

# EFSA GMO Panel: Workshop on allergenicity assessment

## Protein Digestion

Alan Mackie

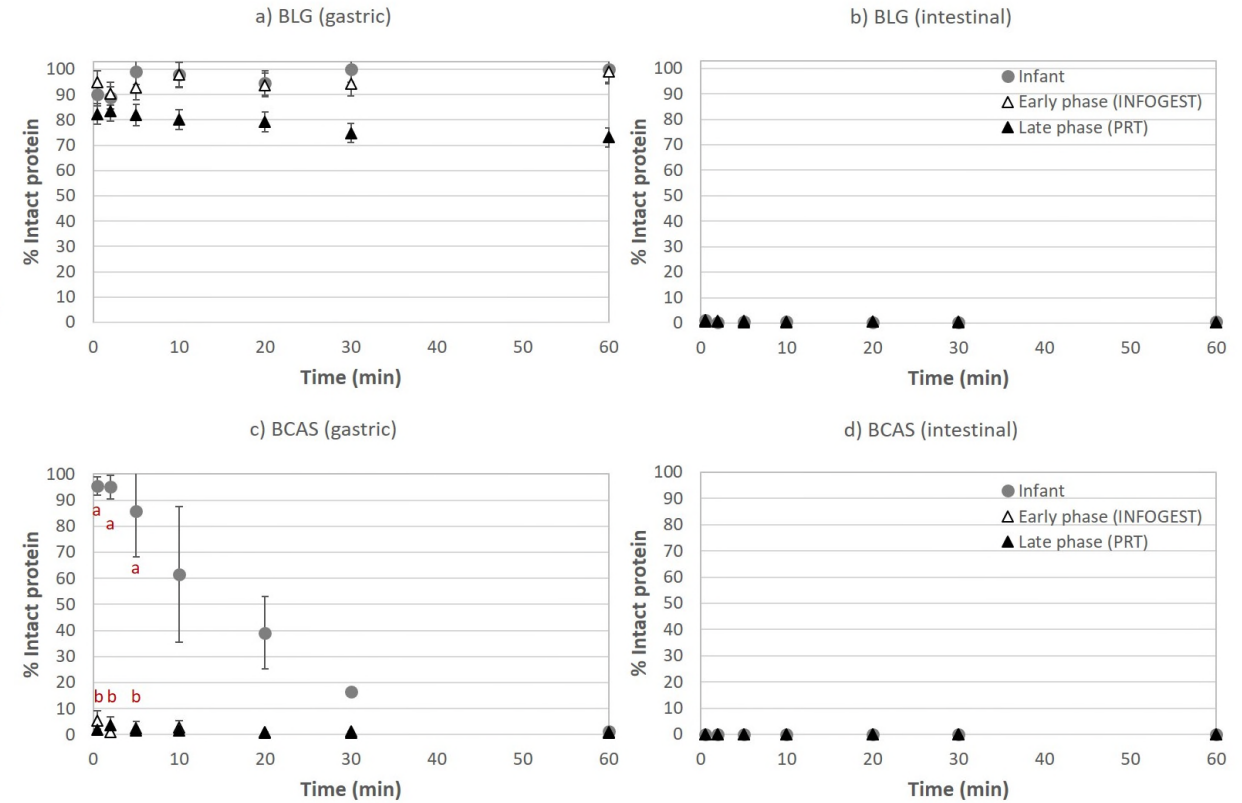
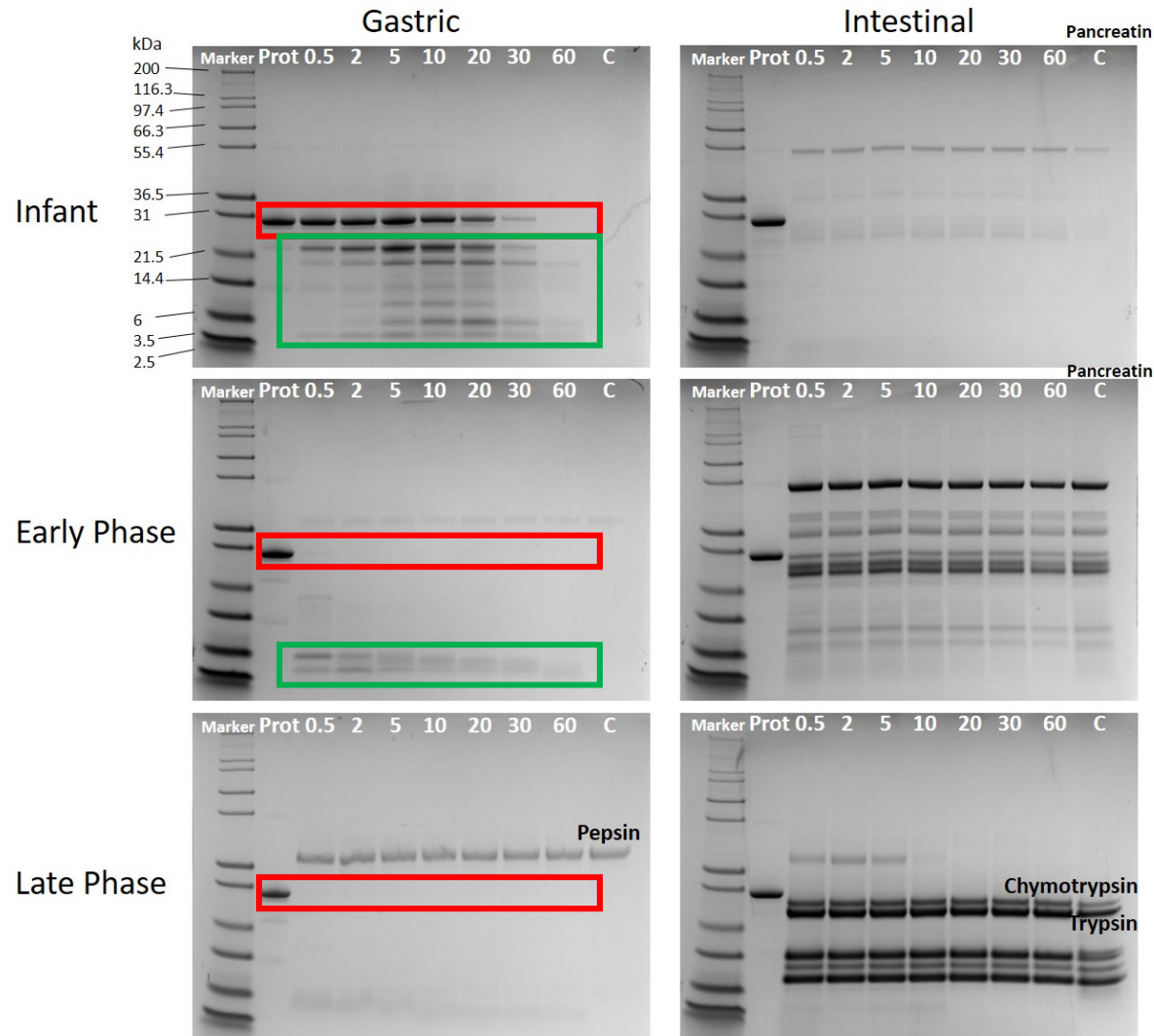
# Introduction

Protein digestion is assumed to be a risk factor for allergenicity on the grounds that amino acids and small peptides are not allergenic:

- Can physiologically relevant tools be developed and effectively applied?
- Can new protocols offer differences or advantages from the current pepsin resistance test (PRT) with respect to persistent fragments larger than 9 amino acids?
- Are these differences valuable for risk assessment taking into account Annex B and Figure B.2 (Persistent peptides larger than 9 amino acids)?

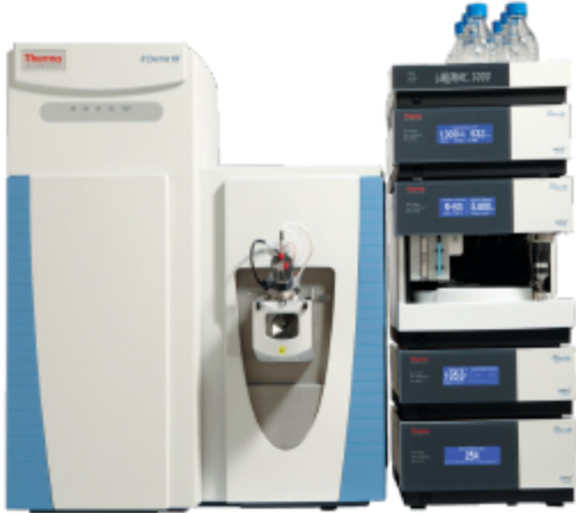


# Static Models: $\beta$ -casein in milk as an example

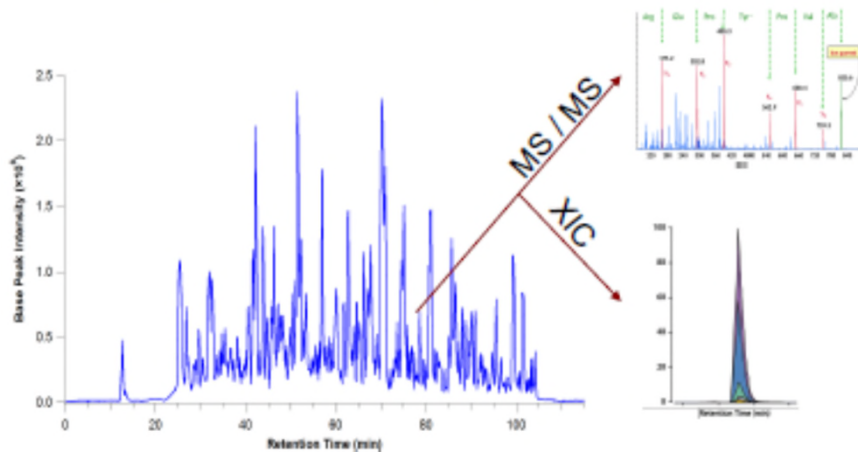


# Static Models: Mass Spectrometry

## NanoLC-MS/MS: Peptide identification and quantification



Mass spectrometry is not inherently quantitative because proteolytic peptides exhibit a wide range of physicochemical properties such as size, charge, hydrophobicity, etc

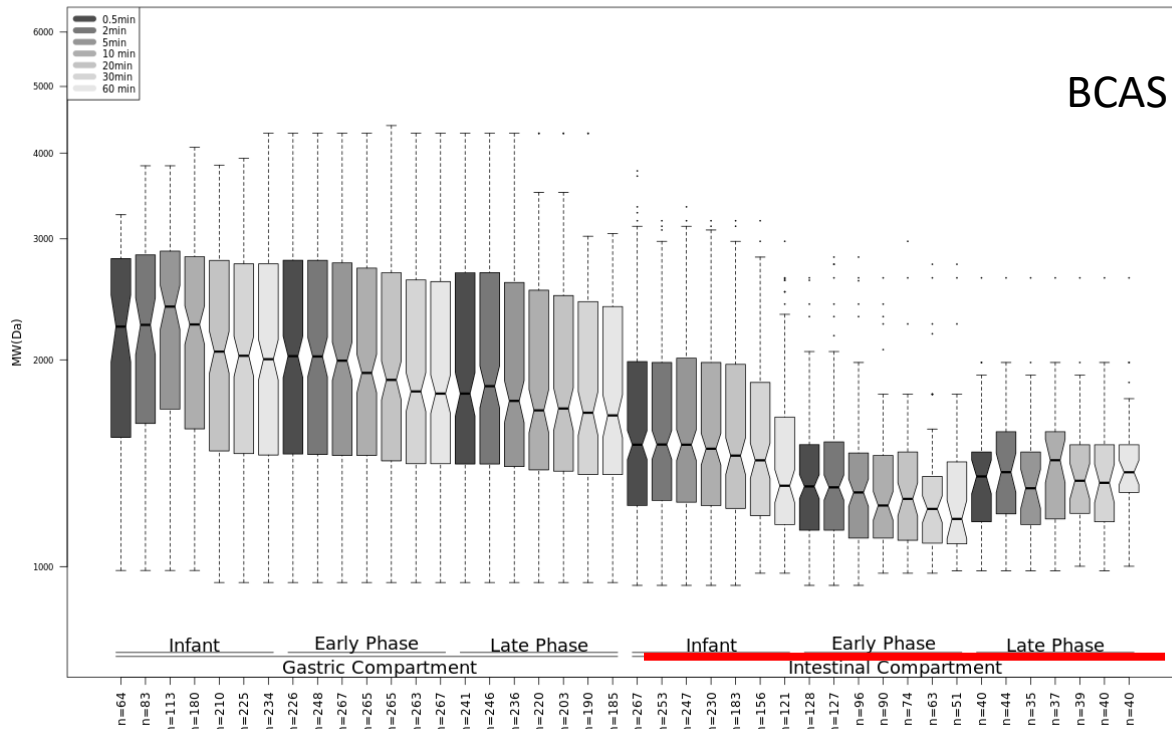


Bantscheff, M., Schirle, M., Sweetman, G. et al. Anal Bioanal Chem (2007) 389: 1017



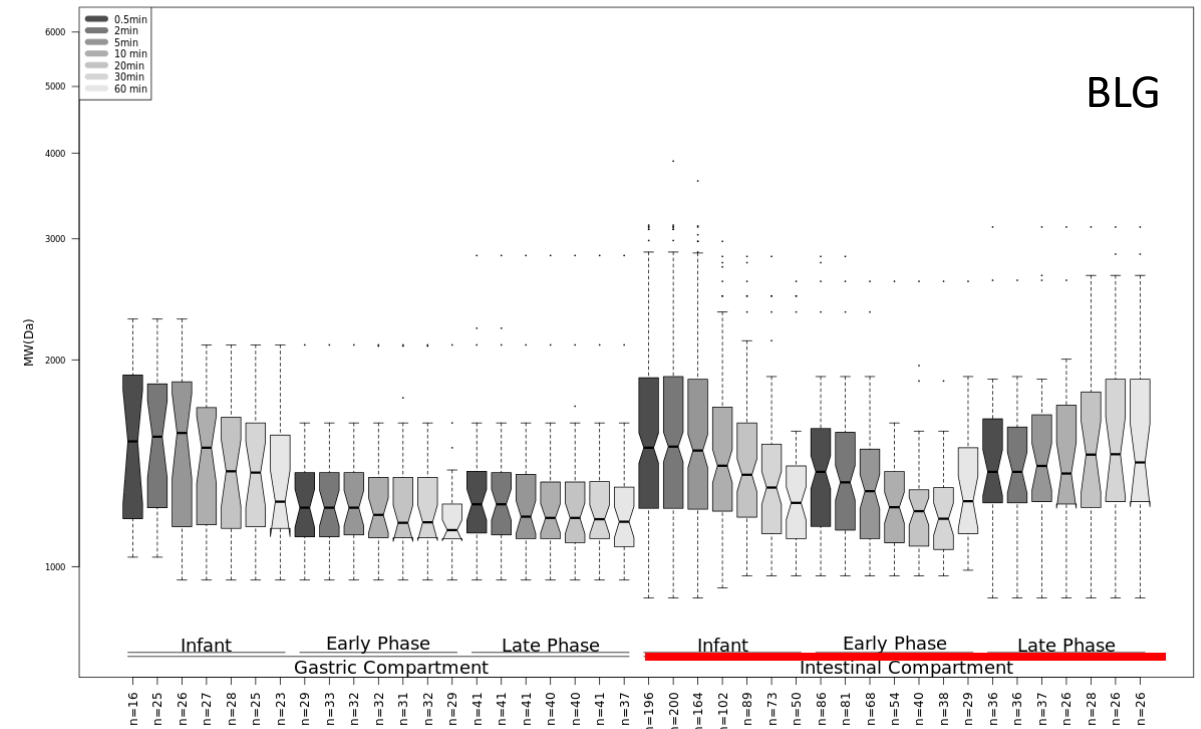
# Static Models: LC-MS

Molecular weight of detected peptides  
Beta-Casein



**More** (n=64-270) and **larger** (median ~2kDa, upper bound ~4kDa) gastric peptides

Molecular weight of detected peptides  
Betalactoglobulin

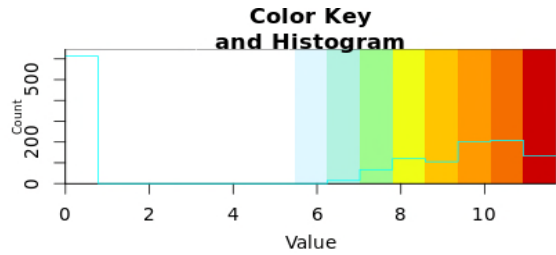


**Fewer** (n=16-32) and **smaller** (median ~1.5kDa, upper bound ~2kDa) gastric peptides

**This is reversed for intestinal peptides**

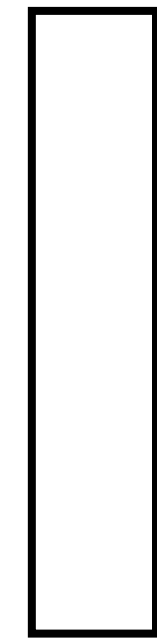


# Static Model: LC-MS

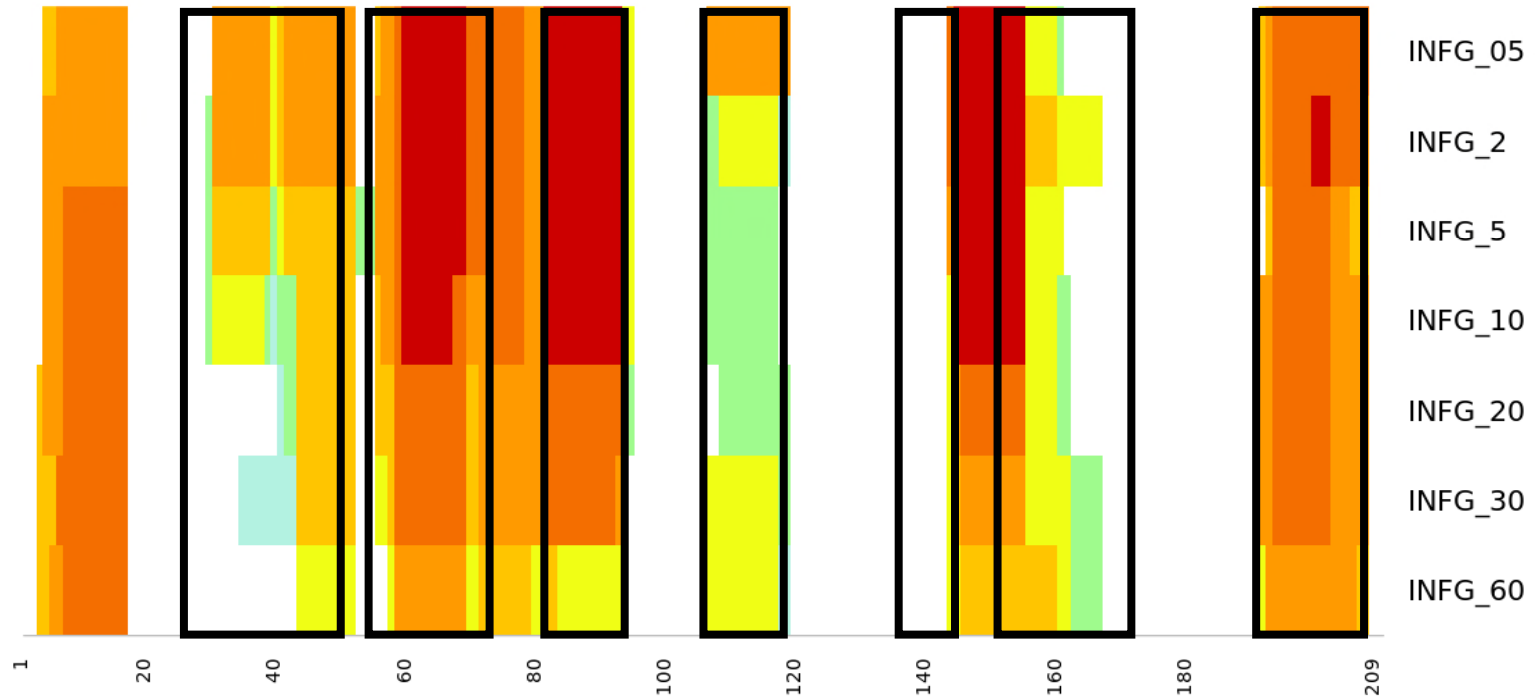


**Beta-Casein  
Intestinal compartment**

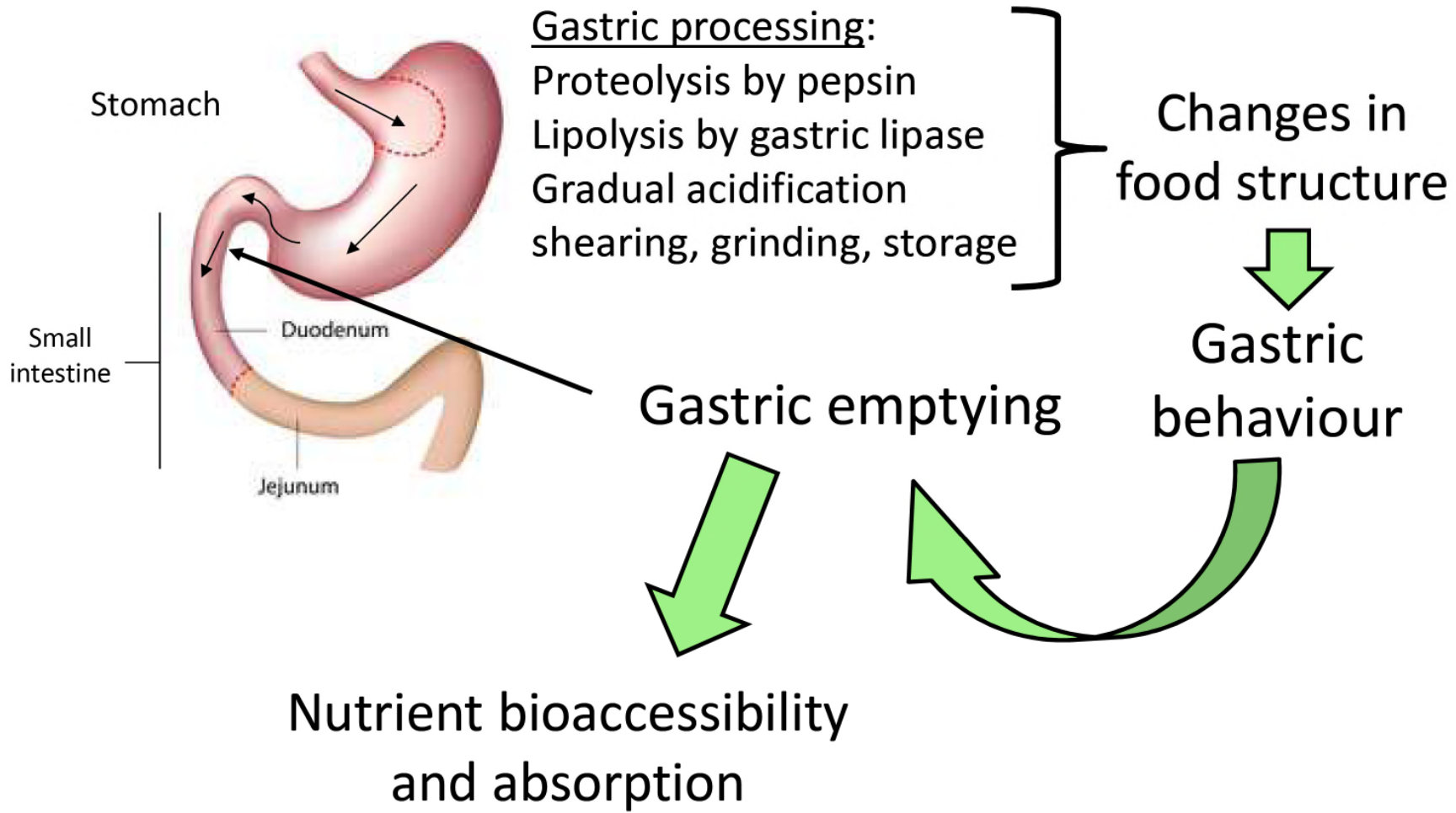
**All identified early  
phase intestinal  
peptides (n=99)**



Epitopes

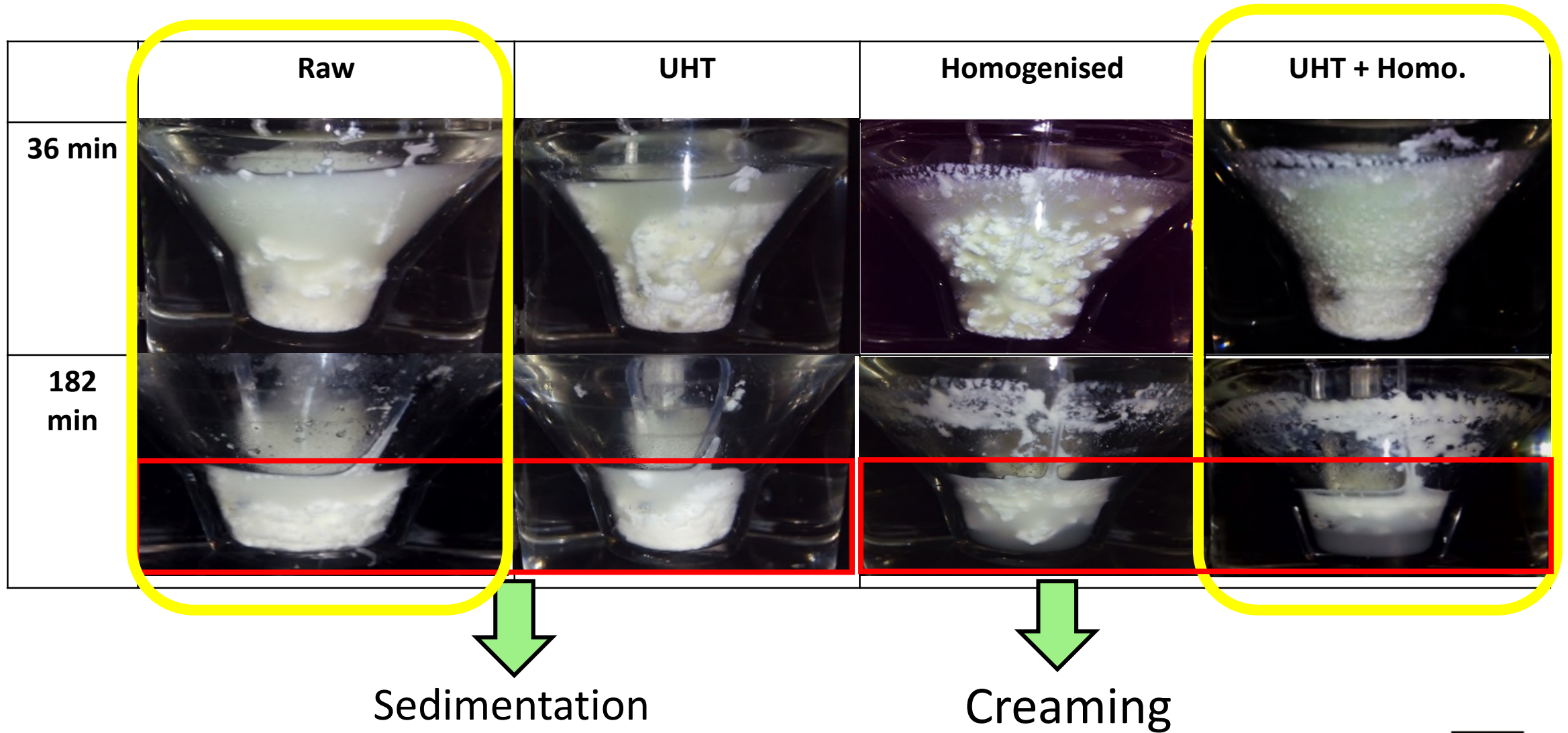


# More complex models including kinetics



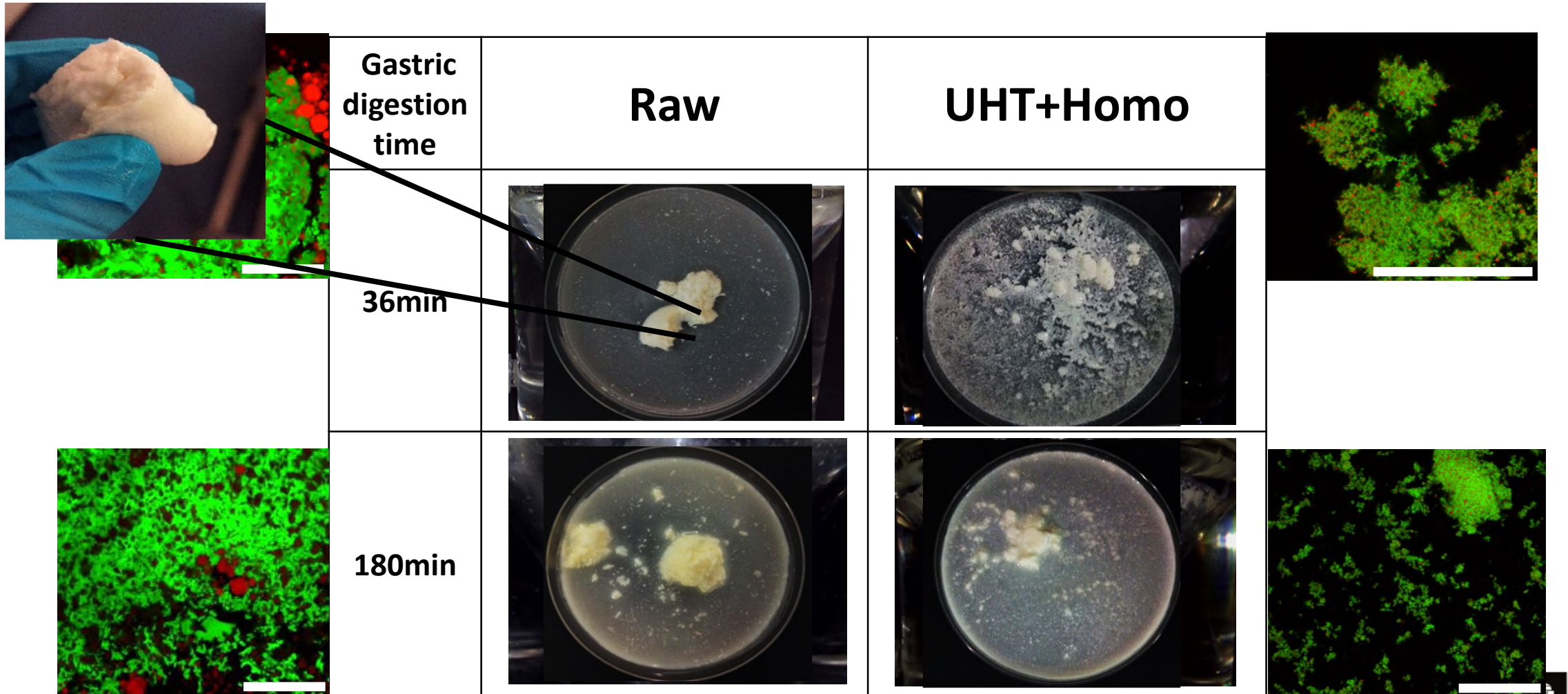


# Gastric behaviour

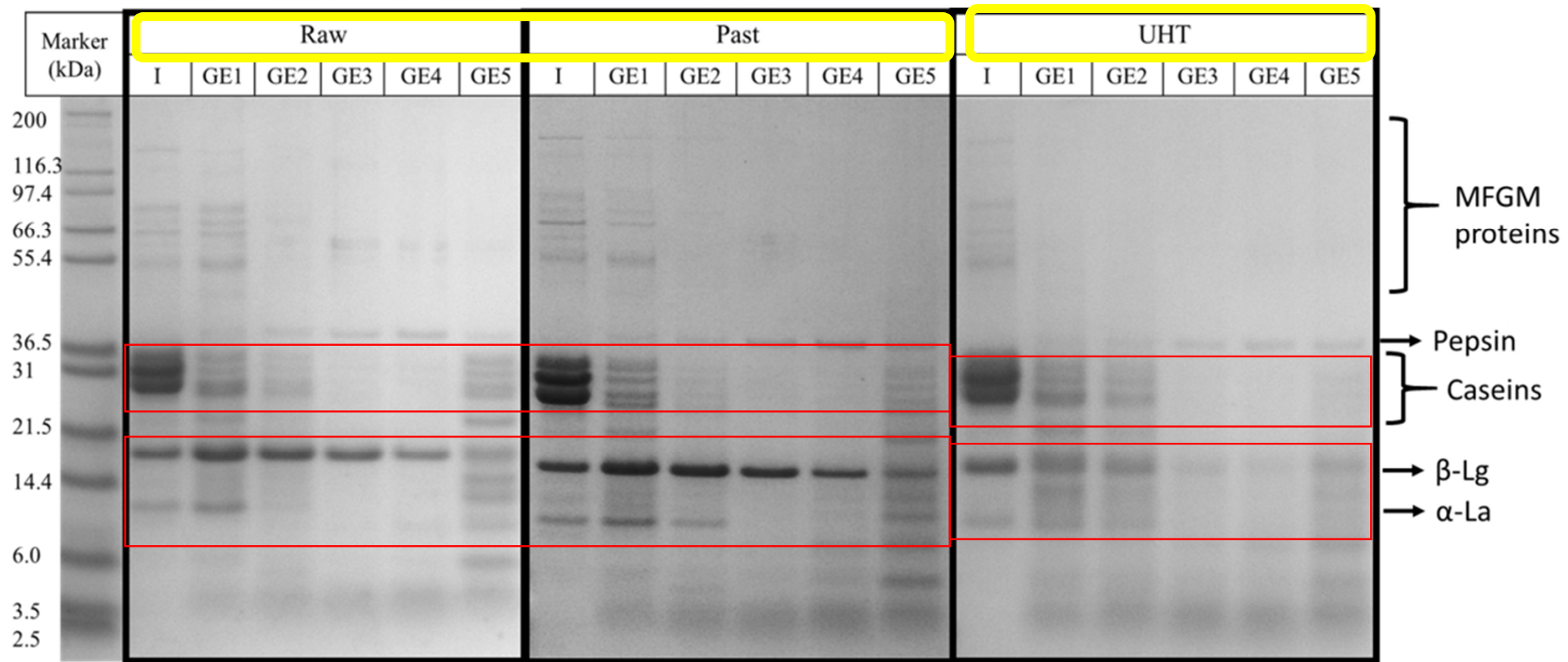




# Gastric structural changes



# Protein digestion affected by gastric behaviour



- Caseins → delayed (except UHT treated samples)
- β-lg → resistant to pepsin (except UHT treated samples)
- UHT but not Pasteurised accelerated protein digestion



# Conclusions

- Early phase adult and infant scenarios (Static) showed increased persistence of intact protein and peptides over PRT for some proteins.
- For **some** proteins there was a correlation between the peptide abundance and known epitopes
- Gastric conditions have a large influence over hydrolysis for some proteins so kinetics can be important and not just endpoints
- Hydrolysis of pure proteins may not be relevant to the real risks
- Do we need to model peptide concentrations throughout the gut or can we just model concentration vs time for key locations?
- How do microbiota in the small intestine influence protein digestion and the immune response?



# Acknowledgements

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