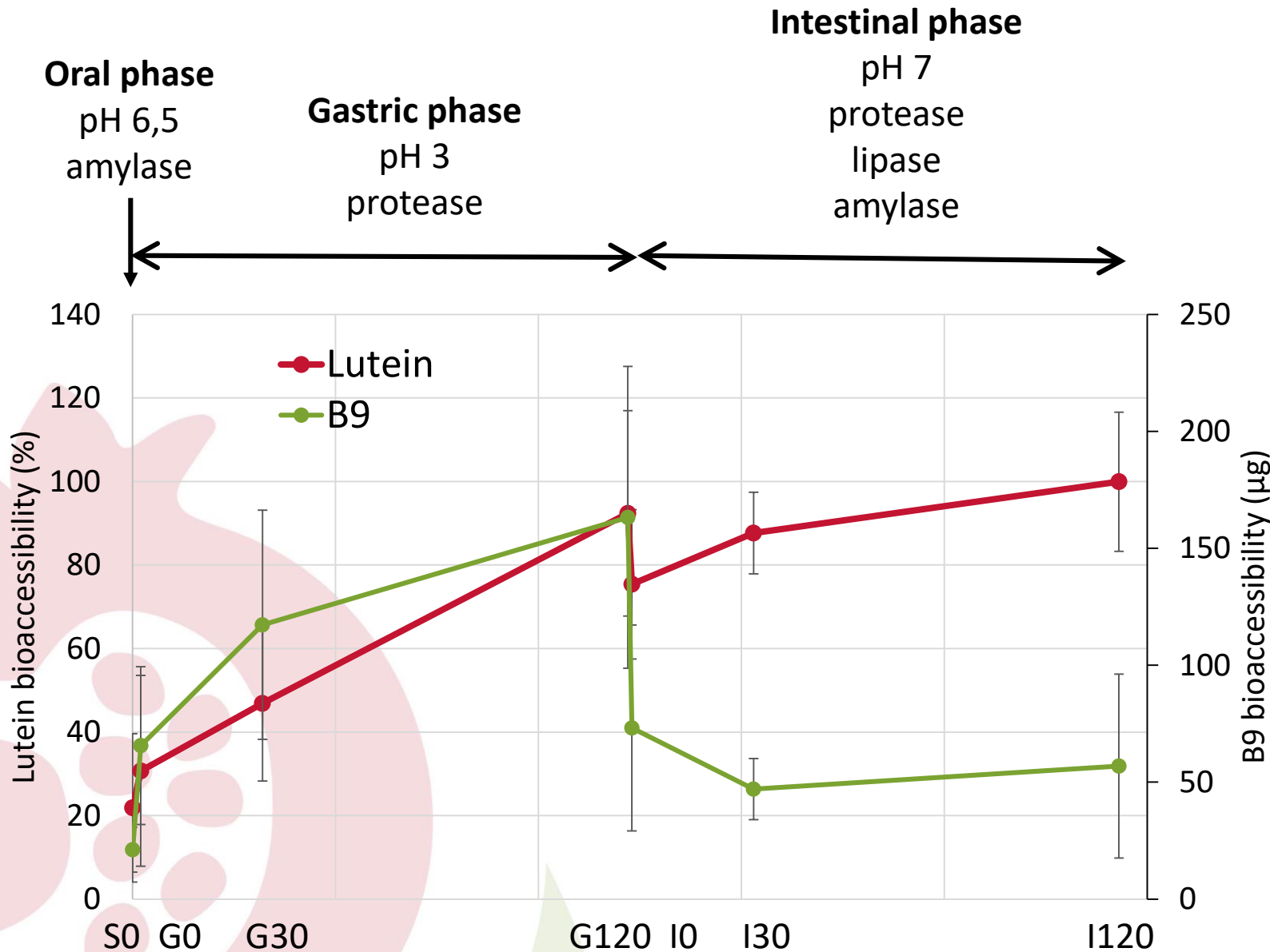
Vitamin B9 correlates with :

- 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



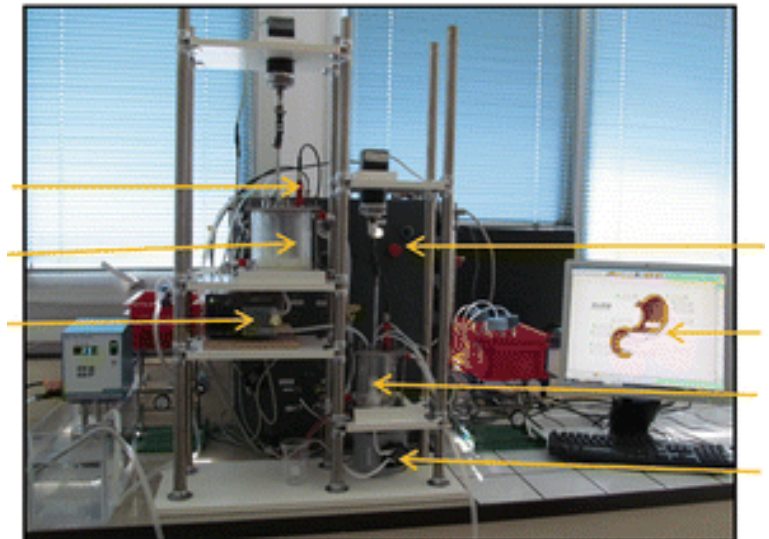
Food structure impacts kinetics of matrix disintegration during digestion



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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Isabelle Savary-Auzeloux  
Caroline Buffière

**Thank you  
for your  
attention**



Nathalie Meunier  
Ruddy Richard



Bruno Pereira



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Olivia Menard  
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Poster n°378/214, session 2