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## In situ disintegration of protein gels (egg white) by pepsin based on time-lapse confocal microscopy

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# *In situ* disintegration of protein gels (egg white) by pepsin based on time-lapse confocal microscopy

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SLAMM  
Mon 12 – Thu 15 Nov 2018  
Hyères, France

# Context



FOOD ↔ NUTRITION



HEALTH



How **food characteristics** can modulate the digestion and **nutrient**

↓  
**FOOD MATRIX  
STRUCTURE**

**utilization** by the human body?

↓  
**PROTEIN DIGESTION**

# Previous results



The extent of ovalbumin *in vitro* digestion and the nature of generated peptides are modulated by the morphology of protein aggregates

Kéra Nyemb<sup>a,b</sup>, Catherine Guérin-Dubiard<sup>a,b</sup>, Didier Dupont<sup>a,b</sup>, Julien Jardin<sup>a,b</sup>, Françoise Nau<sup>a,b,\*</sup>



Investigating the impact of egg white gel structure on peptide kinetics profile during *in vitro* digestion

Kéra Nyemb<sup>a,b</sup>, Julien Jardin<sup>a,b</sup>, David Causeur<sup>c</sup>, Catherine Guérin-Dubiard<sup>a,b</sup>, Didier Dupont<sup>a,b</sup>, Shane M. Rutherford<sup>d</sup>, Françoise Nau<sup>a,b,\*</sup>

The structure of OVALBUMIN AGGREGATES and of EGG WHITE GELS drive the proteolysis extent and the pattern of peptide fractions generated during *in vitro* digestion

Kéra Nyemb<sup>a,b</sup>, Catherine Guérin-Dubiard<sup>a,b</sup>, Stéphane Pézenec<sup>a,b</sup>, Julien Jardin<sup>a,b</sup>, Françoise Nau<sup>a,b,\*</sup>, Shane M. Rutherford<sup>c</sup>, Didier Dupont<sup>a,b</sup>

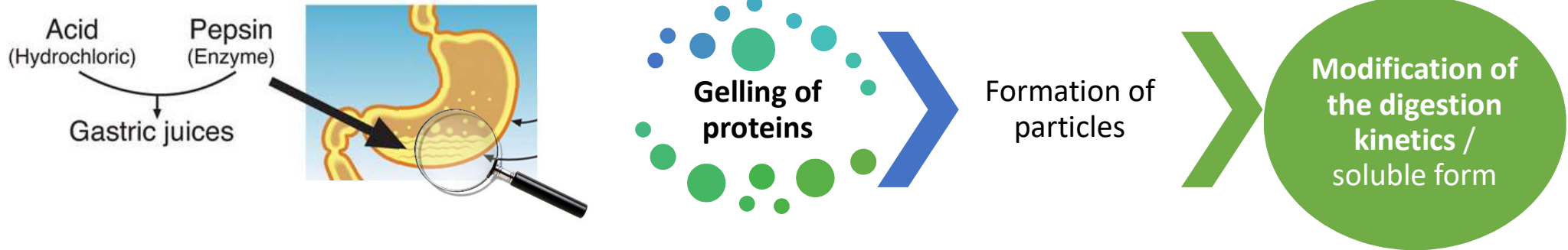


Investigating the impact of egg white gel structure on peptide kinetics profile during *in vitro* digestion

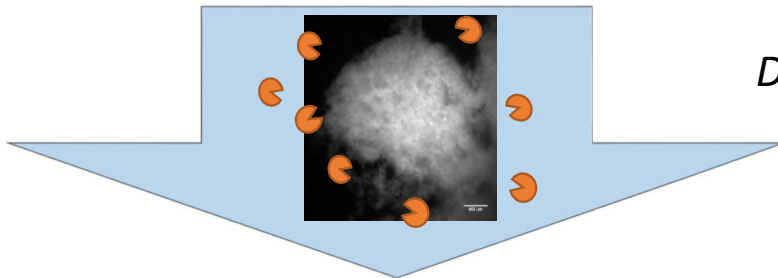
Kéra Nyemb-Diop<sup>a,b</sup>, David Causeur<sup>c</sup>, Julien Jardin<sup>a,b</sup>, Valérie Briard-Bion<sup>a,b</sup>, Catherine Guérin-Dubiard<sup>a,b</sup>, Shane M. Rutherford<sup>d</sup>, Didier Dupont<sup>a,b</sup>, Françoise Nau<sup>a,b,\*</sup>

**Food matrix structure is a deciding parameter for food disintegration during digestion**

The gastric phase is a key step in the digestion of semi-solid to solid foods, including protein gels

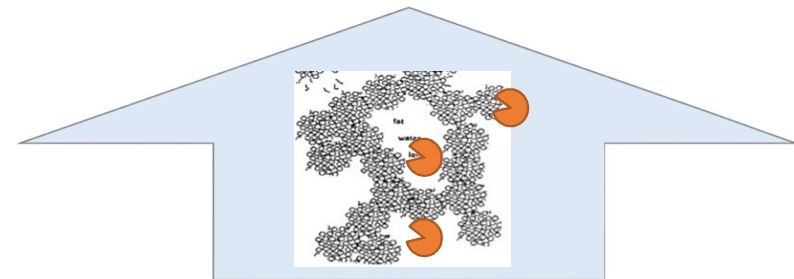


How do gastric acid secretion and pepsin reach their substrates in the stomach?



*Do they **degrade the protein substrates at the surface of the matrix**, which thus gradually erodes, releasing products from enzymatic reaction in the stomach?*

*Or do they **diffuse within the protein gel particles** to reach their substrates?*

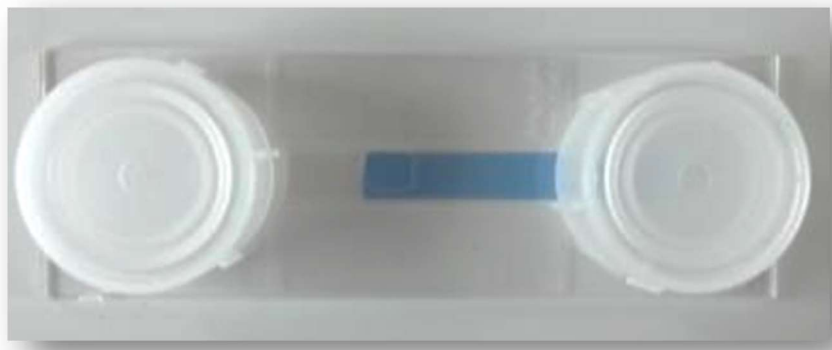
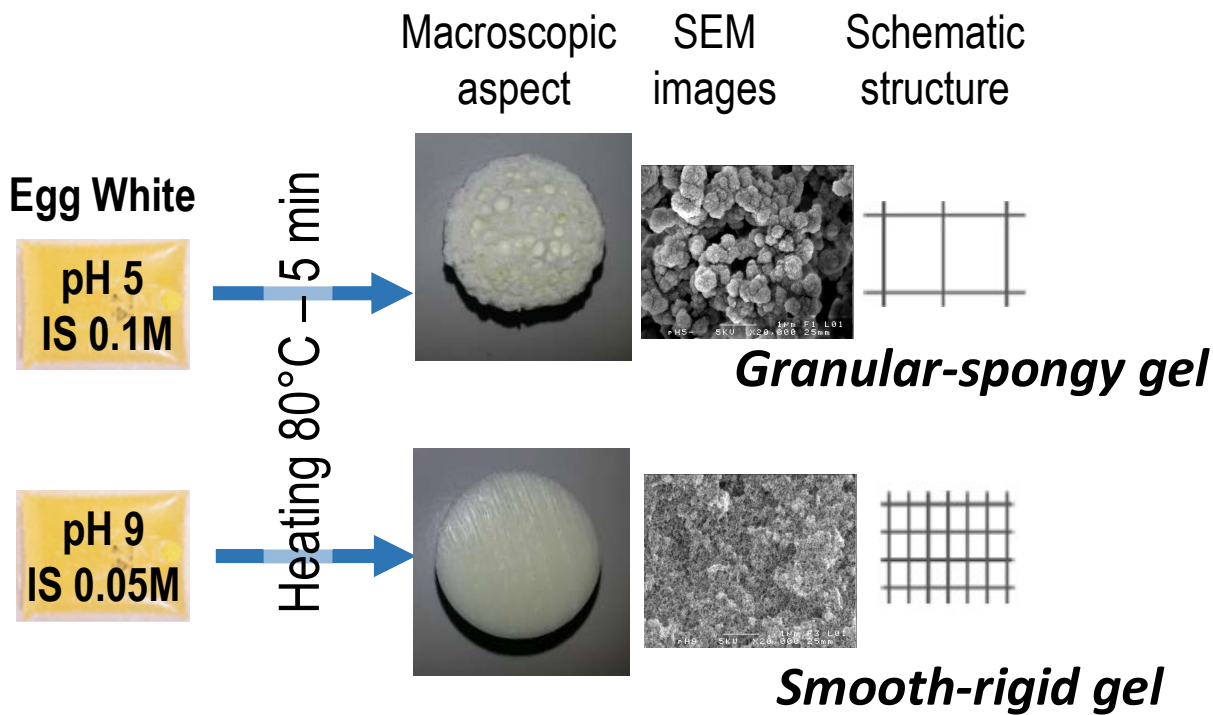


# Strategy

Two egg white gels (EWG) [1]

➔ Identical composition : 10w/w% protein

➔ **Different microstructures :**



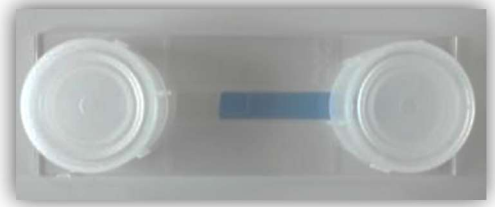
- **100 μL EW** in an IBIDI μ-Slide system
- **Fast Green** added as a protein marker ( $\lambda_{ex}$  633 nm)
- Heat gelation at 80°C/5min

[1] Nyemb et al. (2016) Food Hydrocolloids, 54B, 315-327

# Strategy

EWG disintegration during gastric digestion was observed using the confocal microscope **ZEISS LSM880** equipped with the new **Airyscan detector**

→ **high spatial & temporal resolution**



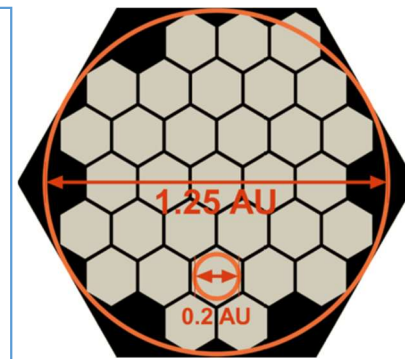
Addition of **150 μL SGF\***  
+ pepsin (**8000 U/ml**)



*\*Simulated Gastric Fluid*

**Airyscan detector**

- ✓ Each of the 32 detector elements acts as its own small pinhole
- ✓ **1.7× higher resolution & increases signal-to-noise ratio** by 4–8× compared with conventional CSLM using an optimized 1-AU pinhole (Airy Unit)

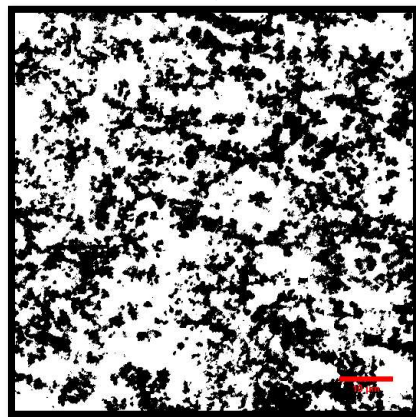
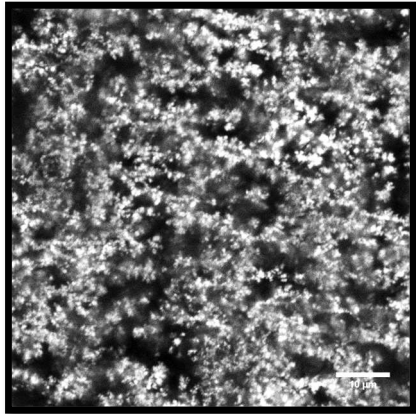


Tile scan set up to cover **1 mm field of view from the gel surface:**

- 15 images captured with the **x63 oil objective**
- every 15 min over a **120 min digestion period (37°C)**

Treatment & controls	EWG pH 5 & 9
SGF* pH2 + pepsin	3 rep
SGF* pH2 (no pepsin)	1 rep
Water only	1 rep
Photo-bleaching	1 rep

# Image analysis of the gel microstructures



Black – Particles ; White - Background

## White top hat filter

→ to enhance protein structures

## Smooth

→ to remove acquisition noise

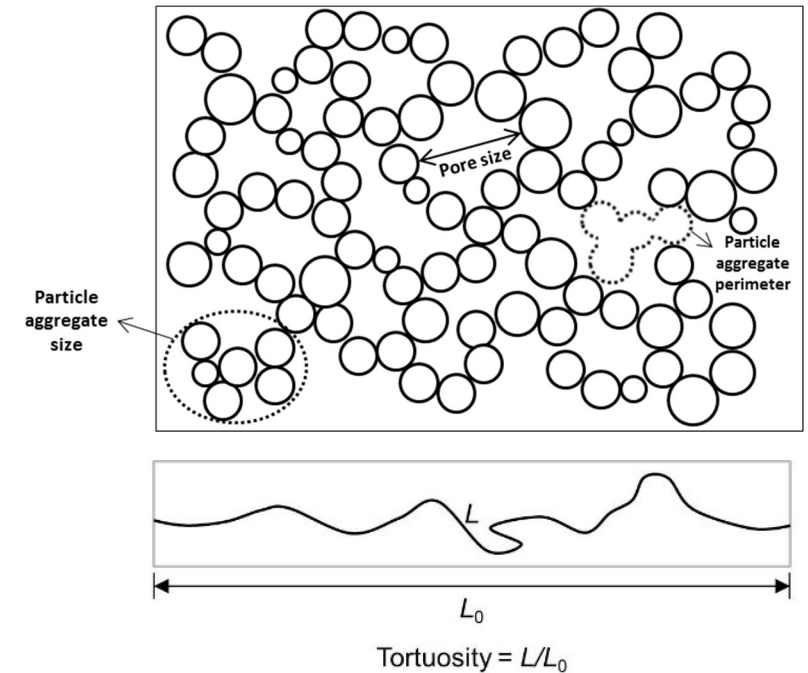
## Otsu threshold

→ to get binary image

## Quantification of microstructural parameters using specific plugins:

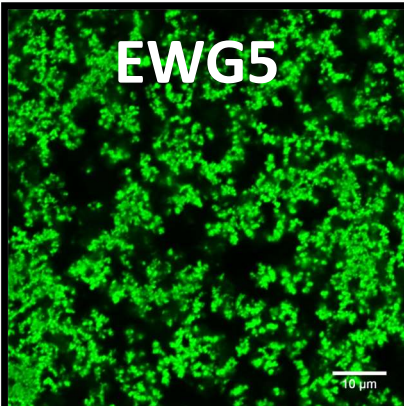
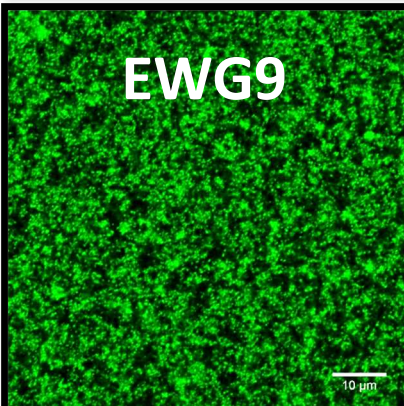
- *MorphoLibJ*
- *Granulometry*
- *Geodesics*

- ✓ Area fraction of particles
- ✓ Perimeter density of particle aggregates
- ✓ Pore size distribution
- ✓ Particle size distribution
- ✓ Tortuosity



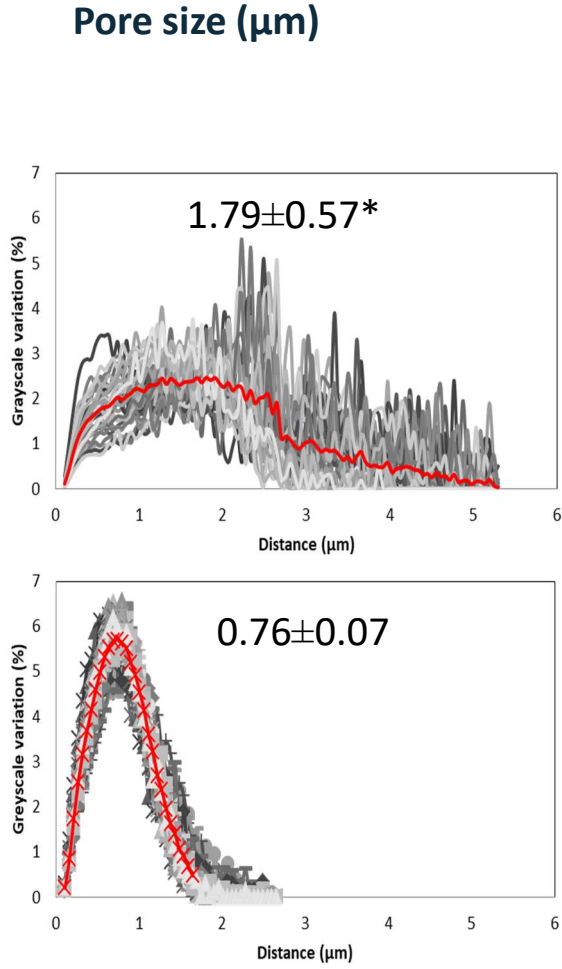
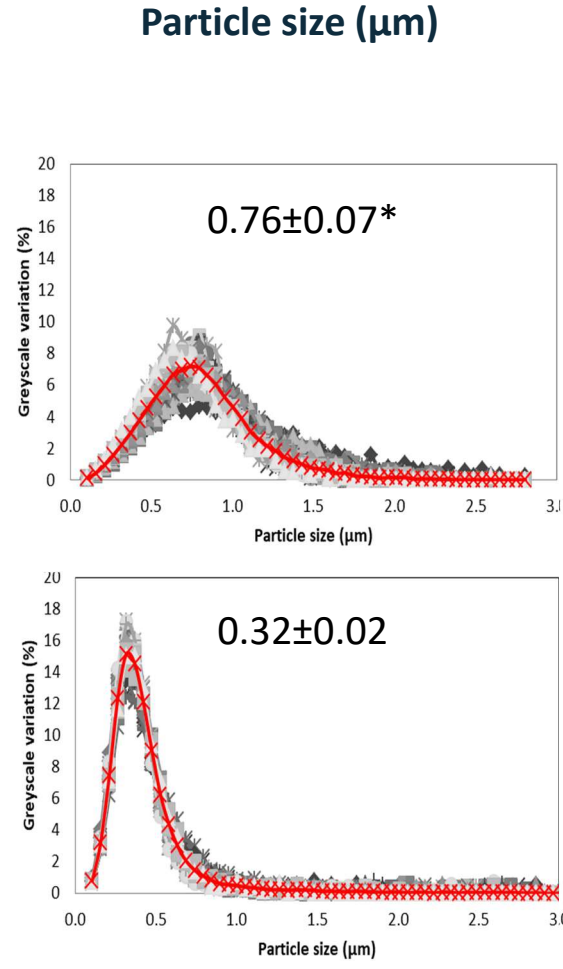
*Silva et al., Food Hydro. 2015*

# Results. Initial microstructure of the gels

	Area fraction of particle	Perimeter density of particle ( $\mu\text{m}/\mu\text{m}^2$ )	Tortuosity
	$0.31 \pm 0.08$	$0.92 \pm 0.20$	$1.14 \pm 0.14^*$
	$0.29 \pm 0.02$	$1.93 \pm 0.11^*$	$1.07 \pm 0.01$

Protein particles in green, scale bar 10 $\mu\text{m}$

\*  $p\text{-value} < 0.05$

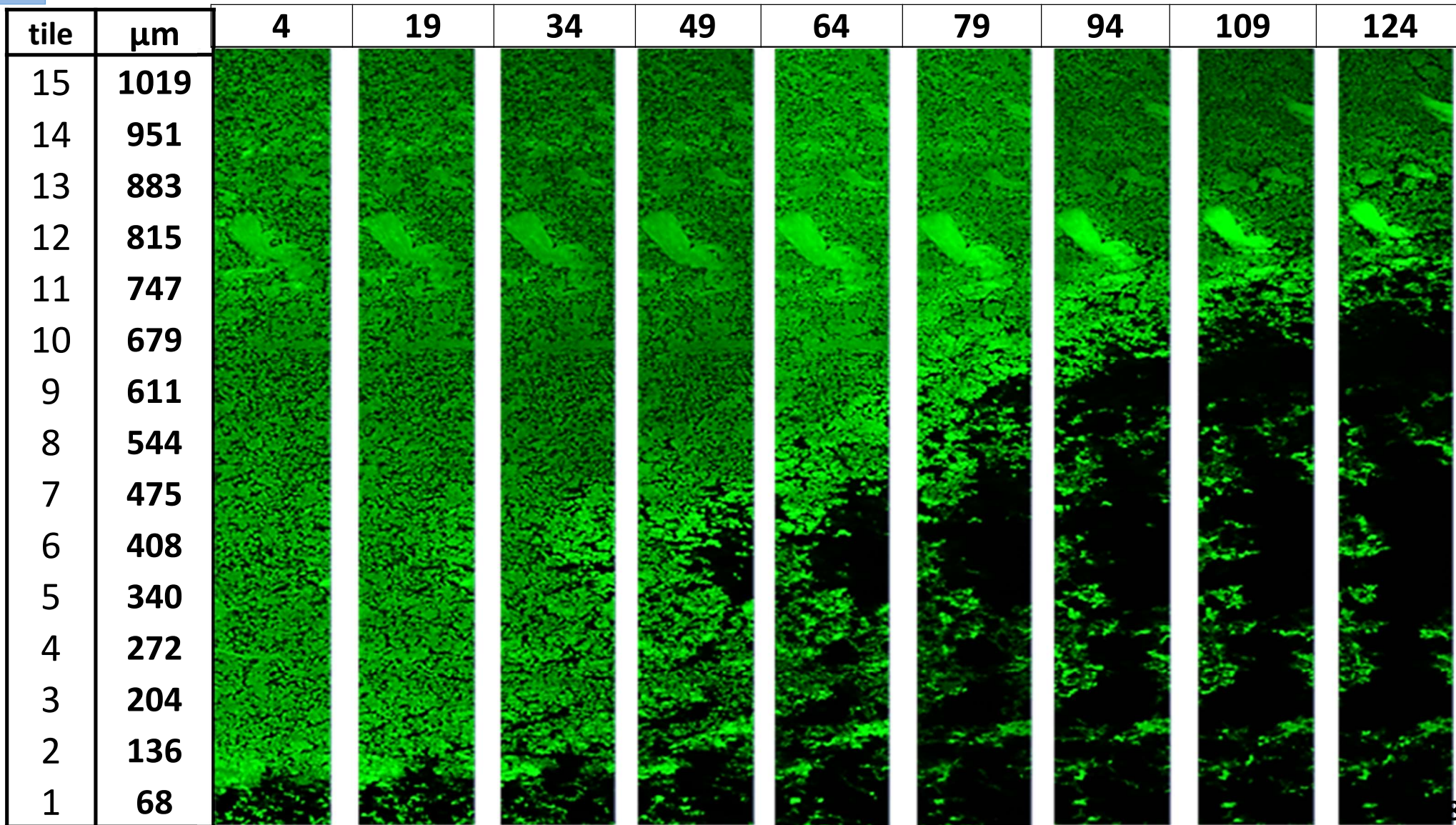




**EWG5**

Digestion time (min)

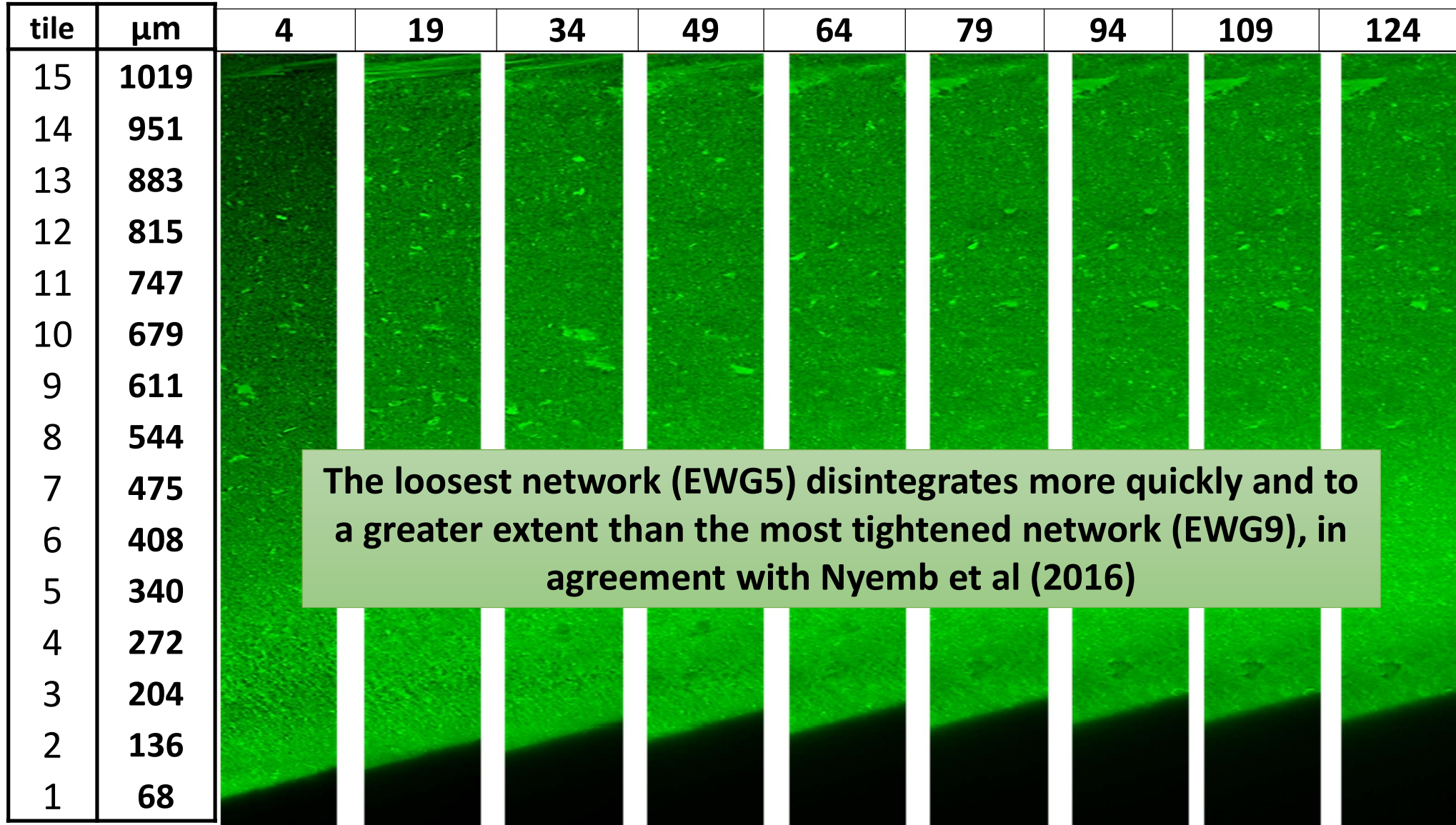
Distance from gel surface ( $\mu\text{m}$ )



**EWG9**

**Digestion time (min)**

**Distance from gel surface ( $\mu\text{m}$ )**

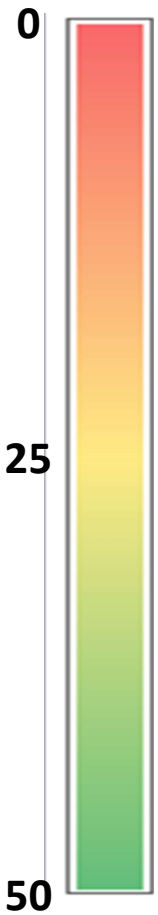


The loosest network (EWG5) disintegrates more quickly and to a greater extent than the most tightened network (EWG9), in agreement with Nyemb et al (2016)

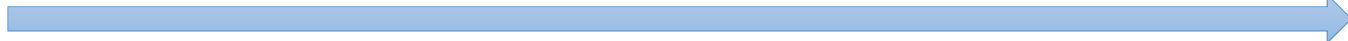
**EWG5**

**Digestion time (min)**

Particle area fraction (%)



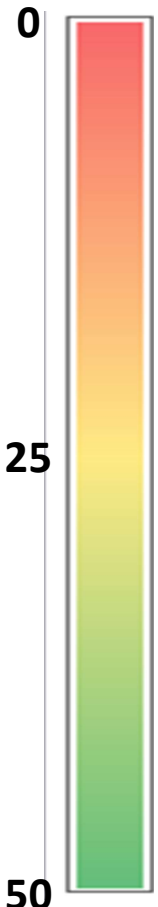
Distance from gel surface (μm)



Tile	Distance (μm)	Native gel	4 min	19 min	34 min	49 min	64 min	79 min	94 min	109 min	124 min
15	1019	Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green
14	951	Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green
13	883	Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green
12	815	Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green
11	747	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green
10	679	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green
9	611	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green
8	544	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green
7	475	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green
6	408	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green
5	340	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green
4	272	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green
3	204	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green
2	136	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green
1	68	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green

**EWG9**

Particle area fraction (%)



Distance from gel surface (μm)

		Native gel	Digestion time (min)								
tile	μm		4 min	19 min	34 min	49 min	64 min	79 min	94 min	109 min	124 min
15	1019										
14	951										
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5	340										
4	272										
3	204										
2	136										
1	68										

## Discussion

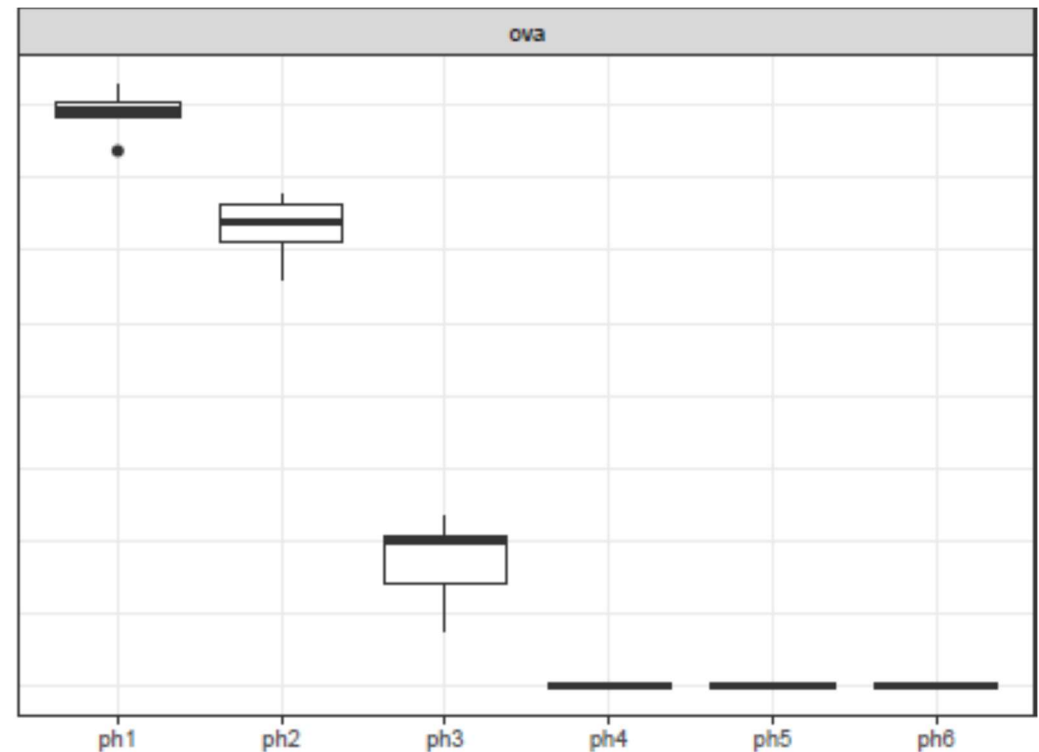
Diffusion coefficient of FITC-pepsin measured by FRAP (20°C)

Medium	$D_{\text{eff}}$ ( $\mu\text{m}^2 \text{s}^{-1}$ )
Water	$104.5 \pm 10.7^{\text{a}}$
pH5-granular EWG	$52.5 \pm 5.3^{\text{b}}$
pH9-smooth EWG	$44.2 \pm 6.1^{\text{c}}$

It is not possible to conclude if proteolysis is limited by :

- the **diffusion of pepsin inside the gels**
- the **local pH**, which is of **paramount importance on pepsin activity**

Activity of pepsin on ovalbumin in function of the pH



## Perspectives

These technique is valuable to get a better picture on how food characteristics can modulate digestion kinetics

BUT :

Improvement of the proposed methodology is needed to **decouple the acidification phase by SGF from the enzymatic action by pepsin.**

Moreover, more research is required **to produce different microstructures of protein gels with both identical composition and pH.**