Pepsin diffusivity in whey protein gels and its effect on digestion

Qi Luo*, Remko M. Boom, Anja E.M. Janssen
Food Process Engineering, Wageningen University, The Netherlands

Introduction

Food structures are important in the digestion process. We previously found that the gel structure was hindering the hydrolysis of protein. However, the hindrance is not simply slowing down the hydrolysis, but also altering the enzyme kinetics to some extent (1). We assumed that pepsin can penetrate the gel microstructure and hydrolyze the protein in the gel matrices, and inferred that the diffusion limitation in the gel matrices had led to the difference in hydrolysis kinetics.

Objective

In this research, our aim is to study the diffusivity of pepsin in food microstructure and *vice versa*, the effect of pepsin diffusion on the microstructure and digestion.

Methods

- WPI gel was used as a model for protein based food matrix
- Fluorescence Correlation Spectroscopy (FCS) was employed to investigate the diffusivity of pepsin in gels
- Size-Exclusion Chromatography (HPSEC) was used to characterize the hydrolysis in the gel digestion
- Scanning Electron Microscopy (SEM) was used to observe the microstructure of the protein gels.

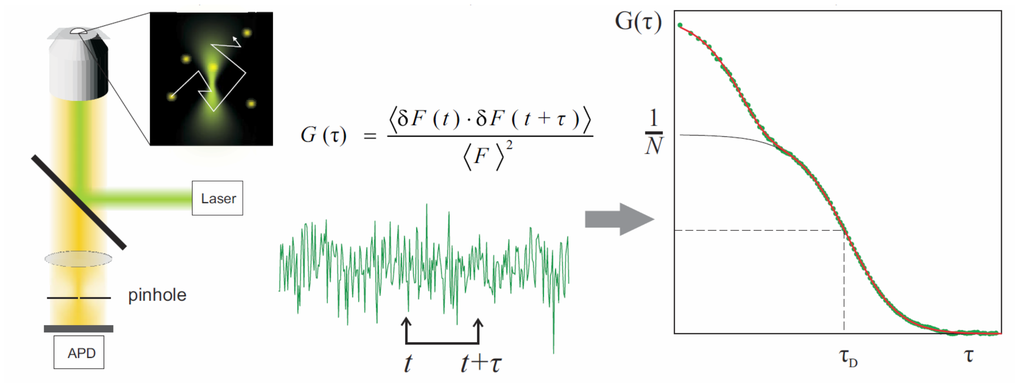
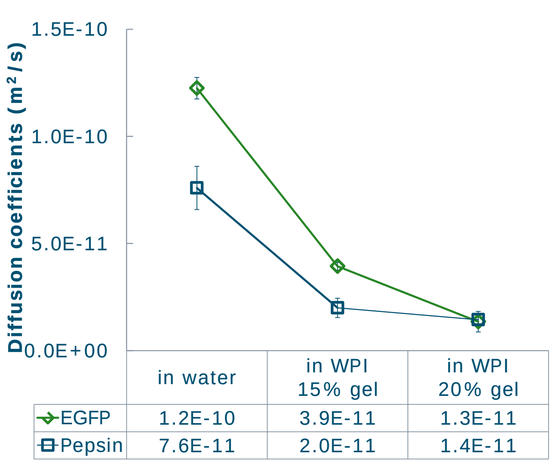


Figure 1. Principle of FCS, from Ries and Schwille, Bioessays, 2012

Results: Fluorescence Correlation Spectroscopy



	in water	in WPI 15% gel	in WPI 20% gel
EGFP	1.2E-10	3.9E-11	1.3E-11
Pepsin	7.6E-11	2.0E-11	1.4E-11

Figure 2. Diffusion coefficients of Pepsin and Enhanced Green Fluorescent Protein in water and WPI gels

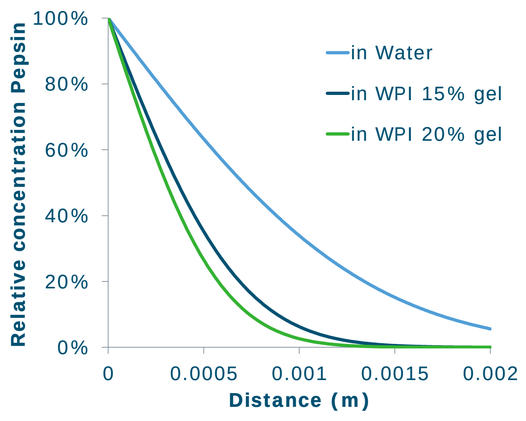


Figure 3. Relative pepsin concentration along the diffusion distance (0~2mm) in one dimension after 2h, using Fick's second law.

The diffusion coefficients of pepsin (Fig.2) can be used to estimate the enzyme concentration gradient (Fig.3). It showed that the hydrolysis of gel matrix could be constrained within a thin layer.

Result: Scanning Electron Microscopy

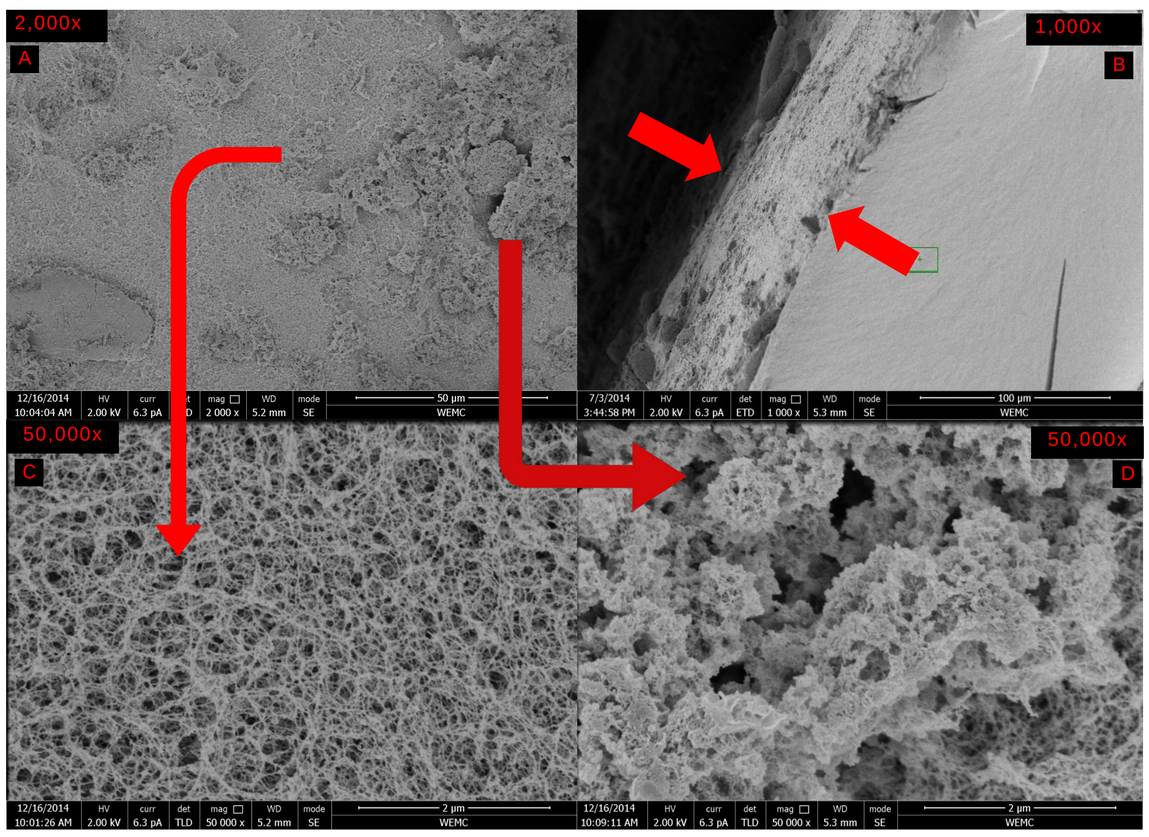


Figure 4. Scanning electron microscopy image of WPI 15% gel surface (A, C, D) and cross-section (B), after 1h of digestion

SEM image of Digested WPI 15% gel showed "rough" (C) and "smooth"(D) area on the surface. The cross-section of the gel (B) showed a weakened gel layer on the edge of the gel.

Results: Size-Exclusion Chromatography

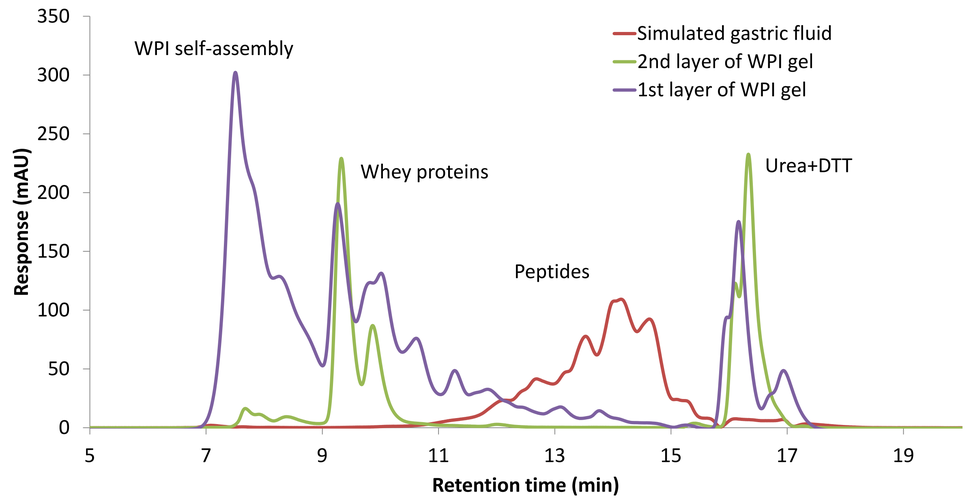


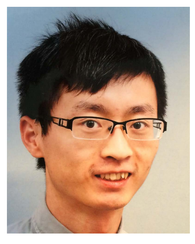
Figure 4. Gel composition analysis by HPSEC. WPI 15 % gels were digested, sliced and dissolved in urea+DTT

The first 2mm of the WPI gel showed the existence of hydrolysates while deeper layers showed none, which is in accordance with the estimation based on the enzyme diffusion.

Conclusions

- Diffusion study and gel composition analysis proved that the digestion process is affected by food microstructure, partly due to the diffusion limitation.
- SEM shows that the surface layer of the protein gel is weakened and consequently "worn-away" during digestion.

Food Process Engineering, Wageningen University
P.O. Box 17, 6700 AA Wageningen
Contact: qi.luo@wur.nl
T +31 (0) 317 48 08 51
www.wageningenUR.nl/fpe



Reference

1 Q. Luo, R. M. Boom and A. E. M. Janssen, LWT - Food Science and Technology, 2015, 63, 161–168.