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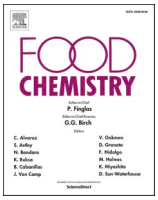
Food Chemistry 389 (2022) 133132

Contents lists available at ScienceDirect

Food Chemistry

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



Spatial-temporal mapping of the intra-gastric pepsin concentration and proteolysis in pigs fed egg white gels

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ARTICLE INFO

Keywords:
Food structure
In vivo digestion
Protein gel
Amino acid absorption

ABSTRACT

While there is a consensus that food structure affects food digestion, the underlying mechanisms remain poorly understood. A previous experiment in pigs fed egg white gels of same composition but different structures evidenced such effect on food gastric disintegration. In this study, we detailed the consequences on intra-gastric pH, pepsin concentration and proteolysis by sampling throughout the stomach over 6 h digestion. Subsequent amino acid absorption was investigated as well by blood sampling. While acidification was almost homogeneous after 6 h digestion regardless of the gel, pepsin distribution never became uniform. Pepsin started to accumulate in the pylorus/antrum region before concentrating in the body stomach beyond 4 h, time from which proteolysis really started. Interestingly, the more acidic and soft gel resulted in a soon (60 min) increase in proteolysis, an earlier and more intense peak of plasmatic amino acids, and a final pepsin concentration three times higher than with the other gels.

1. Introduction

There is a scientific consensus with respect to the impact of food structure on the digestion process, with potential consequences on nutrient bioavailability (Norton et al., 2014; Parada and Aguilera, 2007; Turgeon and Rioux, 2011). This has been established for carbohydrate digestion (Björck et al., 1994), lipid digestion (Marciani et al., 2009; Meena et al., 2014), as well as protein digestion. In particular, the way proteins aggregate or gel, depending on the physicochemical conditions during food processing, has proven to have a large impact on *in vitro* proteolysis of whey proteins (Macierzanka et al., 2012) and egg white proteins (Nyemb et al., 2016; Nyemb et al., 2014a; Nyemb et al., 2014b; Nyemb-Diop et al., 2016). Similarly, consequences on *in vivo* protein digestion and amino acid bioavailability have been evidenced for dairy proteins (Barbé et al., 2014; Barbé et al., 2013). An assumption is that the gastric digestion process, which is the first step of proteolysis, is directly impacted by food structure. This was confirmed by comparing the fate, during gastric digestion in pigs, of three egg white gels (EWGs) of identical composition but differing in structure and texture (Nau et al., 2019). This *in vivo* study exhibited different profiles of both EWG disintegration and intra-gastric acidification kinetics, which suggest that the physical properties of the gels is a determinant factor influencing the ingress of gastric fluid into the chyme. The impact of EWG structure on the progress of gastric proteolysis could be assumed to arise from the different gastric acidification kinetics as pH is a key parameter for pepsin activity (Luo et al., 2018). However, besides pH, pepsin concentration is another determinant factor for protein hydrolysis. Yet, although it is accepted that gastric digestion is a complex process during which pepsin concentrations vary over the stomach and over time (Kondjoyan et al., 2015), to the best of our knowledge, no *in vivo* data have been reported on the intra-gastric distributions of both pepsin concentration and proteolysis.

To deepen our understanding of protein digestion in the stomach, this study aimed to complete the previously quoted study in which three EWGs of identical composition but different in structure and texture were fed to pigs, in order to assess the impact of food structure on the

Abbreviations: AA, amino acid; DH, degree of hydrolysis; DTT, dithiothreitol; EW, egg white; EWG, egg white gel; OPA, ortho-phtalaldehyde; IS, ionic strength; EAA, essential amino acid.

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https://doi.org/10.1016/j.foodchem.2022.133132
Received 7 January 2022; Received in revised form 7 April 2022; Accepted 29 April 2022
Available online 2 May 2022

digestion process (Nau et al., 2019). These EWGs were prepared under different pH and ionic strength (IS) conditions during heat gelation to produce: a granular-spongy EWG (pH 5; IS 1 M), an intermediate EWG (pH 7; IS 1 M), and a smooth-rigid EWG (pH 9; IS 0.05 M). The present study describes, for the first time, a detailed spatial–temporal mapping of the pepsin concentration and proteolysis in the stomach, throughout a 6 h postprandial period in pigs. The impact of food structure on both phenomena (pepsin and proteolysis distributions) is investigated, and the consequences on the amino acid bioavailability are evaluated.

2. Material and methods

2.1. Experimental diets

The experimental diets were composed of three different egg white gels (EWGs) of the same nutrient composition but different in terms of pH, ionic strength, textural and structural features. The EWGs were prepared applying different combinations of pH and ionic strength (IS) before heat gelation, in order to obtain either a granular-spongy gel (pH 5, IS 1 M, hereinafter referred to as EWG-pH5); an intermediate gel (pH 7, IS 1 M, hereinafter referred to as EWG-pH7); or a smooth-rigid gel (pH9, IS 0.05 M, hereinafter referred to as EWG-pH9). The macroscopic, microscopic and rheological characteristics of these gels are described in Nyemb et al. (2016). Egg white solutions were filled in synthetic bags (1 kg per bag, 30 mm thick), and heated in a water-bath at 90 °C for 150 min for a complete and homogeneous gelation (Nau et al., 2019). The EWGs were cooled immediately after cooking, and stored at 4 °C until being cut up into 1 cm^3 pieces before given to the animals. EWGs were systematically prepared the day before the test.

2.2. Animal trial and sampling procedure

The samples used for the present study were collected from the animal trial described in Nau et al. (2019), for which all experimental methods have been approved by the Animal Ethics Committee at Massey University, Palmerston North, New Zealand. Briefly, growing entire male pigs (around 25 kg) were randomly allocated to receive a single meal (around 1 kg, 87 g proteins) of one of the three EWGs, and to one of the five postprandial sampling times (20, 60, 120, 240, and 360 min) for each diet. The animals were then sedated, and were euthanized at the pre-defined postprandial sampling time. Once the pig was killed, the stomach was carefully removed, clamped at the both ends, washed with deionized water, and then opened from the oesophageal to pyloric ends, according to a procedure adapted from Bornhorst et al. (2013).

The pH of the gastric chyme was then immediately measured at different locations around the proximal and distal regions of the stomach. Samples were collected from the same locations, added with pepstatin (100 μL of a 0.5 mg/mL pepsatin solution in around 20 g of chyme) before freeze-drying and stored at 4 °C until further analyses. Because of the cumbersome and costly immunochemical analysis for pepsin quantification, the intra-gastric pepsin concentration was measured only in 63 pigs among the 95 pigs initially included in the animal trial, that is 4 to 5 pigs per diet for each digestion time. In total, n = 20 for EWG-pH5, n = 23 for EWG-pH7, and n = 20 for EWG-pH9. For the same reasons, only eight intra-gastric locations were considered in the present study, among the ten considered in Nau et al. (2019). The data presented here for intra-gastric pH and proteolysis resulted from these 63 pigs and eight intra-gastric locations.

Blood samples were also collected when killing pigs, and plasma was separated immediately by centrifugation at 3,000*g* for 15 min at 4 °C. Plasma samples were frozen at −80 °C until further analysis. The data on plasma presented here resulted from 85 pigs including 52 pigs out of the 63 mentioned above, that is 5 or 6 pigs per diet for each digestion time. In total, n = 28 for EWG-pH5, n = 28 for EWG-pH7, and n = 29 for EWG-pH9.

2.3. Measurement of pepsin concentration

Inhibition ELISA to measure pepsin concentration in the gastric chyme samples was adapted from Rolet-Répécaud et al. (2015). A NUNC 96-wells microplate was coated with 100 μL/well of porcine pepsin (Sigma P6887; 0.5 μg/mL) diluted in carbonate buffer (0.1 M, pH 9.6) and incubated for 1 h at 37 °C. After three successive rinses with 250 μL/well of PBS-T (phosphate saline buffer [0.05 M, pH 7.2, 0.15 M NaCl] with 0.05% Tween-20), the remaining binding sites were saturated with 250 μL/well of gelatin (Sigma 1.04070; 10 g/L) diluted in PBS-T and incubated for 1 h at 37 °C. The plate was then rinsed as described above.

Serial dilutions of a porcine pepsin solution (1 mg/mL) were prepared in PBS-T in order to obtain a concentration range of 5.10^{-5} μg/mL to 50 μg/mL as standards. One hundred and fifty microliters of diluted standard or sample and 150 μL of primary antibodies (anti-pepsin polyclonal antibodies from goat; Genetex GTX39360; 1:12000 in PBS-T) were mixed in microtubes before incubation for 1 h at 37 °C. The mixture (100 μL) was then added into each well of the microplate, incubated for 1 h at 37 °C, and the plate was rinsed as described above. Lastly, 100 μL of secondary antibodies (alkaline phosphatase conjugated to anti-goat IgG (Fc specific) from rabbit; Sigma SAB3700260; 1:7000 in PBS-T) were added in each well of the microplate, incubated for 1 h at 37 °C, and rinsed as described above. Both negative and positive controls were added in microplates. Negative controls contained PBS-T and primary antibodies. Positive controls were prepared with a known concentration of pepsin.

The absorbance at 405 nm was measured against a blank in each well after addition of 100 μL of the reagent solution and incubation for 45–100 min at 37 °C. The reagent solution was prepared by diluting 10 mg *para*-nitro-phenyl-phosphate [Eurobio Scientific, 50-80-01] in 10 mL of a di-ethanolamine solution (di-ethanolamine 1 M [Sigma D83303], $MgCl_2$ 1 mM, $Zn[OOCCH_3]_2$ 0.1 mM, pH 9.3). The pepsin concentration in gastric chyme samples was calculated from the standard curve, using a logistic regression with four parameters performed with the Gen5 data analysis software (Biotek). Each sample was analysed in triplicate after 10-fold to 1000-fold dilution, each dilution being measured in duplicate.

2.4. Proteolysis measurement

The rate of proteolysis in gastric samples was assessed by measuring the free primary amino groups in the soluble fraction using the o-phtalaldehyde (OPA) spectrophotometric assay, according to a method adapted from Church et al. (1983) and Nielsen et al. (2001). The freeze-dried samples of gastric chyme were solubilized at 10 mg/mL in 0.1 M sodium phosphate buffer pH 7.5 with 1% SDS and 1% β-mercaptoethanol, under stirring (250 rpm) overnight at 25 °C. After centrifugation at 6,000*g* for 20 min, the supernatants were collected, and then diluted in distilled water (1:4). Fifty μL of each diluted solution were dropped into a well of a 96-wells microplate in which 100 μL of the OPA reagent was added. The OPA reagent was prepared by mixing 2.5 mL SDS 20% (w/v), 2.5 mL OPA at 10 mg/mL in ethanol, 700 μL dithiothreitol (DTT) 1 M, and 20 mM sodium tetraborate buffer pH 9.5 qsp 100 mL. The absorbance at 340 nm was measured after 10 min of incubation at room temperature. The free primary amino groups were quantified using a standard curve prepared with methionine at a concentration range of 0 to 2 mM (i.e. 0 to 32 mg/L free amino groups). All samples and standards were analysed in triplicate.

In order to avoid the bias due to the progressive dilution of the gastric content over time, the free NH_2 concentration was expressed on dry matter basis. Dry matter content of the gastric chyme was determined by drying overnight at 120 °C (Nau et al., 2019).

2.5. Construction of the spatial-temporal maps and intra-gastric data calculation

The intra-gastric spatial distribution of the measured pH, pepsin

concentration, free amino group concentration, and dry matter are illustrated using 2D colour maps. These maps were built using the Matlab software with a 2D contour plot ("contour" function with the "jet" option) of experimental data interpolated on the stomach wall geometry, as previously proposed by Bornhorst et al. (2014). The experimental values considered correspond to the median of the measurements at each sampling location for a given diet at a given digestion time (i.e. 4 or 5 pigs), and data interpolation was performed using a grid density of 400 and a 2D bi-harmonic spline interpolation ("griddata" function with the "v4" method). The eight sampling locations (i.e. true experimental data) are illustrated by small circles in the 2D maps. Maria J. Ferrua has developed these programs and kindly provided them for the purpose of the present study.

In order to compare the spatial–temporal dynamics associated to the three diets, the mean values of each intra-gastric characteristics (pepsin concentration, pH, and free amino groups concentration) were also calculated based on data at the eight intra-gastric locations, for all the pigs of the same batch (diet × digestion time). Moreover, to illustrate the spatial heterogeneity of the measured quantities over the entire stomach, a gradient score as well as a heterogeneity score were built at each digestion time. The gradient of each intra-gastric characteristics was defined as the difference between the maximum and minimum values (i.e. the range of values). The heterogeneity was defined as the gradient divided by the mean (i.e. the normalized range of values). The gradient and heterogeneity were calculated first for each pig separately, before the mean values were calculated for all the pigs of the same batch.

2.6. Plasmatic AA measurement

Free amino acids were quantified in plasma samples by exchange chromatography, using an Automatic Amino Acid Analyzer (Biochrom 30, Biochrom Ltd., Cambridge, UK).

After thawing at room temperature, 600 μL plasma were added with 60 μL DTT 10%, and stored for 2 min at room temperature before adding 60 μL sulfosalicylic acid 0.5 g/mL. The mixture was then homogenized, stored for 1 h on ice, and centrifuged at 2,000*g* and 4 °C for 20 min. The supernatant was collected, filtered on 0.45 μm, and the filtrate was then diluted (1:2) in 0.2 M lithium citrate buffer pH 2.2.

Amino acid elution was performed using successive lithium citrate buffers, with post-column derivatization with ninhydrine (Ultra Ninhydrin Reagent Kit, Biochrom). Absorbance was measured at 440 nm and 570 nm. Amino acid quantification was achieved by measuring each peak area and using an external calibration curve. Each plasma sample was analysed only once; independent replications were obtained since 2 to 5 pigs were analysed per diet for each digestion time.

2.7. Statistical analysis

All measurements were reported in the text as mean values ± SD. Statistical analysis were performed using Rcmdr package (version 2.7–1) of R software (version 4.1.0). A Kruskal-Wallis test was first performed in order to test the digestion time effect. The Wilcoxon rank sum test was applied to perform multiple comparisons of means. A p-value < 0.05 was required to consider a difference was significant.

3. Results and discussion

3.1. Distribution of pepsin is heterogeneous in the stomach at all time points

The measurement of pepsin concentration at eight intra-gastric locations over the 6 h postprandial period revealed different spatial–temporal maps of pepsin distribution according to the three EWGs the pigs were fed with (Fig. 1**A**). The first result is that pepsin was never uniformly distributed over the stomach. It should be noted that the pictures were very similar regardless pepsin concentration was expressed on a fresh matter or a dry matter weight basis.

At 20 min of digestion, pepsin concentration was close to zero almost everywhere, regardless the type of EWG (Fig. 1**A and B**). From 20 min to 60 min, the mean intra-gastric pepsin concentrations slightly increased for all EWGs, with statistical significant effect in pigs fed EWG-pH7 ($p = 0.018$) and EWG-pH9 ($p = 6.72e\text{-}05$) (Fig. 1**B**, Suppl. Data 1). This increase originated from localised high concentrations of pepsin (Fig. 1**A**).

From 60 min to 120 min digestion, the mean pepsin concentration continued to slightly increase with all diets (Fig. 1**B**), with a statistical significant effect observed in pigs fed EWG-pH5 only ($p = 0.021$) (Suppl. Data 1). The area with the highest pepsin concentrations also seemed to extend towards the entrance of the stomach (Fig. 1**A**). This explains why despite some additional pepsin secretions, the gradients of intra-gastric pepsin concentration (i.e. max–min over the eight locations) did not significantly change (Fig. 1**C**, Suppl. Data 3), and even seemed to be reduced on average with all diets. Consequently, the heterogeneity scores of intra-gastric pepsin concentration (i.e. gradient/mean ratio) tended to decrease regardless the EWG (Fig. 1**D**); the decrease was significant in pigs fed EWG-pH5 only ($p = 0.029$; Suppl. Data 4). These results indicate a certain mixing of the chyme, likely due to the progressive recovery of the migrating motor complex (MMC) which is known to be interrupted by feeding in pigs fed only once or twice a day (Deloose et al., 2012).

It is notable that the maps indicate a pepsin accumulation at the pylorus/antrum region at 60 min and 120 min of digestion (Fig. 1A), suggesting a pepsin streaming along the stomach wall from the chief cells from which pepsinogen is secreted, and which are mainly found in the body (Somaratne et al., 2020). The antral contractions waves (ACW) that are supposed to start after meal ingestion (for 20 min in humans) (Ferrua and Singh, 2010) likely contribute to the accumulation at the pylorus region, despite the pylorus is not at the bottom of the stomach when the pigs are standing up. Conversely, the lowest pepsin concentrations were logically measured in the proximal region of the stomach (Suppl. Data 2). Therefore, decreasing pepsin concentrations were observed from the pylorus region to the entrance of the stomach regardless the EWG, despite the area of high pepsin concentration was much broader in pigs fed EWG-pH9 at 120 min of digestion (Fig. 1A).

From 120 min to 360 min digestion, pepsin distribution throughout the stomach evolved from a pepsin abundant pylorus/antrum area towards a pepsin abundant body area for the three EWGs. However, this transition seemed to occur earlier in pigs fed EWG-pH5 and EWG-pH7 (before 240 min) than in pigs fed EWG-pH9 (after 240 min) (Fig. 1**A**). Knowing that the three EWGs exhibited identical composition but differed in structure and texture (Nyemb et al., 2016), these features may be responsible for the differences in chyme mixing and pepsin distribution all over the stomach. Since the soft and fragile EWG-pH5, and to a lesser extent the intermediate EWG-pH7, were shown to be largely disintegrated from 120 min (Nau et al., 2019), it is indeed likely that pepsin could more rapidly diffuse into the gastric content of the proximal region with both of these gels. On the contrary, the cohesive and elastic EWG-pH9 was still poorly disintegrated at this digestion stage (Nau et al., 2019), likely limiting pepsin ingress into the chyme and therefore still favouring pepsin streaming along the stomach wall. Such a mechanism appears, therefore, consistent with a delayed and less pronounced establishment of pepsin abundant area in the upper part of the stomach in this latter case.

At 360 min, the most striking result was that the mean intra-gastric concentration of pepsin was about three times higher with EWG-pH5 than with the two other gels (Fig. 1**B**). In pigs fed EWG-pH7 and EWG-pH9, the mean intra-gastric concentrations of pepsin increased similarly up to 240 min digestion, from which it remained constant (EWG-pH9) or slightly decreased (EWG-pH7; $p = 0.002$) (Fig. 1**B**, Suppl. Data 1). This suggests a slowdown, and even a stoppage of the pepsin secretion from this moment and up to the end of the 6 h postprandial period for both of these EWGs. On the contrary, pepsin concentration still grew dramatically ($p = 1.94e\text{-}13$) beyond 240 min digestion in pigs

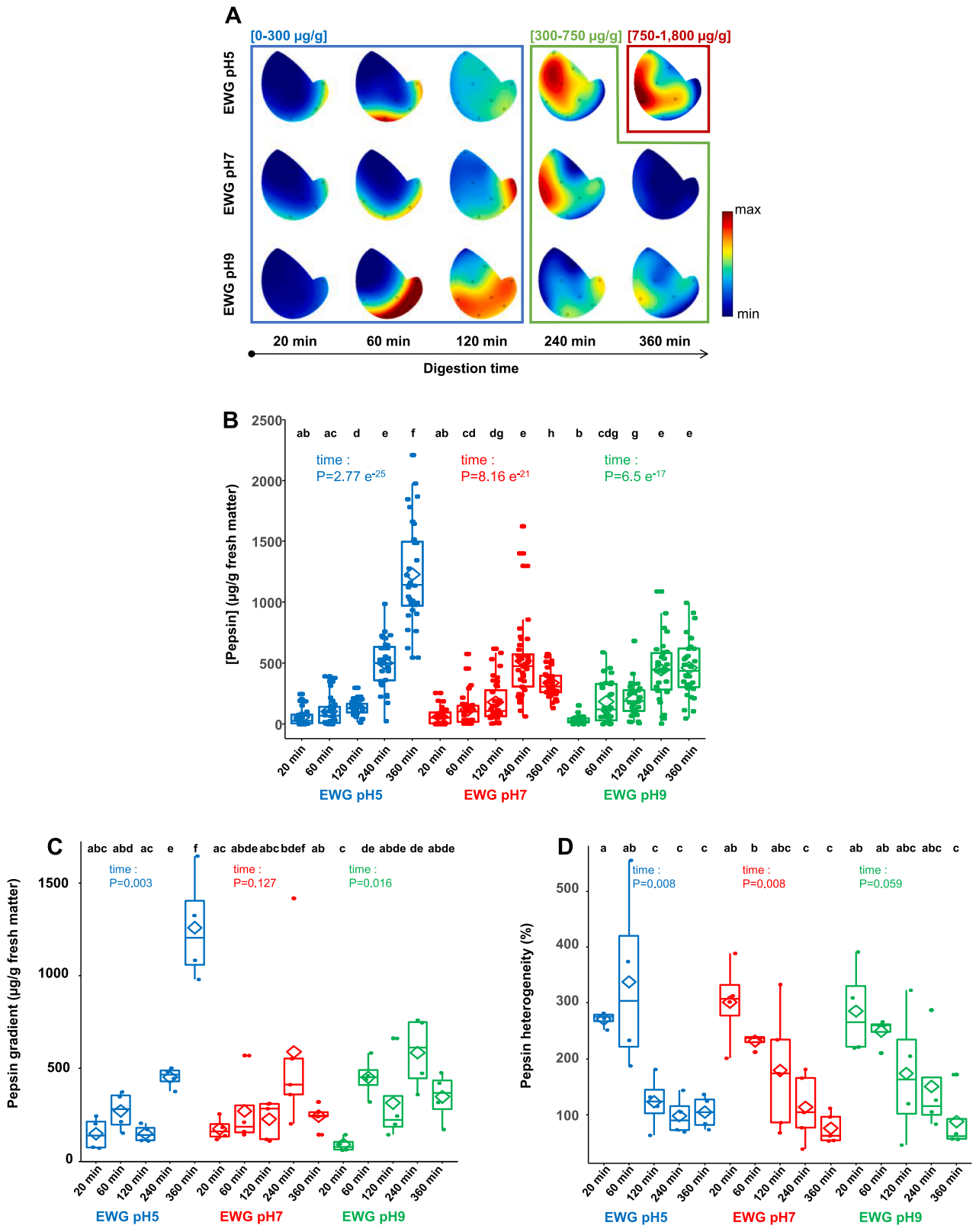

Fig. 1. Intra-gastric pepsin distribution and concentration over the 6 h postprandial in pigs fed either granular-spongy (pH5), intermediate (pH7) or smooth-rigid (pH9) forms of egg white gel (EWG). (**A**) Mapping of the pepsin concentration reconstituted from quantification at eight intra-gastric locations (empty circles). Colour-coded scales correspond to median pepsin concentrations from 0 to 300 µg/g of fresh chyme for 20 min, 60 min and 120 min digestion time, and from 300 to 750 µg/g for 240 min and 360 min digestion time, except for EWG-pH5 at 360 min digestion time (from 750 to 1,800 µg/g). Medians (horizontal bars), means (diamonds), and 25th-75th percentiles (boxes) of (**B**) pepsin concentration calculated from the eight sampling locations and all pigs of a given condition (diet × digestion time) (n = 32 or 40), **(C)** pepsin gradient (concentration range for a given pig; n = 4 or 5), and (**D**) pepsin heterogeneity (normalized gradient for a given pig; n = 4 or 5). Time effect (Kruskal-Wallis test) is indicated for each EWG (B, C and D). Different letters indicate a significant difference ($p < 0.05$, Wilcoxon test).

fed EWG-pH5 (Fig. 1**B**, Suppl. Data 1), leading to a substantial increase ($p = 0.029$) of the gradient of pepsin concentration (Fig. 1**C**, Suppl. Data 3). This singular behaviour therefore suggests that the pepsin secretion rate was much higher and/or remained high for a much longer period with EWG-pH5.

Regarding the overall evolution of the intra-gastric heterogeneity scores, it considerably decreased ($p = 0.029$) from 60 min to 120 min of digestion in pigs fed EWG-pH5, before remaining almost constant up to the end of the 6 h postprandial period. However, it decreased more gradually over the whole digestion in pigs fed EWG-pH7 and EWG-pH9 (Fig. 1**D**, Suppl. Data 4). At the end of the experiments, similar heterogeneity scores were reached regardless the EWG (Fig. 1**D**), despite different absolute levels of pepsin concentration (Fig. 1**B**) and intra-gastric gradients (Fig. 1**C**), suggesting overall similar mixing regardless the EWG. It is also noticeable that even after 6 h of digestion, pepsin was not yet uniformly distributed over the stomach (Fig. 1**A**), despite an indisputable homogenisation of the gastric chyme occurred, as revealed by the decrease of intra-gastric heterogeneity from around 300% to 80% (Fig. 1**D**). Albeit contrary to the traditional idea of a rapid and complete homogenisation of the meal, these observations are consistent with the modelling of intra-gastric fluid dynamics that concludes to a moderate mixing of highly viscous meals (Ferrua and Singh, 2010). Similarly, Goetze et al. (2009) reported a very incomplete mixing of test meals with gastric secretion in humans, as demonstrated by MRI. It should be noted that the heterogeneity reported here for pepsin concentration, even after 6 h of digestion, is not in contradiction with the homogeneous distribution of dry matter content, particle size and viscoelasticity of the chyme, previously reported (Nau et al., 2019). Indeed, these latter directly depend on the initial EWG features and in particular on their softness, which resulted in a mechanical disintegration, mainly during mastication and swallowing. Thus, the physical characteristics of the gastric chyme were homogeneous all over the stomach, unlike pepsin concentration that depends on pepsin secretion by the stomach wall and pepsin ingress in the chyme.

Lastly, our experimental data can also be compared with the existing literature on the rate of pepsin secretion. The mean intra-gastric concentration of pepsin on fresh chyme weight measured in the present study ranged from 103.0 μg/g (for pigs fed EWG-pH5) to 183.5 μg/g (for pigs fed EWG-pH9) after 60 min of digestion. Knowing that the test diets consisted in 1 kg EWG, and gastric emptying was around 20% after 1 h digestion (Nau et al., 2019), the mean flow of secreted pepsin over the whole stomach was then around 2 mg/min during the first hour of digestion. This order of magnitude is actually very consistent with the values reported by Malagelada et al. (1979) for pepsin output (around 25 mg per 10 min during the first hour of digestion) in humans fed with a complete meal previously homogenized. Moreover, when looking at longer digestion times, it is noteworthy that the intra-gastric pepsin concentrations measured in the present study were very close to that measured in humans by Kalantzi et al. (2006). Thus, at 240 min digestion, the mean values ranged from 0.44 mg/g to 0.52 mg/g in pigs fed EWG-pH9 and EWG-pH5, respectively, *vs* a mean value of 0.58 mg/mL reported by these authors after 210 min digestion. Note that the data provided by Kalantzi et al. (2006) were used to develop the standardised static *in vitro* digestion method proposed by Minekus et al. (2014).

3.2. pH spatial–temporal maps: similarities and differences with the pepsin maps

Hydrochloric acid and pepsin are two major components of the gastric juice, which plays a key role in the protein digestion process. Both components are secreted by different glandular cells of the stomach epithelium: pepsin is secreted as pepsinogen by the chief cells present in the body, whereas HCl is secreted by the parietal cells mainly present in the fundus area (Somaratne et al., 2020). However, both secretions are interrelated, since pepsin secretion has been proven to be stimulated by gastric mucosal acidification (Johnson, 1973). Moreover, proteolysis needs simultaneous pepsin and acid release in the stomach lumen, since HCl is required for the activation of the proenzyme pepsinogen into its active form of pepsin, as well as for acidification required for pepsin activity (Luo et al., 2018). It thus seemed appropriate to compare intra-gastric maps of pH and pepsin concentration over the postprandial period.

The pH spatial–temporal maps are presented in Fig. 2**A**. It is noteworthy that none unbuffered acid pocket at the cardia/gastroesophageal junction was observed during the postprandial period, regardless the EWG, contrary to what is sometimes observed in humans (Fletcher et al., 2001; Simonian et al., 2005). Moreover, a progressive acidification was observed in all cases, with very low pH values (pH 2–3) reached after 360 min of digestion within the entire stomach volume (Fig. 2**A and 2B**), time at which 31% to 42% of the dry matter ingested still remained in the stomach (Nau et al., 2019). This suggests high HCl secretion over the 6 h postprandial, in particular when considering that EWGs have a high buffering capacity.

When pepsin maps and pH maps are compared, the first striking similarity is that acidification also started in the pylorus region (Fig. 2**A**), just as pepsin accumulation (Fig. 1**A**). As mentioned above, both phenomena might result from the streaming of the gastric juice along the stomach wall at the beginning of the digestion. This can be attributed to the fact that the stomach was still very full since only 20% of the dry matter of the meal were emptied from the stomach at 60 min of digestion, and that the food was not yet thoroughly disintegrated (Nau et al., 2019). Moreover, the MCC activity was likely still interrupted because of the distension of the stomach (Deloose et al., 2012).

A second similarity lies in the progressive homogenization of the intra-gastric pH, in the distal and proximal regions (Fig. 2**A**). This homogenization extended over a long period (more than 240 min in pigs fed EWG-pH7 and EWG-pH9), consistently with Simonian et al. (2005) who reported lower pH in the distal region in comparison to the proximal region up to 4 h after meal ingestion in humans.

However, some differences also exist between intra-gastric pH and pepsin concentration changes. At the very beginning of the postprandial period and regardless the EWG, the intra-gastric pHs remained very homogeneous (Fig. 2**A and 2D**) and close to the meal pHs (Fig. 2**B**), most certainly because of the high buffering effect of the protein gels. Thereafter, the intra-gastric pH gradient (Fig. 2**C**) and pH heterogeneity (Fig. 2**D**) increased up to 60 min (EWG-pH5), or even 120 min (EWG-pH7 and EWG-pH9) of digestion, before decreasing upon chyme mixing. These bell-shaped trends observed for the gradient and heterogeneity, thus simply reflect that the pH was very uniformly distributed at both the start (20 min) and the end (360 min) of the investigated time window. Yet, it is noteworthy that the gradient and heterogeneity increased much more sharply ($p = 0.029$) from 20 min up to 120 min in pigs fed EWG-pH9 (Fig. 2**C and 2D**; Suppl. Data 7 and 8). This led to much higher maximum gradient and heterogeneity scores in pigs fed EWG-pH9 (4.92 $\pm$ 0.31 pH units; 100.8% $\pm$ 19.1) than in pigs fed EWG-pH7 (2.76 $\pm$ 1.44 pH units; 51.0% $\pm$ 25.5) or EWG-pH5 (2.61 $\pm$ 1.09 pH units; 49.5% $\pm$ 23.2). This is consistent with the difference in initial pH between the three meals, assuming that HCl secretion did not depend on the type of EWG ingested, and knowing that the decrease in gastric pH mainly depends on the meal buffering capacity (Weinstein et al., 2013). Yet, most of the buffering capacity of EWGs occurs below pH 5 (Mennah-Govela et al., 2019). Nevertheless, after 6 h of digestion, the pH gradient was again very low and not significantly different from one EWG to another (0.38 $\pm$ 0.29, 0.15 $\pm$ 0.03, and 1.16 $\pm$ 1.6 pH units in pigs fed with EWG-pH5, EWG-pH7 and EWG-pH9, respectively) (Fig. 2**C**). This is not really surprising as pH is not proportional to H^+ concentration, but to its logarithm, which erases small H^+ concentration changes.

Other dissimilarities can be noted in the evolution of the mean pepsin concentration and the mean pH when considering the studied diets individually. As previously mentioned, the mean pepsin concentration continuously increased over the 6 h postprandial period in pigs fed EWG-pH5, whereas a plateau was reached at 240 min in pigs fed EWG-

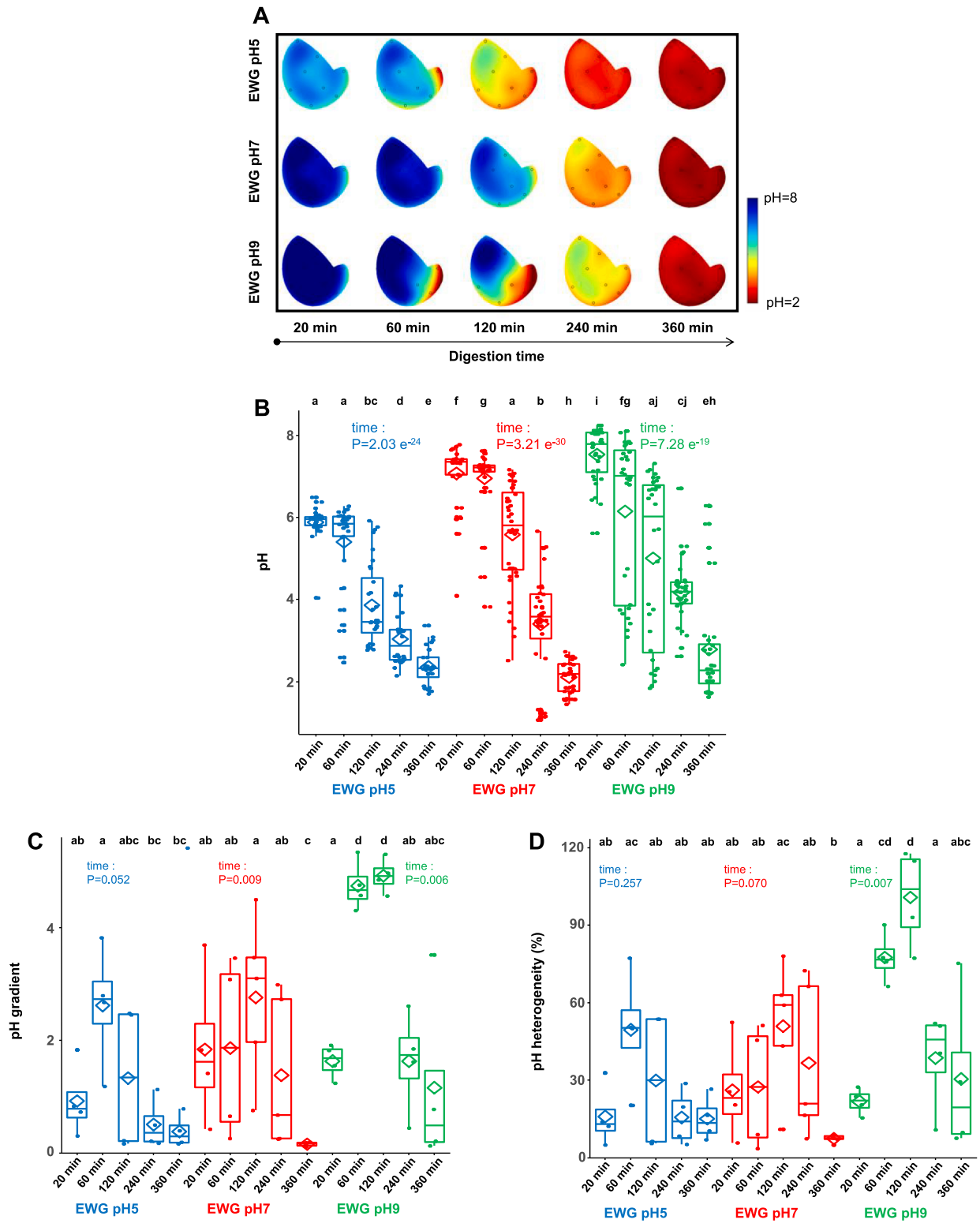

Fig. 2. Intra-gastric pH distribution over the 6 h postprandial in pigs fed either granular-spongy (pH5), intermediate (pH7) or smooth-rigid (pH9) forms of egg white gel (EWG). (**A**) Mapping of the pH reconstituted from measurements at eight intra-gastric locations (empty circles). Colour-coded scales correspond to median pH from acidic values (in red) to alcalin values (in blue), on the range pH2-pH8. Medians (horizontal bars), means (diamonds), and 25th-75th percentiles (boxes) of (**B**) pH calculated from the eight sampling locations and all pigs of a given condition (diet × digestion time) (n = 32 or 40), **(C)** pH gradient (pH range for a given pig; n = 4 or 5), and (**D**) pH heterogeneity (normalized gradient for a given pig; n = 4 or 5). Time effect (Kruskal-Wallis test) is indicated for each EWG (B, C, and D). Different letters indicate a significant difference ($p < 0.05$, Wilcoxon test). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

pH7 and EWG-pH9 (Fig. 1**B**). However, the mean pH regularly and significantly decreased over the 6 h postprandial regardless the EWG, to reach similar pH end values (pH = 2.35 ± 0.42, pH = 2.11 ± 0.39, and pH = 2.79 ± 1.37 in pigs fed EWG-pH5, EWG-pH7 and EWG-pH9, respectively) (Fig. 2**B**, Suppl. Data 5). This resulted in that the combined evolution of both intra-gastric parameters followed a different pathway depending on the EWG (Fig. 3). While pepsin concentration increased overall linearly when pH decreased for EWG-pH9 (R^2 = 0.71) and EWG-pH7 (R^2 = 0.62), the increase in pepsin concentration was better modelled by a power law for EWG-pH5 (R^2 = 0.74). For pH above 3.0, the significantly lower pH values in pigs fed EWG-pH5 during the first half of the postprandial period (Fig. 2**B**) induced a shift towards the left in the plot pepsin *vs* pH in comparison to EWG-pH7 and EWG-pH9 (Fig. 3), suggesting similar and simultaneous pepsin and HCl secretions, regardless the EWG. However, for pH below 3.0 (i.e. beyond 240 min of digestion, Fig. 2**B**), the intra-gastric pepsin concentration dramatically increased in pigs fed EWG-pH5, strongly contrasting with the much more gradual trend observed in pigs fed EWG-pH7 and EWG-pH9 (Fig. 3). In other words, this suggests that HCl and pepsin secretions are not necessarily synchronized until the very end of gastric digestion, contrary to what has been previously reported during a 2 h monitoring of the postprandial period in humans (Malagelada et al., 1979). Actually, acid and pepsinogen secretions would initially react *in vivo* to the same physiological stimuli, in particular food ingestion due to vago-vagal reflexes resulting from distension of the stomach, but both processes have been shown to be regulated independently (Gritti et al., 2000). Moreover, food characteristics such as physical properties are also susceptible to modulate gastric secretions (Bornhorst, 2017; Malagelada et al., 1979). Yet, the three EWGs differed in pH, ionic strength (1 M for EWG-pH5 and EWG-pH7, 0.05 M for EWG-pH9), texture and structure. Since it has previously been established that EWG-pH5 and EWG-pH7 similarly disintegrated over the gastric phase (Nau et al., 2019), it can be assumed that the food pH, and consequently the mean intra-gastric pH, is the main reason for the higher secretion of pepsin in pigs fed EWG-pH5 from 240 min digestion. This is consistent with the demonstration that HCl stimulates pepsin secretion very efficiently in dogs (Johnson, 1973). The stimulating effect of an acidic drink on gastric secretion has also been reported with a bread meal in humans (Freitas et al., 2022), though without HCl and pepsin secretions being distinguished.

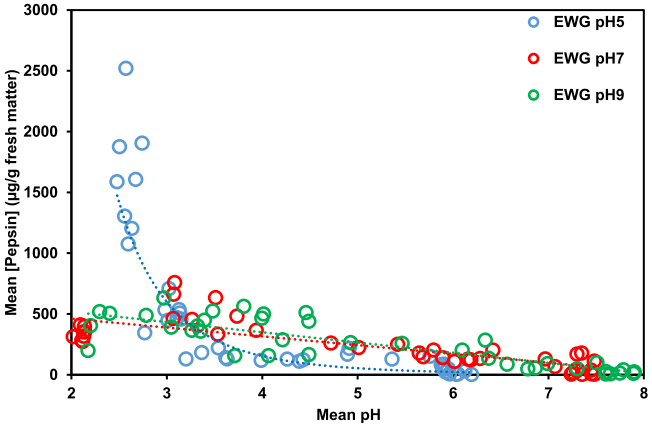

Fig. 3. Combined evolution of intra-gastric pH and pepsin concentration (on fresh matter basis) over the 6 h postprandial in pigs fed either granular-spongy (pH5), intermediate (pH7) or smooth-rigid (pH9) forms of egg white gel (EWG). Each circle is the mean value for a given intra-gastric location and a given EWG at a given digestion time (n = 4 or 5). The dotted lines indicate linear regressions (for EWG-pH7 and EWG-pH9) or a regression to a power law (for EWG-pH5).

3.3. The spatial–temporal map of proteolysis is not a simple combination of the pepsin and pH maps

In order to follow the progress of the intra-gastric proteolysis, the concentration of free NH_2 groups was measured over the stomach and over the 6 h postprandial period. The NH_2 concentrations were expressed on the dry matter basis to avoid the bias due to the progressive dilution of the gastric content (Fig. 4**A**).

The first striking result was that, except in pigs fed EWG-pH5, the free NH_2 concentration remained almost constant at its initial value up to 120 min digestion. Thereafter, a low increase occurred from 120 min to 240 min in pigs fed EWG-pH7 (p = 4.43e-04) and EWG-pH9 (p = 8.65e-05), before a higher increase from 240 min to 360 min (p = 2.07e-17 in pigs fed EWG-pH5, p = 4.01e-18 in pigs fed EWG-pH7, and p = 3.79e-04 in pigs fed EWG-pH9) (Fig. 4**A and 4B**; Suppl. Data 9).

Because the free NH_2 concentrations are expressed on a dry matter basis, their evolution directly reflects the kinetics of gastric proteolysis. To facilitate quantitative comparisons across studies, these data were also converted into degrees of hydrolysis (DH) by assuming that proteins in EW are around 85% of dry matter, the average molecular weight of amino acid residues is 120 g/mol, and the stoichiometry between amino acid and NH_2 is 1:1 (i.e. omitting the side chain of the lysine residues). Moreover, it is more than likely that no significant proteolysis did actually occur during the first 20 min of digestion, because of the high quantity of EWG ingested by the pigs (around 1 kg), the slow pepsin secretion (Fig. 1**B**), and the high intra-gastric pH at this stage of digestion (Fig. 2**B**). Therefore, the overall average of the intra-gastric concentrations of free NH_2 measured in all pigs at 20 min digestion was regarded as the baseline level. For this reason, all the DH values were calculated considering the free NH_2 concentration measured for a given pig minus this base concentration corresponding to 0% DH.

As mentioned above, the intra-gastric concentration of free NH_2 showed briefly a different pathway at the very beginning of the digestion in pigs fed EWG-pH5, with a significant increase (p = 5.1e-10) between 20 min and 60 min of digestion (Fig. 4**B**, Suppl. Data 9), in particular in the proximal region of the stomach (Suppl. Data 10). During these forty minutes, the mean estimated DH increased up to 1.03% ± 1.01 (5.72 ± 1.18 mg NH2/g dry matter) in these pigs (p = 1.44e-07). Since pepsin concentration was the same regardless the EWG during the two first hours of digestion, throughout the stomach and especially in the proximal region (Suppl. Data 2), intra-gastric pH might be responsible for this discrepancy between pigs fed EWG-pH5 and the others. Actually, the mean pH of about 6.0 measured at 20 min and 60 min digestion in the proximal region in pigs fed EWG-pH5 (Suppl. Data 6) corresponds to the pH threshold below which pepsin can start to hydrolyse EW proteins (Salelles et al., 2021). Therefore, a small extent of pepsin hydrolysis in the early stages of the gastric digestion of EWG-pH5 is not inconceivable, and could be explained by the presence of slightly more acidic local pH. On the contrary, the mean pH values measured in the proximal region during the same digestion stage in pigs fed EWG-pH7 (around 7.3) and EWG-pH9 (around 7.7) were higher than the pH threshold for porcine pepsin activity. Therefore, it can be assumed that in these conditions, a large part of the pepsin secreted was rapidly inactivated, explaining that no proteolysis could occur (Fig. 4**A and 4B**).

Surprisingly, between 60 min and 120 min of digestion, the mean proteolysis significantly (p = 2.5e-05) decreased in pigs fed EWG-pH5 (5.72 ± 1.18 *vs* 4.92 ± 0.37 mg NH_2/g dry matter). At the same time, it did not significantly change in pigs fed EWG-pH9 (3.96 ± 0.71 *vs* 4.41 ± 1.10 mg NH_2/g dry matter), and slightly increased (p = 0.023) in pigs fed EWG-pH7 (4.27 ± 0.75 *vs* 4.63 ± 0.60 mg NH_2/g dry matter) (Fig. 4**B**; Suppl. Data 9). At 120 min, the mean proteolysis was only slightly higher in pigs fed EWG-pH5 than that in pigs fed EWG-pH7 and EWG-pH9 (p = 0.029 and p = 0.047, respectively). Actually, proteolysis decrease observed between 60 min and 120 min in pigs fed EWG-pH5 was mainly controlled by the values measured in the proximal region (Suppl. Data 10). Then, the decrease of the mean proteolysis might result

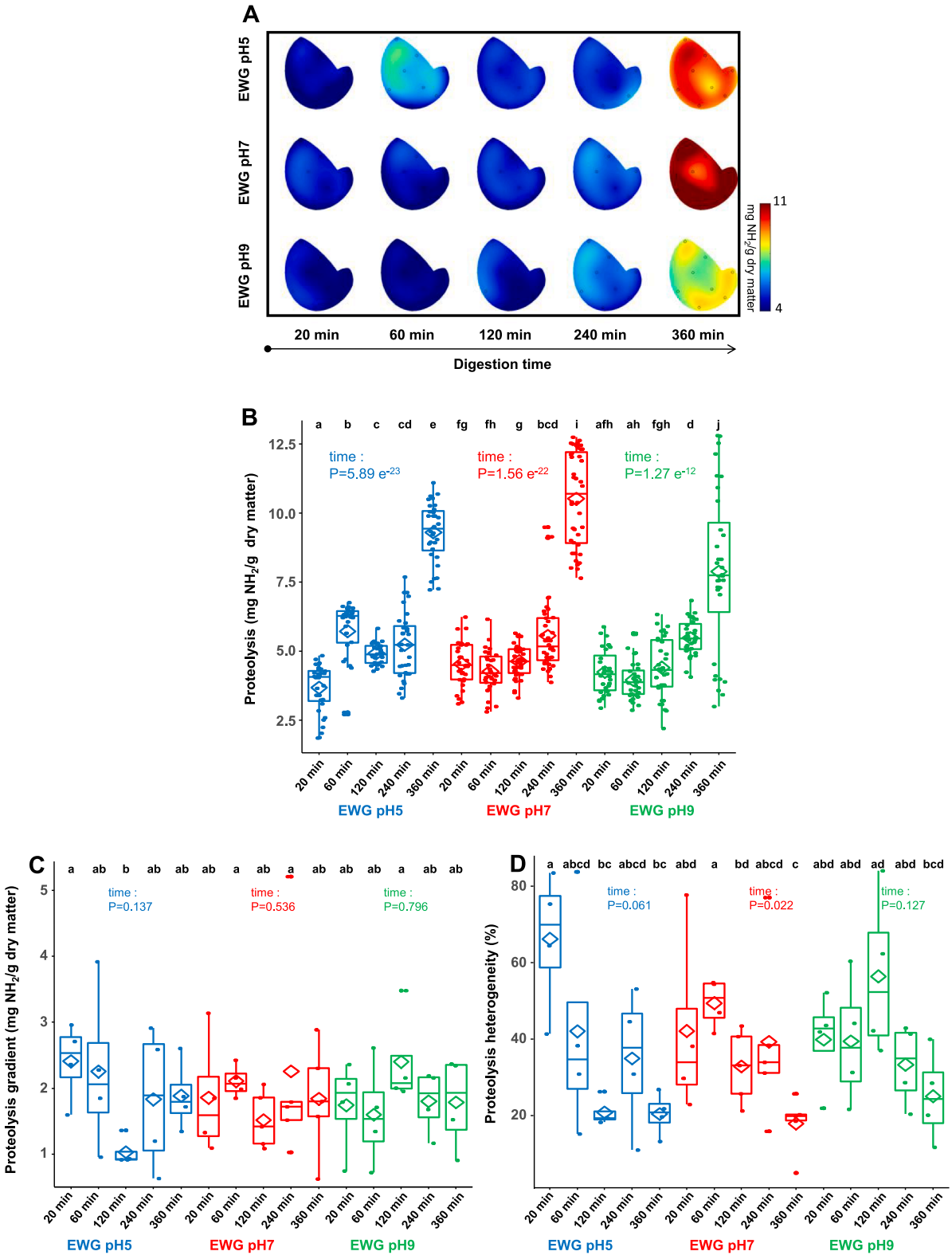

Fig. 4. Intra-gastric proteolysis (expressed as concentration of free NH_2 on dry matter basis) distribution over the 6 h postprandial in pigs fed either granular-spongy (pH5), intermediate (pH7) or smooth-rigid (pH9) forms of egg white gel (EWG). (**A**) Mapping of the proteolysis reconstituted from measurements at eight intra-gastric locations (empty circles). Colour-coded scales correspond to median proteolysis from 4 to 11 mg NH_2/g dry matter. Medians (horizontal bars), means (diamonds), and 25th-75th percentiles (boxes) of (**B**) proteolysis calculated from the eight sampling locations and all pigs of a given condition (diet × digestion time) (n = 32 or 40), **(C)** proteolysis gradient (proteolysis range for a given pig; n = 4 or 5), and (**D**) proteolysis heterogeneity (normalized gradient for a given pig; n = 4 or 5). Time effect (Kruskal-Wallis test) is indicated for each EWG (B, C, and D). Different letters indicate a significant difference ($p < 0.05$, Wilcoxon test).

from a higher mixing of the gastric chyme in pigs fed EWG-pH5 in comparison to those fed EWG-pH7 and EWG-pH9. This assumption is supported by the more pronounced drop of the free NH_2 concentration gradient (Fig. 4**C**) and heterogeneity (Fig. 4**D**) in pigs fed EWG-pH5, and is consistent with the quicker homogenisation of the intra-gastric pepsin concentration (Fig. 1**D**) and pH (Fig. 2**D**) observed with this diet.

At 240 min, neither the mean (Fig. 4**B**, Suppl. Data 9) nor the local proteolysis (Suppl. Data 10) significantly differed between the three EWGs. This may seem surprising since the mean intra-gastric pH was still significantly higher in pigs fed EWG-pH9 compared with EWG-pH7 ($p = 0.003$) and EWG-pH5 ($p = 1.95e\text{-}07$) (Fig. 2**B**, Suppl. Data 5). Yet, EW proteolysis by pepsin linearly increases from pH 6.0 to pH 1.0 (Salelles et al., 2021). Actually, proteolysis had not really started yet at this stage, as evidenced by the free NH_2 measurements (Fig. 4**B**). Pepsin concentration certainly increased from 120 min to 240 min of digestion (Fig. 1**B**), and the mean intra-gastric pH was lower than 5.0 in all conditions, but still higher than 3.0 (Fig. 2**B**). It is therefore conceivable that lower pH are required for a more extensive activity of pepsin, not forgetting that it should take time to obtain statistically significant differences with regard to proteolysis.

Lastly, the largest increase of proteolysis occurred between 240 min and 360 min for the three EWGs (Fig. 4**B**). After 6 h of digestion, the gastric chymes were quite homogeneous with regard to the free NH_2 concentration, regardless the EWGs (Fig. 4**D**). Since intra-gastric pepsin concentration was higher ($p = 0.013$) in pigs fed EWG-pH9 in comparison with EWG-pH7 (Fig. 1**B**), the lower ($p = 4.19e\text{-}05$; Suppl. Data 9) proteolysis measured in pigs fed EWG-pH9 may result from the mean intra-gastric pH slightly higher (Fig. 2**B**). Indeed, at 360 min, all intra-gastric pHs were in a pH range (pH 2.0 to pH 3.0) where pepsin activity increases when pH decreases (Salelles et al., 2021). Similarly, the higher ($p = 0.004$; Suppl. Data 9) intra-gastric free NH_2 concentration in pigs fed EWG-pH7 in comparison with those fed EWG-pH5 (Fig. 4**B**) may be the consequence of a slightly lower ($p = 0.045$) intra-gastric pH in the former (Fig. 2**B**), despite a mean pepsin concentration about three times higher ($p = 2.1e\text{-}19$) in pigs fed EWG-pH5 (Fig. 1**B**). Another assumption could be that, despite a higher pepsin secretion in the latter conditions, no extra proteolysis occurred because it had come to an end in the absence of remaining cleavage sites. This assumption is supported by the estimated DH values calculated at 360 min in pigs fed EWG-pH7 and EWG-pH5, namely 2.54% ± 0.86, and 2.52% ± 0.57, respectively. Indeed, these values are not statistically different, and quite close to the maximum DH value reported by Mat et al. (2018) at the end of the gastric phase of *in vitro* digestion (maximum DH = 3.0%). Note that at the same digestion time, the estimated DH was only 1.45% ±1.23 in pigs fed EWG-pH9.

3.4. Differences in intra-gastric proteolysis result in differences in the appearance of plasmatic amino acids

Given the differences observed between the three EWGs with respect to the intra-gastric proteolysis, the question arose whether amino acid (AA) bioavailability should be impacted. As expected and regardless the EWGs, the total plasmatic AA content increased in the first stage after the meal ingestion (Fig. 5**A**), as the consequence of the rapid gastric emptying which occurred from the start of the postprandial stage (Nau et al., 2019). Up to 60 min of digestion, no significant difference was observed between the EWGs. However, after 120 min, the plasmatic AA content was significantly higher in pigs fed EWG-pH5 (Suppl. Data 13) in comparison with EWG-pH7 ($p = 0.004$) and EWG-pH9 ($p = 0.009$), whereas gastric emptying was similar for the three EWGs up to 240 min (Nau et al., 2019). Therefore, it can be assumed that this observation arose from the higher intra-gastric proteolysis observed at 60 min digestion in pigs fed EWG-pH5 (Fig. 4**B**). Thus, a more extensively hydrolysed gastric chyme delivered to the duodenum could accelerate proteolysis by the pancreatic enzymes, resulting in a quicker AA transfer into the blood stream. Another assumption could be related to the more fragile texture of the EWG-pH5 (Nyemb et al., 2016), which would result in a quicker proteolysis in the duodenum. In any event, the delay observed between the occurrences of the higher intra-gastric proteolysis in pigs fed EWG-pH5 (60 min of digestion) and the highest plasmatic level of free AA (120 min) likely indicates that the time required for intestinal proteolysis and AA absorption was shorter than 60 min. This delay seems consistent with the interval of time between which protein concentration in the duodenum and plasmatic AA concentration were maximum (15 min and 30 min after the meal, respectively) in pigs fed dairy products (Barbé et al., 2013). Indeed, in the present study, hydrolysed proteins had to be first emptied in the duodenum before transfer to the blood stream.

The maximum plasmatic level of free AA was similarly reached at 120 min postprandial in pigs fed EWG-pH7, but was surprisingly delayed at 240 min digestion in pigs fed EWG-pH9 (Fig. 5**A**). Yet, no significant difference could be observed between pigs fed EWG-pH9 and EWG-pH7

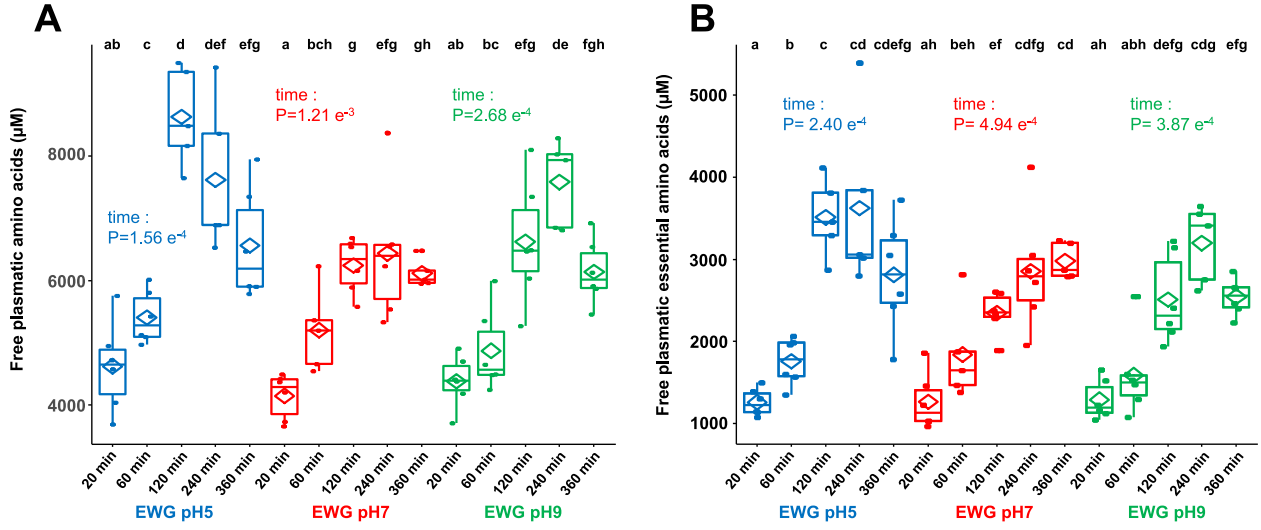

Fig. 5. Plasmatic free amino acids over the 6 h postprandial in pigs fed either granular-spongy (pH5), intermediate (pH7) or smooth-rigid (pH9) forms of egg white gel (EWG). (**A**) Total amino acids, and (**B**) essential amino acids. Data are medians (horizontal bars), means (diamonds), and 25th–75th percentiles (boxes) of plasmatic free amino acid concentration (n = 5 or 6). Time effect (Kruskal-Wallis test) is indicated for each EWG. Different letters indicate a significant difference ($p < 0.05$, Wilcoxon test).

with respect to gastric emptying (Nau et al., 2019), mean intra-gastric proteolysis except at 360 min digestion (Fig. 4**B**), or intra-gastric heterogeneity of proteolysis (Fig. 4**D**) throughout the postprandial period. On the contrary, the particle size in the gastric chyme has proved to be larger in pigs fed EWG-pH9 in comparison with the others, up to 240 min and in both distal and proximal regions of the stomach (Nau et al., 2019), probably because of the higher cohesiveness and elasticity of EWG-pH9 (Nyemb et al., 2016). Moreover, in pigs fed EWG-pH9, the gastric chyme itself was described as the most elastic and the most cohesive of the three (Nau et al., 2019). Therefore, it may be assumed that in pigs fed EWG-pH9, the gastric chyme entering the duodenum was slightly more difficult to hydrolyse by the pancreatic enzymes, thus leading to a delayed AA absorption and transfer into the blood stream.

After reaching the maximum value, the plasmatic AA content either decreased, in pigs fed EWG-pH5 and EWG-pH9, or remained constant, in pigs fed EWG-pH7, to finally reach similar level regardless the EWGs (Fig. 5**A**). The decrease of plasmatic AA level is the logical consequence of the protein anabolism. The reduction in dry matter content over the digestion in the gastric chyme (Suppl. Data 14), and therefore of the dry matter flux into the duodenum, likely contributed also to this decrease, despite intra-gastric proteolysis significantly increased between 240 min and 360 min of digestion (Fig. 4**B**).

At the end, it is noteworthy that the soft and fragile EWG-pH5 resulted in the more massive and rapid transfer of AA in the blood stream. At the opposite, the elastic and cohesive EWG-pH9 resulted in a less and delayed absorption of AA. As regards to the intermediate EWG-pH7, an intermediate behaviour was observed, that is AA absorption as rapid as, but smaller than that measured with EWG-pH5, and associated with a more stable level of plasmatic AA from 120 min up to 360 min postprandial (Fig. 5**A**).

The conclusions drawn here were applicable to the essential amino acids (EAA) as well. In particular, there was no significant difference between the three EWGs up to 60 min, and at 120 min of digestion, the plasmatic EAA content was significantly higher in pigs fed EWG- pH5 in comparison with those fed EWG-pH7 ($p = 0.0043$) and EWG-pH9 ($p = 0.0173$). Moreover, the highest mean value was observed earlier in pigs fed EWG-pH5 (120 min) in comparison with pigs fed EWG-pH9 (240 min) (Fig. 5**B**). The only difference related to the kinetics of EAA absorption in pigs fed EWG-pH7, which tended to increase throughout the 360 min of digestion, while a stagnation was observed from 120 min up to 360 min for total AA in these animals. This increase resulted from valine, threonine, and phenylalanine, the absorption kinetics of which similarly increased (data not shown). However, the plasmatic EAA content measured after 360 min of digestion was not significantly different in pigs fed EWG-pH7 in comparison with pigs fed EWG-pH5, but significantly higher than in pigs fed EWG-pH9 ($p = 0.0173$).

4. Conclusion

A previous *in vivo* study during which three EWGs different in texture, structure, pH and ionic strength, but with identical composition, were fed to pigs, highlighted an impact from the features of protein gels on their disintegration in the stomach and suggested consequences on the ingress of gastric secretions into the chyme (Nau et al., 2019). In the present study, the more detailed analysis of the samples collected during this previous experiment demonstrated that the physicochemical features of protein gels also influence the progress of gastric proteolysis and, beyond, the AA absorption.

In particular, the comprehensive and original investigation based on a detailed and simultaneous mapping of intra-gastric pH, pepsin concentration and proteolysis supports the hypothesis that the gastric fluids distribute more quickly over the stomach when the EWG is softer and rapidly disintegrated (namely EWG-pH5). However, the beginning of the postprandial period (first hour) was characterized by HCl and pepsin accumulation in the pylorus/antrum area regardless the EWG. This is probably due to the high volume of the meals, which resulted in very full stomachs, and therefore favouring the streaming of the gastric fluids along the stomach wall, likely exacerbated by the shutdown of the MCC. Above all, this study revealed that the gastric chyme homogenisation was slow and still incomplete even after 6 h of digestion, and even with the most fragile EWG-pH5. Nevertheless, the easier distribution of pepsin into the chyme with this latter EWG, combined with lower intra-gastric pH, resulted in a faster, while limited, intra-gastric proteolysis. This could explain the earlier and more massive appearance of AA in the blood with EWG-pH5, unless intestinal proteolysis was also facilitated because of the more extensively disintegrated structure of the gastric chyme obtained from this more acidic, soft and fragile EWG. In any event, it thus appeared that such attributes could be beneficial when body protein loss should be limited, such as in the elderly. On the contrary, when slow protein digestion is preferred, for example for protein gain in young people (Dangin et al., 2003, Dangin et al., 2002), an alkaline, elastic and cohesive gel (such as EWG-pH9) could be preferred. Moreover, it is noteworthy that in the specific conditions applied in the present study, intra-gastric proteolysis only really took off after 4 h of digestion. This likely resulted from the combination of high meal volumes, which required large quantities of pepsin secreted, and the high buffering capacity of EWGs, which required large quantities of HCl secreted to reach pH favouring pepsin activity.

Another outstanding outcome of this study is that, despite measurements have been performed on different animals for the different digestion times and the different diets, the results are remarkably consistent. This tends to support the robustness of the phenomena described in this study and, by extension, the credibility of the related assumptions proposed. Moreover, several extrapolations of present results regarding intra-gastric pepsin concentration and proteolysis were consistent with literature data. Hence, the low pepsin secretion during the first hour after the meal (2 mg/min) and intra-gastric pepsin concentration after 4 h of digestion (around 0.5 mg/g) are very close to measurements performed in humans, thus also confirming the relevance of the pig model for human digestion.

CRediT authorship contribution statement

Françoise Nau: Conceptualization, Methodology, Validation, Formal analysis, Writing – original draft, Supervision, Project administration, Funding acquisition. **Steven Le Feunteun:** Formal analysis, Writing – review & editing. **Yann Le Gouar:** Formal analysis, Investigation, Writing – review & editing. **Gwénaële Henry:** Formal analysis, Investigation, Writing – review & editing. **Maryvonne Pasco:** Formal analysis, Investigation. **Catherine Guérin-Dubiard:** Writing – review & editing, Supervision. **Kéra Nyemb-Diop:** Methodology, Formal analysis, Investigation. **Didier Dupont:** Conceptualization, Methodology, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors wish to warmly thank Dr Shane M. Rutherfurd (Riddet Institute, Massey University, Palmerston North, New Zealand) for his cooperation in the conception and supervision of the animal experiment from which the samples analysed in the present study are coming from. They also wish to thank Thiébaud Boucher who contributed to this work during his Master internship, and Maria J. Ferrua for kindly sharing with us the Matlab programs she developed.

Funding

This work was supported by the Ministère de l'Enseignement Supérieur et de la Recherche de la République française, the Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement (INRAE), and the Association pour le Développement de la Recherche sur l'Œuf et les Ovoproduits (ADRO Ouest).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2022.133132.

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