Comparison of *biceps femoris* muscle proteome in dry-cured Xuanwei ham with different quality grades

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Abstract— **Two-dimensional gel electrophoresis and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry were used to investigate the proteome in *biceps femoris* muscles from Chinese traditional dry-cured Xuanwei ham with different quality grades. The quality of Xuanwei ham was determined by sensory evaluation and the amount of 3-methylbutanal which was the key aroma-active compound in dry-cured Xuanwei ham. At the end of ripening, the 3-methybutanal was 178.53 μg/kg muscle and 107.78 μg/kg muscle in high quality ham and low quality one, respectively. Eleven proteins or fragments which were differentially expressed between *biceps femoris* muscles with different quality grades were identified. The expression levels of myosin-1, carbonic anhydrase 3 (CA3), fructose-bisphosphate, aldolase C-A (FBA), phosphoglycer-ate mutase 2 (PGM2), pyruvate kinase isozymes M1/M2 isoform 3 (PKI3) were more in high quality grade ham. Conversely, α-actin, albumin, aldehyde dehydrogenase, alpha-actinin-3-like isoform 1 (α-actinin3-1), beta-enolase, glycerol-3-phosphate dehydrogenase [NAD+] were overexpressed in low quality grade ham. In particular, the quantity of CA3, FBA, PGM2, PKI3, α-actin, albumin and α-actinin3-1 correlated well with the amount of 3-methylbutanal. The results indicated that CA3, FBA, PGM2, PKI3, α-actin, albumin and α-actinin3-1 might be potential dry-cured ham quality biomarkers and their identification provided new insight into the molecular mechanisms and pathways associated with dry-cured ham quality.**

Keywords— **muscle proteome, xuanwei ham, meat quality**

I. INTRODUCTION

The acceptance of dry-cured ham by consumers is mainly determined by their sensory quality, especially their unique and characteristic flavor. 3-methylbutanal, with low threshold value and pleasant "cured" flavor, is contributed positively to the dry-cured ham flavor [1]. Proteolysis plays an important role in the formation of some critical volatile compounds attributed to the release of free amino acids and reaction of strecker degradation in the long ripening process. There are tens and thousands of proteins in meat muscle, which are essential to palatable quality of meat due to the potential capability of denaturation and proteolysis [2]. Proteomics is a powerful approach to monitor the innumerable proteins simultaneously and precisely, which is helpful to elucidate the molecular mechanism between muscle proteins and meat quality.

Xuanwei ham is one of the most famous traditional Chinese dry-cured hams produced in Yunnan province [3-4]. The objective of this investigation was to compare the proteomes between dry-cured Xuanwei hams with different quality, and identify the major proteins for good quality.

II. MATERIALS AND METHODS

Black Dahe pig is a typical local breed (Yunnan, China). In this experiment, Xuanwei hams originated from the black Dahe pigs (90~100 kg) were produced by Dongheng Group (Yunnan, China) according to the standard processing. The green hams were held at 0-5 ˚C and 70-80 % relative humidity (RH) for 36 h. Then, the hams were rubbed with 3 % (w/w) NaCl on the muscle surface, placed on platform and held for one week at 0-5 ˚C and 85-95 % RH. The hams were salted again with 2 % (w/w) NaCl and held further for 3 weeks at 0-5 ˚C and 85-90 % RH. After salting, the hams were hung with straw strings in a chamber at 0-5 ˚C and 70-85 % RH for post-salting. After 60 days, the hams were washed the excess salt on the surface and hung on strings in a ventilated chamber for drying (10~15℃, 60~70% RH, 60 days) and

ripening (15~20℃, 65~75% RH, 180 days). At the end of ripening, the hams were grade to different categories according to aroma quality primarily by "three probes", which indicated to insert a bovine bone probe into a ham on three different locations on the muscle surface, and smell the probe quickly when removing it. If the three probes were all aromatic, the ham was classified to high quality grade. Supposing that only one or two probes were aromatic, the ham was belonged to low quality grade. Depending on the quality assessing method, *biceps femoris* muscles were sampled from six hams with high quality and low quality, respectively.

Sarcoplasmic and myofibrillar were extracted by the methods described by Laville [5] and Kim [6]. Samples of approximately 800 μg were loaded onto immobilized pH gradient (IPG) strips (pH 5~8, 17 cm, Bio-Rad), and run the isoelectric focusing with a PROTEAN IEF SYSTEM, COMPLETE (Bio-Rad). The second dimension was performed on 12 % SDS-polyacrylamide gels with P Ⅱ XL cell (Bio-Rad). After Coomassie Brilliant Blue G-250 staining, image analysis and in-gel trypsin digestion, the digested protein samples were analyzed using an AUTOFLEX Ⅱ TOF-TOF (Bruker Daltonics, Germany). The peptides were analyzed by MASCOT software and the proteins were identified in non-redundant protein database of NCBI.

The analysis of hexanal was performed by dynamic headspace combined with GC-MS (6890N/5975C, Agilent, USA).

III. RESULTS AND DISCUSSION

Proteomic profiles by two-dimensional gel electrophoresis of biceps femoris muscle from dry-cured Xuanwei ham with different quality grades were shown Figure 1. Approximate 350 spots were detected in the Coomassie-stained 2DE gels. Eleven spots were differentially expressed twofold or greater differences in density of coomassie staining between the high quality and low quality Xuanwei ham. Six protein spots stained with higher densities in the high quality grade group and the other six protein spots stained with higher densities in the low quality grade group (Figure 1). The twelve spots were identified as eleven known proteins (Table 1). The expression levels of myosin-1, carbonic anhydrase 3 (CA3), fructose-bisphosphate aldolase C-A (FBA), phosphoglycerate mutase 2 (PGM2), pyruvate kinase isozymes M1/M2 isoform 3 (PKI3) were more in high quality grade ham. Conversely, α-actin, albumin, aldehyde dehydrogenase (AD), alpha-actinin-3-like isoform 1 (α-actinin3-1), beta-enolase, glycerol-3-phosphate dehydrogenase [NAD+] (G3PD) were overexpressed in low quality grade ham.

Table 1 The identification results for the differential expression proteins of *biceps femoris* muscles from Xuanwei ham with high quality and low quality

Spot no.	Identified proteins	experimental pI/Mr	Mascot score	accession no.	H vs. L
28	alpha-actinin-3-like isoform 1	5.31/103802	230	gi|311247190	-
29	mitochondrial aldehyde dehydrogenase 2	6.43/57340	176	gi|187370719	-
31	albumin	5.92/71362	216	gi|833798	-
32	glycerol-3-phosphate dehydrogenase [NAD+], cytoplasmic	6.01/38339	128	gi|298160993	-
34	beta-enolase	8.05/47443	219	gi|113205498	-
38	pyruvate kinase isozymes M1/M2 isoform 3	7.98/65446	274	gi|311260850	+
39	pyruvate kinase isozymes M1/M2 isoform 3	7.98/65446	357	gi|311260850	+
41	fructose-bisphosphate aldolase C-A	8.45/39925	161	gi|156120479	+
42	carbonic anhydrase 3	7.72/29678	179	gi|56711366	+
43	phosphoglycerate mutase 2	8.86/28830	160	gi|201066358	+
45	Actin, alpha 1, skeletal muscle	5.23/42338	179	gi|134024776	-
49	myosin-1	5.60/223947	180	gi|157279731	+

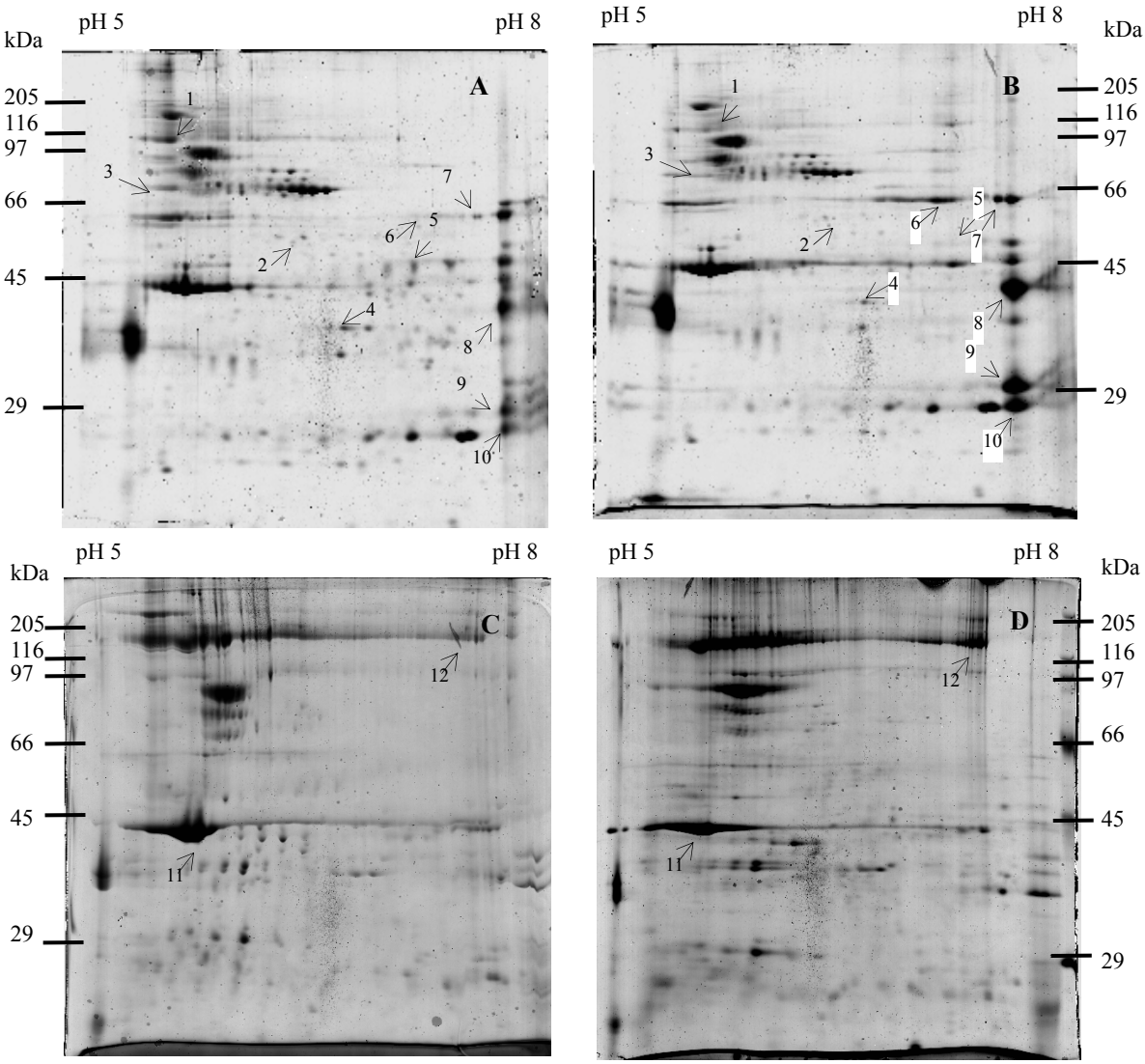

Figure 1. 2-DE proteome maps of *biceps femoris* muscles from Xuanwei ham with high quality or low quality. A, sarcoplasmic with low quality; B, sarcoplasmic with high quality; C, myofibrillar with low quality; D, myofibrillar with high quality.

Proteolysis is a major factor affecting meat flavor due to the weakening and degradation of myofibril in the muscle. Actin and myosin are the main myofibrillar fragments, and the degradation of myofibril fragmentation can increase the meat quality, such as tenderness and flavor. In this study, α-actin was lower in high quality grade group, while myosin-1 was higher. The differential expressions of α-actin and myosin-1 might cause more structure proteins degrade into free amino acids, which promoted some chemical and enzymatic reactions related to the flavor. 3-methybutanal, the key aroma compound in dry-cured Xuanwei ham, was 178.53 μg/kg muscle and 107.78 μg/kg muscle in high quality ham and low quality one, respectively. CA3, FBA, PGM2, PKI3, AD, beta-enolase, G3PD are some regulating proteins,

which were correlated to the metabolisms of structure and function proteins. The differential expressions of these regulating and metabolism enzymes lead to the different proteolysis of myofibril fragments, which contributed to the quality of dry-cured Xuanwei ham significantly.

IV. CONCLUSIONS

The differential proteome profiles between dry-cure Xuanwei ham with high quality and low quality indicated that CA3, FBA, PGM2, PKI3, α-actin, albumin and α-actinin3-1 might be potential dry-cured ham quality biomarkers, and their identification provided new insight into the molecular mechanisms and pathways associated with dry-cured ham quality.

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