LWT - Food Science and Technology 63 (2015) 161–168

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Digestion of protein and protein gels in simulated gastric environment

Qi Luo*, Remko M. Boom, Anja E.M. Janssen

Wageningen University, Food Process Engineering Group, P.O. Box 17, 6700 AA Wageningen, the Netherlands

ARTICLE INFO

Article history:
Received 20 November 2014
Received in revised form
16 March 2015
Accepted 21 March 2015
Available online 11 April 2015

Keywords:
Gastric digestion
Pepsin
Whey protein isolate
Egg white protein
Protein gel

ABSTRACT

Despite the increasing attention to food digestion research, food scientists still need to better understand the underlying mechanisms of digestion. Most *in vitro* studies on protein digestion are based on experiments with protein solutions. In this study, the digestion of egg white protein and whey protein isolate in solution and in gels was investigated using simulated gastric conditions. The digestion process was followed via the dry matter loss, degree of hydrolysis and peptide distribution. We showed that the performance of pepsin is an important factor in protein digestion, and hydrodynamic force effectively disintegrated the gel particles and enhance the hydrolysis of protein. The gel microstructure had shown to be a hindrance for the digestion of protein. However, the hindrance is not simply slowing down the hydrolysis, but also altering the apparent enzyme kinetics to some extent: while the dissolved proteins were hydrolysed through a 'zipper' type mechanism, the gels showed a slower 'one-by-one' mechanism. Overall, we believe that the digestion of the protein gels is influenced by the microstructure of food matrices, caused by the immobilisation of the substrate in the network, and the steric hindrance in the diffusive ingression of pepsin and egression of peptides.

1. Introduction

The gastric digestion of food into dissolved or dispersed nutrients is an essential step in human nutrition and health. However, regarding the digestion of solid food, limited knowledge is available, especially on the underlying mechanism of the process (Bornhorst & Singh, 2014). By understanding the disintegration dynamics of solid food in the stomach, the digestion process can be better pictured. As a result, food products may be better designed towards the targeted consumer group.

In the mouth, the solid food is masticated, mixed with saliva and formed into a cohesive mass, *i.e.* the food bolus. The bolus is then swallowed, and undergoes a process of swelling, hydrolysis, disintegration and dissolution in the stomach (Bornhorst & Singh, 2012; Kozu et al., 2014). In the stomach, the peristaltic waves promote the disintegration of the bolus by grinding and mixing, which reduces diffusion distances and enlarges the interfacial area. The disintegration of the matrix is dependent on the physical and chemical condition of the digestion environment as well as the density and coherence of the food matrix (Bornhorst & Singh, 2012).

When the particles in the bolus are able to pass the pylorus which acts as a sieve, they are selectively emptied to the duodenum (Guerra et al., 2012). The food is then further digested and absorbed in the small intestine (Whitney, Cataldo, & Sharon, 1998).

Protein is one of the most important macronutrients in food. The digestion of protein is mostly facilitated by the acid and pepsin in the stomach and subsequently by the pancreatic and intestinal enzymes in the small intestine (Whitney et al., 1998).

Pepsin (EC 3.4.23.1) is the major enzyme in gastric fluid. It is an aspartic protease and has a broad specificity with a preference for hydrophobic residues (Rawlings & Salvesen, 2012), especially the aromatic amino acid residues tyrosine and phenylalanine (Fruton & Bergmann, 1939).

The enzymatic hydrolysis of proteins, including the peptic hydrolysis, has been extensively studied. With regard to the kinetics of the enzymatic hydrolysis, some models are proposed to characterise the reaction during the proteolysis, including the widely used Linderstrøm-Lang's model.

Linderstrøm-Lang introduced two extreme types of reactions for native globular proteins: the "one-by-one" type and the "zipper" type (Adler-Nissen, 1976; Linderstrøm-Lang, 1952, also see Supplementary Material). In the "one-by-one" type, as soon as a

Abbreviations: WPI, whey protein isolate; EWP, egg white protein; SD, standard deviation; DH, degree of hydrolysis; MW, molecular weight; SEC, size-exclusion chromatography; SGF, simulated gastric fluid.

* Corresponding author. Tel.: +31 317480851.
E-mail addresses: qi.luo@wur.nl (Q. Luo), remko.boom@wur.nl (R.M. Boom), anja.janssen@wur.nl (A.E.M. Janssen).

http://dx.doi.org/10.1016/j.lwt.2015.03.087
0023-6438/

protein is attacked by protease, the protein tend to be hydrolysed in one sequence to the final products, and thus intermediate products can scarcely be detected. In the "zipper" type, the initial stage of hydrolysis is fast, but the subsequent steps are much slower, which results in a wide range of peptides in solution (Ortiz & An, 2000). Most proteases will act in between the two extreme models (Adler-Nissen, 1976). Choisnard et al. observed a 'one-by-one' mechanism for the peptic hydrolysis of native haemoglobin and a 'zipper' type reaction for the hydrolysis of denatured haemoglobin (Choisnard et al., 2002).

Generally, studies into the kinetics and mechanism of enzymatic proteolysis are mostly based on the reaction in solutions, whereas most of the proteins in our food are present in solid foods (even casein in milk tends to precipitate and form a solid mass in the stomach (Lambers, Bosch, & Jong, 2013)). It is interesting to study the effect of the solid matrix on the digestion and thus on the behaviour of pepsin. Barbé et al. (2014) found that acid and rennet gels show different bioavailability and kinetics in digestion. Guo et al. (Guo, Ye, Lad, Dalgleish, & Singh, 2014) made simulated boluses of soft and hard gels and studied the gastric digestion. In this study we aim at the digestion of well-defined solid, protein matrices in a simulated gastric environment. Protein gels were used for this. We focus on the role of pepsin and its interaction with other factors (e.g., pH and matrix properties).

2. Materials and method

2.1. Materials

Pepsin from porcine gastric mucosa(≥400 activity units/mg protein), mucin from porcine stomach (Type III) and all other chemicals were purchased from Sigma–Aldrich, Inc. (St. Louis, USA). Milli-Q water (resistivity 18.2 MΩcm at 25 °C, Merck Millipore, Billerica, USA) was used in all experiments. Whey Protein Isolate (WPI) (Bipro, lot no. JE 034-70-440-6) was purchased from Davisco Food International, Inc. (Le Sueur, USA) This batch of WPI was reported to have a protein content of 97.9 g/100 g dry solid. Chicken fresh shell eggs were purchased from a local supermarket. The egg white was separated from the whole egg and gently mixed. Afterwards, the egg white was lyophilised and ground. The egg white protein (EWP) powder thus obtained was stored under dry conditions at room temperature. The protein content was 89.3 g/100 g dry solid measured by DUMAS in duplicate.

2.2. Preparation of gel and simulated digestion fluids

Egg white gel and whey protein gel with 15 g protein/100 g gel or 20 g protein/100 g were made as follows: egg white protein powder and WPI were respectively dissolved in water (15 g protein/100 g or 20 g protein/100 g) and stirred at room temperature for at least 2 h. The solutions were centrifuged at 1000 rpm (1 rpm = 2π rad/min, approximately 200 × *g* relative centrifuge force for the swing-bucket rotor) for 10 min to eliminate air bubbles, and were poured into Teflon tubes which were then sealed. The Teflon tubes were rotated at 50 rpm heated in a 90 °C water bath for 30 min. After that, they were immediately cooled in an ice-water bath. The gels were sealed with multiple layers of kitchen plastic wrap (cling foil) and stored at 4 °C. Visual inspection showed that gel with a concentration of 20 g protein/100 g is much more compact than that gel with a concentration of 15 g protein/100 g. The gels were stored 1–5 days prior to use.

The simulated gastric fluid (SGF) and simulated saliva were prepared based on the composition of human gastric juice and saliva, following Kong & Singh (2008). The simulated saliva contained gastric mucin (1 g/L), α-amylase (2 g/L), NaCl (0.117 g/L), KCl (0.149 g/L), and $NaHCO_3$ (2.1 g/L). The SGF comprised of pepsin (1 g/L), mucin (1.5 g/L), NaCl (8.775 g/L), and pH 1.8 to 2.0 adjusted with 2 mol/L HCl. The SGF without pepsin had the same composition except that no pepsin was added. SGF prepared at pH 3 were used to study the effect of pH in dry matter loss of WPI gel.

2.3. Dry matter loss of WPI gel

Static soaking system A based on the design of Kong and Singh (2008) was built (Fig. 1A) to apply stirring and track the dry matter loss hourly. The system was kept at 37 °C, agitated at 100 rpm.

The gel samples (cylindrical, Ø 7 mm × 9 mm approximately, original weight (0.25 ± 0.01) g (mean ± standard deviation (SD), N = 24)) were first dipped into simulated saliva at 37 °C for 15 s to mimic the oral process, afterwards they were fixed on the needles and soaked in 300 mL SGF so that direct contact of the gel and magnetic bar was avoided. Four gel samples were tested in one batch, each time point was an individual experiment. This set-up was used to measure the effect of gel concentration and the effect of pH.

2.4. Study of peptic hydrolysis in simulated gastric fluid

2.4.1. Protein solutions and protein gels in static soaking

The hydrolysis of protein solutions and protein gels in SGF was studied in static soaking system B (Fig. 1B).

For solution experiments, 0.1 g protein (EWP or WPI) was dissolved in 1.9 mL Milli-Q water. These solutions underwent the heat treatment at 90 °C, 30 min, 1400 rpm in a pre-heated Eppendorf thermomixer (Eppendorf AG, Hamburg, Germany). Each heat-treated protein solution was added to one of the vessels containing 50 mL of SGF, stirring at 100 rpm.

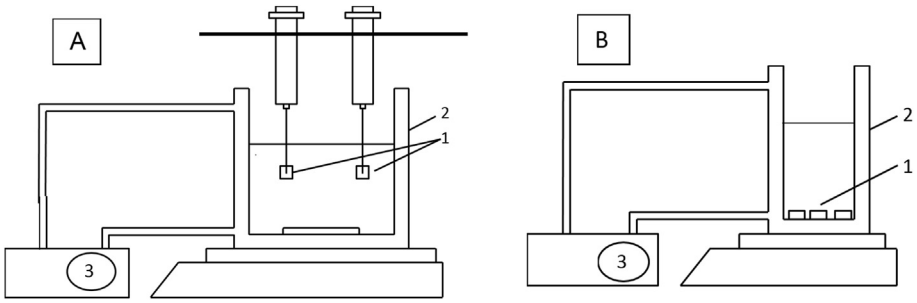

Fig. 1. Static soaking systems A for studying the dry matter loss of whey protein isolate gel and systems B for studying protein hydrolysis: 1. Gel samples in simulated gastric juice (SGF) (System A: 4 of Ø 7 mm × 9 mm cylindrical samples in 300 mL SGF, system B: 10 of Ø 5 mm × 5 mm cylindrical samples in 50 mL SGF) 2. Water-jacketed beaker 3. Heat circulator.