Food Chemistry 249 (2018) 111–118

Contents lists available at ScienceDirect

Food Chemistry

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

Combined *in vivo* and *in silico* approaches for predicting the release of bioactive peptides from meat digestion

T. Sayd[a], C. Dufour[b], C. Chambon[a], C. Buffière[c], D. Remond[c], V. Santé-Lhoutellier[a,*]

[a] INRA, UR370 Qualité des Produits Animaux, F-63122 Saint Genès Champanelle, France
[b] INRA, UR SQPOV, F-84000 Avignon, France
[c] Université Clermont Auvergne, INRA, UNH, F-63000 Clermont-Ferrand, France

ARTICLE INFO

Keywords:
Peptide
Mass spectrometry
Meat
Digestion
Bioactive peptides
Quantification

ABSTRACT

We studied the kinetics of peptide release during the gastric digestion of meat proteins *in vivo*, in view to predicting the release of bioactive peptides further on in the digestive tract. Six mini pigs fitted with gastric cannulas received a meal with cooked beef as protein source. Digesta was collected at regular time intervals up to 5½ h. The peptides generated by the gastric digestion of meat were identified and quantified using label-free LC MS, thereafter subjected to *in silico* digestion mimicking the action of intestinal enzymes. Three clusters of proteins presenting similar evolutions according to their dynamic hydrolysis were obtained. This study clearly improves the *in silico* prediction of the intestinal release of bioactive peptides by mapping meat protein degradation in the stomach in an *in vivo* model. Knowledge of the conformation of the peptides released in the stomach further improves this prediction.

1. Introduction

Meat is a source of good qualityprotein for humans, due to its balanced composition in essential amino acids, and its high digestibility (Oberli et al., 2015). Nowadays, the concept of nutritional quality of proteins fits within a larger definition that includes the potential to release, during digestion, peptides able to exert various physiological effects beneficial for human health (Caron et al., 2016). These bioactive peptides contain 2 to 20 amino acid residues. They can either have local effects on the digestive tract or be absorbed through the intestine, pass intact into the bloodstream and play a physiological role in peripheral tissues. Depending on the sequence of the amino acids, these peptides can exhibit diverse antioxidant, antimicrobial, immunomodulatory, antithrombotic and antihypertensive properties. Among them, the generation of angiotensin I-converting enzyme (ACE) inhibitory peptides (antihypertensive properties) and dipeptidyl peptidase enzyme (DPP-IV) inhibitory peptides (management of postprandial hyperglycemia) is well documented. The potential of meat to release ACE inhibitory peptides during digestion has been thoroughly demonstrated using *in vitro* models (Arihara, Nakashima, Mukai, Ishikawa, & Itoh, 2001; Katayama et al., 2008; Escudero, Sentandreu, & Toldra, 2010; Vercruysse, Van Camp, & Smagghe, 2005), and recently in milk and soy (Capriotti et al., 2015; Piovesana et al., 2015; Zenezini Chiozzi et al., 2016a). Few studies have been conducted *in vivo*; nevertheless, Bauchart et al. (2007) demonstrated in pigs, that after meat ingestion, some of the peptides among the wide variety of those released in the small intestine contained bioactive sequences, mainly corresponding to antihypertensive activity. Progress made in bioinformatics and knowledge of the amino acid sequences of food proteins have made it possible to detect bioactive sequences and thus determine the potential release of bioactive peptides (Zenezini Chiozzi et al., 2016b). However, this potential offered by *in silico* analyses requires additional information on the kinetics of peptide release in the gastrointestinal compartments, as well as the quantification of the peptides to identify their possible physiological effect. Recently a label-free method to quantify peptide release during the *in vitro* digestion process of beef meat was developed (Sayd, Chambon, & Sante-Lhoutellier, 2016). In this study we evaluate the digestion of beef meat proteins as precursors of biologically active peptides, by combining the study of the kinetics of peptides released *in vivo* in the gastric compartment with an *in silico* approach that reproduces the lower part of the digestive tract, in view of detecting potentially bioactive peptides.

2. Material and methods

All the procedures described were conducted in accordance with the guidelines formulated by the European Community for the use of experimental animals (2010/63/EU), and the study was approved by the

* Corresponding author.
E-mail address: veronique.sante-lhoutellier@inra.fr (V. Santé-Lhoutellier).

https://doi.org/10.1016/j.foodchem.2018.01.013
Received 15 September 2017; Received in revised form 7 December 2017; Accepted 1 January 2018
Available online 02 January 2018

Local Committee for Ethics in Animal Experimentation (n CE24-10; Comité d'Ethique en Matière d'Expérimentation Animale d'Auvergne, Aubière, France).

2.1. Animals

The study involved 6 female Göttingen mini pigs (Ellegaard, Denmark) (9–10 months old; 21.9 ± 1.1 kg body weight). At least 3 weeks before initiating the intervention, the mini pigs were surgically fitted with a permanent cannula (silicone rubber; 12-mm i.d., 17-mm o.d.) in the body of the stomach, in the middle of the long axis of the greater curvature. The cannula was ventrally exteriorized on the left flank, just after the last rib. The mini pigs were housed in individual pens (1 × 1.5 m), separated by Plexiglass walls, in a ventilated room with controlled temperature (20–23 °C). Apart from sampling days, they were fed once daily, at 08:15, with 400 g of a commercial feed (Porcyprima: 18% protein, 2% fat, 5% cellulose, 6% ash; Sanders Nutrition Animale, France), and had free access to water. In order to ensure the rapid and complete ingestion of the test meals during the sampling days, they were accustomed to receiving this type of meal before starting the experiment.

2.1.1. Test meal

The protein source was provided by 120 g of beef meat. The muscle *Triceps brachii* was obtained from a 15-month-old Charolais bull, aged for 15 days, and cooked under vacuum at 70 °C for 30 min in a water bath. Before cooking the muscle was minced with an 8-mm diameter grinder. The meal was prepared by mixing the minced meat with *N*-free ingredients (starch, lipid, and fiber). The composition of the test meal is shown in Table 1. In order to ensure the rapid and complete ingestion of the test meals during the sampling days, the mini pigs were accustomed to receiving this type of meal before starting the experiment.

2.1.2. Experimental procedure

The evening before the day of sampling, the stomach was flushed by intragastric injection of 200 mL of warm water followed by free evacuation of the chyme through the cannula. On the day of sampling, the mini pigs did not receive the commercial feed and were exclusively offered test meals. The test meal was given at 09:00 and the animals always consumed the whole meal in less than 15 min. The digesta (average volume 60 mL) was collected gravimetrically in a graduated beaker 30 min before and 15, 45, 90, 150, 240, and 330 min after test meal delivery. The collected digesta was immediately homogenized (30 s) with an Ultra-Turrax homogenizer (T25 Digital; IKA Werke GmbH & Co. KG, Staufen, Germany), aliquoted and immediately frozen in liquid nitrogen before analysis. All samples were kept at −80 °C until analysis.

2.2. Analytical methods

2.2.1. Peptide extraction

After defrosting, 3 mL of each collected digesta were diluted with 2.5 mL of 50 mM sodium bicarbonate (pH 8). After vortexing, the mixed sample was centrifuged at 4000*g* for 15 min. The collected supernatant was filtered through gauze compresses. The resulting filtrate was immediately put on ice and the proteins were precipitated with cold trichloroacetic acid (15% final concentration) for 1 h. Then, the tubes were centrifuged at 4000*g*, at 4 °C, for 15 min. The following peptide extraction was conducted on the gastric samples (Sayd et al., 2016). Briefly, peptide extraction was performed using porous silica nanoparticles MCM-41 (Sigma) according to Tian, Ren, Ma, Li, et al. (2007), with some modifications (25 mg of MCM-41 nanoparticles were hydrated with 1 mL of 3% TCA). The resulting slurry was mixed and processed ultrasonically. Immediately, 1 mL of the gastric sample resulting from the TCA precipitation described above was added and shaken for 2 h at 4 °C. Then the suspension was centrifuged for 15 min at 4000*g* and the supernatant was removed. The silica nanoparticles were then washed 3 times with 1 mL H_2O. The peptides retained on the MCM-41 porous silica nanoparticles were eluted with 1 mL of 80% acetonitrile. A reference sample was produced by mixing the 36 gastric samples at equal volume (50 µL) to constitute a "gastric sample mix". The samples were kept at −20 °C until used.

Table 1
Composition of test meal.

ingredients	quantity
cooked meat (g)	120
sunflower oil (mL)	40
cellulose (g)	7
starch (g)	70
egg yolk (g)	3
pectin (g)	1
Water (mL)	30

2.2.2. Liquid chromatography and mass spectrometry

An UltiMate 3000 Rapid Separation LC (RSLC) system (ThermoFisher-Dionex, France) was used to separate the peptides extracted. First, 8 µL of extracted digesta were automatically loaded on to a precolumn (300 µm × 5 mm) equilibrated with a solution of 0.05%TFA for desalting and concentration. After 6 min, the concentration column was placed on line with the separation nanocolumn (Pepmap100, Acclaim, 75 µm × 150 mm, ThermoFisher) and the peptides were separated with a gradient from 4% to 70% ACN/H_2O (80/20) at a flow rate of 400 nL/min for 50 min. The eluted peptides were electrosprayed in positive-ion mode at 2.7 kV through a CaptiveSpray ion source in the mass spectrometer QTOF (Impact II, Bruker). The CID mode was selected to acquire the maximum number of MS/MS possible in three seconds after the full MS scans (*m/z* 150–2200).

2.2.3. Label-free peptide quantification

The acquired spectra of each sample were loaded into the Progenesis QI software (nonlinear Dynamics, Waters Company). Using personal peak-modeling algorithm, the software transformed the data of the MS and MS/MS scans into a peak list comprising positional information (*m/z* and retention time) and quantification (peptide abundance). To compare all the expression profiles and compensate for between-run variations, the "sample mix" was used as a reference, using automatic alignment to align the retention times of all the others. Only the features with two or three charges were kept for analysis.

2.2.4. Peptide identification and data search

After run alignment and ion detection (http://www.nonlinear.com/qi-for-proteomics/how -it-works/), the MSMS list of all the peaks of interest was exported from the Progenesis software as a mascot file (.mgf) and used for peptide identification with MASCOT (V 2.2) in the NCBI_Bos taurus database (24,207 sequences). The search parameters used were: no enzyme, 20 ppm peptide mass tolerance and 0.05 Da fragment mass tolerance. Methionine oxidation was allowed as a variable modification. Only peptides with ion scores of 30 and above were considered and re-imported into the Progenesis software. Protein abundance was calculated by summing the peptide abundances allocated to the respective protein. We chose to set a minimum of two unique peptides to validate protein identification.

2.2.5. In silico digestion and prediction of bioactive peptide release

To mimic the intestinal digestion of each peptide identified in the gastric chyme we used the "enzyme action" tool of BioPep database (Minkiewicz, Dziuba, & Michalska, 2011). We selected the action of three intestinal proteinases: trypsin (EC 3.4.21.4) chymotrypsin (EC 3.4.21.1) and pancreatic elastase (EC 3.4.21.36). Then, the theoretical intestinal peptides obtained were subjected to the "search for active