PROTEIN MARKER FOR DISCRIMINATION IN COOKED MEAT USING THE PROTEOMIC ANALYSIS

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Abstract – The purpose of this study was to find discrimination markers in cooked meat for major four species such as beef, pork, chicken and duck. Myofibrillar proteins isolated from each meat were analyzed by one dimensional (1D) gel electrophoresis and some proteins were identified by LC-MS/MS analysis. Although species-specific proteins were not found from cooked meat and meat mixtures, we confirmed that troponin I which were commonly distributed in all meat species, could be useful markers for discrimination of mammals from poultry by their different electrophoretic mobility. Species-specific peptides identified by LC-MS/MS spectra allow identification of each species regardless same protein. Therefore, it could be easy to discriminate between mammals and poultry by comparing electrophoretic mobility of troponin I and each species could be identified by LC-MS/MS analysis.

Key Words – Electrophoresis, myofibrillar, cooked meat

I. INTRODUCTION

In meat industry, more rapid and accurate as well as inexpensive technologies for discrimination of meat species origin are demanded. In point of view, a few reports recently conducted are inspire and valuable for application in meat industry: Stamoulis et al. (2010) found two mitochondrial DNA molecules for poultry meat identification in food; Montowska and Pospiech (2012) introduced proteomic method for meat discrimination in raw and cooked meat as well as meat products; Rapodi et al. (2015) introduced multispectral image spectroscopy as a new, rapid and non-invasive technique to detect beef and pork in raw meats. Even so, various aspects on identification of species origin in meat products are needed to develop rapid, accurate, simple and cheap methods, and apply easily to the meat industry.

Therefore, the hypothesis of this study is that same proteins distributed in skeletal muscles from different species have different characteristics such as electrophoretic mobility rate and amino acid sequence. A few proteins were found as markers for discrimination of cooked meat species by 1D gel electrophoresis and confirmed whether these proteins could be candidates for developing rapid and simple discrimination method by LC-MS in this study.

II. MATERIALS AND METHODS

The longissimus muscle from beef and pork and pectoralis major muscle from chicken and duck were purchased from commercial slaughterhouses (5 replicates per meats) and ground followed by trimming the connective tissue and visible fat. Each meat was mixed for 5 min using a mixer (KP26M, KitchenAid®, St. Joseph, MI, USA). The blended samples prepared to 500g total weight and cooked on 80°C during 30 min. Samples (4g) were taken from all mixture and homogenized with 30 ml of rigor buffer. The homogenates were centrifuged at 10,000 g for 10 min and then supernatant was collected. This process was repeated three times with fresh rigor buffer. The remained pellet was homogenized with 30 ml of fresh rigor buffer and used for myofibrils. The protein concentration was determined according to Bradford (1974) method with BSA as a standard. The final concentration of samples adjusted to 2 mg/ml for 1D gel electrophoresis analysis.

Myofibrillar proteins were mixed with sample buffer. 1D gel electrophoresis was performed using 10% SDS-polyacrylamide gel and a 4% stacking gel at a 20 mA constant current per gel.

The gel was stained using Coomassie blue. The gels were scanned using a scanner (2100XL, UMAX®, TX, USA).

After 1D gel bands of which had different electrophoretic mobility among the species were selected and each band was excised from the stained gel. In-gel digestion was performed according to the Kim (2014) method with modification.

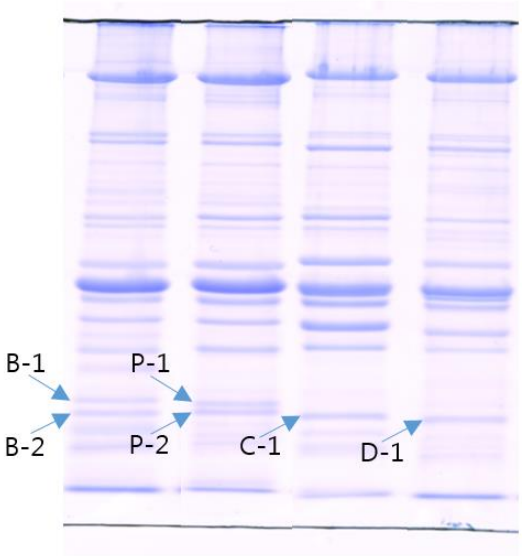
LC-MS/MS was performed using a nano-LC and LTQ mass spectrometer (Applied Biosystems, CA, USA) with capillary column (150 mm × 0.075 mm, Proxeon, Odense M, Denmark) and a Magic C18 stationary phase resin (5 μm, 100 Å pore, Michrom BioResources, CA, USA) as described by Lee et al. (2014).

Proteins separated by 10% 1D gel were transferred onto polyvinylidene difloride (PVDF) membranes (Millipore, MA, USA) and blocked with Tris buffered saline with Tween 20 containing 5% skim milk. A primary antibody whose specificities for Troponin I (sc-8118) and secondary antibody (anti-goat IgG-HRP, sc-2768) was purchased from Santa Cruz Biotechnology, Inc. (TX, USA).

III. RESULTS AND DISCUSSION

1. 1D gel electrophoresis

Figure 1. 1D gel electrophoresis image of cooked meat species.



Myofibrillar proteins from each meat species were separated by 1D gel electrophoresis as shown in Fig. 1. Many bands showing the different electrophoretic mobility between meat species were observed myofibrillar. For, myosin and actin were observed as clear bands and their intensities are very strong. Bands B-1 and P-1 from bovine (B) and porcine (P) muscles, respectively, showed same mobility, however there was no band in the duck (D) lane and a weak band was observed in chicken (C) lane.

2. LC-MS/MS

Table 1. Myofibrillar proteins identified by LC-MS/MS after separation by 1D gel electrophoresis

Band number	Protein name[a]	Species	Theoretical p*I*/Mw (Da)[b]	Score[c]	Peptides identified
B-1	Unknown				
B-2	Troponin I type 2 (skeletal, fast)	*Bos taurus*	8.88/21575	425	23
P-1	Troponin I, fast	*Sus scrofa*	9.02/21505	290	12
P-2	Unknown				
C-4	Troponin I, fast	*Gallus gallus*	8.74/20736	443	16
D-4	Troponin I, fast skeletal muscle	*Anas platyrhynchos*	9.42/20763	298	15

[a] Protein name and accession number were derived from the NCBI database.
[b] Theoretical molecular weight and p*I* recorded in NCBI database.
[c] The MASCOT baseline significant score is 70, and for the proteins identified in more than one spot, the highest score was presented.

Myofibrillar proteins from each meat species identified by LC-MS/MS after separation by 1D gel electrophoresis as shown in Table 1. All the proteins were identified with high MASCOT scores ranged from 290 to 443, 12-23 peptides. Proteins name of all bands were Troponin I. Even though the name of the protein to same, there were meat species differences of mole weight and p*I*. Because the contraction and relaxation mechanism of muscle varies species to species.

IV. CONCLUSION

Summary, protein marker was investigation using the proteomic analysis. Although same proteins there were differences of electrophoretic mobility and mole weight. Though the name of these proteins are same, therefore this it could be used to identify the mammals and poultry of raw meat by protein marker.

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