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

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► To cite this version:

Manon Hiolle, Beatrice Gleize, N. Meunier, B. Pereira, R. Richard, et al.. Investigating in vitro digestion of food models to explain in vivo micronutrient bioavailability. 6.International Conference on Food Digestion, Apr 2019, Grenade, Spain. , 2019. hal-02100429

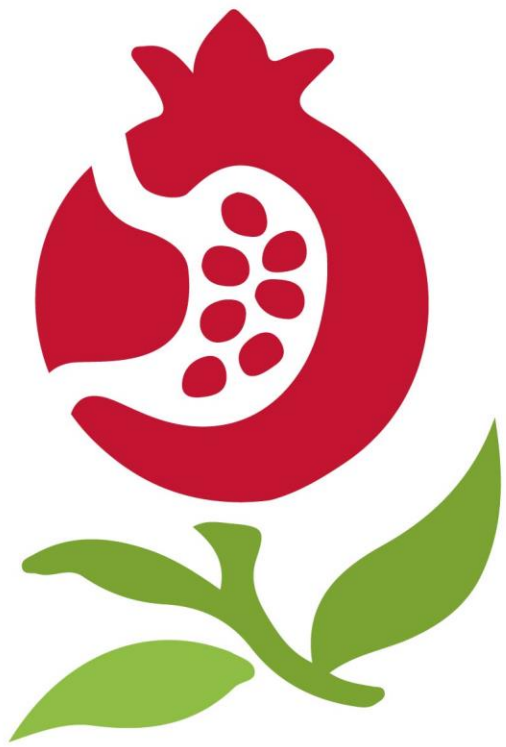
HAL Id: hal-02100429

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Submitted on 2 Jun 2020

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6th INTERNATIONAL
CONFERENCE
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GRANADA 2019
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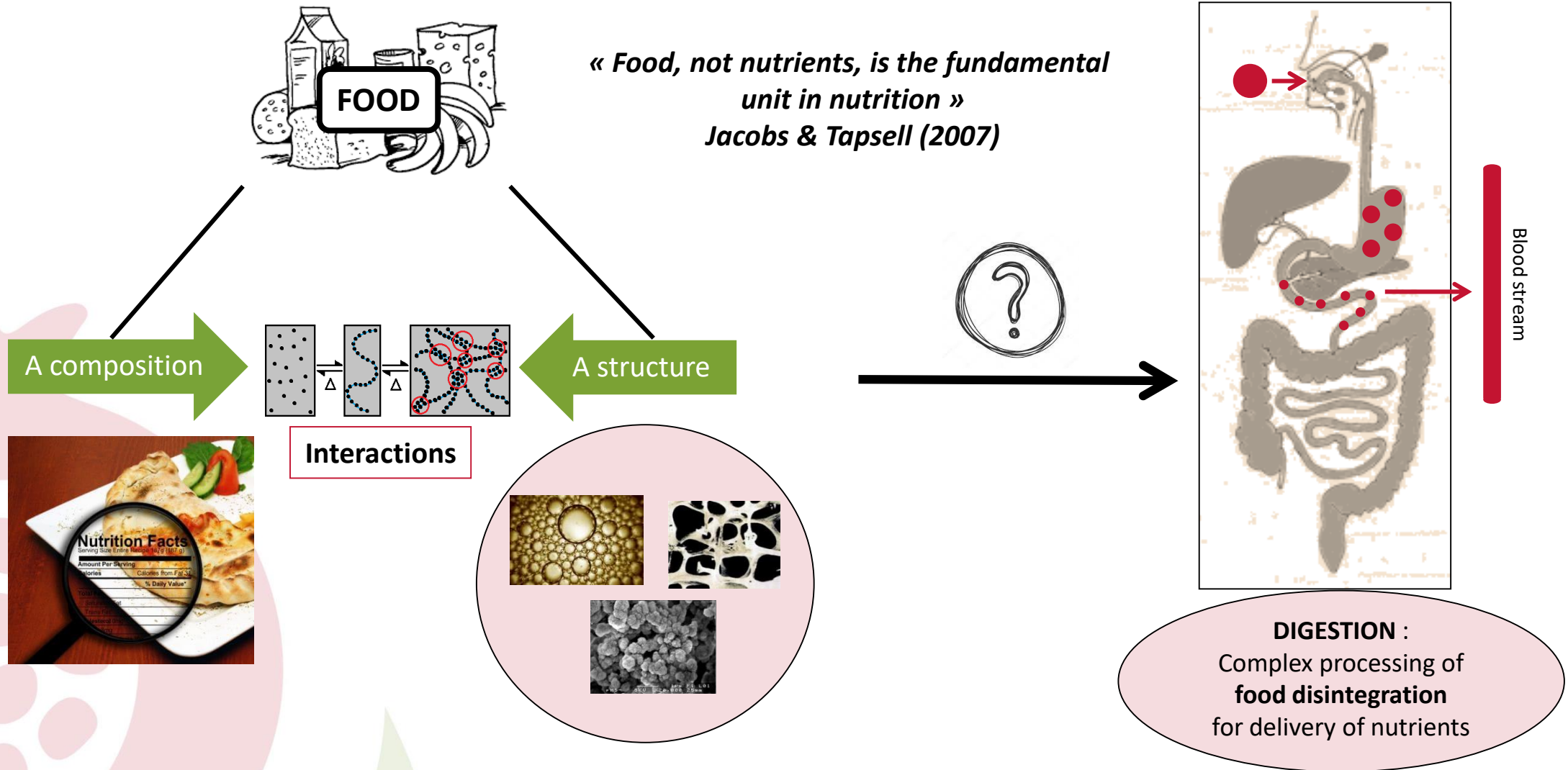
GRANADA
CIENCIA E INNOVACIÓN

INRA
SCIENCE & IMPACT

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

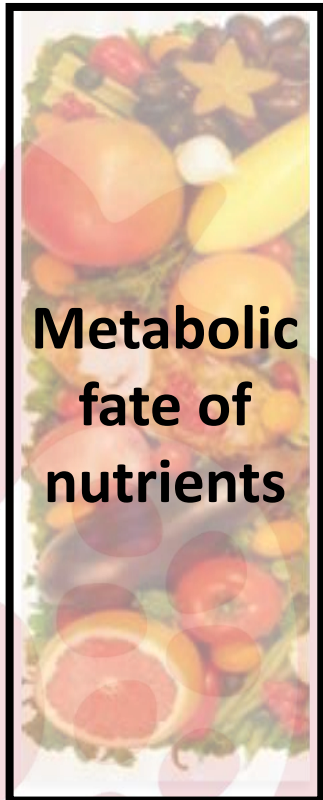
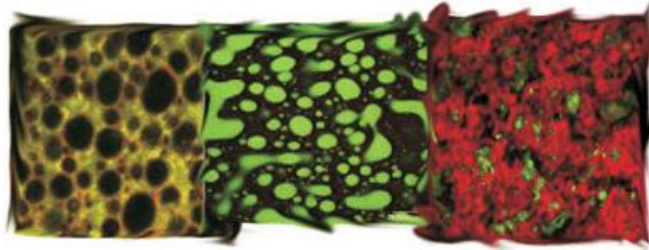
Speaker : Manon Hiolle

Nutritional quality : beyond food composition



Nutritional quality : beyond food composition

Food structure



Metabolic fate of nutrients

Bioaccessibility

Bioavailability

Kinetics

Metabolic responses

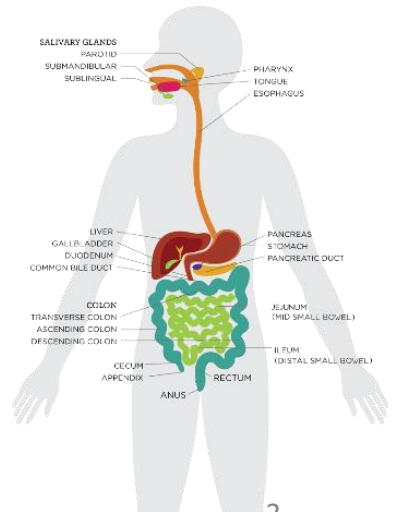
Macronutrients :

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



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

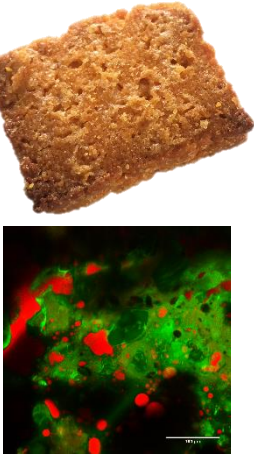
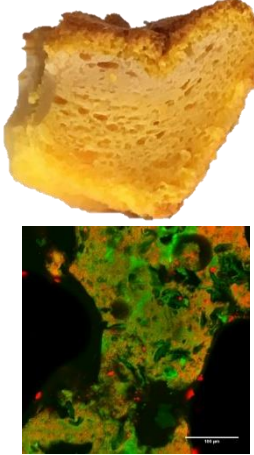

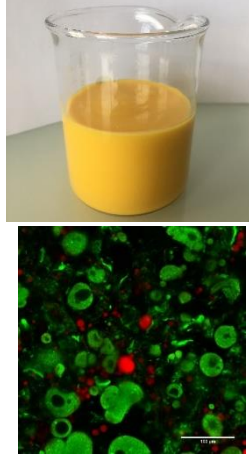
- Absorption kinetics: quantitative analysis of micronutrients in plasma or chylomicrons

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

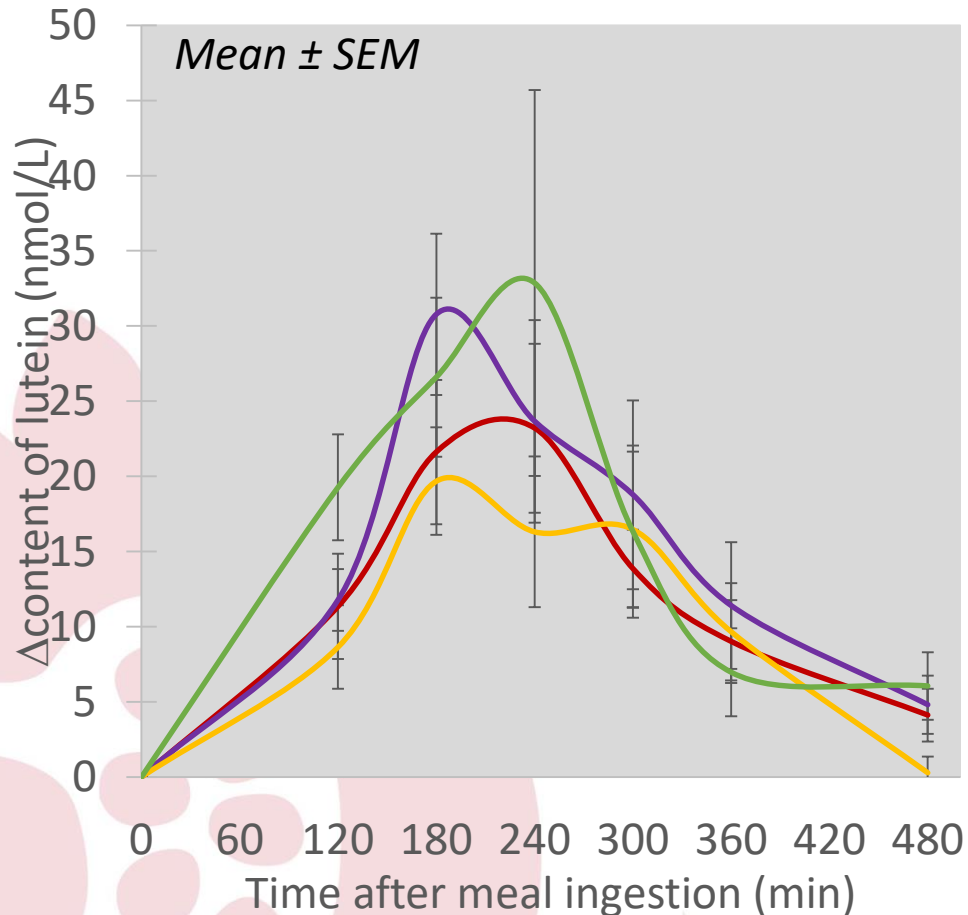
✓ Enriched in (per portion) :

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

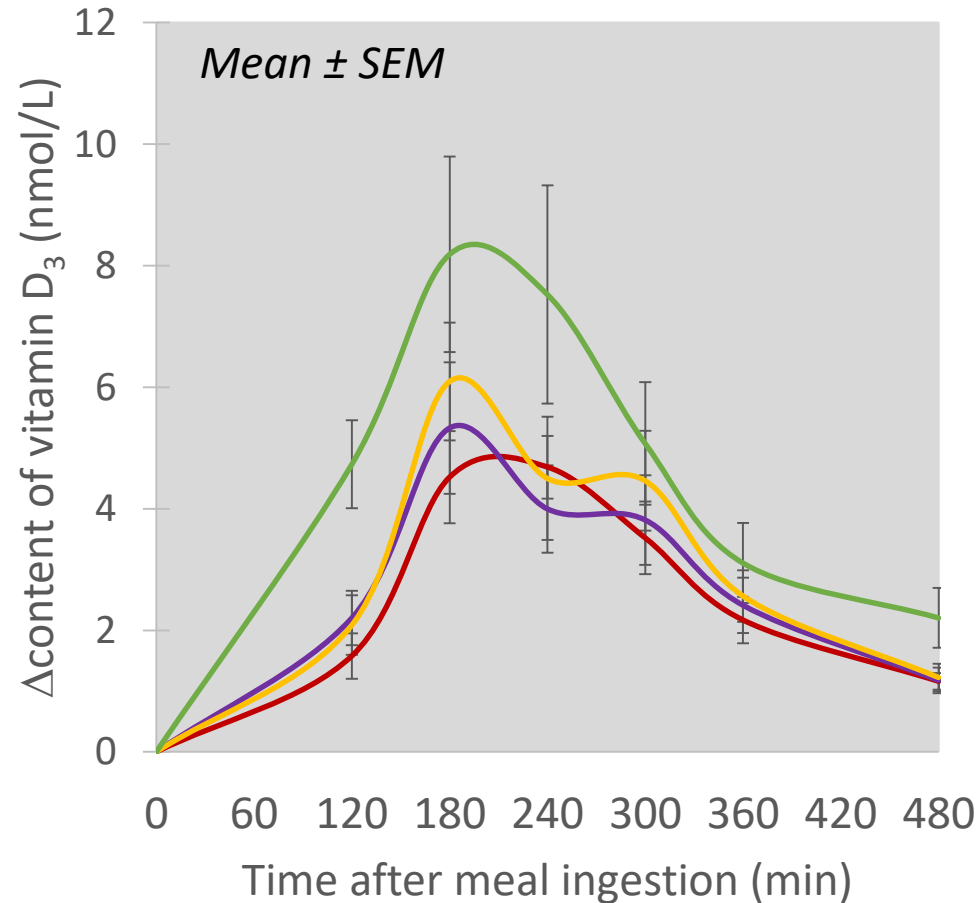
■ : lipids (Red Nile)
■ : proteins (Fast Green)

Results - clinical study

Lutein



Vitamin D

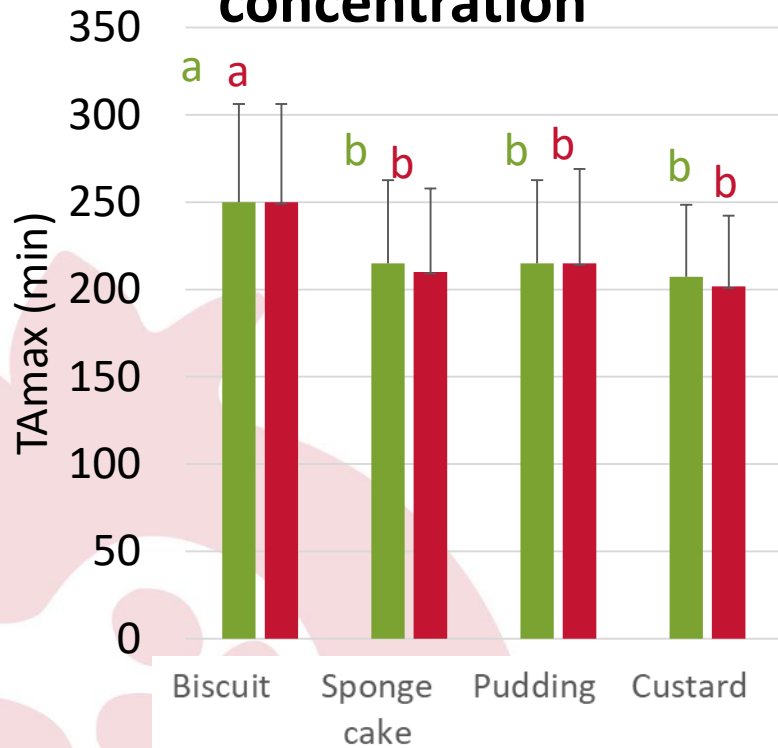


- Extraction of 2 parameters from the curves :
- T_{Am} : Time of maximal absorption
 - AUC : Area under the curve

— Biscuit — Sponge cake — Pudding — Custard

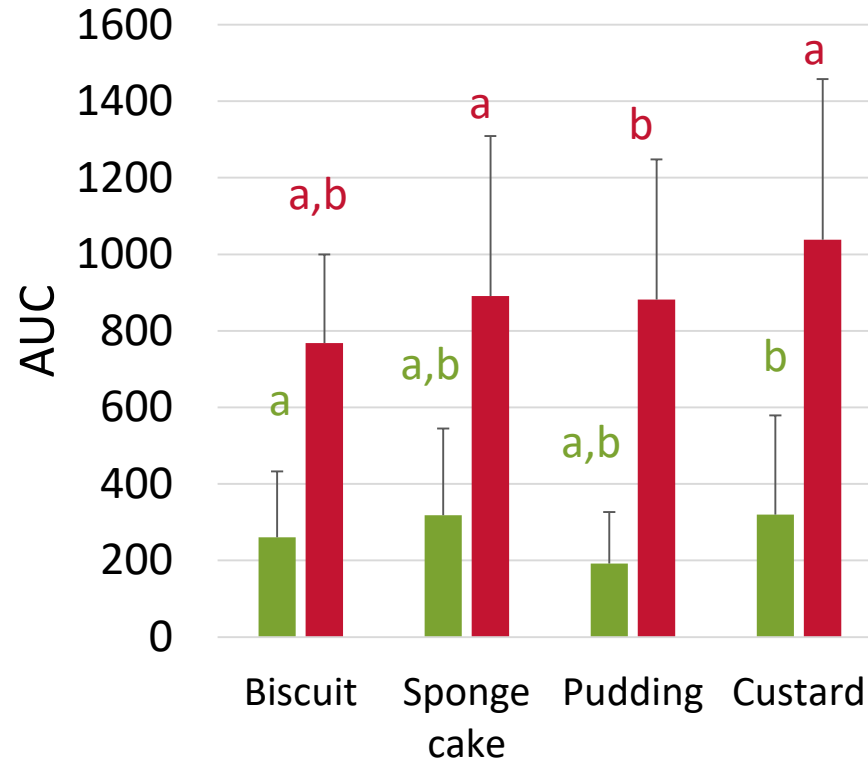
Results - clinical study

Time of maximal concentration



+ -
Hardness

Area under the curve



+ -
Hardness

■ Vitamin D
■ Lutein

- ✓ Later T_{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

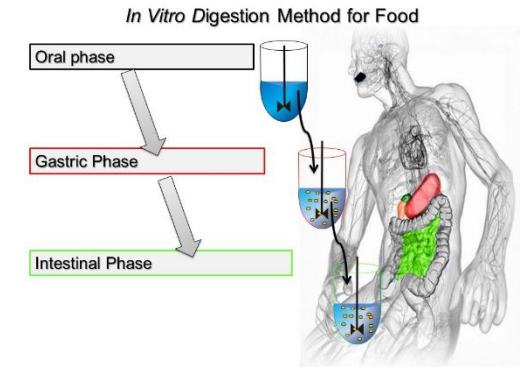
Can we explain *in vivo* digestion mechanisms by using an *in vitro* digestion model ?

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 :

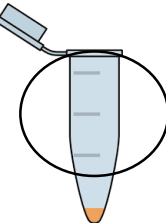
- ✓ Matrix disintegration
 - Proteolysis
 - Lipolysis
 - Amylolysis
- ✓ Release of micronutrients (lutein and vitamin B9)



(Minekus, 2014)

Digestion kinetics

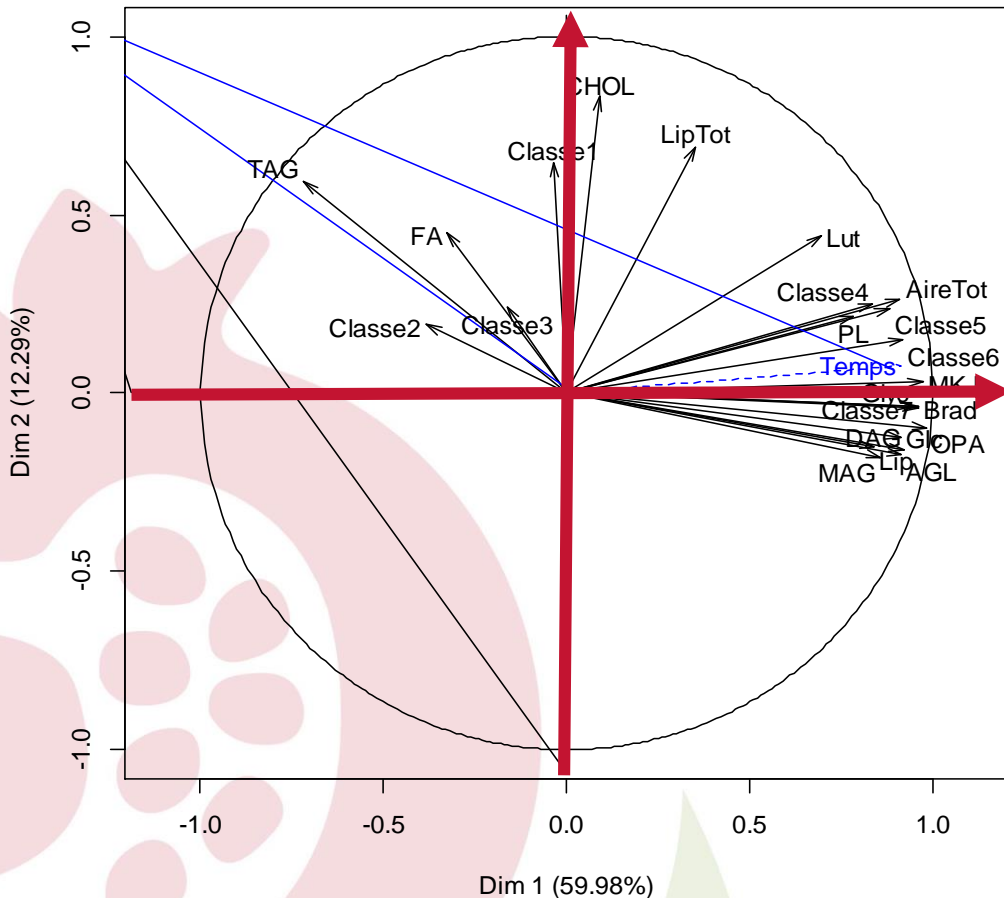
(S0, G0, G30, G120, I0, I30, I120)



Principal component analysis – graph of variables

Axis n°2 :

Solubilization of macronutrients
in the supernatant



Axis n°1 :
Hydrolysis of
macronutrients

Axis n°1 :

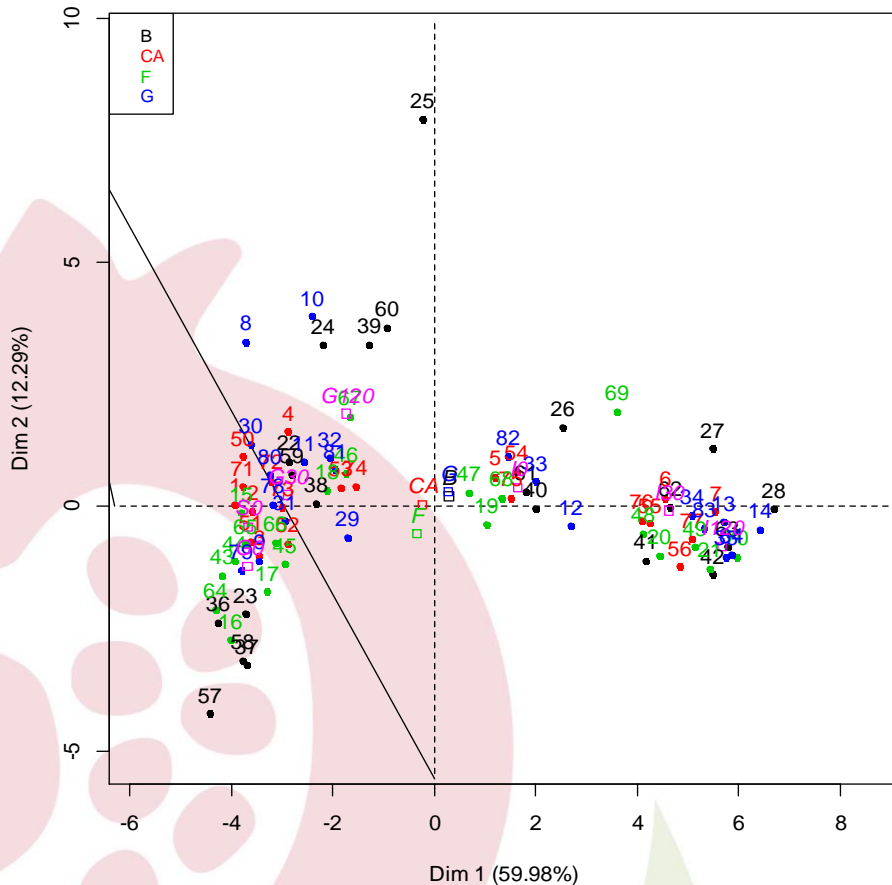
- Low molecular weight peptides (< 25 kDa) and free amino group content
- FFA, MAG and DAG
- Carbohydrates less than 10 glucose units equivalent
- **Hydrolysis of macronutrients**

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

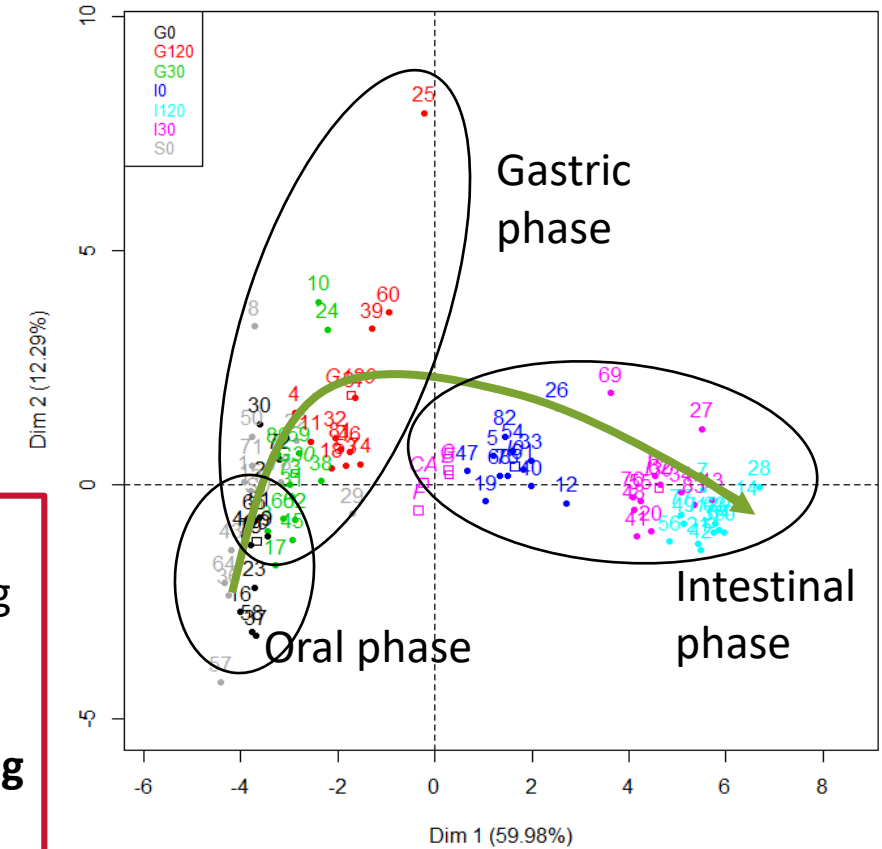
Axis n°2 :
Solubilization of macronutrients
in the supernatant



1°) Grouped by matrix : no matrix effect highlighted

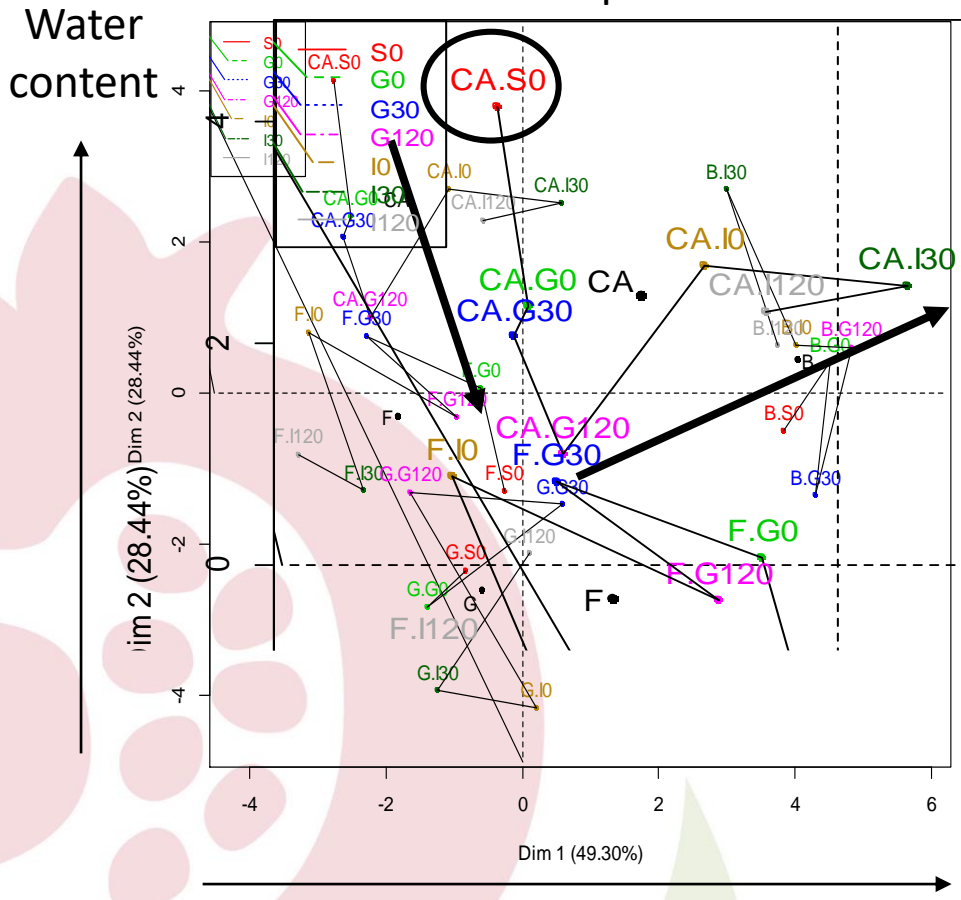
Axis n°1 :
Hydrolysis of macronutrients

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

Heat
treatment

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	

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	

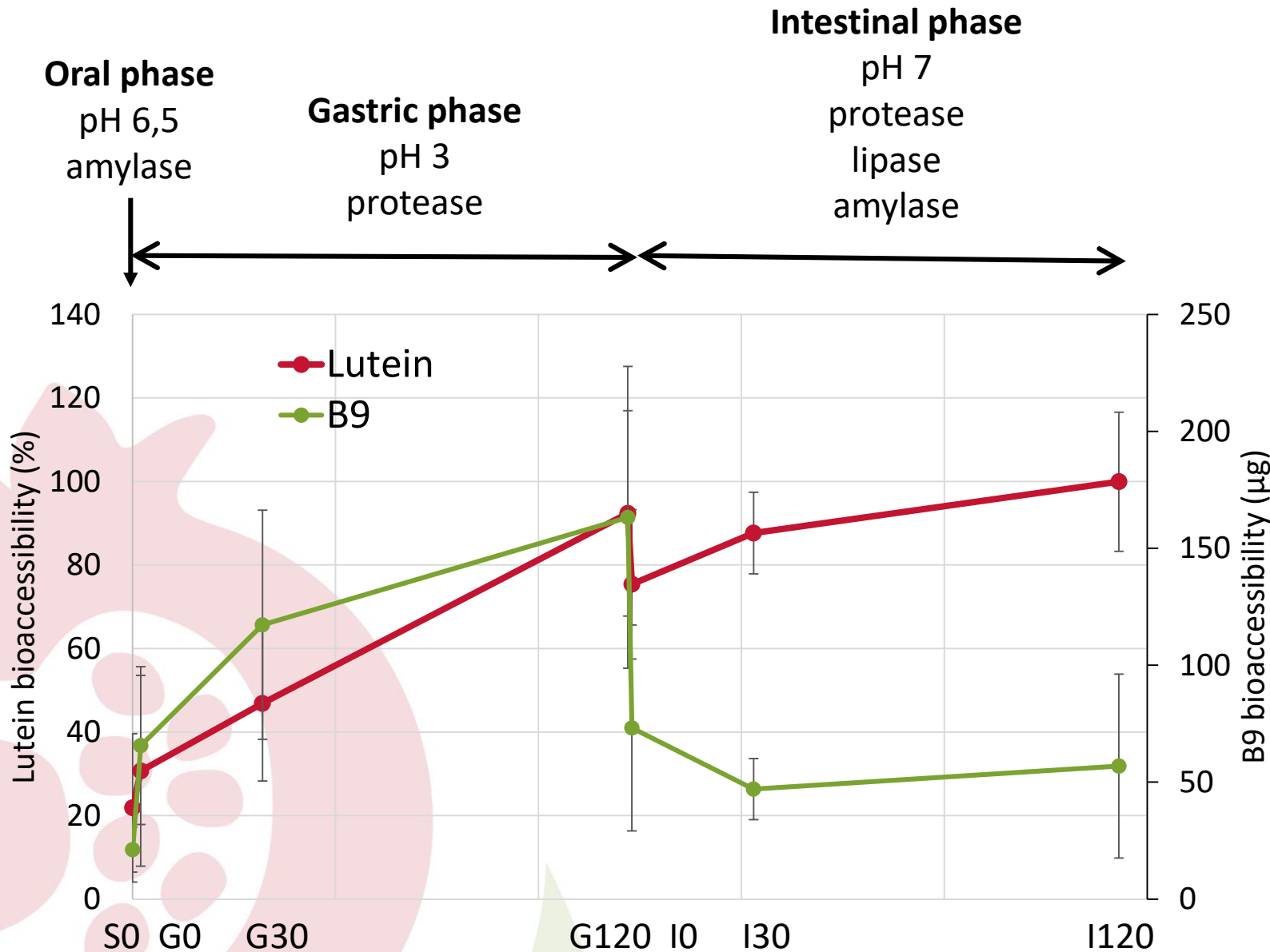
Vitamin B9 correlates with :

- 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



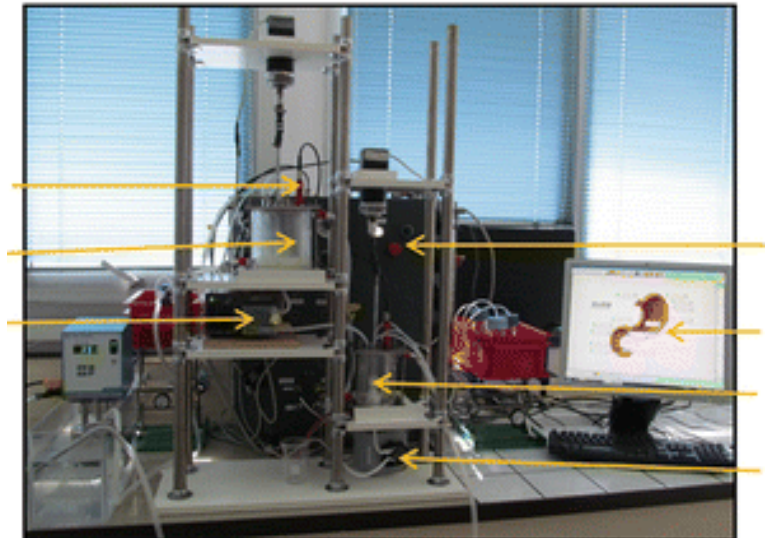
Food structure impacts kinetics of matrix disintegration during digestion



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