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Investigating the impact of egg white gel structure on peptide kinetics profile during *in vitro* digestion

Kéra Nyemb-Diop [a,b], David Causeur [c], Julien Jardin [a,b], Valérie Briard-Bion [a,b], Catherine Guérin-Dubiard [a,b], Shane M. Rutherfurd [d], Didier Dupont [a,b], Françoise Nau [a,b,*]

[a] INRA, UMR1253 Science et Technologie du Lait et de l'Œuf, 65 rue de Saint-Brieuc, 35042 Rennes Cedex, France
[b] Agrocampus Ouest, UMR1253 Science et Technologie du Lait et de l'Œuf, 65 rue de Saint-Brieuc, 35042 Rennes Cedex, France
[c] Agrocampus Ouest, UMR6625 IRMAR CNRS, 65 rue de Saint-Brieuc, 35042 Rennes Cedex, France
[d] Riddet Institute, Massey University, University Ave., Palmerston North 4474, New Zealand

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ABSTRACT

This study aimed to explore how food structure, in the form of different egg white gel matrices, impacts the peptide kinetics profiles using an *in vitro* model that simulated digestion in the adult gastrointestinal tract. Four different gel matrices were prepared by heating egg white gel solutions using different combinations of pH and ionic strength. Label-free quantitative peptidomics and statistical analysis of the resulting kinetics profiles were performed. The 3231 identified peptides were distributed in 7 clusters based on "Gel structure × Digestion time" interaction coefficients, indicating that peptide kinetics profiles were greatly influenced by the gel structure. Moreover, the protein within the gel from which the peptides were released was highly significantly correlated with the peptide clusters, demonstrating that the influence of the gel structure varied according to the protein in question. Such findings may have nutritional relevance *in vivo* as they imply that the peptides reaching the intestine are different according to the initial EW gel structure.

1. Introduction

The food matrix plays a very important, but often under-appreciated role in the nutritional quality of foods because of its influence on digestion within the gastrointestinal tract (Fardet, Souchon, & Dupont, 2013). This is because nutrients are contained within larger continuous matrix often comprising specialized microstructures that are either formed naturally in the food or produced as a result of processing, where the nutrients may interact at both macro- and micro-scale with the components and structures of the food matrix (Parada & Aguilera, 2007). Thus, from the food matrix to the ultimate health effects within the body, the fate of food in the digestive tract has to be considered, since it determines the bioaccessibility of nutrients, their intestinal absorption, their metabolism and finally their potential health effects. Although nutrient composition has long been the main criteria for assessing the nutritional quality of foods, it is now recognized that the food matrix, and in particular, the food microstructure, must also be an important consideration (Parada & Aguilera, 2007). Consequently, an increasing number of studies are appearing in the scientific literature that focus on the impact of food microstructure on digestion progress, using food products with the same composition but different microstructures as model systems (Aguilera, 2005; Kaufmann & Palzer, 2011; Turgeon & Rioux, 2011). As an example, combining label-free quantitative peptidomics and multivariate data analysis, we have previously investigated the impact of ovalbumin (the major egg white protein) aggregate morphology on *in vitro* protein digestion; thus, we have shown that, at the end of intestinal digestion, the initial aggregate morphology influenced the amount of each peptide released (Nyemb, Jardin, et al., 2014). We also highlighted that the structural characteristics of egg white gels impact the extent of *in vitro* protein digestion and the nature of peptides released (Nyemb et al., 2016). Using dairy matrices, Barbé et al. (2013) also demonstrated the impact of food structure on protein digestion and bioavailability.

Beyond the impact of food matrix on digestion at the end point, the kinetics of digestion should also be considered, because of its potential impact on metabolic effects. Related to the concept of slow and fast proteins (Boirie et al., 1997), it has been established that the rate of protein digestion impacts the postprandial utilization of dietary amino acids by the body (Dangin, Boirie, Guillet, & Beaufrère, 2002; Dangin et al., 2003; Lacroix et al., 2006; West et al., 2011). Especially, because of different postprandial kinetics in plasma amino acids and hormones resulting from different digestive kinetics observed for whey protein and casein, the issue of potential difference between these two types of dairy

* Corresponding author at: Agrocampus Ouest, UMR1253 Science et Technologie du Lait et de l'Œuf, 65 rue de Saint-Brieuc, 35042 Rennes Cedex, France.
E-mail addresses: david.causeur@agrocampus-ouest.fr (D. Causeur), julien.jardin@rennes.inra.fr (J. Jardin), valerie.briard@rennes.inra.fr (V. Briard-Bion), catherine.guerin@agrocampus-ouest.fr (C. Guérin-Dubiard), S.M.Rutherfurd@massey.ac.nz (S.M. Rutherfurd), didier.dupont@rennes.inra.fr (D. Dupont), francoise.nau@agrocampus-ouest.fr (F. Nau).

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proteins regarding their satiating effect has been addressed, despite still controversial (Hall, Millward, Long, & Morgan, 2003; Marsset-Baglieri et al., 2014). But according to Juvonen et al. (2011), food structure would be more effective than the type of dairy proteins in modulating the kinetics of postprandial release of gastrointestinal hormones. Moreover, the kinetics of digestion may influence the nature of the released peptides and the concentration of these peptides in the intestinal lumen, some of them being potential biologically active peptides; the potential of food to provide such peptides has been proposed as a new parameter to assess the nutritional value of proteins (Bauchart et al., 2006). Then, to reinforce the reality of food matrix importance for the assessment of nutritional quality of foods, it is necessary to investigate the impact of food matrix structure on the process of digestion from a kinetics perspective.

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 unique functional (foaming, gelling, emulsifying) properties, egg white proteins (EWP) are extensively used in food systems and especially in food gels (Doi, 1993; Mine, 1995; Mine & Zhang, 2013). A diverse range of egg white gel structures can be generated by altering the heat-induced gelation process, especially by varying the physicochemical parameters like pH and ionic strength before heat treatment (Croguennec, Nau, & Brule, 2002; Handa, Takahashi, Kuroda, & Froning, 1998; Ziegler & Foegeding, 1990).

Using egg white gels (EWGs) as a model food, the present study aimed to explore the impact of gel structure on what peptides were released and how the peptide content changed during the whole digestion (referred to here as the "peptide kinetics profile"). This was performed by using an *in vitro* digestion model that simulated digestion in the gastrointestinal tract, and by using label-free quantitative peptidomics analysis and statistical data analysis.

2. Materials and methods

2.1. Chemicals and materials

Pasteurized egg white was obtained from 3 Vallées processing plant (Ambrières les Vallées, 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 was the pasteurized egg white; EWUF, which was the partially desalted EW.

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) equipped with a multi-tubular mineral membrane (1 m^2, 8 kDa molecular weight cut off; TAMI, Nyons, France). The ultrafiltration was performed at 20 °C up to a volume reduction ratio equal to 2.7. The retentate was then diluted 2.7-fold with distilled water to obtain EWUF. The protein concentration (N × 6.25) 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 ionic strength was 1 M and 0.05 M in EW and EWUF, respectively. The protein concentration was 10.5% and 10.9% in EW and EWUF, respectively.

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. 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. In vitro digestion model

The *in vitro* digestion model was designed to simulate oral, gastric and intestinal digestion in the adult human and was adapted from that described by Nyemb, Guérin-Dubiard, et al. (2014), based on an *in vitro* digestion model described by Dupont et al. (2010). Simulated oral digestion was performed by cutting gel samples into 3 mm cubes, which is the size of the food bolus that has been observed after mastication of EWG (Jalabert-Malbos, Mishellany-Dutour, Woda, & Peyron, 2007). The gels were distributed in 5 vials (1 g per vial) and simulated digestion was carried out for 5 different times up to 90 min using 1 vial per time point. Briefly, simulated gastric digestion was performed by adding porcine pepsin (182 U per mg protein) to simulated gastric fluid (SGF) at pH 2.5 containing 10 $mg \cdot ml^{-1}$ of EWP. Pepsinolysis was stopped at each time point by raising the pH to 7.0 using 0.5 M ammonium bicarbonate. For simulated gastric plus intestinal digestion, the pH of the reaction mixture was raised to 8.0 after the gastric digestion phase, in order to irreversibly inactivate pepsin, and then adjusted to 6.5. Bile salts and pancreatic enzymes were then added to give final concentrations as follows: 0.0625 M sodium taurocholate, 0.065 M sodium glycodeoxycholate, 0.4 U bovine α-chymotrypsin per mg of EWP and 34.4 U porcine trypsin per mg of EWP. Proteolysis was stopped by adding 0.06 mM soybean Bowmann–Birk trypsin–chymotrypsin inhibitor. All digestions were carried out in duplicate.

2.5. Mass spectrometry

Any particulate matter remaining after digestion was broken by sonication using a microtip probe sonicator (Q700, QSonica, Newtown, USA) working at 20% amplitude (3 × 1.5 s). The samples were then centrifuged at 6000*g* for 30 min (Microcentrifuge 5415C, Eppendorf, Hamburg, Germany), and the supernatants filtered (0.2 μm, Sartorius stedim biotech, Goettingen, Germany) in preparation for liquid chromatography and mass spectrometry (LC–MS/MS) analysis.

2.5.1. Identification of peptides

Mass spectrometry (MS) analysis was adapted from that described by Nyemb et al. (2016), using a nanoRSLC Dionex U3000 system fitted to a Q Exactive mass spectrometer (Thermo Scientific, San Jose, USA) equipped with a nanoelectrospray ion source. Briefly, samples were first concentrated using a μ-precolumn pepMap100 (C18 column, 300 μm i.d. × 5 mm length, 5 μm particle size, 100 Å pore size; Dionex, Amsterdam, The Netherlands), before peptide separation was performed on a PepMap RSLC column (C18 column, 75 μm i.d. × 150 mm length, 3 μm particle size, 100 Å pore size; Dionex); column temperature was maintained at 35 °C along peptide separation that was performed at a flow rate of 0.3 $\mu L \cdot min^{-1}$ using solvent A (2% (v/v) acetonitrile, 0.08% (v/v) formic acid and 0.01% (v/v) TFA in deionized water) and solvent B (95% (v/v) acetonitrile, 0.08% (v/v) formic acid and 0.01% (v/v) TFA in deionized water). Elution gradient was as follows: a first rise from 5 to 35% solvent B over 80 min, followed by a second rise from 35 to 85% solvent B over 5 min. Eluted peptides were directly electrosprayed into the mass spectrometer operating in positive ion mode with a voltage of 1.8 kV. The mass spectra were recorded in full MS mode using the m/z range 250–2000. The resolution of the mass analyzer for a m/z of 200 a.m.u (atomic mass unit) was set to 70,000 in the acquisition method. For each scan, the ten most intense ions were selected for fragmentation. MS/MS spectra were recorded

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