

RESEARCH ARTICLE

Discrimination of in vitro and in vivo digestion products of meat proteins from pork, beef, chicken, and fish

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In vitro digestion products of proteins were compared among beef, pork, chicken, and fish. Gastric and jejunal contents from the rats fed these meat proteins were also compared. Cooked pork, beef, chicken, and fish were homogenized and incubated with pepsin alone or followed by trypsin. The digestion products with molecular weights of less than 3000 Da were identified with MALDI-TOF-MS and nano-LC-MS/MS. Gastric and jejunal contents obtained from the rats fed the four meat proteins for 7 days were also analyzed. After pepsin digestion, pork, and beef samples had a greater number of fragments in similarity than chicken and fish samples, but the in vitro digestibility was the greatest ($p < 0.05$) for pork and the smallest for beef samples. After trypsin digestion, the species differences were less pronounced ($p > 0.05$). A total of 822 and 659 peptides were identified from the in vitro and in vivo digestion products, respectively. Our results could interpret for the differences in physiological functions after the ingestion of different species of meat.

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1 Introduction

In recent years, some epidemiological studies have associated overconsumption of red meat with the development of cardiovascular disease and colon cancer [1–4]. However, the critical role of meat in human diet may not be underestimated because it provides the human a lot of nutrients, especially

of high-quality proteins [5]. It has been known that proteins undergo complex degradation in the digestive tract and this can result in a variety of digestion products with different fates; amino acids, and some small peptides can be absorbed in their natural states in the duodenum and the jejunum, but the undigested proteins, and medium and large size peptides may be utilized by gut bacteria or excreted directly [6, 7]. A previous study showed that the ingestion of proteins from beef, pork, turkey meat, casein, and soybean affected triacylglycerol level in rat liver and plasma, and the VLDL level in the plasma [8]. A recent study in our group also indicated the significant difference in body weight gain and plasma amino acid profiles in rats fed pork, beef, chicken, and fish proteins (Shangxin Song, personal communication).

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Abbreviations: **CHCA**, α -cyano-4-hydroxycinnamic acid; **C-terminus**, carboxyl terminus; **F**, phenylalanine; **FA**, formic acid; **HCD**, Higher-energy collisional dissociation; **L**, leucine; **N-terminus**, amino terminus; **PCA**, principal component analysis; **Y**, tyrosine

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Significance of the study

In vitro digestibility of meat protein varies with meat species. Pepsin digested products of pork and beef samples had a greater number of fragments in sequence similarity than chicken and fish samples, but the in vitro pepsin digestibility between pork and beef was significantly different. In vivo protein digested products differed from in vitro ones.

To interpret for the difference, it is crucial to characterize the complex proteins from raw or cooked meat and the complex digestion products of meat proteins when they go through the stomach and the intestine. In recent years, MS-based methods have been applied to identify the in vitro digestion products of proteins from different food sources [9–11]. Of the digestion products, oligopeptides account for a large proportion that can be degraded by the brush border peptide hydrolases and taken up into intestinal epithelial cells where the size of oligopeptides may be critical [12]. However, few data are available on the nutrition aspects of digestion products from different meat proteins.

The objectives of the present study were to characterize the in vitro digestion products of cooked pork, beef, chicken, and fish treated with pepsin and trypsin, and to characterize gastric and jejunal contents obtained from the rats fed the four meat proteins for 7 days.

2 Materials and methods

2.1 Materials

Pork, beef, chicken, and fish were used. Pork *longissimus dorsi* muscle (the number of biological replicates, n equals 8, muscle pH 5.83 ± 0.18), beef *longissimus dorsi* muscle ($n = 8$, pH 5.46 ± 0.08), chicken *pectoralis major* muscle ($n = 8$, pH 5.84 ± 0.05) and fish muscle from silver carps ($n = 8$, pH 6.67 ± 0.17) were obtained from a commercial meat packing company. Pepsin from porcine gastric mucosa (P7125) and trypsin from porcine pancreas (T7409) were purchased from Sigma Aldrich (USA). BCA protein assay kit (23225) was purchased from Thermo Scientific (USA). The 4–12% Bis-Tris Criterion precast gels (345-0125), XT sample buffer (161-0791), XT MES running buffer (161-0789), and broad molecular weight calibration marker (Precision Plus Protein Dual Xtra Standard, cat. no. 161-0377, molecular range 2–250 kDa) were purchased from Bio-Rad (USA). Ultracel-3 Membrane (Amicon Ultra-0.5, 3 kDa) (UFC500396) and Zip-Tip C18 pipette tips (ZTC18S096) were purchased from Millipore (USA).

2.2 Sampling and cooking procedure

All visible fat and connective tissue were removed from pork, beef, and chicken muscles. Bones, scale, and fat were re-

moved from fish. Pork and beef muscles were cut vertically into 2 cm-thick pieces, whereas chicken and fish muscles were minced and prepared for 2 cm-thick pies. All samples were packed in plastic pouches and cooked in a 72°C water bath (Crystal Industries, USA) until the center temperature of meat pieces or pies reached 70°C and cooking time was 30 min approximately.

2.3 In vitro digestion

Cooked meat was in vitro digested according to the procedures of Escudero et al. (2010) with some modifications [10]. Briefly, 0.5 g of cooked meat sample was separately homogenized (IKA, T25, Germany) in 2 mL of distilled water for 2×30 s at 9500 rpm and 2×30 s at 13 500 rpm with 30 s cooling between bursts. The homogenates were adjusted to pH 2.0 with 1 M HCl and pepsin was added at a ratio of 1–31.25 based on the mass of meat (substrate). The reaction mixture was kept at 37°C for 2 h with continuous shaking, and then the enzyme was inactivated by adjusting the pH to 7.5 with 1 M NaOH. One milliliter of the resulting digestion mixture was removed for further analysis and the remaining (approximately 1.5 mL) was kept at 37°C for the trypsin digestion. Trypsin was added at a ratio of 1–50 based on the mass of meat (substrate). The reaction mixture was maintained under the same conditions as above. After 2 h of trypsin digestion, enzyme activity was terminated by heating at 95°C for 5 min, and then 1 mL of the resulting digestion mixture was taken out for further analysis. Both the pepsin and pepsin/trypsin treated samples were deproteinized by adding three volumes of ethanol and storing for 12 h at 4°C. The samples were then centrifuged at $10,000 \times g$ for 20 min at 4°C. The supernatant and the precipitate were separated and stored at -18°C for electrophoresis and MS analysis.

The in vitro digestibility was evaluated on a dry weight basis. Briefly, cooked meat was chilled, freeze-dried, and ground into powder. Intramuscular fat was removed by using a mixture of solvent methylene chloride/methanol (V/V = 2:1) and organic solvent was volatilized in a fume hood. The dried mixtures contained greater than 90% of protein. Two portions of protein powder weighing 1 g were taken from the same replicate. One portion was only treated with pepsin, and the other one was treated with pepsin and followed by trypsin. The digestion procedures were the same as above expect for pepsin added at a ratio of 4–31.25 and trypsin at a ratio of 4–50 based on the mass of protein powder (substrate). After digestion, the resulting mixture was centrifuged at $10,000 \times g$ for 20 min at 4°C and the supernatant was discarded. The precipitate was dried until constant weight. The degree of digestibility was calculated as follows (1):

$$DT = \left(1 - \frac{W_i}{W_t}\right) \times 100\% \quad (1)$$

DT: digestibility (%); W_i : weight of dried insoluble protein; W_t : total weight of dried meat protein power before digestion.

2.4 In vivo digestion of meat

To evaluate the changes of meat proteins in the digestive tract, animal experiment was performed. Briefly, cooked meat was chilled, freeze-dried, and ground into powder. Intramuscular fat was removed by using a mixture of solvent methylene chloride/methanol (V/V = 2:1) and organic solvent was volatilized in a fume hood. The dried mixtures contained greater than 90% of protein. The proteins were incorporated into the chow diets at a percentage of 20%. The chow diets were prepared according to AIN-93G formula including protein 20%, cornstarch 39.75%, sucrose 13.2%, soybean oil 7%, fiber 5%, mineral mix (AIN-93-G-MX) 3.5%, vitamin mix (AIN-93-VX) 1%, L-cystine 0.3%, choline bitartrate 0.25%, and tert-butylhydroquinone 0.0014% [13].

All animal experimental protocols were approved by the Animal Care Committee of Nanjing Agricultural University. Forty male Sprague-Dawley rats of 21 days old were pre-fed a chow diet for 7 days and then changed with meat protein diets. The rats were reared individually in stainless steel cages with 12-h light-dark cycles and provided with diet and water ad libitum. After 7-day feeding, the animals were withdrawn with food for 4 h and killed by decapitation. The contents in the stomach and jejunum were carefully collected, and heated at 95°C for 5 min to inactivate the enzymes. In order to remove fat and oil from the collections to facilitate the peptides characterization, one hundred milligrams of the stomach and jejunal contents in 100 μ L of Milli-Q water were mixed with 50 μ L of petroleum ether and vortexed for 20 s and centrifuged at 10,000 g for 5 min [14]. The lower aqueous phase was pooled. The extraction step was repeated 3 times. Three volumes of ethanol were added into the pooled aqueous portion and then centrifuged at 10,000 $\times g$ for 20 min at 4°C. Each ethanol-soluble fraction was filtered through an ultracel-3 membrane centrifugal filter unit to remove peptides greater than 3 kDa, and then through the Zip-Tip C18 pipette tips for desalting. The resulting peptides were stored at -18°C for further analysis with nano-LC-MS/MS system.

2.5 Gel electrophoresis

SDS-PAGE was performed to characterize the protein profiles before and after in vitro digestion of cooked meat.

For undigested samples, 1 g of cooked sample was homogenized in 4 mL of extraction buffer (2% SDS, 10 mM sodium phosphate buffer, pH 7.0) with an Ultra Turrax homogenizer (IKA T25 Digital, Germany). The homogenate was centrifuged (Allegra™ 64R, Beckman Coulter, USA) at 1500 $\times g$ for 15 min at 4°C. The supernatant was retained. For digested samples, the ethanol-precipitated proteins from the digestion products were dissolved in 0.4 mL of the same extraction buffer. Protein concentration in the supernatants and the

digestion products were determined with a BCA protein assay kit. Only proteins or peptides with large molecular weights may be precipitated in ethanol and separated on SDS-PAGE gels. Correspondingly, ethanol-soluble portions are peptides from protein digestion and have smaller molecular weights so that they are difficult to separate on SDS-PAGE gels. And thus, we applied SDS-PAGE to profile intact or partially digested proteins and MALDI-TOF-MS/LC-MS-MS to profile digestion products.

Protein samples were adjusted to a final protein concentration of 0.5 μ g/ μ L with XT sample buffer and heated at 95°C for 5 min. Ten microliters of each sample was loaded on 4–12% Bis-Tris Criterion precast gels and run in 900 mL of XT MES running buffer at 150 V for approximately 1 h. Protein bands were visualized with Coomassie blue R250 and images were captured with an image scanner (GE Healthcare, USA). The band intensities were quantified with the Quantity One software (Bio-Rad, USA). The intensity of each band was calculated as its actual intensity relative to that of the 25 kDa band in the calibration marker lane.

A total of ten visual bands differing with species were further identified. In brief, gel bands were destained, cleaned, and digested with trypsin (Promega, Madison, WI, USA). The extracted peptide mixture was analyzed by MALDI-TOF-MS/MS (ultrafleXtreme Bruker, Germany). All peptide spectra were submitted to the online MASCOT program (<http://www.matrixscience.com>) for data searching against the NCBI databases. The search parameters were set as follows: 0.12 Da mass tolerances for peptides and 0.6 Da mass tolerance for TOF fragments; one allowed trypsin miscleavage; carbamidomethyl of cysteine as fixed modification; and oxidation of Met and pyro-Glu formation of N-terminal glutamine as variable modifications. Only significant hits were accepted, as defined by the MASCOT probability analysis ($p < 0.05$).

2.6 Characterization of the in vitro digestion products with MALDI-TOF-MS

The ethanol-soluble fractions of the pepsin and trypsin digestion products were characterized by MALDI-TOF-MS in duplicate. An aliquot (1 μ L) of each sample was spotted on the target plate with an equal volume of matrix solution (α -cyano-4-hydroxycinnamic acid, 5 mg/mL prepared in 50% ACN/0.1% TFA). The mixture was allowed to dry before analysis. Positive ion (MH^+) spectra were acquired in the linear MALDI-TOF mode. The m/z signals were recorded between 700 and 3500 amu for peptide detection.

The peak data of the MS spectra were subjected to hierarchical clustering using the ClinProTools 2.2 software (Bruker Daltonics, Germany). Dendrograms and PCA were generated using the unsupervised clustering function of the program.

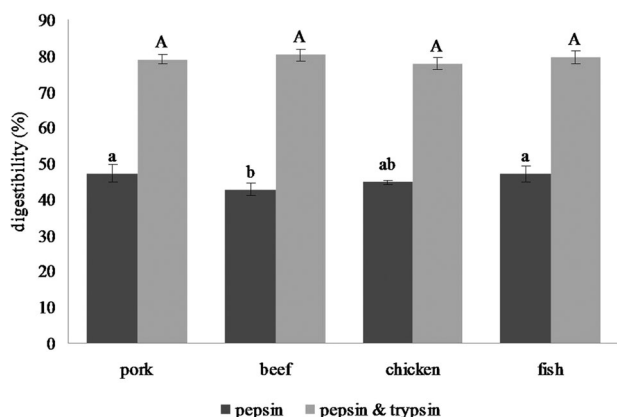


Figure 1. In vitro digestibility of cooked pork, beef, chicken, and fish. Black column, samples were treated with pepsin; gray column, samples were treated with pepsin followed by trypsin. Different letters on the same color column indicate statistically significant difference among the values.

2.7 Characterization of the in vitro digestion products and intestinal contents with nano-LC-MS/MS

The ethanol-soluble fractions of the pepsin and trypsin digestion products were also analyzed with a hybrid quadrupole orbitrap mass spectrometer equipped with a nano-electrospray ionization source (Q-Exactive, Thermo Fisher Sci-

entific, USA). The ethanol-soluble fractions were filtered through ultra-0.5 mL centrifugal filter units with ultracel-3 membrane (Amicon Ultra, Millipore, Ireland) under centrifugation at $15,000 \times g$ for 15 min. Peptide mixtures were then fractionated on an on-line nano-LC system (EASY-nLC 1000, Thermo Scientific, USA). Samples were loaded onto a C18 column (7.5×150 mm, $2 \mu\text{m}$) at a maximum pressure of 500 bar. Peptides were separated using a mobile phase changing from 0.1% formic acid (FA) in water (buffer A) to 0.1% FA in ACN (buffer B). A step-gradient elution at a flow rate of 350 nL/min was applied with an increasing concentration of buffer B: (1) 10 min from 0 to 8%, (2) 25 min from 8% to 20%, (3) 15 min from 20% to 30%, (4) 5 min from 30% to 90%, and finally kept at 90% until the procedure ended. The hybrid quadrupole orbitrap mass spectrometer was operated in a data-dependent mode, and a scan cycle was initiated with a full-scan MS spectrum (from 100 to 1500 amu). The top 20 abundant ions were selected for higher energy collisional dissociation fragmentation in the linear ion trap, and the exclusion time was set as 60 s. The extracts from intestinal contents were characterized with the same method.

MS/MS spectra of peptides were matched using the Proteome Discoverer (Thermo Fisher Scientific, USA) against the Swiss-Prot database, including subsets *Sus scrofa* for pork, *Bos taurus* for beef, *Gallus gallus* for chicken, and *Teleostei* for fish (<http://www.uniprot.org/taxonomy/complete-proteomes>). It is notable that the databases for fish may not be complete and herein we may not get enough information.

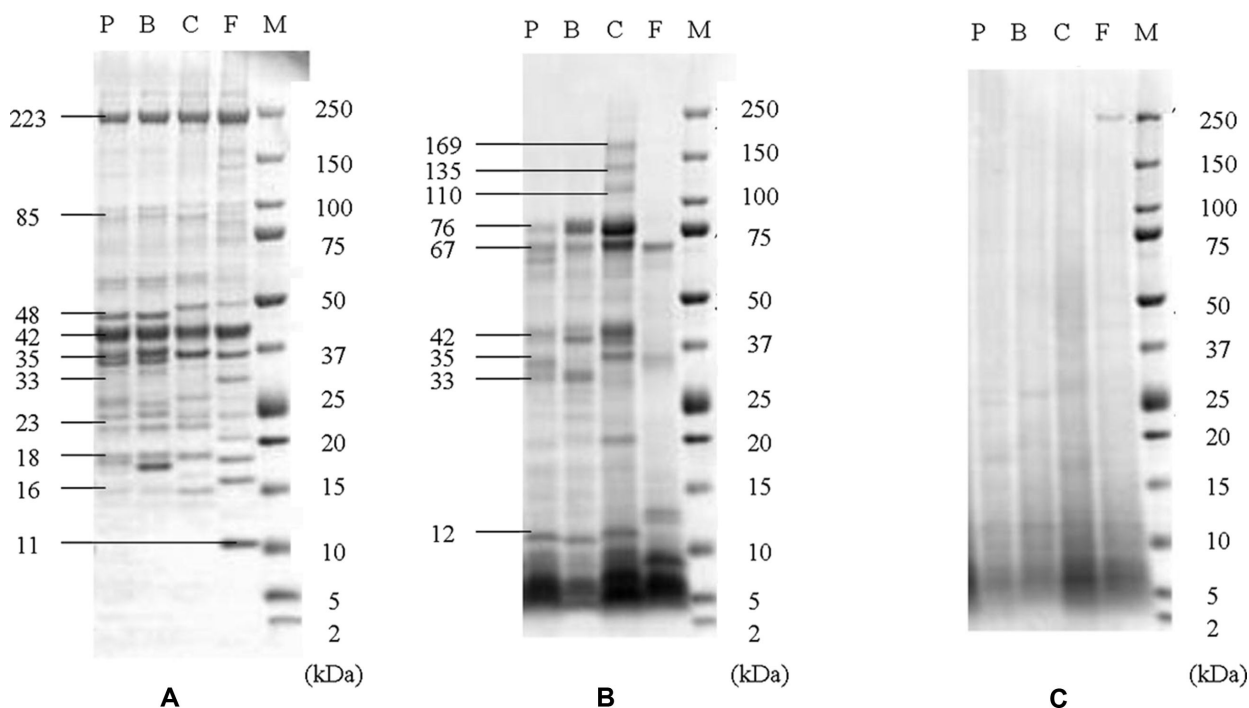


Figure 2. SDS-PAGE profiling of total proteins from the four species before and after digestion. (A) Total proteins in undigested cooked meat; (B) pepsin-digested, ethanol-insoluble fraction; (C) pepsin/trypsin-digested, ethanol-insoluble fraction. Lanes P/B/C/F/M: pork/beef/chicken/fish/marker.

Table 1. Quantification and qualification of protein bands from nondigested cooked meat on SDS-PAGE

Bands	Relative band intensity ²⁾ , mean±SD				Protein name	Proteins identified by MALDI-TOF-MS				Score
	Pork	Beef	Chicken	Fish		Accession no.	peptides	Mw _t ³⁾ (KDa)	Sequence coverage (%)	
223	0.77 ± 0.23 ^a	0.72 ± 0.23 ^a	0.59 ± 0.20 ^a	0.62 ± 0.27 ^a	Myosin-2	gi 55741490 <i>Sus scrofa</i>	2	223.92	4.00	365
					Myosin heavy chain 4	gi 66472732 <i>Danio rerio</i>	7	223.09	5.00	545
85	0.07 ± 0.03 ^b	0.10 ± 0.02 ^a	0.07 ± 0.03 ^b	0.03 ± 0.01 ^c	Muscle glycogen phosphorylase	gi 106073338 <i>Sus scrofa</i>	4	84.38	8.00	126
48	0.56 ± 0.05 ^a	0.50 ± 0.05 ^b	0.40 ± 0.05 ^c	0.19 ± 0.05 ^d	beta-enolase	gi 113205498 <i>Sus scrofa</i>	3	47.44	8.00	218
					beta-enolase	gi 47551317 <i>Danio rerio</i>	2	47.84	9.00	76
42	1.30 ± 0.32 ^a	1.09 ± 0.26 ^{ab}	1.26 ± 0.24 ^{ab}	1.06 ± 0.20 ^b	alpha-cardiac actin	gi 6636384 <i>Danio rerio</i>	4	42.31	18.00	421
35	0.16 ± 0.07 ^d	0.29 ± 0.09 ^c	0.73 ± 0.12 ^a	0.43 ± 0.07 ^b	Glyceraldehyde-3-phosphate dehydrogenase	gi 65987 <i>Sus scrofa</i>	2	35.91	8.00	177
					Glyceraldehyde-3-phosphate dehydrogenase	gi 169403947 <i>Danio rerio</i>	3	35.99	13.00	162
33	0.17 ± 0.04 ^b	0.19 ± 0.06 ^b	0.09 ± 0.03 ^c	0.30 ± 0.03 ^a	Not identifiable	-	-	-	-	-
23	0.20 ± 0.03 ^a	0.23 ± 0.04 ^a	0.12 ± 0.05 ^b	0.20 ± 0.06 ^a	Not identifiable	-	-	-	-	-
18	0.30 ± 0.06 ^b	0.35 ± 0.09 ^a	0.24 ± 0.01 ^c	0.16 ± 0.02 ^d	Not identifiable	-	-	-	-	-
16	0.19 ± 0.01 ^c	0.17 ± 0.02 ^c	0.32 ± 0.02 ^b	0.45 ± 0.06 ^a	Not identifiable	-	-	-	-	-
11	0.00 ^b	0.00 ^b	0.00 ^b	0.87 ± 0.10 ^a	Not identifiable	-	-	-	-	-

1) The figures are estimated molecular weights of proteins on SDS-PAGE based on the bands calibrated to standard markers.

2) Relative band intensity was calculated as its actual intensity relative to that of the 25 kDa band in the calibration marker lane, and means with different superscripts on the same row differed significantly with species ($p < 0.05$).

3) The figures are the theoretical molecular weights of the identified proteins. Proteins marked with “-” are bands that could not be identified by the MALDI-TOF-MS method.

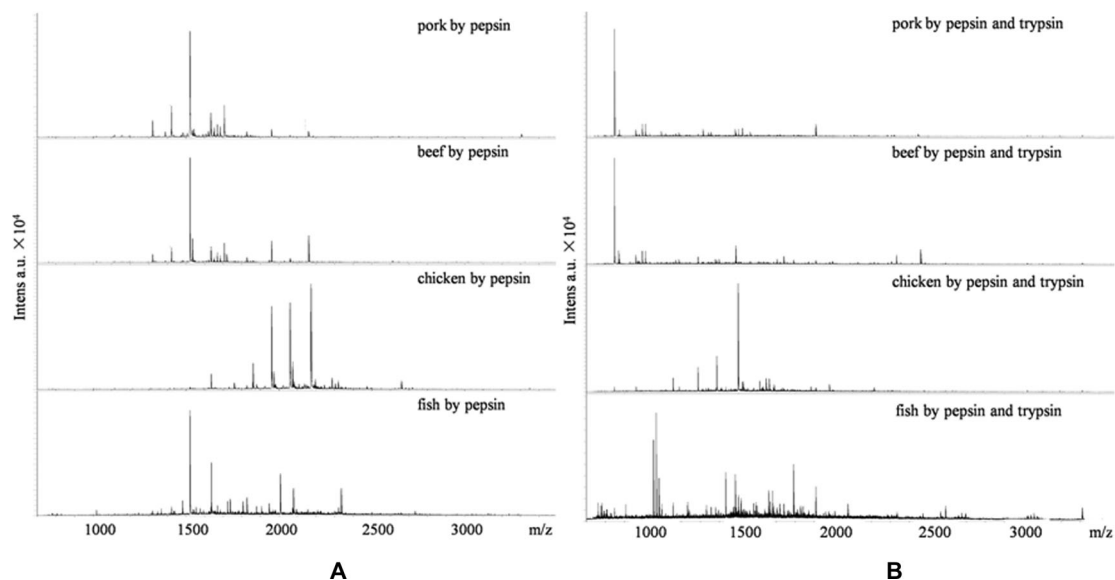


Figure 3. Representative MALDI-ToF-MS spectra of ethanol-soluble fragments from cooked pork, beef, chicken, and fish after treatment with pepsin and trypsin. (A) Pepsin-digested, ethanol-soluble fraction; (B) pepsin/trypsin-digested, ethanol-soluble fraction.

However, our MS data provide powerful evidence that different meat proteins undergo digestion to different extents. Data matching was performed with a fragment ion mass tolerance of 0.8 Da and a parent ion tolerance of 10 ppm. An unspecific enzyme was used in peptic peptides database search, while both nonspecific enzyme and trypsin were used in tryptic peptides search. Oxidation of methionine and pyro-Glu formation of N-terminal Gln was chosen as dynamic modifications, respectively, and two missing cleavages were allowed. Peptide identifications were accepted if they could be established at a PeptideProphet probability greater than 95%. Protein matching was accepted

if they could be established at greater than 95% probability and contained at least two identified spectra. Venn diagrams (<http://bioinfo.gp.cnb.csic.es/tools/venny/index.html>) were applied to analyze the similarity of peptides from the four types of meat.

2.8 Statistical analysis

One-way analysis of variance and Duncan’s multiple-range test were performed to test the difference in band intensity and digestibility among four species with the SAS

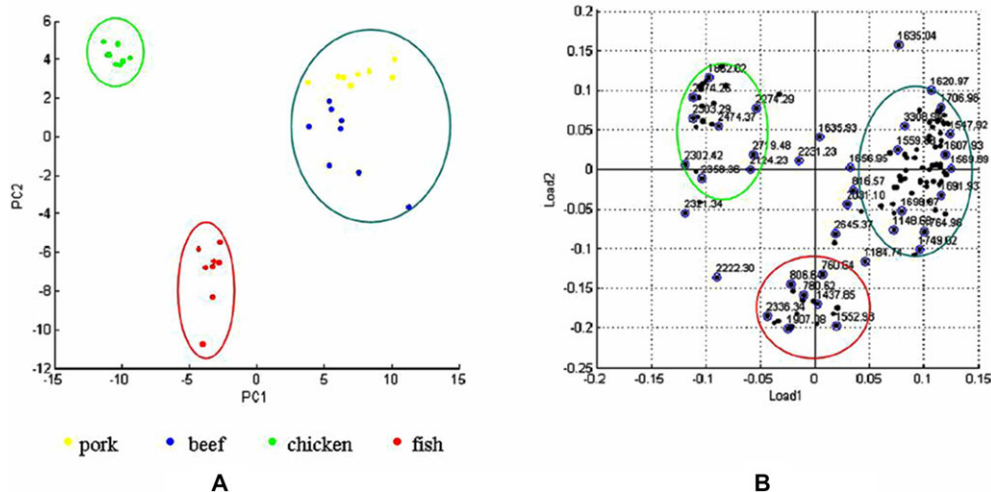


Figure 4. Scores plot (A) and loadings plot (B) for the peptides obtained from cooked pork, beef, chicken, and fish treated with pepsin. Plots in circles with the same color (from A and B) have a close relationship to each other.

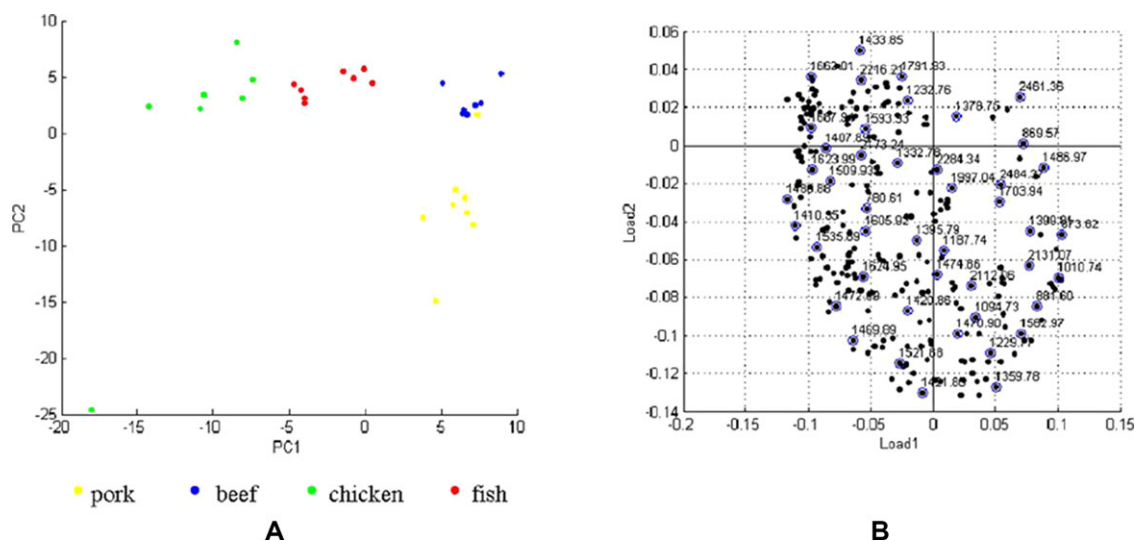


Figure 5. Scores plot (A) and loadings plot (B) for the peptides obtained from cooked pork, beef, chicken, and fish treated with pepsin and trypsin.

program (SAS Institute Inc., USA). Clustering and principal component analyses were performed for spectral data, as described in Section 2.6.

3 Results and discussion

3.1 Protein profiling and in vitro digestibility vary with meat species

The bioavailability of dietary proteins was normally evaluated by the extent of the digestibility, that is, the ease of protein breakdown and the size of the products (peptides) after digestion with digestive enzymes, especially of pepsin and trypsin [15, 16]. In the present study, the in vitro digestibility (%) of meat proteins (Fig. 1) showed a significant difference ($p < 0.05$) among species after pepsin treatment. This could be attributed to different levels of collagen in fresh meat (0.41, 0.59, 0.28, and 0.28% for pork, beef, chicken, and fish meat, respectively). The digestibility of pork and fish was significantly greater ($p < 0.05$) than that of beef (47.22%, 46.98% versus 42.75%). Chicken (44.67%) did not differ from any of the other three groups. After pepsin and trypsin digestion, the digestibility was not significantly different ($p > 0.05$) between any two groups. In another study, we found that the concentration of plasma total amino acids was significantly different among the rats fed pork, beef, chicken, and fish proteins (Shangxin Song, personal communication). This indicates that the composition of the digestion products in the digestive tract could result in the difference in the absorption of amino acids in the small intestine. The in vitro digestibility may not be enough to explain the bioavailability of dietary proteins.

SDS-PAGE revealed the difference in protein composition of cooked pork, beef, chicken, and fish, in particular for the band intensities of glycogen phosphorylase (85 kDa), beta-enolase (48 kDa), and glyceraldehyde-3-phosphate dehydrogenase (35 kDa) ($p < 0.05$, Fig. 2A, Table 1). Our data showed that beta-enolase and proteins with estimated molecular weights of 23 kDa and 18 kDa were highly expressed in beef and pork compared to chicken and fish. In contrast, glyceraldehyde-3-phosphate dehydrogenase was highly expressed in chicken and fish. For beef, muscle glycogen phosphorylase was the least expressed ($p < 0.05$). This could be because it is acetic acid but not glycogen that is the main energy source for the ruminant animals [17].

After pepsin digestion, myosin heavy chain (223 kDa) in all species was degraded into smaller fragments (Fig. 2B). Three new bands of 110 kDa, 135 kDa, and 169 kDa appeared specifically in chicken protein samples. We speculate that these bands may come from the degradation of proteins of molecular weight greater than 200 kDa, such as myosin heavy chain. We did not perform identification of these degraded bands because of technical problems. The 42 kDa protein (actin) seemed not to be completely degraded in the pork, beef, and chicken samples, but it was degraded in the fish sample. The 35 and 33 kDa proteins seemed partially resistant to the pepsin. The other proteins were degraded into fragments smaller than 12 kDa. When pepsin-treated samples were further incubated with trypsin, almost all of the bands disappeared (Fig. 2C).

Pork and fish were found to be easier to degrade by pepsin than beef and chicken on the basis of electrophoresis analysis and in vitro digestibility assay. This could be associated with protein sequences and structure varying with species.

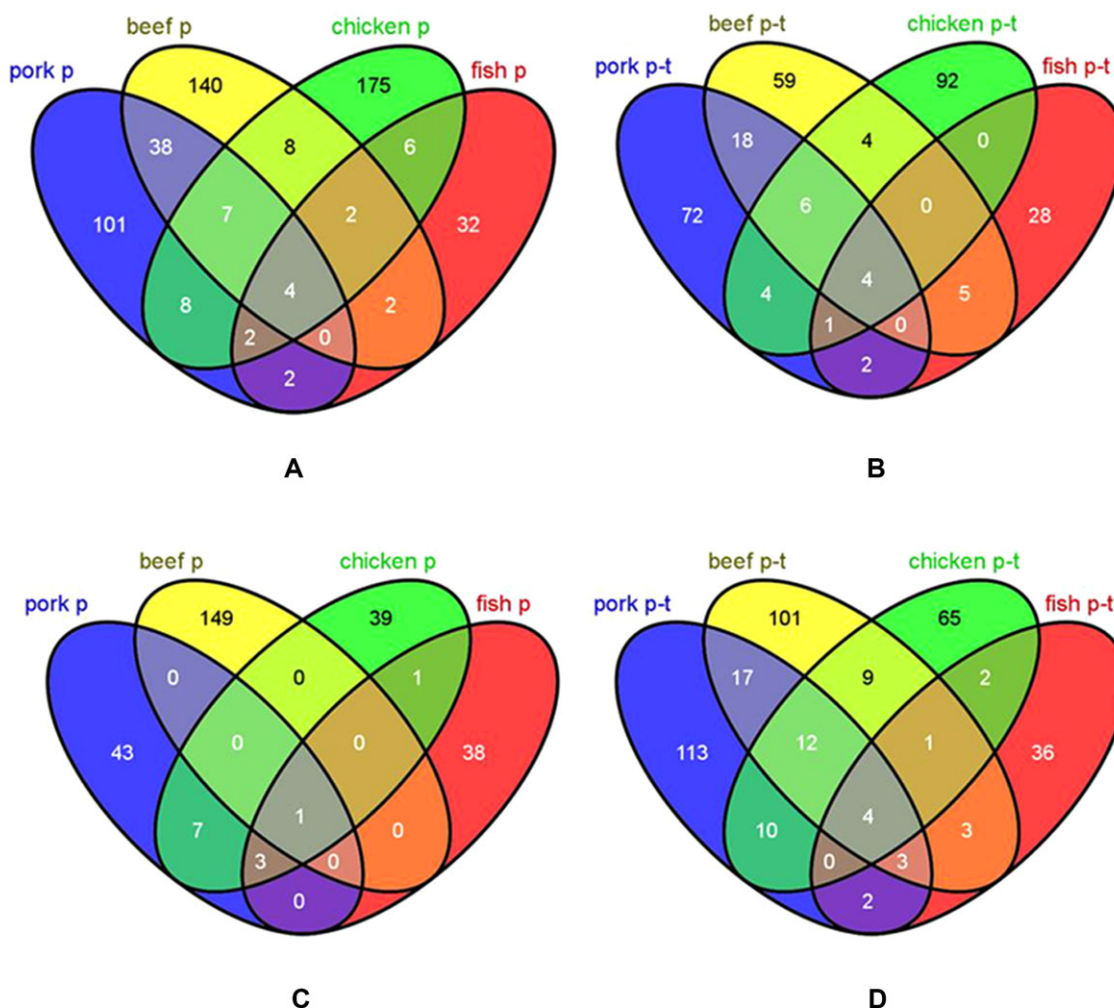


Figure 6. Venn diagrams of peptides obtained from pork/beef/chicken/fish meat. Pork p, beef p, chicken p, and fish p represent the peptides from pork, beef, chicken, and fish treated with pepsin; Pork p t, beef p t, chicken p t, and fish p t represent the peptides from pork, beef, chicken, and fish treated with pepsin and trypsin. (A) In vitro assay, treated with pepsin; (B) in vitro assay, treated with pepsin and trypsin; (C) in vivo assay, gastric contents; and (D) in vivo assay, jejunal contents.

3.2 Peptide profiling of pepsin and trypsin treated meat differed with species

To further explore the difference in protein digestibility among species, MS-based analysis was performed. MALDI-TOF-MS analysis (Fig. 3) indicated that pork and beef samples treated with pepsin had a similar peptide profile with an m/z range between 1300 and 2200 amu and those treated with pepsin and trypsin had an m/z range from 850 to 1050 amu. Chicken samples showed a greater m/z value than pork and beef samples, whereas fish had a broader m/z range than any of the other meat species in either treatment.

Principal component analysis showed a high similarity between beef and pork samples and also between chicken and fish samples after pepsin treatment (Fig. 4A). However, the differences among species decreased after trypsin digestion (Fig. 5A). This confirmed the above observation.

The scores plot revealed significant differences ($p < 0.05$) among chicken, fish, and the other two meat species after pepsin digestion (Fig. 4B), but no significant difference ($p > 0.05$) existed between pork and beef (Fig. 5B). The loadings plot indicated that the pork and beef samples were inclined to be degraded by pepsin into fragments of around 1500 amu, with fragments around 2000 amu for the chicken samples and fragments between 2000 amu and 800 amu for the fish samples. Again, when the samples were treated with both pepsin and trypsin, species difference in the fragments from the ethanol-soluble fractions was weak, but species were still well separated. Unfortunately, it was difficult to get any useful information from the loadings plot, which meant that their products became more uniform after trypsin digestion.

The nano-LC-MS/MS system identified 527 peptides from the pepsin treated samples and 295 peptides from pepsin and trypsin treated samples (Fig. 6). The Venn diagrams showed

aspects: (1) myosin is more susceptible to degrade than actin, L-lactate dehydrogenase, and creatine kinase, and its digestion products could be faster absorbed by or pass through the gastrointestinal tract; (2) physiological conditions in the gastrointestinal tract is more complex than the in vitro model, in particular for gastric emptying pattern and intestinal microbial ecosystem [20–24].

4 Concluding remarks

Pork, beef, chicken, and fish meat are common sources for protein and other nutrients. However, protein composition varies with these species and this affects protein digestion in the digestive tract. The present study indicates that pork and beef have a great similarity, but a great difference from chicken and fish in the peptide profiling of pepsin and trypsin-treated samples. This could be attributed to the difference in contents of amino acids methionine, tyrosine, phenylalanine, leucine, arginine, or lysine that are specific for pepsin and trypsin cleavage. Our results could, to a certain context, interpret for the differences in physiological response after ingestion of different meat proteins.

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