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The structural properties of egg white gels impact the extent of *in vitro* protein digestion and the nature of peptides generated

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ABSTRACT

The impact of egg white gel (EWG) structure on the process of digestion was investigated using an *in vitro* digestion model and a multi-scale characterization of the EWG structure. Four different structures of EWG were prepared combining various pH and ionic strength conditions before heating. The extent of digestion, estimated by the appearance of soluble peptides, was greater for the particulate gel comprising spherical aggregates as compared to the filamentous gel comprising linear aggregates. This result may be explained by an alteration of enzyme diffusion due to the microstructural characteristics of the gel, or an alteration of digestive enzyme adsorption or catalytic activity due to local differences in pH within the gel matrices. Peptide identification using LC-MS/MS highlighted that the aggregate morphology modulated ovotransferrin hydrolysis which resulted in the release of specific peptides depending on the EWG structure. This work illustrates the links existing between two multi-step processes, protein structuration into gel matrices and matrix destructuration by digestive enzymes. Such quantitative and qualitative differences reinforce the importance of the food matrix on the digestibility of food and its subsequent nutritional quality.

1. Introduction

From the understanding of influencing parameters to underlying mechanisms, including structural characterization, gelation of food proteins has generated a considerable interest over the last years (Aguilera & Stanley, 1993; Banerjee & Bhattacharya, 2012; Harris, 1990; Totosaus, Montejano, Salazar, & Guerrero, 2002; Ziegler & Foegeding, 1990). Recently, it has been recognized that food structure is an important parameter that influences digestion within the gastrointestinal tract (Fardet, Souchon, & Dupont, 2013). More specifically, the importance of food protein gelation on the nutritional properties of human foods has been investigated and the potential of protein gelation for developing food structures that modulate digestion in now recognized (Norton, Espinosa, Watson, Spyropoulos, & Norton, 2015).

Heating of protein solutions triggers conformational changes that affect the folded structure of proteins and causes the formation of aggregates which join together to form gels (Ma & Holme, 1982; Weijers, Barneveld, Stuart, & Visschers, 2003). Using an *in vitro* digestion model, we have previously shown that the morphology of ovalbumin (OVA) aggregates strongly influences the extent of digestion, nature and amount of generated peptides, and that non-aggregated OVA is more resistant to enzymatic proteolysis than aggregated OVA (Nyemb, Guérin-Dubiard, et al., 2014; Nyemb, Jardin, et al., 2014). Similar work on heat-induced aggregated whey protein isolates (WPI) reported that the protein's susceptibility to digestion by gastric enzymes differed according to the physicochemical properties of the aggregates (Zhang & Vardhanabhuti, 2014). Moreover, several studies have suggested that the rate of digestion of gelled protein systems depends on the

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gel structure. Using a static *in vitro* gastrointestinal model, Macierzanka et al. (2012) reported that filamentous gels of WPI were digested to a greater extent than particulate gels, whilst filamentous gels formed at pH 2.5 were resistant to pepsinolysis. The results showed that particulate gels formed at 70 °C for 24 h were less resistant to proteolysis than particulate gel formed at 85 °C for 30 min. Using a different *in vitro* gastrointestinal model, Remondetto, Beyssac, and Subirade (2004) similarly suggested that the structural properties of β-lactoglobulin gel formed by cold-set gelation with iron, influenced the course of proteolysis. As a matter of fact, filamentous and particulate gels showed equivalent proteolysis, but particulate gels released more iron than filamentous gels during the gastric phase, and the opposite was observed during the duodenal digestion phase. In contrast, Guo, Ye, Lad, Dalgleish, and Singh (2014) have shown, using a dynamic *in vitro* gastric model, that the release of oil droplets from a filamentous whey emulsion gel was higher when compared to a particulate gel due to greater disintegration and therefore greater pepsin accessibility to cleavage sites.

Egg white (EW) is more or less a colloidal suspension of different proteins in water, with a total protein content of about 10% (Mine & Zhang, 2013; Strixner & Kulozik, 2011). Due to their functional and nutritional properties, egg white proteins (EWP) are extensively used in food systems and especially in food gels (Doi, 1993; Mine, 1995; Mine & Zhang, 2013). The structural properties of these gels depend on the aggregate morphology such as linear strands or spherical particles, which in turn depends on pH, ionic strength, protein concentration and heating time and temperature (Croguennec, Nau, & Brule, 2002; Doi, 1993). EW is an interesting model to investigate protein gelation because it can form a wide range of gel structures while keeping a constant chemical composition.

The present study aimed to investigate the impact of EW gel structure on the process of digestion from both a quantitative (extent of digestion) and qualitative (nature of peptides generated) perspective. The quantitative perspective is essential since the efficiency of digestion is directly linked to the nutritional quality of foods. But the qualitative perspective also deserves to be considered since the nature of peptides generated can be related to some specific bioactivities. In that aim, this study was achieved by applying an *in vitro* model that simulated digestion in the stomach and small intestine, on different EW gel structures prepared with heat treatment using different combinations of pH and ionic strength. A multi-scale characterization of these gels was performed.

2. Materials and methods

2.1. Reagents and chemicals

Pasteurized egg white was obtained from 3 Vallées processing plant, France. Unless otherwise stated, chemicals were purchased from Sigma (St Louis, MO, USA). Ultrapure water was purified using a Milli-Q system (Millipore, Molsheim, France).

Two egg white preparations were used: EW, which is the pasteurized egg white; EWUF which is the EW that was partially desalted using ultrafiltration.

2.2. Preparation of low ionic-strength egg white (EWUF)

In order to produce the EWUF, EW was concentrated by ultrafiltration using a pilot plant ultrafiltration system (TIA, Bollene, France). The system was equipped with a multi-tubular mineral membrane (1 m^2) having an 8 kDa molecular weight cut off (TAMI, Nyons, France). The ultrafiltration was performed at 20 °C until the solution was concentrated 2.7-fold. The retentate was then diluted with distilled water (1:2.7 v:v) to obtain EWUF. The protein concentration (N X 6.25) in the EWUF was determined using the Kjeldahl method. The chloride concentration was quantified using a potentiometer (Chloride analyzer 926, Ciba Corning Diagnostic, Halstead, U.K.). Magnesium and sodium concentrations were determined by atomic absorption spectrometry as described by Brulé, Maubois, and Fauquant (1974). The EW had a ionic strength of 1 M and a protein concentration of 10.5% while the EWUF had a ionic strength of 0.05 M and a protein concentration of 10.9%; initial pH of both solutions was 9.2.

2.3. Heat-induced gelation of egg white

The pH of EW was adjusted to 5.0 and the pH of EWUF was adjusted to either 2.0, 7.0 or 9.0 with 2 M HCl in order to obtain a wide range of EWG structures and properties. Variations in sample volumes due to the addition of acid were minimized by adding sufficient ultrapure water to obtain a protein concentration of 10%. Solutions (40 ml) were each poured into synthetic casing with a 1.43 cm radius (r) (Krehalon, Deventer, Holland). Samples were heated at 80 °C for 1 h in a temperature-controlled water bath. After heating, the samples were immediately cooled in ice water and kept at 4 °C for 10 min. The casings were then removed and the gel equilibrated to room temperature before further analysis.

2.4. Multi-scale characterization of egg white gels (EWG)

2.4.1. Textural properties

The egg white gels (EWG) were cut into 20 mm long sections using two parallel metal wires. Measurements of syneresis were adapted from the method of water release measurement described by Croguennec et al. (2002). Briefly, a gel section was placed vertically on a previously weighed plate containing Whatman filter paper (Whatman 541, PolyLabo, France). Measurements of water release were performed using the TA.XT2 Texture Analyzer (Stable Micro Systems, Surrey, UK) with a 5 N load cell and equipped with a 4-mm-dia stainless steel plate. The plate was lowered at a constant speed of 10 mm. min^{-1} to a distance of 3 mm above the plate and the gel deformation was maintained for 3 min. After compression, the plate and the wet Whatman filter were weighed in order to calculate the weight of water released. The syneresis was defined as the weight of water released from a determined volume of EWG (expressed in kg m^{-3}).

An instrumental texture profile analysis (TPA) was performed using the same TA.XT2 Texture Analyzer (Stable Micro Systems, Surrey, UK) with a 5 N load cell. The gels were compressed twice at 10 mm s^{-1} to a 25% compression rate. The results are reported as the means of triplicate tests. Cohesiveness was obtained from the TPA curve as described by Pons and Fiszman (1996) from the work of Bourne (1978). Cohesiveness is defined as “the ratio of the positive area during the second compression portion to that during the first compression, excluding the area under the decompression portion in each cycle”.

2.4.2. Rheological properties

An AR2000 Rheometer (TA Intruments, Guyancourt, France) equipped with a 2°/40 cone-plane geometry was used for measuring the rheological properties of egg white samples. The rheological procedure aimed at reproducing the heat changes when liquid EW samples are placed in the water bath at 80 °C. Egg samples were coagulated directly on the rheometer plate with a temperature program from 40 °C to 80 °C in 3 min, and then maintained at 80 °C for 20 min. The plate was equipped with a Peltier temperature control. A moisture trap was used to prevent