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## Spatial-temporal mapping of the intra-gastric pH, pepsin concentration and proteolysis in pigs fed egg white gels

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## 7<sup>th</sup> International Conference on Food Digestion

# ➤ Spatial-temporal mapping of the intra-gastric pH, pepsin concentration and proteolysis in pigs fed egg white gels

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## > Context



PROTEIN DIGESTION



How **food characteristics** can modulate the digestion process and **nutrient utilization** by the human body?



FOOD MATRIX  
STRUCTURE

### A KEY ROLE OF THE GASTRIC PHASE ?

# ➤ Previous *in vitro* results on Egg white gels



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>, Shane M. Rutherford<sup>c</sup>, Françoise Nau<sup>a,b,\*</sup>



The structure of egg white gels 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>, 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>



Investigating the impact of ovalbumin aggregate morphology on *in vitro* ovalbumin digestion using label-free quantitative peptidomics and multivariate data analysis

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>



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>



INRAE

Spatial-temporal mapping of the intra-gastric pH, pepsin concentration and proteolysis in pigs fed egg white gels

3<sup>rd</sup> May 2022 / 7<sup>th</sup> ICFD, Cork, Ireland / Steven Le Feunteun

# ➤ Previous *in vivo* results on Egg white gels (EWG)



Spatial-temporal changes in pH, structure and rheology of the gastric chyme in pigs as influenced by egg white gel properties

Françoise Nau<sup>a,b,\*</sup>, Kéra Nyemb-Diop<sup>a,b</sup>, Valérie Lechevalier<sup>a,b</sup>, Juliane Floury<sup>a,b</sup>, Chloé Serrière<sup>c</sup>, Natascha Stroebinger<sup>c</sup>, Thiébaud Boucher<sup>a,b</sup>, Catherine Guérin-Dubiard<sup>a,b</sup>, Maria J. Ferrua<sup>c</sup>, Didier Dupont<sup>a,b</sup>, Shane M. Rutherford<sup>c</sup>

<sup>a</sup>Agrocampus Ouest, UMR1253 Science et technologie du lait et de l'œuf, 65 rue de Saint-Brieuc, 35042 Rennes, France

<sup>b</sup>INRA, UMR1253 Science et technologie du lait et de l'œuf, 65 rue de Saint-Brieuc, 35042 Rennes, France

<sup>c</sup>Riddet Institute, Massey University, University Avenue, Palmerston North 4474, New Zealand

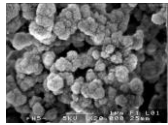


3 egg white gels: different structures, same composition

Egg White

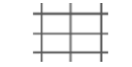
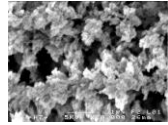
pH 5  
IS 0.1M

Heating 90°C – 150 min



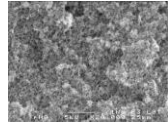
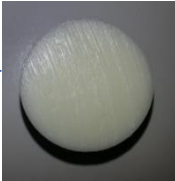
Granular-spongy  
gel

pH 7  
IS 0.05M



Intermediate  
gel

pH 9  
IS 0.05M



Smooth-rigid  
gel

Test meals: 1 kg



n=99

Euthanization

20 min

60 min

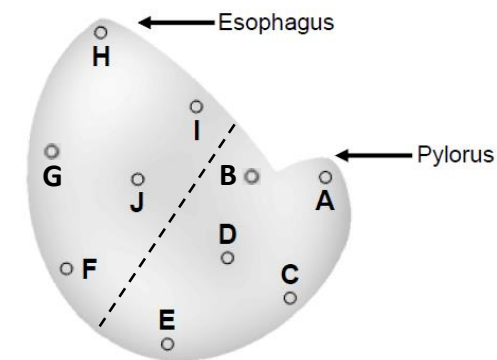
120 min

240 min

360 min

1 trial = 1 EWG x 1 time  
5 to 6 pigs / trial

Gastric sampling  
in 10 locations



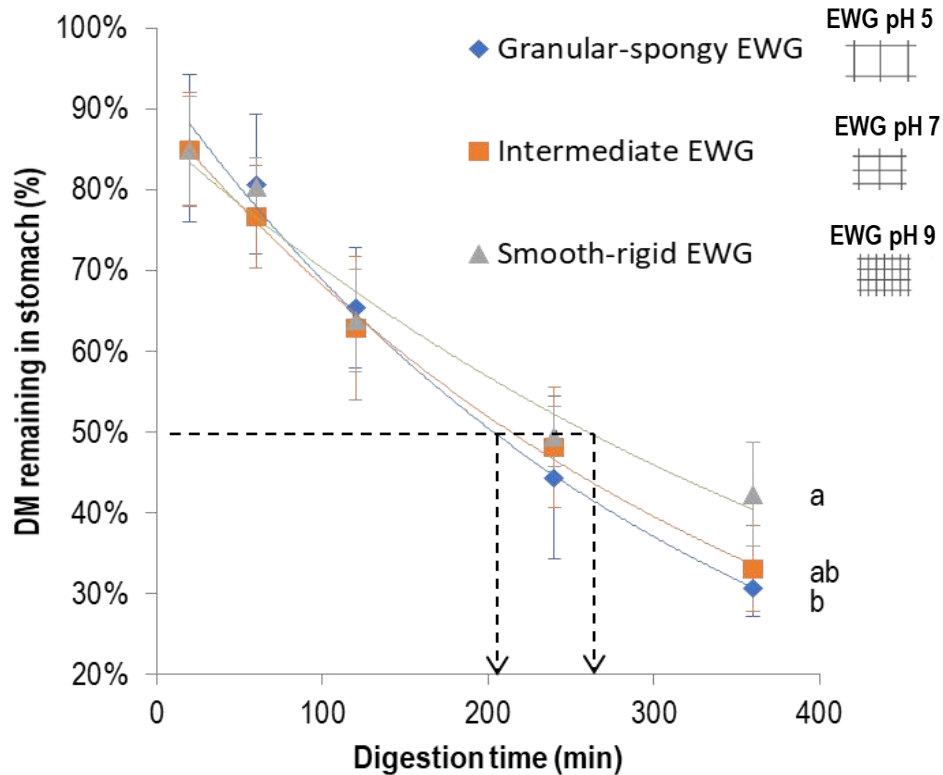
- pH
- Wet and dry masses
- Granulometric distribution
- rheological properties

## ➤ Previous *in vivo* results on Egg white gels (EWG)

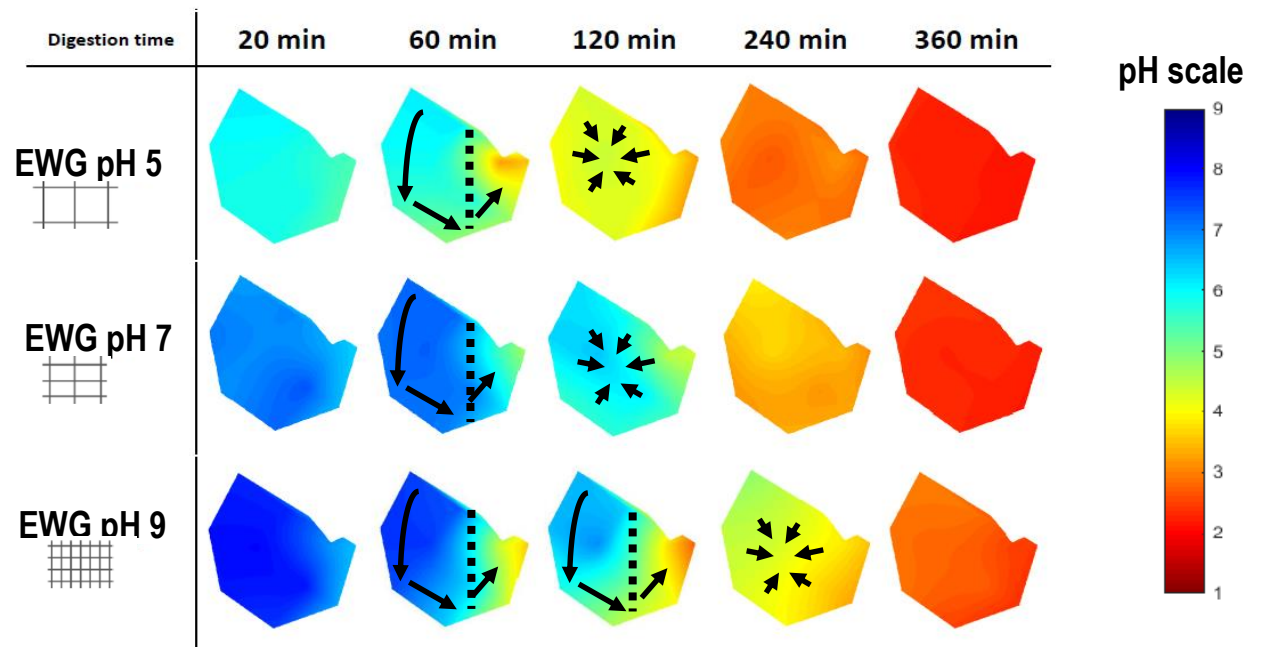
Spatial-temporal changes in pH, structure and rheology of the gastric chyme in pigs as influenced by egg white gel properties

Françoise Nau<sup>a,b,\*</sup>, Kéra Nyemb-Diop<sup>a,b</sup>, Valérie Lechevalier<sup>a,b</sup>, Juliane Floury<sup>a,b</sup>, Chloé Serrière<sup>c</sup>, Natascha Stroebinger<sup>c</sup>, Thiébaud Boucher<sup>a,b</sup>, Catherine Guérin-Dubiard<sup>a,b</sup>, Maria J. Ferrua<sup>c</sup>, Didier Dupont<sup>a,b</sup>, Shane M. Rutherford<sup>c</sup>

<sup>a</sup>Agrocampus Ouest, UMR1253 Science et technologie du lait et de l'œuf, 65 rue de Saint-Brieuc, 35042 Rennes, France  
<sup>b</sup>INRA, UMR1253 Science et technologie du lait et de l'œuf, 65 rue de Saint-Brieuc, 35042 Rennes, France  
<sup>c</sup>Ridlet Institute, Massey University, University Avenue, Palmerston North 4474, New Zealand



- ✓ Gastric emptying profiles are influenced by EWG characteristics, but not in a great extent ( $t_{1/2} \sim 200\text{-}250$  min  $\Rightarrow \sim 4$ h)

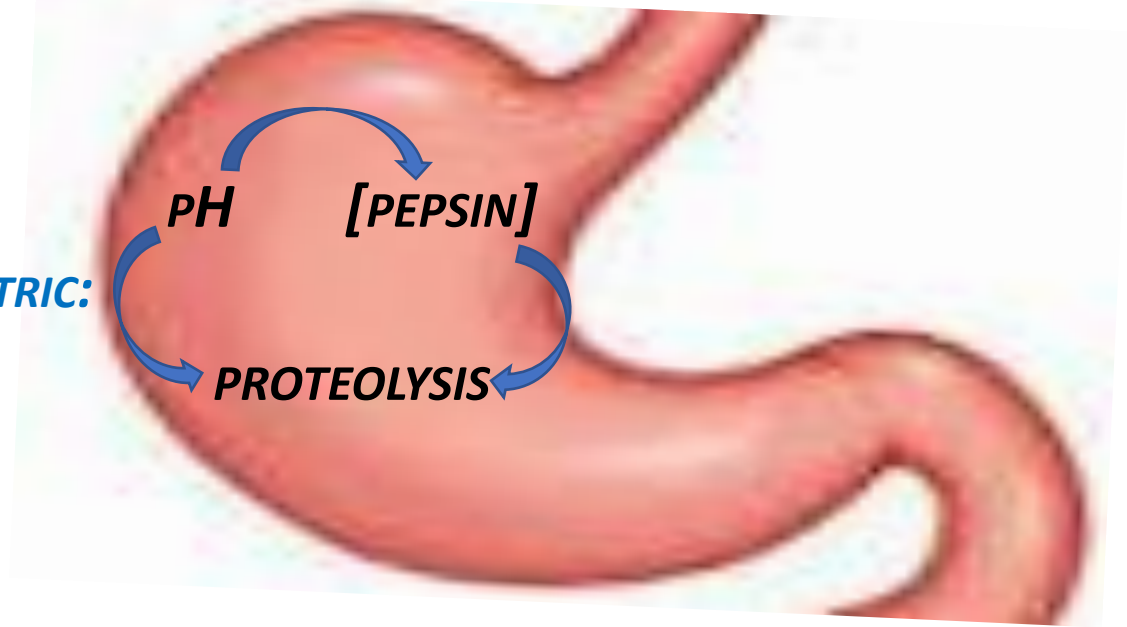


- ✓ Acidification starts in the pylorus region (HCl streaming along stomach walls)
- ✓ Beyond the initial pH, the gel structure impacts the mixing kinetics
- ✓ Consistent with (Bornhorst et al., *Food Biophysics*, 2014) on almonds and rice

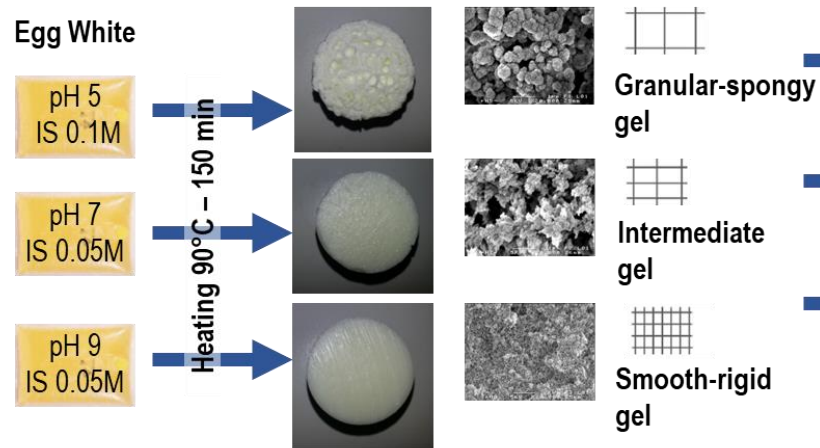
# ➤ Objectives of the present study

COMPLEMENTARY ANALYSES OF THE SAME SAMPLES TO:

- ❑ FURTHER STUDY THE RELATIONSHIPS BETWEEN INTRAGASTRIC:
- ❑ THE CONSEQUENCES ON SUBSEQUENT AMINO-ACID ABSORPTION



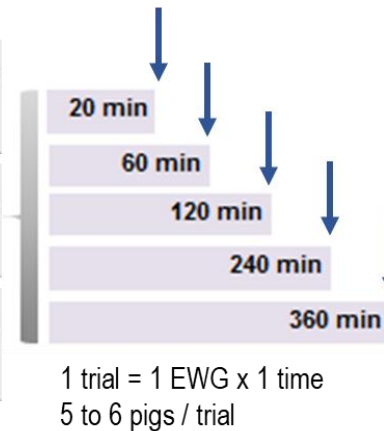
3 egg white gels: different structures, same composition



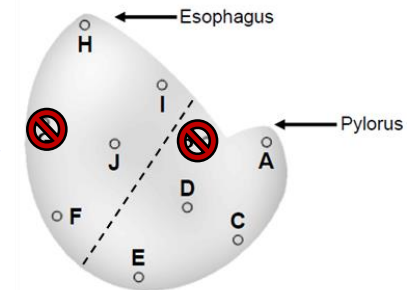
Test meals: 1 kg



Euthanization



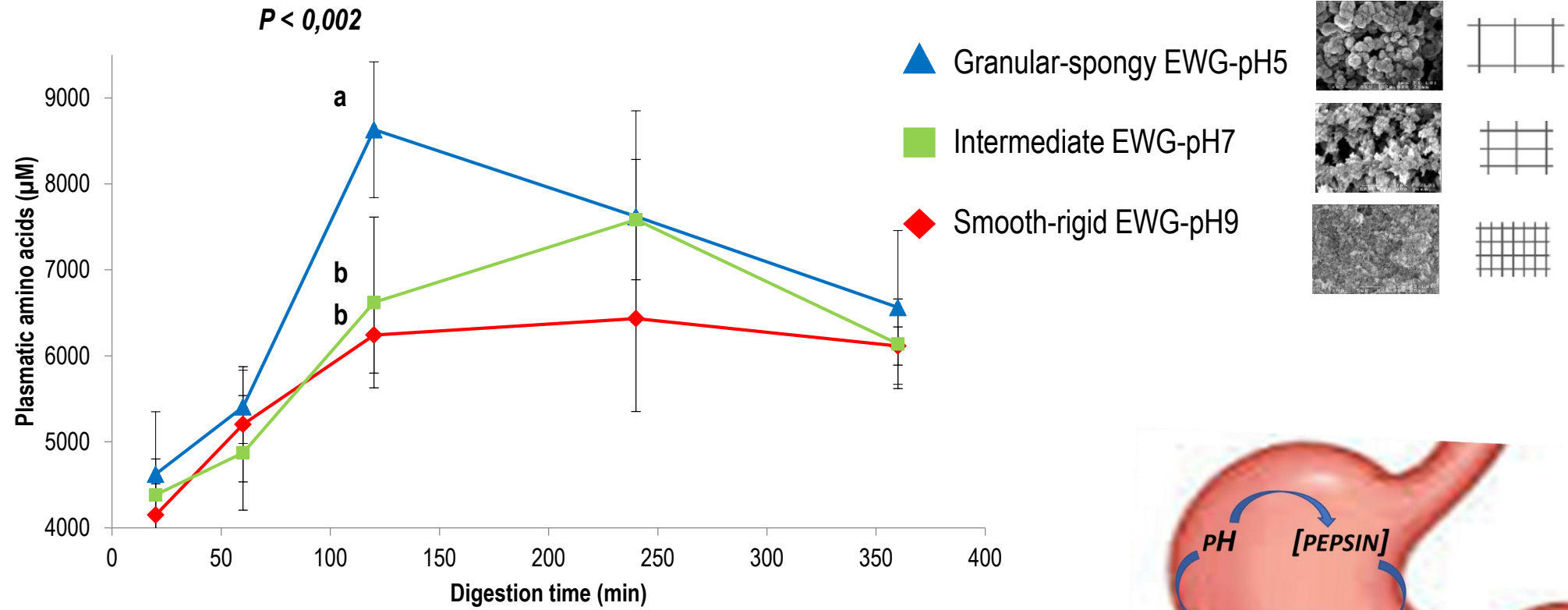
Gastric sampling in 8 locations



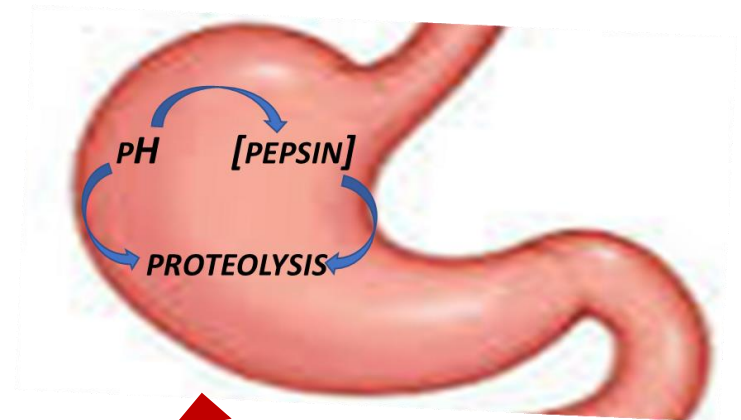
- pH
- Wet and dry masses
- Granulometric distribution
- rheological properties
- Pepsin concentration (ELISA)
- Proteolysis (OPA)

- Free amino acid content (Peripheral blood)

# ➤ Peripheral blood aminoacidemia



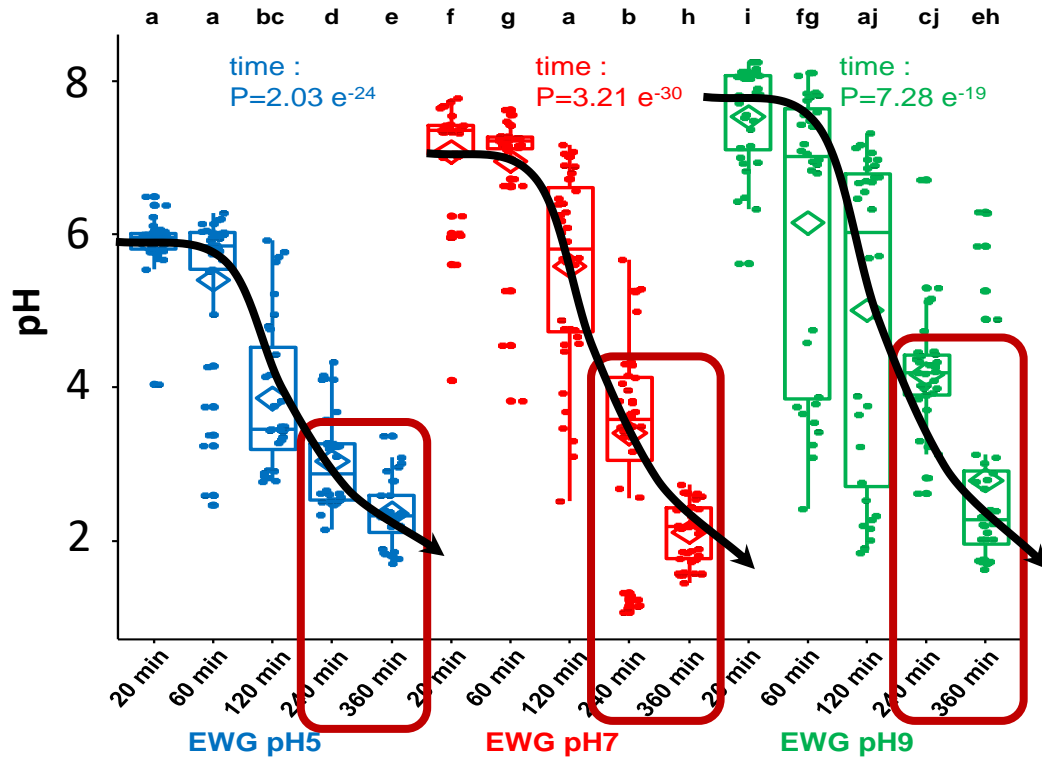
✓ The loosest protein network is the more quickly digested & leads to the highest and earliest plasmatic amino acid peak



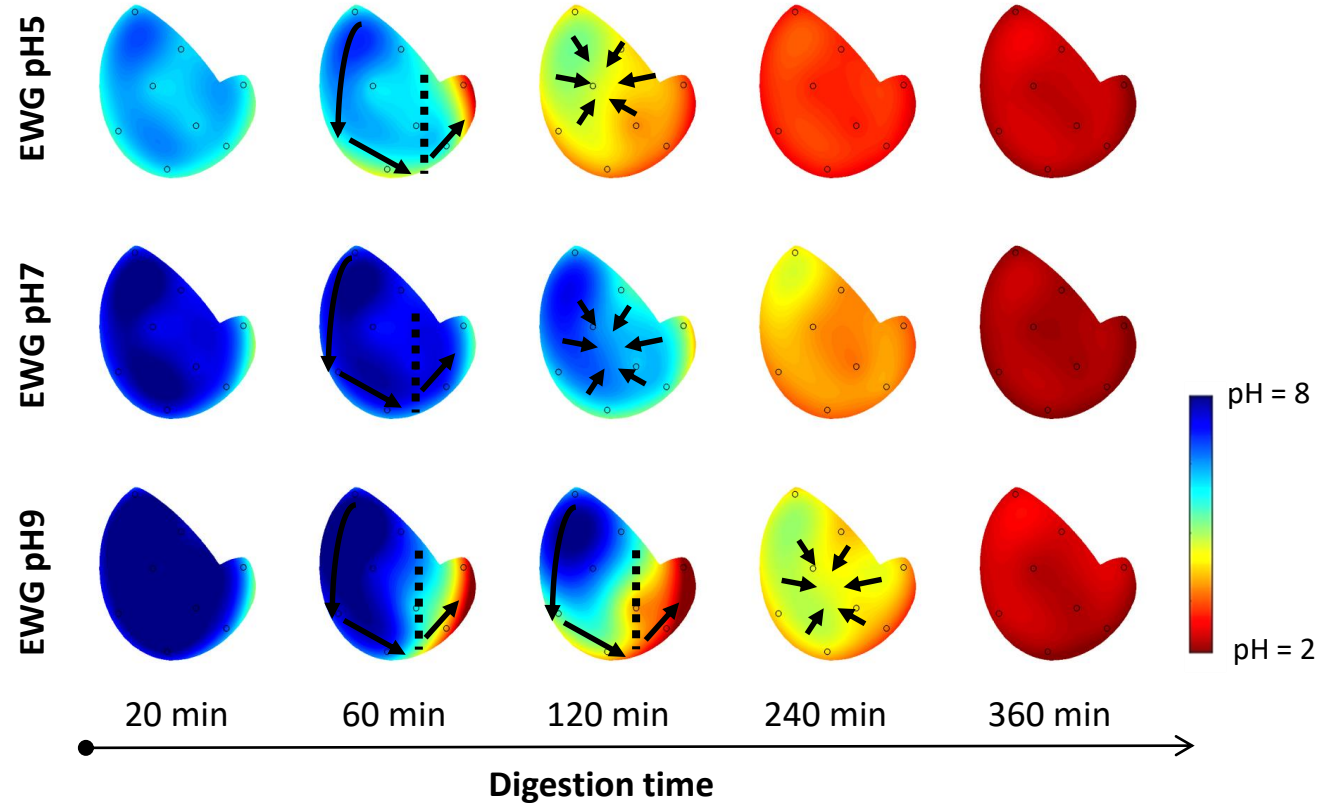


# > pH results

All values



Maps

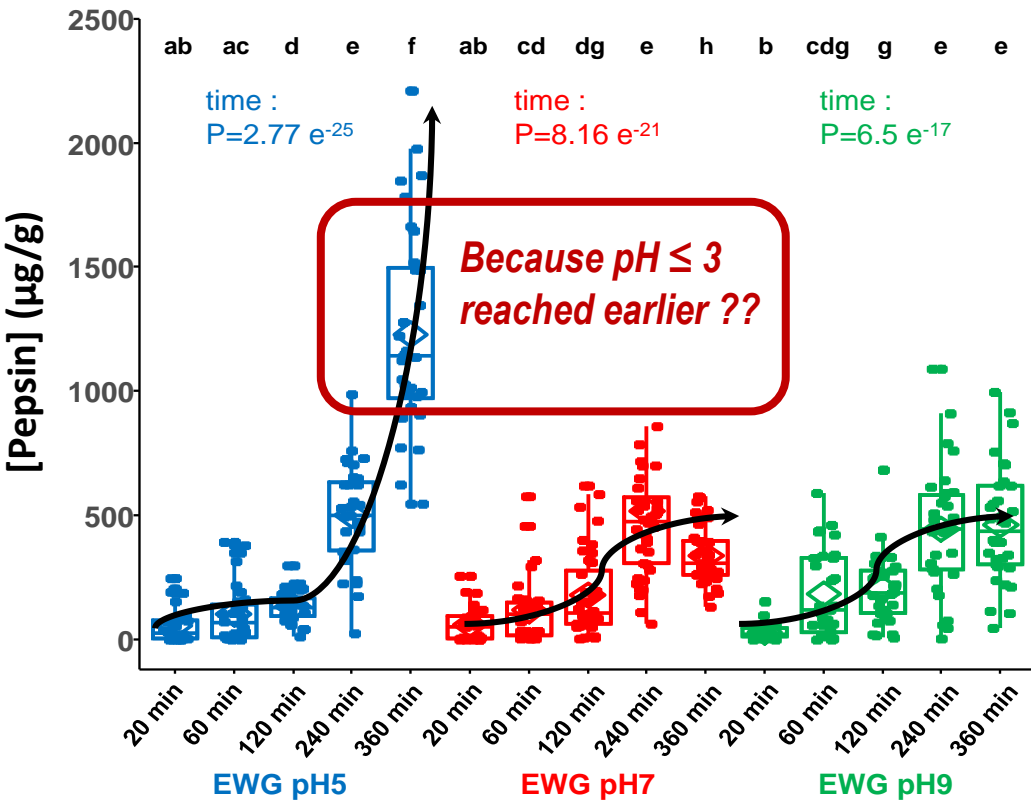


✓ At  $t_{1/2}$  (~ 4h), pH range between 3-4

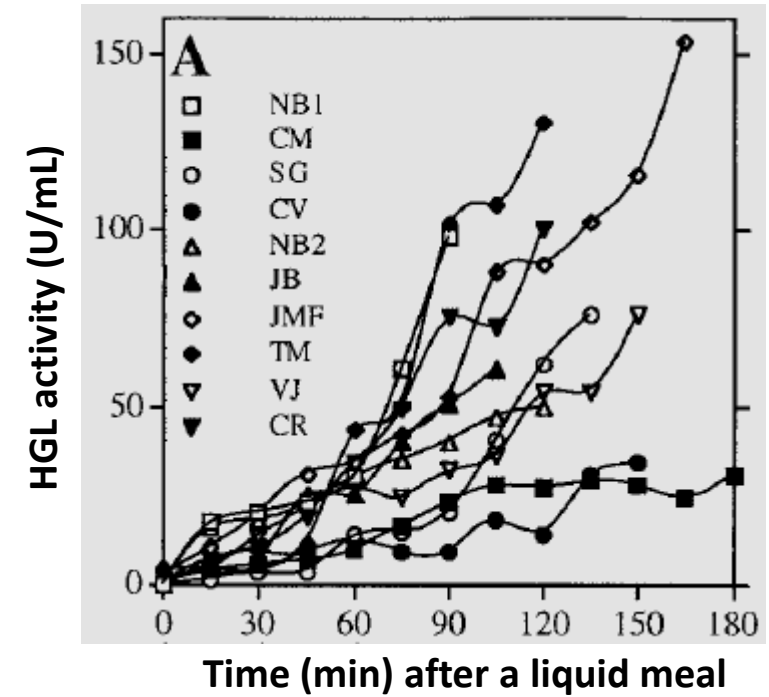
✓  $1.5 \times t_{1/2}$  (6h) to reach pH 2

# ➤ Pepsin results ( $\mu\text{g/g}$ of wet chyme)

All values



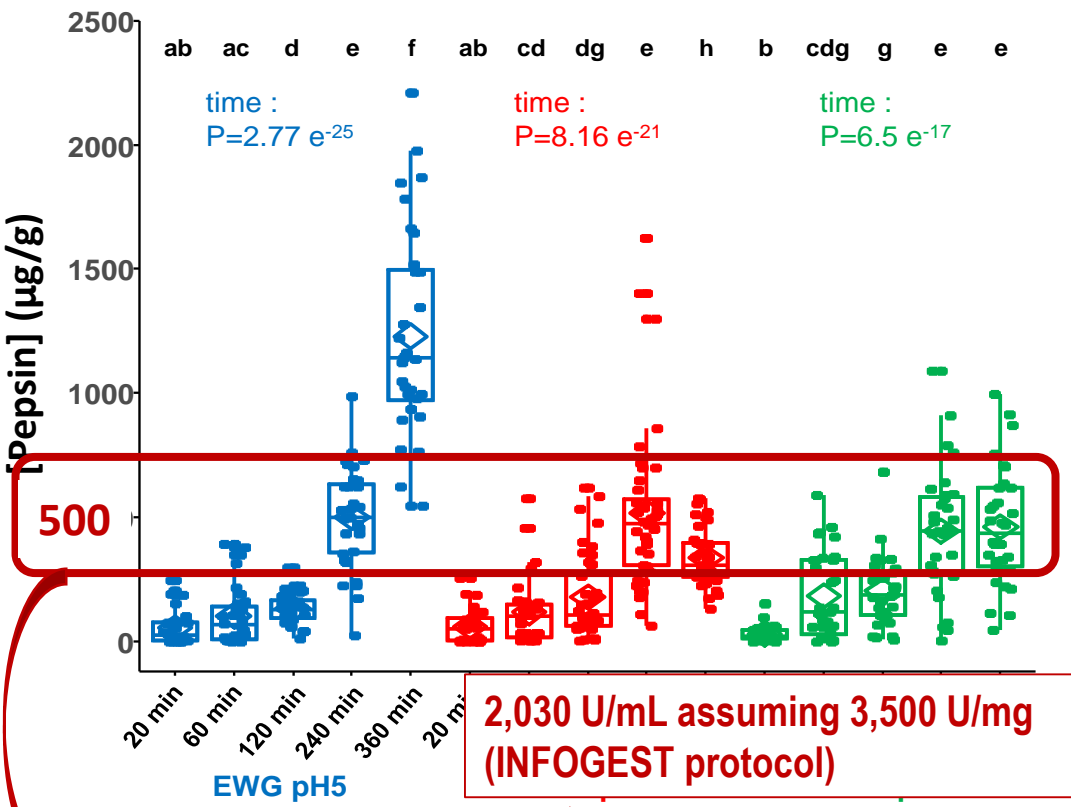
- ✓ Max [Pepsin] reached at  $\geq t_{1/2}$  (4-6h, as for  $pH \leq 3$ )
- ✓ 2 shapes of concentration profiles depending on the gels



- ✓ Consistent with previous reports on Gastric Lipase in Humans (*Carrière et al., Digestion, 2001*)

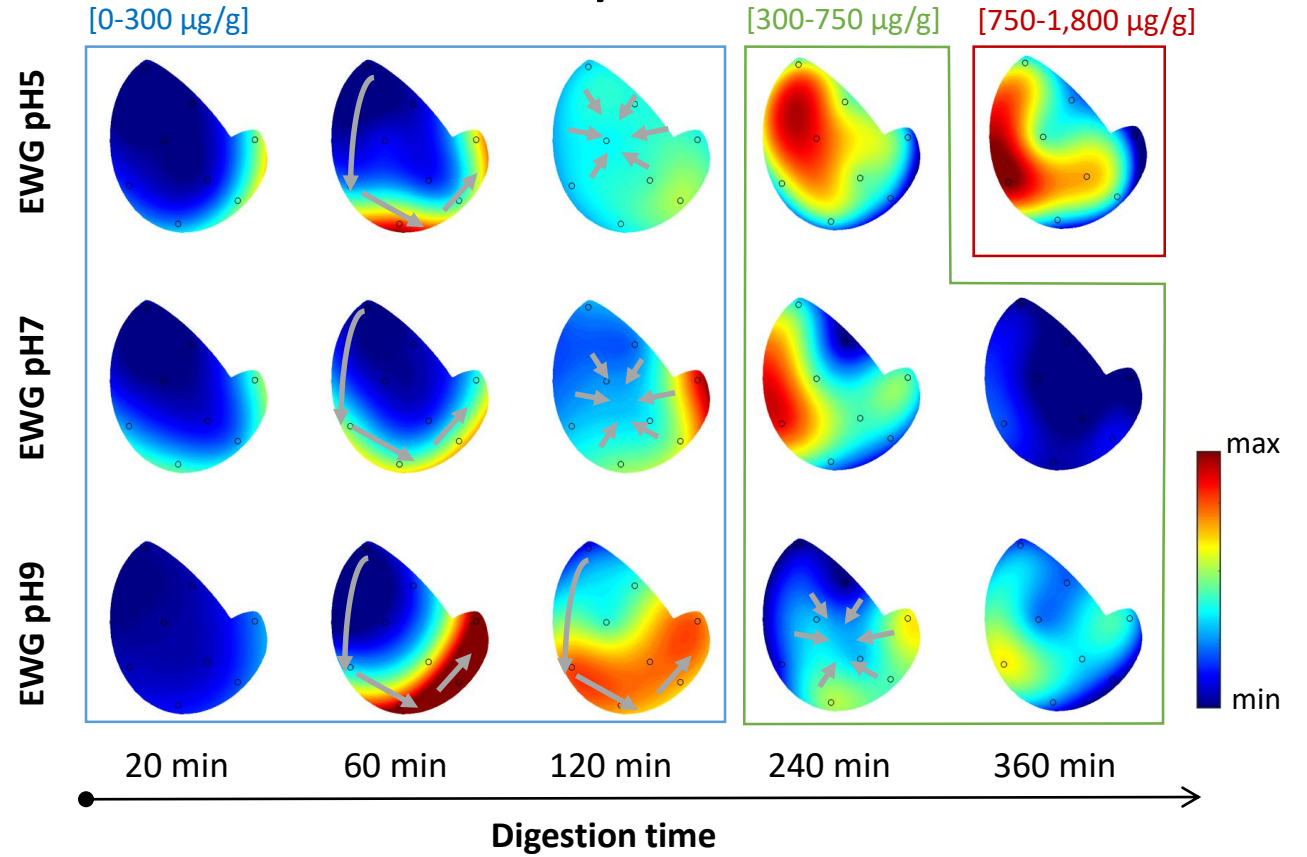
# ➤ Pepsin results ( $\mu\text{g/g}$ of wet chyme)

All values



- ✓ 500  $\mu\text{g/g}$  at 240 min  $\approx$  580  $\mu\text{g/g}$  at 210 min in humans (Kalantzi et al., Pharm. Res., 2006)
- ✓ Mean flow rate of 2.1 mg/min  $\approx$  2.5 mg/min in humans (Malagelada et al., Dig. Dis. Sci., 1979)

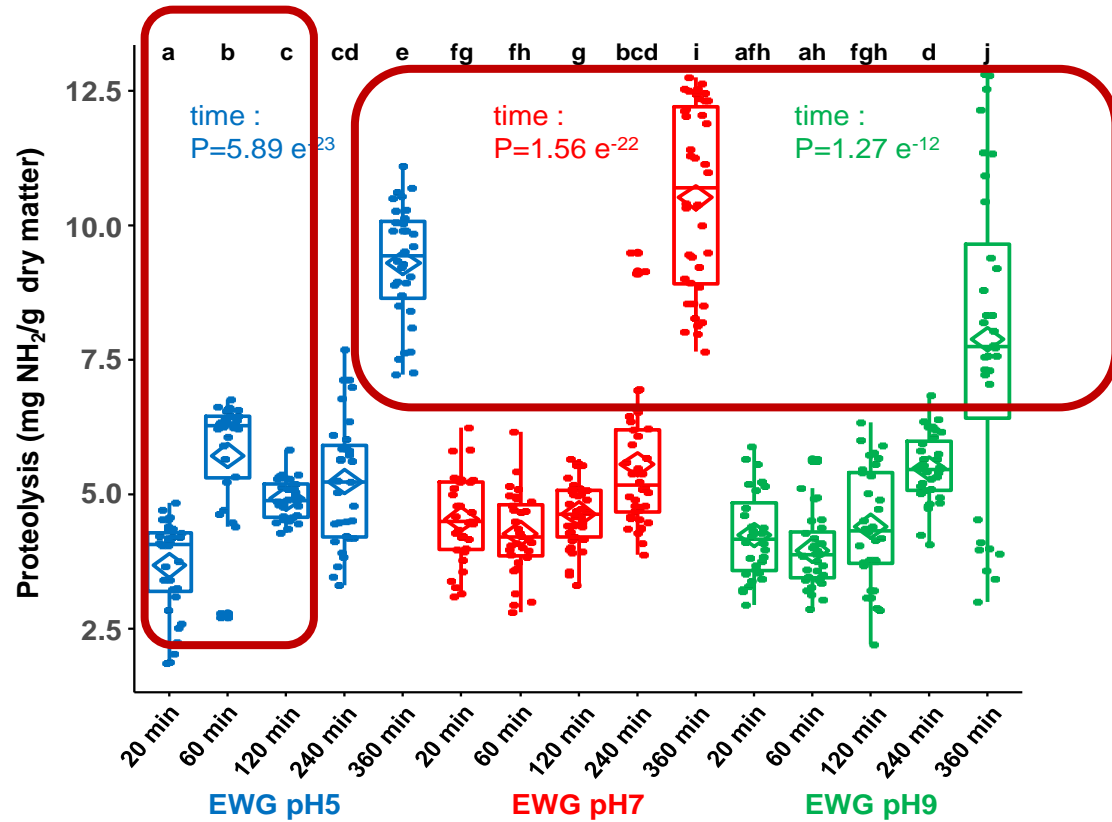
Maps



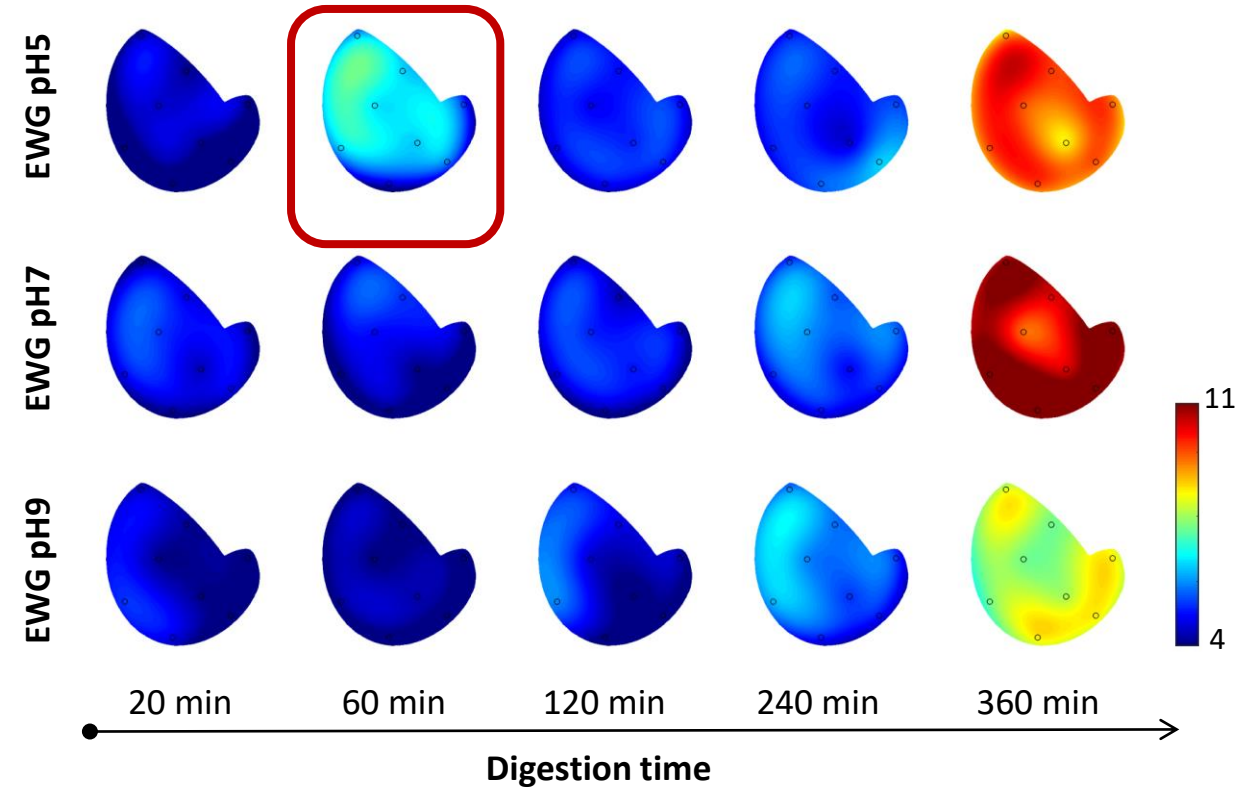
- ✓ Very similar as for the pH (pepsin streaming along the walls)...
- ✓ ... before it turns to accumulate in the proximal region
- ✓ pH and pepsin maps are  $\neq$  in the end because:
  - pH is not a concentration (it is a Log)
  - Food buffers


# ➤ Proteolysis results (mg of free NH<sub>2</sub> / g of dry matter)


All values



Maps



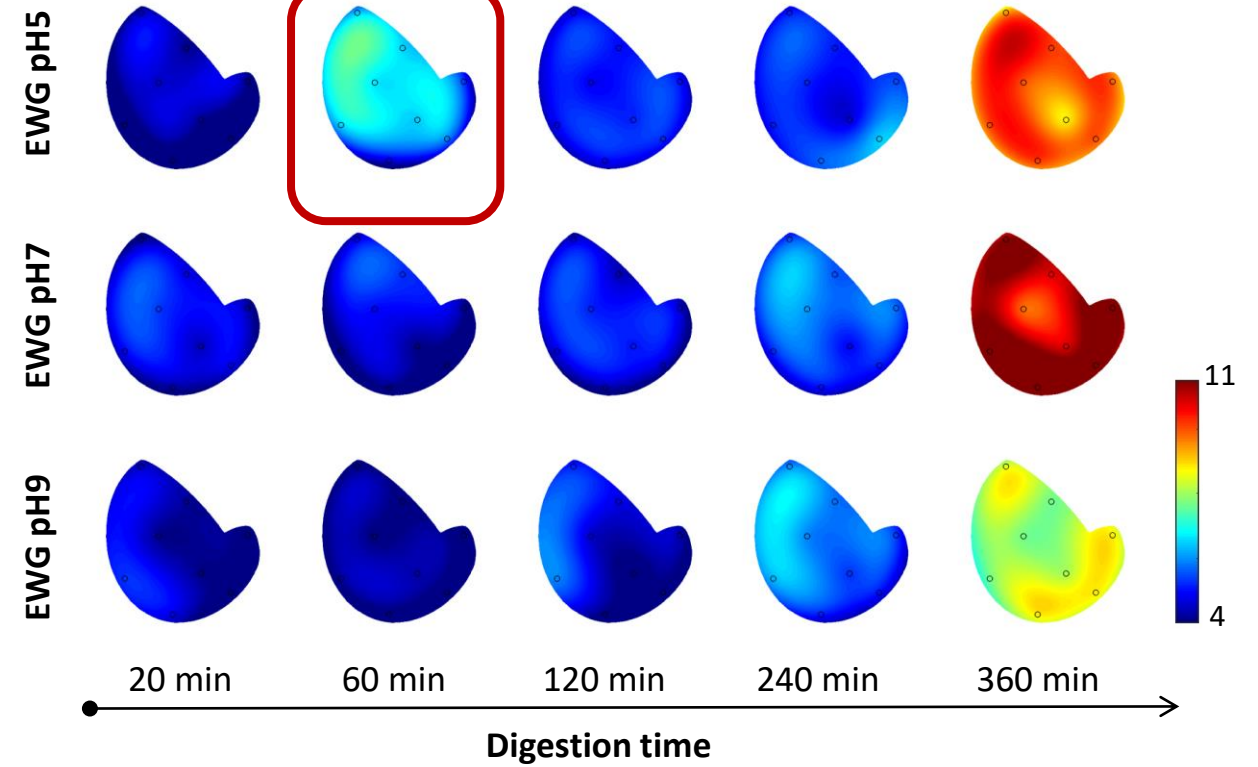
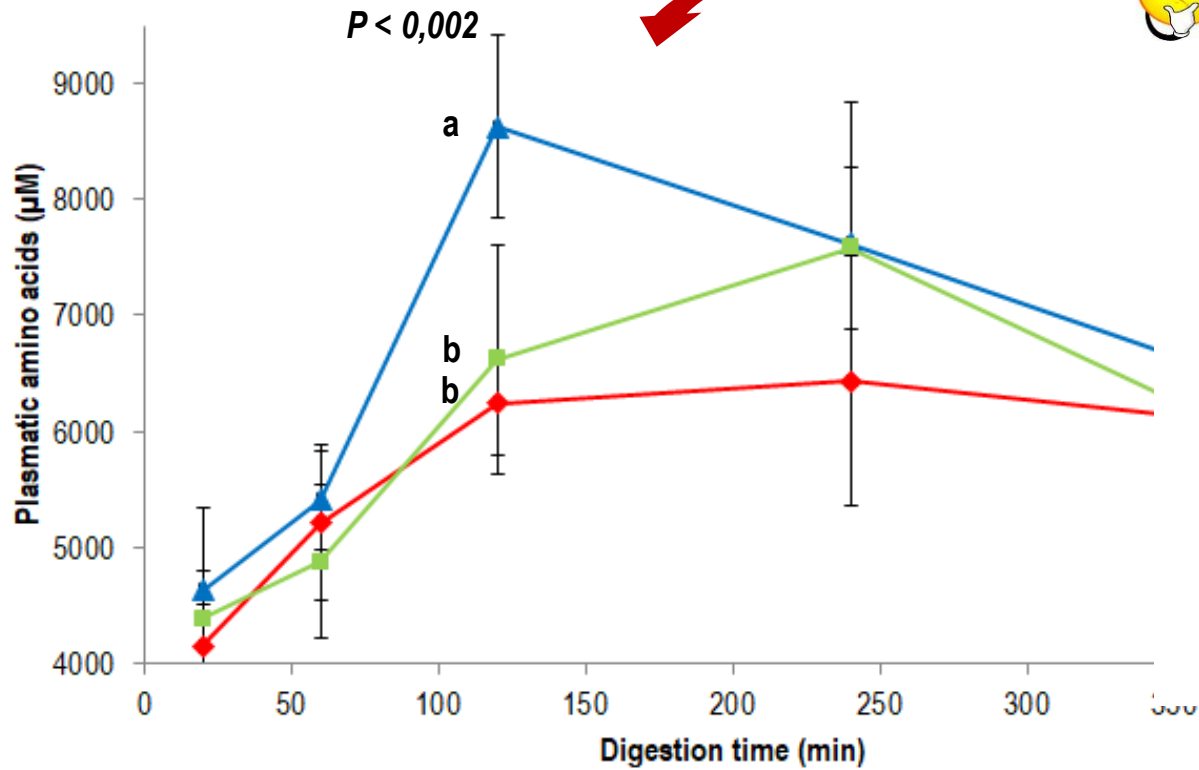
- 
 A clear increase in proteolysis only at 6h (not 4h) !  
 A matter of time (kinetics) / of pepsin real activity ?
- Similar extents of proteolysis despite variable pH and [Pepsin] ??

- 
 Nothing special in the pylorus region (0-120 min) & only slightly in the proximal region (from 240 min) ??
- Significant increase at 60 min for EGW-pH5 ??  
 (pH ≥ 4 & [Pepsin] range ≤ 300 µg/g)

# ➤ Proteolysis results (mg of free NH<sub>2</sub> / g of dry matter)

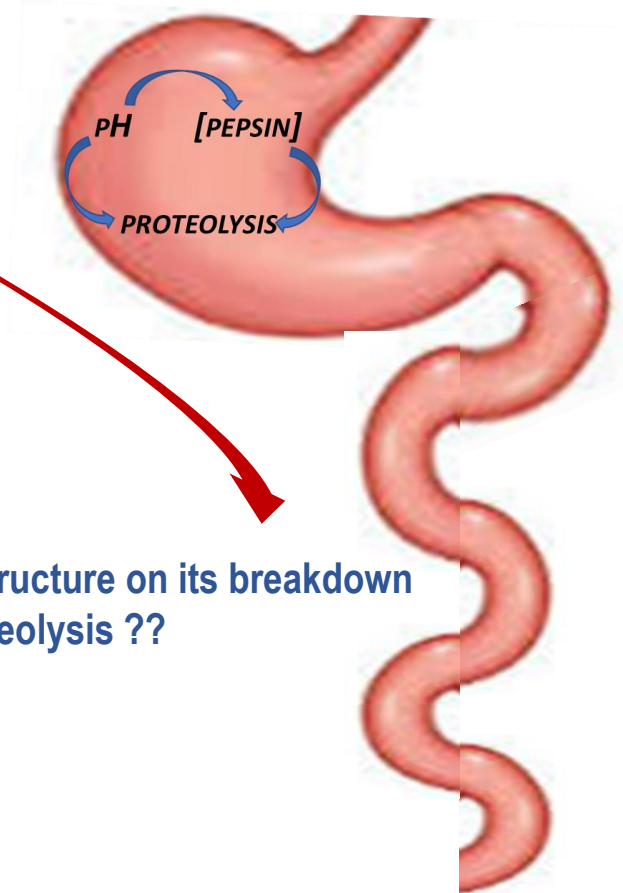
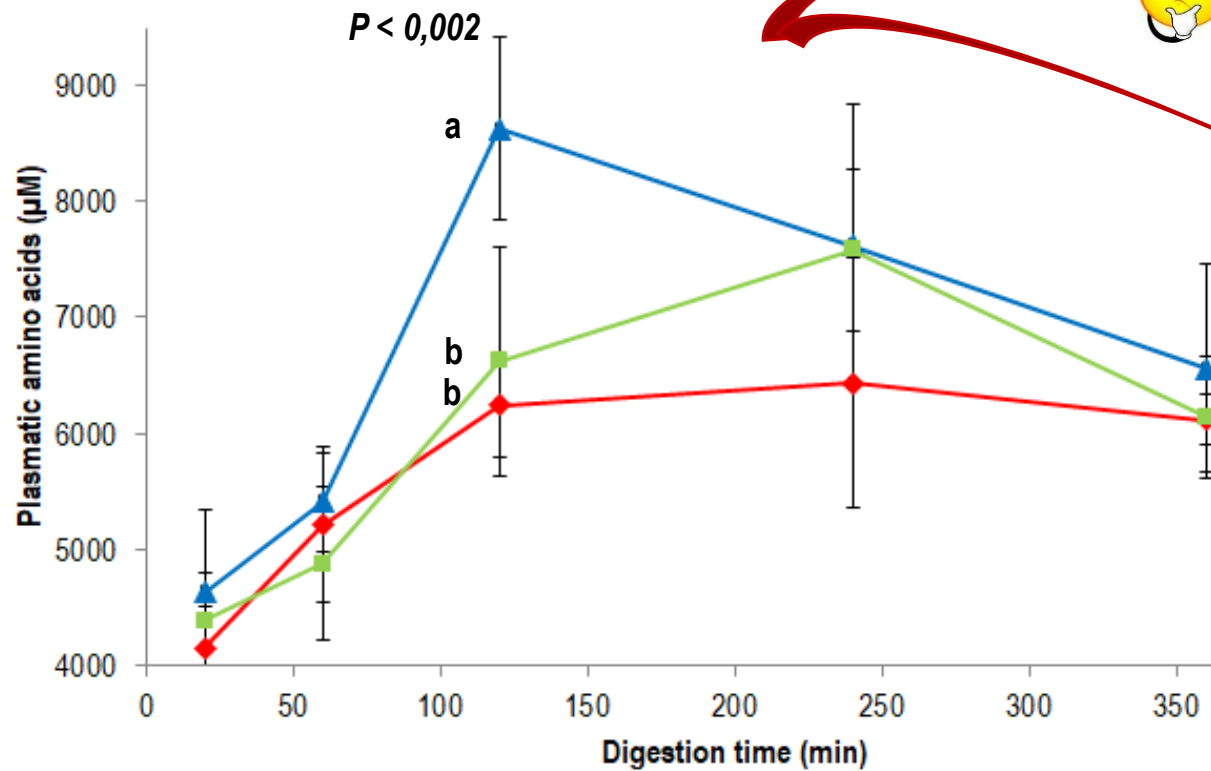
**Plasma aminoacidemia**

**Maps**



## ➤ Proteolysis results (mg of free NH<sub>2</sub> / g of dry matter)

### Plasma aminoacidemia



Effect of food structure on its breakdown & intestinal proteolysis ??

## ➤ Main Conclusions

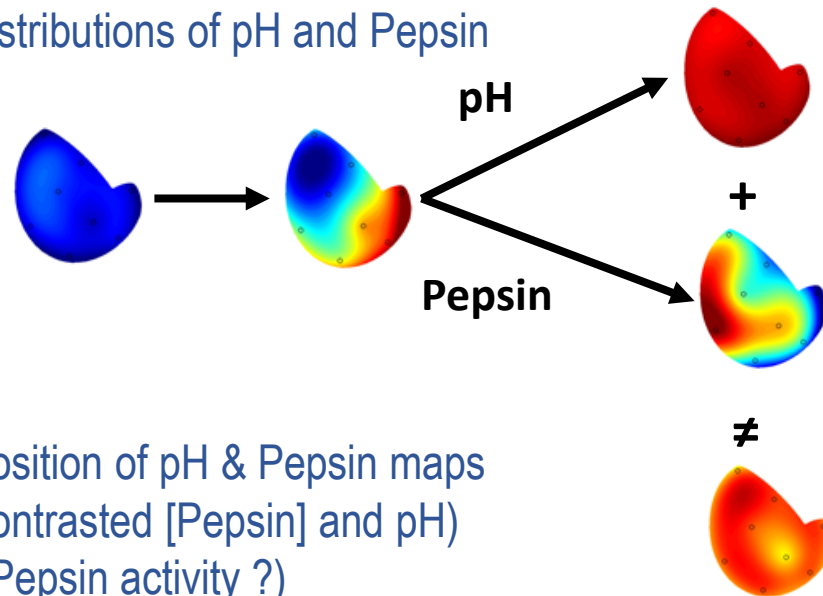
✓ **pH and [Pepsin] values are consistent with:**

- The available literature
- INFOGEST static and semi-dynamic protocols



✓ **Effects of EWG structure were observed on:**

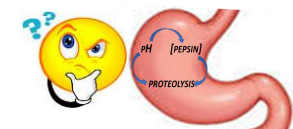
- Gastric emptying
- Gastric mixing ➡ Intra-gastric distributions of pH and Pepsin



✓ **Gastric proteolysis was:**

- Not simply explained by a superimposition of pH & Pepsin maps
- Robust (similar for all gels despite contrasted [Pepsin] and pH)
- Late (a matter of: time ? [Pepsin] ≠ Pepsin activity ?)

✓ **Gastric proteolysis does not seem the one key to understand the effect of EWG structure on plasmatic AA (=> intestinal behaviour ?)**



Nau et al., just accepted in



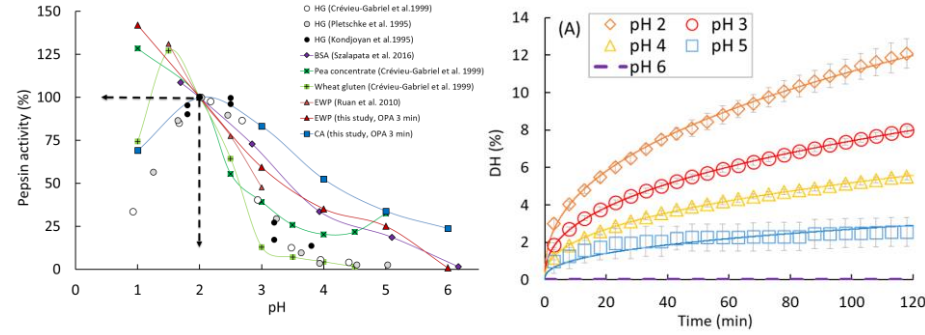
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**Pepsin activity as a function of pH, substrates & time:**  
(Salelles et al., Food Funct., 2021)  
**Poster 12 (Session 1, Sherrard Suite)**



$$DH(\%) = \alpha \times time^\beta$$



**Thank you for your attention !**

