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In vitro protein digestibility of pork products is affected by the method of processing

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ABSTRACT

Processing contributes to different flavors and textures of pork products. However, processing methods have also showed a great impact on meat nutrition. In this study, protein digestibility and digested products were compared among four kinds of processed pork products (cooked pork, emulsion-type sausage, dry-cured pork and stewed pork). Cooked samples were homogenized and digested by pepsin and trypsin. The digestibility of meat proteins was evaluated by particle size measurement, SDS–PAGE, and LC-MS/MS. Emulsion-type sausage had the highest digestibility and the lowest particle size ($P < 0.05$), while stewed pork showed the opposite results ($P < 0.05$). Band profiling on SDS-PAGE gels were significantly different before and after digestion, and between pork products as well. LC-MS/MS analysis revealed that stewed pork samples had the greatest number of 750– 3500 Da Mw peptides in digested products, while emulsion-type sausage had the smallest number of peptides between 750 and 3500 Da. Long-time salting and drying, and long-time and high-temperature cooking may induce pork proteins to being less susceptible to pepsin digestion.

1. Introduction

Pork is the largest meat species of production and consumption (FAO, 2015). Pork is also an important source for several minerals (e.g., iron and selenium) and vitamins (e.g., V_A, V_{B12} and V_{B11}) and provides a high amount of protein to diet (Biesalski, 2005). Some pork products like emulsion-type sausage and dry-cured ham are popular over the world, while such pork products as stewed pork are popular in some Asian countries. Processing has been shown to affect protein digestibility and nutritional values of pork products (Remond, Savary-Auzeloux, Gatellier, & Sante-Lhoutellier, 2008). For example, long-time salting and drying, characteristic of dry curing, has been shown to induce pork proteins to oxidation and degradation (Bermúdez, Franco, Carballo, Sentandreu, & Lorenzo, 2014; Gallego, Mora, Aristoy, & Toldra, 2015; Zhao et al., 2005). Chopping and cooking of emulsion-type sausage is accompanied with protein gelling and emulsification (Cofrades & Jiménez-Colmenero, 1998; Han, Zhang, Fei, Xu, & Zhou, 2009; Xiong, 1994). Such changes affect protein bioavailability in gastrointestinal tract. Protein oxidation and aggregation induced by processing could result in different digestibility of pork and chicken products (Hur, Lim, Decker, & McClements, 2011; Liu & Xiong, 2000). There are two kinds of factors to cause protein aggregation, i.e., disulphide bridges and covalent inter-protein links, existing in different pork products (Di Luccia et al., 2015). In most cases, cooking is critical to make pork and pork products edible, in which cooking temperature and time determine protein oxidation and aggregation (Santé-Lhoutellier, Astruc, Marinova, Greve, & Gatellier, 2008; Bax et al., 2012; Promeyrat, Gatellier, Lebret, Kajak-Siemaszko, Aubry & Sante-Lhoutellier, 2010), and moderate cooking increases the digestibility in gastrointestinal tract (Wen, Zhou, Li et al., 2015; Wen, Zhou, Song et al., 2015).

Protein oxidation may change in vitro digestibility by altering protein hydrophobicity, aggregation and secondary structure (Sun, Zhou, Zhao, Yang, & Cui, 2011). Cooking condition affects in vitro protein digestion but extended cooking at 100 °C could not increase digestibility (Kaur, Maudens, Haisman, Boland, & Singh, 2014). In practice, the processing of pork products is a combination of different treatments, e.g., curing, tumbling, chopping, smoking, frying, or steaming. Different combinations of these preparations may lead to different extents of protein digestibility when we eat pork products. However, it is still less known about the differences in protein digestion among different pork products.

In this study, we compared the protein digestibility of four types of processed pork products (cooked pork, emulsion-type sausage, dry-cured pork and stewed pork) through use of particle size measurement, SDS-PAGE analysis, and LC-MS/MS analysis. The aim was to examine how the different methods of processing affected protein digestibility of these products.

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2. Materials and methods

2.1. Meat product preparation

Pork products were made with pork *longissimus dorsi* muscles from the same carcasses. Cooked pork was prepared according to the following steps: pork muscle was cut vertically into 15 × 10 × 5 cm pieces that were packed in retort pouch and directly cooked in water bath till the center temperature reached 72 °C. Emulsion-type sausage was prepared according to the following formulation: pork muscle and back fat at a ratio of 4 to 1, salt (1.8%) and tripolyphosphate (0.4%). Meat and fat were chopped using a high speed chopper during which salt and tripolyphosphate were mixed, and the batter was stuffed into 48-mm-diameter plastic casings. The sausages were cooked till the center temperature reached 72 °C. Dry-cured pork was prepared as follows: curing with 5% salt and sun-drying for one month. The dry-cured pork was softened in hot water and cooked to the center temperature of 72 °C. Stewed pork was prepared according to the following formulations: pork muscle was vertically divided into strips (5 cm width) and cooked according to the procedure of Li et al. (2016) with minor modifications. Briefly, pork strips were blanched in boiling water for 5 min, chilled and cut into 5 × 5 × 5 cm cubes. The cubes were pan-fried (180 °C) for 5 min with soybean oil (10 g kg^{-1} of meat) on a pot-induction surface. The cubes were fried and turned twice at an interval of 60 s (skin side not fried) and then cooked in boiling water (water/meat: 1/4) for 5 min. After that, the cubes were stewed at 100 °C for 150 min. Eight replicates were applied for each product.

2.2. In vitro digestion

Pork products were digested according to the procedure of Wen, Zhou, Song et al. (2015) with minor modifications. Meat sample (0.5 g) was homogenized (2 × 30 s at 9500 × *g* and 2 × 30 s at 13,500 × *g* at 4 °C) in 2 mL of PBS (10 mmol/L Na_2HPO_4-NaH_2PO_4, pH 7.0) with 30 s cooling between bursts. After homogenization, the homogenates were adjusted to pH 2.0 with 1 mol/L HCl and gastric pepsin was added at a ratio of 1 to 31.25 on a meat weight basis. The reaction mixture was incubated at 37 °C for 2 h and then the reaction was ended by adjusting the pH to 7.5 with 1 mol/L NaOH. Then, trypsin was added at a ratio of 1 to 50 on the meat weight basis. The mixture was maintained at 37 °C for another 2 h and then heated at 95 °C for 5 min. To remove undigested proteins from the digestion mixtures, 3 folds of ethanol (V:V) were added and then kept at 4 °C for 12 h. The resulting mixtures were centrifuged (10,000 × *g*, 20 min, 4 °C). The supernatant and precipitate were separated for use in further analysis.

The in vitro digestibility was expressed as the percentage of the difference in protein contents before and after digestion. From each meat sample, two aliquots (about 1.0 g) were taken. One aliquot was reacted with pepsin, and the other one was digested with pepsin and subsequently with trypsin. The digestion procedures were described as above. Then, the resulting mixtures were centrifuged at 10,000 × *g* for 20 min at 4 °C and the protein contents in the precipitates were detected by the BCA method with a commercial kit (Thermo Scientific, Rockford, IL, USA). The degree of digestibility was calculated as follows:

$$Digestibility(\%) = \frac{W_0 - W_1}{W_0} \times 100\%$$

where W_1: protein content (g) in the precipitate after gastric or pancreatic digestion; W_0: protein content (g) in the untreated product before digestion.

2.3. Gel electrophoresis

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was performed according to the procedure of Li et al. (2012) to characterize the total protein profiles before and after digestion.

A total of 1.0 g of cooked meat was homogenized in 4 mL of extraction buffer (2% SDS and 0.01 mol/mL PBS at pH 7.0). The homogenate was centrifuged at 1500 × *g* for 15 min at 4 °C and the supernatant was retained. The protein concentration was measured with a commercial BCA protein assay kit (Thermo Scientific, Rockford, IL, USA). A certain volume of supernatant was taken and mixed with 12.5 μL of XT sample buffer (Bio-Rad, Hercules, CA) and made up to a total volume of 50 μL with ultrapure water and the final protein concentration of 1 μg/μL. The mixtures were heated at 95 °C for 5 min to induce protein denaturation. A 12 μL of protein sample was loaded on a precast gel (4–12% Bis-Tris Criterion, Bio-Rad, Hercules, CA). The gels were run in 950 mL of XT MES running buffer (Bio-Rad, Hercules, CA) at 150 V for 1.5 h. Proteins were then stained with Coomassie Blue R250 for 3 h and destained until the bands were clear.

Gel images were captured using an image scanner (GE Healthcare, Little Chalfont, SE), and the band intensities were quantified with the Quantity One software. The intensity of each band was calculated as its actual intensity relative to the intensity of the 190 kDa band in the prestained calibration marker.

2.4. Profiling of digested products

The ethanol-soluble fractions of the pepsin and trypsin digested products were characterized according to the method of Wen, Zhou, Song et al. (2015). Briefly, the ethanol-soluble fractions were loaded onto ultra-0.5 mL centrifugal filter units (Amicon Ultra, Millipore, Billerica, MA, USA) and centrifuged at 14,000 × *g* for 15 min. The filtrates were concentrated with ZipTip C18 (Millipore, Billerica, MA, USA). The concentrated peptide mixture was loaded onto a C18 column (2 cm × 200 μm, 5 μm), and then passed through a C18 chromatographic column (75 μm × 100 mm, 3 μm) for separation. Peptides were separated by step-gradient elution with buffers A (0.1% formic acid in water) and B (0.1% formic acid in 84% acetonitrile) at a flow rate of 300 nL/min, including 0– 12 min (97%A, 3%B), 12– 100 min (72%A, 28%B), 100– 120 min (45%A, 55%B),122– 144 min (2%A, 98%B) and 144– 160 min (97%A, 3%B). Peptides were identified under a hybrid quadrupole orbitrap mass spectrometer equipped with a nanoelectrospray ionization source (Thermo Fisher Scientific, USA). The data-dependent mode was selected and a scan cycle was initiated with a full-scan MS spectrum (from 300 to 1800 amu).

Under the program of Proteome Discoverer-1.4 (Thermo Fisher Scientific, Palo Alto, CA, USA), MS/MS spectra were matched against the Swiss-Prot database against *Sus scrofa* for pork (http://www.uniprot.org/). Data matching was performed with a parent ion tolerance of 10 ppm, and two missing cleavages were allowed. Pepsin was selected for peptic peptides database search, while both pepsin and trypsin were chosen in peptic/tryptic peptides search. The similarity of peptides between four types of pork products was analyzed by Venn diagrams (http://bioinformatics.psb.ugent.be/webtools/Venn/).

2.5. Particle size measurement

Pork products were homogenized and digested with pepsin and trypsin as described above. The sizes of particles in homogenates were measured according to the method of Sun et al. (2011) with an integrated-laser light scattering instrument (Mastersizer 3000, Malvern, Worcestershire, UK). The data were analyzed by using the Malvern Mastersizer software (version 5.12c, Malvern, Worcestershire, UK). Of the available data, $D_{4,3}$ represents the mean diameter in volume, and $D_{3,2}$ represents the mean diameter in surface; $D_{x(90)}$ represents the particle size for which 90% of the sample particles have a lower size; $D_{x(50)}$