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In vitro protein digestion of pork cuts differ with muscle type

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ABSTRACT

Porcine *trapezius*, *longissimus dorsi* and *biceps femoris* muscles have been shown to contain different types of muscle fibers. Little is known about the differences in digestibility among cooked pork cuts. In this study, we compared the protein losses of porcine *trapezius*, *longissimus dorsi* and *biceps femoris* muscles under *in vitro* digestion conditions. Meat samples were cooked in 72 °C water bath to center temperature of 70 °C. Then meat samples were homogenized and incubated with pepsin alone or followed by trypsin. The *in vitro* protein losses and particle size were measured. The pepsin and trypsin digestion products were separated and identified by SDS-PAGE and Nano LC-LTQ-Orbitrap XL MS/MS. The results indicated that *longissimus dorsi* muscle had the highest losses under both pepsin and trypsin treatments ($P < 0.05$). In untreated samples, *biceps femoris* muscle showed the greatest particle size ($P < 0.05$), but enzymatic treatment decreased particles to the similar size for all the three muscles ($P > 0.05$). Proteome analysis indicated that *biceps femoris* muscle had the highest susceptibility to digestion. Interaction analysis reveals that differential proteins mainly are related to glycolysis and muscle contraction. And thus fiber types could be the key factor to cause the differences in protein composition and their susceptibility to digestion.

1. Introduction

Meat is an important source of high quality protein, vitamins, and minerals (Pereira & Vicente, 2013). Previous studies indicated that pork and veal cuts had different protein content and amino acid profile in cooked meat (Greenfield et al., 2009; Nasvadi, Law, Mackey, & Logue, 1992; Schweigert, Bennett, & Guthneck, 1951). In addition, different cuts are composed of different types of muscle fibers and different amounts of connective tissues, in terms of metabolic enzymes and collagen, which results in different quality characteristics, especially of tenderness (Chang, Femandes, & Goldspink, 1993; Li, Zhou, & Xu, 2007). In addition, the collagen content in different cuts or meat products substantially affects their nutritional values (Laser-Reutersward, Asp, Bjorck, & Ruderus, 1982). In practice, cuts are usually recommended to be cooked in different ways, but sometimes it depends on many factors such as religion, culture and tradition. Generally speaking, people in western countries prefer roasting and grilling with medium doneness, but Asian people prefer stewing and stir-frying with very well doneness. There are great concerns about cooking methods for different cuts, for example, striploins and tenderloins are more suitable for roasting, while cuts from legs are suitable for mincing and long-term cooking (http://beefretail.org/urmis.aspx). For the same cut, minced beef was found to be more rapidly digested and absorbed than beef steak in older men (Pennings et al., 2013). This could be mainly attributed to the cooking temperature effect. In meat, muscle fiber and intramuscular connective tissue shrink during cooking, resulting in the increase of WBSF when the internal temperature is lower than 75 °C, but further cooking will result in the disintegration of perimysial structure, lowing up the increase of WBSF between 75 and 90 °C (Li, Zhou, & Xu, 2010). However, higher cooking temperature usually decreased the protein digestibility (Bax et al., 2013; Wen, Zhou, Song, et al., 2015). In addition, the teeth conditions of older populations are also critical for the selection of cooking methods. It is still little known about the difference in pepsin- and trypsin-treated protein digestion among pork cuts at the same cooking conditions.

In meat, there are > 1000 proteins that can be classified into three types, *i.e.*, myofibrillar proteins, sarcoplasmic proteins and stromal proteins (Lawrie, 2006). Proteomic studies have shown that the protein profiling is different among muscles/cuts, and the amino acid composition is also quite different among individual proteins (Jia, Hollung, Therkildsen, Hildrum, & Bendixen, 2006). The amounts of phenylalanine, tyrosine, tryptophan, lysine and arginine determine the extents of

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protein digestion in the gastrointestinal tract because these amino acids are the target cleavage sites of pepsin or trypsin (Erickson & Kim, 1990; Savoie, Agudelo, Gauthier, Marin, & Pouliot, 2005). In an *in vitro* simulating model, LC-MS-MS technology has been used to characterize the digestion products of proteins from different meat sources (Escudero, Sentandreu, & Toldra, 2010; Gallego, Mora, Aristoy, & Toldra, 2015; Wen, Zhou, Li, et al., 2015). However, little is known about the digestibility and the digestion products of proteins from different cuts.

In this context, the objectives of this study were: (1) to compare the *in vitro* digestibility of pork proteins from *trapezius*, *longissimus dorsi* and *biceps femoris* muscles by measuring protein losses and particle sizes after *in vitro* pepsin and trypsin treatments, and (2) to characterize the degraded products by using SDS-PAGE and LC-MS-MS.

2. Materials and methods

2.1. Reagents

Porcine gastric pepsin (cat. no. P7125) and porcine pancreatic trypsin (cat. no. T7409) were obtained from Sigma Aldrich (St. Louis, MO, USA). BCA protein assay kit (cat. no. 23225) and protein calibration marker (cat. no. 26619) were obtained from Thermo Scientific (Rockford, IL, USA). Amicon Ultracel-3 membrane (UFC500396) and Zip Tip C18 pipette tips (ZTC18S096) were obtained from Millipore (Billerica, MA, USA).

2.2. Sampling and cooking procedure

Three pork cuts, including neck (*m. trapezius*), loin (*m. longissimus dorsi*) and outside (*m. biceps femoris*) were obtained at 24 h post-mortem from 8 Huai black pigs (a native pig breed) with ultimate pH values of 5.54 ± 0.07, 5.44 ± 0.05 and 5.43 ± 0.05 respectively. All visible fat and epimysial connective tissue were removed. Pork muscles were cut vertically into 2 cm-thick pieces (weights: 50 to 65 g each). All samples were packed in plastic pouches and cooked in a 72 °C water bath (Crystal Industries, USA) for about 30 min. The center temperature was tracked by a thermal probe (Pt 100, Testo AG, Germany). When the center temperature reached 70 °C, meat samples were taken out and chilled in air to room temperature (22 °C). Cooking loss was calculated as the percentage of the difference in meat weight before and after cooking divided by initial meat weight.

2.3. Pepsin and trypsin treatment

Cooked meat samples were treated with pepsin and trypsin according to the procedures of Wen, Zhou, Song, et al. (2015). Briefly, cooked meat sample (1.0 g) was homogenized on ice (Ultra Turrax T25 Basic, IKAWerke, Staufen, Germany) in 5 mL of phosphate buffer solution (10 mmol/L Na_2HPO_4-NaH_2PO_4, pH 7.0) at 9600 rpm for 30 s twice and at 13,400 rpm for 30 s twice with 30 s pause between any two homogenization steps. The homogenate was adjusted to pH 2.0 with 1 mol/L HCl and gastric pepsin was added at 1:31 ratio on a meat mass basis (substrate). The mixture was incubated at 37 °C for 2 h with continuous shaking (150 rpm) and the digestion was stopped by adjusting the pH to 7.5 with 1 mol/L NaOH. After pepsin treatment, trypsin was added to the resulting mixture at 1:50 ratio on a substrate basis. The mixture was incubated at 37 °C for 2 h with continuous shaking (150 rpm). The enzyme was inactivated by heating the reaction system in 95 °C water bath for 5 min.

To remove proteins and high molecular weight peptides, all digesta were mixed with 3 volumes of ethanol and kept at 4 °C for 12 h, and then centrifuged at 10,000 × *g* at 4 °C for 20 min. The precipitate was used for SDS-PAGE, while the supernatant was used for LC-MS-MS analysis.

The *in vitro* protein digestion procedures were the same as above.

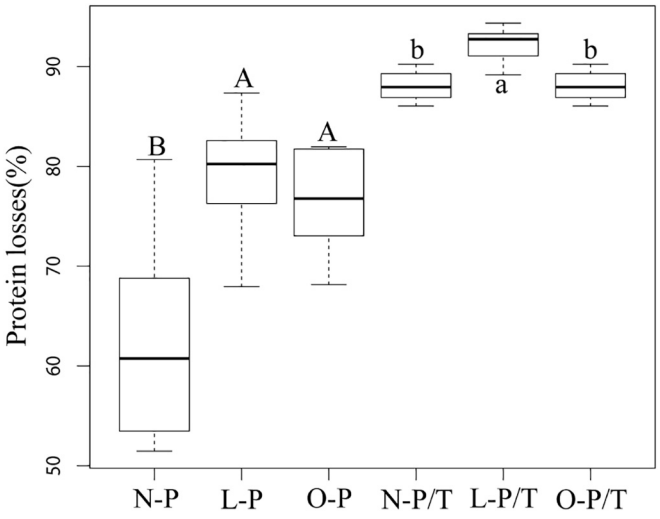

Fig. 1. Protein losses after pepsin and trypsin treatments (n = 8).
N-P, L-P and O-P, protein losses after pepsin treatment for three pork cuts, *i.e.*, the neck, the loin and the outside; N-P/T, L-P/T and O-P/T; protein losses after pepsin and trypsin treatment for three pork cuts, *i.e.*, the neck, the loin and the outside. A, B, averages of pepsin induced protein losses differ significantly with different uppercases ($P < 0.05$); a, b averages of pepsin and trypsin induced protein losses differ significantly with different lowercases ($P < 0.05$).

After pepsin or trypsin treatment, the digestion products were centrifuged at 10,000 × *g* for 20 min at 4 °C and the supernatant was discarded. The protein contents in the ethanol precipitates and in the untreated cooked samples were determined by the BCA protein assay kit (Thermo Scientific, Rockford, IL, USA) according to the manufacturer's instructions. The protein loss was calculated as follows:

$$\text{Protein loss}(\%) = \frac{W_0 - W_1}{W_0} \times 100\%$$

W_1: protein content (g) in the precipitate after gastric or tryptic treatment; W_0: protein content (g) in the cooked meat before pepsin treatment.

2.4. Gel electrophoresis

For untreated cooked samples, samples (1.0 g each) were homogenized on ice (Ultra Turrax T25 Basic, IKAWerke, Staufen, Germany) in 15 mL of extraction buffer (2.0% SDS, 10 mmol/L Na_2HPO_4-NaH_2PO_4, pH 7.0) at 9600 rpm for 30 s twice and at 13,400 rpm for 30 s twice with 30 s pause between any two homogenization steps. The homogenate was centrifuged (Allegra 64R, Beckman Coulter, USA) at 4000 × *g* at 4 °C for 15 min. The supernatant was kept for electrophoresis.

The supernatants from untreated samples and the ethanol precipitates from pepsin and trypsin treated samples were separated on SDS-PAGE gels (4–12% Bis-Tris Criterion precast gels, 26 wells, cat. no. 345-0125, Bio-Rad, USA, with XT MES running buffer, cat. no. 161-0789, Bio-Rad, USA). For digested samples, the ethanol precipitated proteins from the digesta were dissolved in 2 mL of the extraction buffer (10 mmol/L Na_2HPO_4-NaH_2PO_4, pH 7.0). Protein concentration was determined with a BCA protein assay kit (Thermo Scientific, Rockford, IL, USA).

Protein samples were adjusted to a final protein concentration of 1.0 μg/μL with XT sample buffer (cat. no. 161-0791, Bio-Rad, USA) and heated at 95 °C for 5 min, and 12 μL sample (12 μg proteins) was loaded onto each lane. The SDS-PAGE gels were run in 900 mL of XT-MES running buffer at 150 V for approximately 1 h. Proteins were stained with Coomassie blue R250 and destained until the bands were clear. Gel images were captured using an image scanner (GE Healthcare, Little Chalfont, SE). The intensity of each band was quantified with the