Identification of Muscle Proteins Related to Objective Meat Quality in Korean Native Black Pig

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ABSTRACT : This study examined the effects of pre-slaughter fasting, chasing stress and chiller ageing on objective meat quality, and their relations to the proteome profile of longissimus muscle using 20 male Korean native black pigs. Treatments were composed of two levels of pre-slaughter feed withdrawal, two levels of pre-slaughter stress and four chiller ageing times. A 15 min chasing stress immediately prior to slaughter significantly ($p<0.05$) decreased detectable levels of μ-calpain activity during rigor development and chiller ageing, but did not have any direct effect on objective meat quality. On the other hand, pigs fed until the morning of slaughter resulted in significantly ($p<0.05$) higher hunter L* value and cooking loss than those which received an 18 h feed withdrawal prior to slaughter. Cooking loss and hunter L* value were constant during 7 d of chiller ageing, followed by significant increases at 14 d. The fed animals showed a significantly ($p<0.05$) higher hunter a* value at both 3 and 7 d, while the other group maintained a stable redness for 7 d. WB-shear force was not affected by the pre-slaughter treatments, but had significant ($p<0.05$) linear reduction from 1 to 7 d. A gel-based proteome analysis was performed on selected animals for low and high hunter L* values at 1 d. Ten and five spots had greater than two-fold spot densities for the low and high hunter L* groups, respectively. The ten spots included chain A, deoxyribounclease I complex with actin, heat shock protein 27 kDa, a protein similar to cardiac Ca^{2+} release channel, and myosin heavy chain, while the five spots included chain A aldehyde dehydrogenase, glycerol-3 phosphate dehydrogenase, and hemoglobin alpha chain. In general, feeding until the morning of slaughter resulted in more desirable meat color, but appeared to reduce palatability due to increased cooking loss. Proteome analysis demonstrated that various proteins were concomitantly involved in the determination of final meat color. The most noticeable observation in the current study was that various isoforms for a particular protein differed in degradation and/or expression rate depending on meat quality. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 11 : 1599-1607)***

Key Words : Proteolysis, Proteome Analysis, Skeletal Muscle

INTRODUCTION

Pork constitutes approximately a 49% market share in Korea, with overall meat consumption of 35 kg per capita in August 2003 (Korean Ministry of Agriculture and Forest, 2003). Korean native pork (KNP) is one of the most in demand meats, because it has more desirable characteristics such as tenderness, juiciness, redness and brightness than other breeds (Jin et al., 2001). We previously reported that 3,050 out of 6,785 sampled industrial pigs (largely Landrace) showed pale, soft, exudative (PSE) symptom, which was largely related to pre-slaughter animal treatment (Park et al., 2002). On the other hand, it is know that KNP rarely showed PSE-like appearance. The biological basis for this characteristic of KNP has not been known yet, but a previous study of Kim et al. (2001a) implied that the specific color characteristic is related to genetic component (Depreux et al., 2002).

Apart from the genetic component, short-term stress immediately prior to slaughter resulted in lower muscle pH and higher temperature (Henckel et al., 2000; Rosenvold et al., 2002). In addition, the consequence induced protein denaturation and myofibrillar shrinkage (Rosenvold and Andersen, 2003; Bertram et al., 2004). To alleviate the rate and extend of glycolysis postmortem, pre-slaughter fasting has been normally enforced to reduce muscle glycogen stores at the time of slaughter (Eikelenboom et al., 1991; Wittmann et al., 1994). On the other hand, in spite of the particular characteristics of KNP and its economic value, there are only a few available reports on KNP meat quality (Jin et al., 2001; Kim et al., 2001b). Furthermore, to our best knowledge, there is no readily available information about the effect of pre-slaughter treatments such as feed withdrawal and physical stress on meat quality during chiller ageing.

As meat quality is a direct reflection of biophysical status of muscle proteins, proteome analysis of the whole muscle extract is crucial in understanding the biological basis of meat quality traits and their interactions. However, there has been very limited approach until the recently developed high throughput proteome analysis equipment. Hwang (2004) demonstrated that a gel-based proteome analysis was a feasible approach to study postmortem proteolysis in skeletal muscle, and the same group identified 27 protein spots related to meat quality traits of landrace longissimus muscle during chiller ageing (Hwang et al., 2004a). The current study was conducted to evaluate the effects of 18-h feed withdrawal and 15 min chasing stress immediately prior to slaughter on objective meat

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quality during 14 d chiller ageing, and their relations to proteome profile in longissimus muscle of Korean native black pig. Some aspects of this study were reported by Hwang et al. (2004c).

MATERIALS AND METHODS

Animals, experimental design and treatments

A total of 20 male Korean native black pigs (115±18.2 kg, 13 months of age) were sampled from the National Livestock Research Institute (NLRI) breeding program. The pigs were conventionally transported to the NLRI abattoir, approximately 65 km away, with minimum transit stress. The pigs were assigned to a 2×2 factorial which were composed of two pre-slaughter feeding (ten pigs fasted for 18 h, and the other ten pigs fed until the morning of slaughter) and two pre-slaughter stress (two sets of five pigs each from the feeding treatments were chased for 15 min in the lairage paddock immediately prior to slaughter, and two sets of five pigs each from the feeding treatments were handled with minimum stress) treatments, followed by four ageing treatments (1, 3, 7 and 14 d at 1°C). All pigs were stunned by an electronic stunner (230 volts for 2.5 sec), conventionally slaughtered, and placed in a 1°C chiller until the following day.

Sampling and objective meat quality measurements

Muscle temperature was logged at 5 min intervals for 24 h (Thermo Recorder, TR-50C, Japan) using thermocouples inserted into the geometrical center of the muscle between the 3rd and 4th lumbar vertebrae from approximately 30 min after stunning, until the following day. The pH was measured using a portable needle-tipped combination electrode (NWKbinar pH-K21, Germany) at approximately 15 min intervals in the center of the muscle between the 3rd and 4th lumbar vertebrae from approximately 30 min postmortem, until the muscle was judged to have reached ultimate pH. Another measurement was made the following day, approximately 24 h postmortem.

The day after slaughter, *m. longissimus* muscles (from the 7th thoracic vertebrae to the last lumbar vertebrae) were removed, cut into three portions, vacuum packed, and randomly assigned to one of four ageing periods (1, 3, 7 and 14 d) for objective measurements of WB-shear force and meat color. The samples were held at 1°C for the relevant ageing period. WB-shear force was measured on cooked steaks (2.54 cm thick) according to the method described by Wheeler et al. (2000). Sarcomere length was determined at 24 h using a Helium-Neon laser diffraction technique according to the method described by Cross et al. (1981). Cooking loss was determined by calculating percentage of weight loss during cooking (ca. 300 g) for WB-shear force measurement. Objective meat color was determined by a Minolta Chromameter (CR300, Minolta, Japan) on freshly cut surface after a 30 min blooming at 1°C.

Immunoblotting, casein zymology and glycogen content

Approximately 1 g of muscle tissue was taken at the end of the lumbar vertebrae using a home-made biopsy sampler during breeding (i.e., 0 h), 6, 12 and 24 h postmortem. Similar amounts of muscle tissues were also sampled at 3, 7 and 14 d postmortem from the WB-shear force block. Muscle tissue was frozen in liquid nitrogen immediately after sampling, powdered in liquid nitrogen using a mortar-based homogenizer (Warning, Dynamics Corp., USA), and stored at -70°C until analysis. The rate of troponin-T degradation was determined at 0, 6, 24 and 168 h postmortem according to the methods described by Hwang et al. (2004b), with the following differences: 1) Blots were incubated with anti-troponin-T (Clone JLT-12, Sigma, USA) and labeled with goat anti-mouse IgG alkaline phosphatase conjugated secondary antibody (Promega, WI, USA). 2) The bound antibodies were visualized by incubating membranes with BCIP/NBT substrate (Bio-Rad laboratories, CA). Meanwhile, μ-calpain activity was determined at 0, 6, 12, 24, 72 h postmortem by a casein zymology according to the method described by Veiseth et al. (2001), with the following differences: 1) Gels were digitalized by an imaging system (Fluor-S MultiImager, Bio-Rad, USA). 2) Relative density was determined by using an image analysis software (Quantity One, Bio-Rad, USA). Glycogen content was determined on the 0 h sample according to the method described by Henckel et al. (2002)

Two-dimension electrophoresis (2DE) and staining

2DE was performed for four selected animals at 1 d largely according to the method described by Yan et al. (2001), with minor modifications. Fifty mg of powdered tissue was homogenized using a handheld homogenizer for 1 min in 1 mL lysis buffer, containing 7 *M* urea, 2 *M* thiourea, 2% CHAPS, 1% DTT and 0.8% Pharmalyte pH 3-10 nonlinear. After the homogenization, the lysate was vortexed for 1 min and incubated at room temperature for 30 min with rotational shaking. The lysate was then centrifuged at 40,000×g for 1 h at 20°C, and the second layer of supernatant was collected. Protein concentration was determined in triplicates according to the method described by Westermeier and Naven (2002), which was compatible with the sample preparation reagents such as 1% DTT, 8 *M* urea, 2 *M* thiourea, 4% CHAPS, and 2% Pharmalyte (Amersham Pharmacia Biotech, Amersham, UK). Protein concentration was measured spectrophotometrically at 480 nm with 0-50 μg of BSA as standard curve. The samples were diluted with rehydration solution, containing 8 *M* urea, 0.5% CHAPS, 0.2% DTT, and 0.2% Pharmalyte pH 3-10 nonlinear. Isoelectric

focusing was performed using pH 3-10 nonlinear IPG strip (Amersham Pharmacia Biotech, Amersham, UK). The strips were rehydrated for 12 h with the sample mixed rehydration buffer (450 μL/strip) and focused at 50 μA/strip for 70 kVh at 20°C. At the completion of focusing, the strips were equilibrated in 50 m*M* Tris-HCl buffer, pH 8.8, containing 6 *M* urea, 30% glycerol, 2% SDS, 0.002% bromophenol blue, and 1% DTT for 15 min, followed by an additional incubation for 15 min with the same buffer replacing DTT with 4.8% iodoacetamide. The second dimension electrophoresis was performed with 12.5% acrylamide (37.5:1 ratio of acrylamide to bisacrylamide) using the Ettan DALT system (Amersham Pharmacia Biotech, Amersham, UK) at 17 W/gel and 15°C. Gels were stained by 0.1% colloidial Coomassie Brilliant Blue G-250 in 2% phosphoric acid, 10% ammonium sulphate, and 20% methanol for 48 h (Westermeier and Naven, 2002). Gels were digitalized by an imaging system (Fluor-S MultiImager, Bio-Rad, USA).

Image analysis and spot identification

Triplicate gels for each sample were analyzed using a 2DE image analysis software (PDQuest, Bio-Rad, USA) according to the manufacturer's instruction. Spots appearing in more than two gels within 50% of variation in optical density were accepted as analytical spots, and average normal volume was used for the final analysis. To create 2DE reference map, 170 major spots were excised using an automated spot cutter (Proteome Works, Bio-Rad, USA) by a circular plug of 1.5 mm in diameter, and transferred to polypropylene 96 well reaction places. Proteins were subjected to in-gel trypsin digestion (Yan et al., 2001). Excised gel spots were destained with 100 μl of destaining solution (50% methanol in 10% acetic acid) with shaking for 5 min. After removal of the solution, gel spots were incubated with 200 m*M* ammonium bicarbonate for 20 min; this was repeated until colorless. The gel pieces were dehydrated with 100 μL of acetonitrile and dried in a vacuum centrifuge. The dried gel pieces were rehydrated with 20 μL of 50 m*M* ammonium bicarbonate containing 0.2 μg modified trypsin (Promega, Madison, WI, USA) for 45 min on ice. After removal of the remaining solution, 30 μL of 50 m*M* ammonium bicarbonate was added and digestion was performed overnight at 37°C.

Home-made chromatographic columns were used for desalting and concentration of the peptide mixture prior to mass spectrometric analysis. A column consisting of 100-300 nL of Poros reverse phase R2 material (20-30 μm bead size, PerSeptive Biosystems) was packed in a constricted GELoader tip (Eppendorf, Hamburg, Germany). For MALDI-ToF analysis, the column was washed with 0.1% trifluoroacetic acid (TFA), and approximately 5-10 μL of digested peptide fraction was loaded, followed by washing once with 0.1% TFA. Approximately 0.5 μL of peptide sample solution was directly loaded onto the MALDI-ToF MS sample plate together with 0.5 μL of the matrix solution (15-20 g/L α-Cyano-4-hydroxycinnamic acid in 70% acetonitrile). MALDI-MS spectra were obtained using a delayed-extraction reflectron time-of-flight mass spectrometer (Model MALDI-R, Micromass, Manchester, UK).

For MS/MS analysis, peptides were eluted with 1.5 μL elution solution (50% methanol, 49% H_2O, 1% formic acid) directly into a precoated borosilicate nanoelectrospray needle (Micromass, Manchester, UK). MS/MS spectra were generated by nano-ESI on a Q-TOF2 mass spectrometer (Micromass, Manchester, UK). The source temperature was 80°C. A potential of 1 kV was applied to the precoated borosilicate nanoelectrospray needles (EconoTip™, New Objective, USA) in the ion source combined with a nitrogen back-pressure of 0-5 psi to produce a stable flow rate (10-30 nL/min). The cone voltage was 40 V. The quadrupole analyzer was used to select precursor ions for fragmentation in the hexapole collision cell. The collision gas was Ar at a pressure of 6-7×10-5 mbar and the collision energy was 20-30 V. Product ions were analyzed using an orthogonal TOF analyzer, fitted with a reflector, a micro-channel plate detector, and a time-to-digital converter. The data were processed using a Mass Lynx Windows NT PC system. Peptide masses from MALDI-ToF MS were matched with the theoretical peptides of proteins in the NCBI database using MASCOT and/or Profound software. Also, all MS/MS spectra recorded on tryptic peptides were searched against protein sequences from NCBInr and EST databases using the MASCOT search program (www.matrixscience.com). A number of spot was identified by gel comparison with a reference protein (Hwang et al., 2004a).

Statistical analysis

The effects of pre-slaughter treatments and ageing on objective meat quality traits were evaluated by analysis of variance using a mixed model (SAS, 1997). Models included fixed effects of feeding, stress, ageing time, sampling location, and the first order interactions, with animal as a random term. Sampling location was retained in the model as it was necessary to have it adjusted, regardless of the level of probability. The significant difference of means ($p<0.05$) was separated by a least-significant-difference test (a pair-wise t-test).

RESULTS AND DISCUSSION

Effect of fasting, physical stress and chiller ageing on objective meat quality

The current experiment evaluated the response of

Table 1. Least square means, F ratios and significance levels for pH, temperature, sarcomere length and objective meat quality traits as a function of feed withdrawal for 18 h, chasing stress immediately prior to slaughter and chiller ageing

	Treatment										F ratio			
	Feed withdrawal		Stress			Ageing (day)								
	18 h	No	No	15 min	Avg. se	1	3	7	14	Avg. SE	Feed	Stress	Ageing	Feed×ageing
Glycogen (mmol/kg)	67.3	77.2	74.7	69.8	3.27						4.54*	1.14		
pH at 30 min	6.6	6.9	6.9	6.7	0.07						7.86*	2.46		
pH at 1.5 h	6.5	6.6	6.6	6.4	0.07						2.64	3.73		
pH at 24 h	6.2	5.8	6.0	6.0	0.08						10.31**	0.01		
Temp at 30 min (°C)	40.7	39.4	39.4	40.7	0.31						9.41**	7.82*		
Temp at 1.5 h (°C)	33.3	33.4	30.7	36.0	0.78						0.01	23.68***		
Temp at 24 h (°C)	0.6	1.2	0.5	1.3	0.22						4.48*	6.58*		
Sarco (um)	1.84	1.85	1.87	1.83	0.04						0.09	0.52		
Peak force (kg)	6.1	5.5	5.6	6.0	0.49	7.4^{a}	6.1^{b}	5.2^{c}	4.6^{c}	0.40	0.9	0.47	5.50***	
Cooking loss (%)	21.1	26.0	23.3	23.8	1.15	22.6^{a}	23.4^{a}	23.3^{a}	24.9^{b}	0.88	9.08**	0.1	6.09**	
Hunter L*	34.1	38.9	37.4	35.5	0.92	35.5^{a}	36.1^{a}	36.3^{a}	37.9^{b}	0.71	13.54**	2.25	9.65***	
Hunter a*	8.6	9.5	9.0	9.1	0.54	8.6^{a}	9.0^{ab}	9.3^{b}	9.3^{b}	0.42	1.33	0.01	2.62	8.68***
df[1]											1/17	1/17	3/57(3/54)	3/54

			1	3	7	14	Avg. SE
Hunter a*	Feed withdrawal	18 h	8.2^{a}	8.1^{a}	8.5^{a}	9.7^{b}	0.59
		No	9.1^{a}	9.9^{b}	10.1^{b}	8.9^{a}	0.59

[1] df: numerator/denominator degree of freedom (where there was an interaction between feed and ageing).
[a, b, c] Means bearing the same letter within each row did not differ significantly (p>0.05), * p<0.05, ** p<0.01, *** p<0.001.

Korean native black pigs to feed withdrawal for approximately 18 h and to chasing stress for 15 min immediately prior to slaughter in terms of meat quality. Previous studies have shown that short-term stress immediately prior to slaughter resulted in lower muscle pH and higher temperature (Brown et al., 1998; Stoier et al., 2001; Rosenvold et al., 2002). In this study, a 15 min chasing stress immediately prior to slaughter had no effect on WB-shear force, cooking loss, and objective color parameters, as well as on initial glycogen content (Table 1). Henckel et al. (2002) reported that physical stress for 10 min immediately prior to stunning significantly reduced glycogen content in crossbreeds of Duroc and progeny of Danish Landrace and Danish Large White. van der Wal et al. (1999) reported that a period of one min in a stunning pen significantly decreased water-holding capacity in Dutch Yorkshire lines. At first glance, the current result suggests that Korean native black pigs are inherently strapping, hence, can tolerate physical stress. On the other hand, it could not be dispelled that the pigs could really be familiar with physical chasing, as the pigs were sampled from an experimental population. Consequently, the stress treatment was not enough to induce differences in initial glycogen level and the rate of pH decline. Meanwhile, the stress treatment showed a tendency to cause lower μ-calpain activity at 6 h (p=0.08), and significantly (p<0.05) lower activities at 12 and 24 h postmortem (Figure 1). This mirrored that the stress caused early activation of the μ-calpain system, which was reflected in the faster rate of troponin-T degradation (Figure 1). It has been shown that proteolytic activity of μ-calpain is the most likely candidate responsible for meat tenderization (Koohmaraie, 1996; Hwang et al., 2003), and that muscle temperature is a significant determinant of this activity (Hwang and Thompson, 2001a). Given that the stress treatment had no effect on pH, the significantly high muscle temperature at 1.5 and 24 h postmortem (p<0.05) (Table 1) could have contributed to the accelerated μ-calpain activity. Hwang et al. (2001b) showed that a marginal difference in μ-calpain activity early postmortem significantly affected the rate of meat tenderization. However, a negligible effect of μ-calpain activity on WB-shear force was particularly noticeable in this study.

Henckel et al. (2002) reported that glycogen level at the time of stunning had a linear relationship with ultimate pH when the level was below 53 mmol/kg for the progeny of Duroc as sire line and Danish Landrace-Danish Large White as dam line. On the other hand, glycogen content of 67 and 77 mmol/kg resulted in pH at 24 h of 6.2 and 5.8 for Korean native black pigs fasted and fed, respectively. The differences between the two groups were significant (p<0.05) for both measurements. Enfalt et al. (1993) previously reported that PSE meat was related not only to lower initial pH, but also to faster decline in pH. In this study, pigs fed until the morning of slaughter, which had significantly higher initial glycogen reserves, also had a significantly (p<0.05) higher pH at 30 min and a significantly (p<0.05) lower pH at 24 h. This implies that

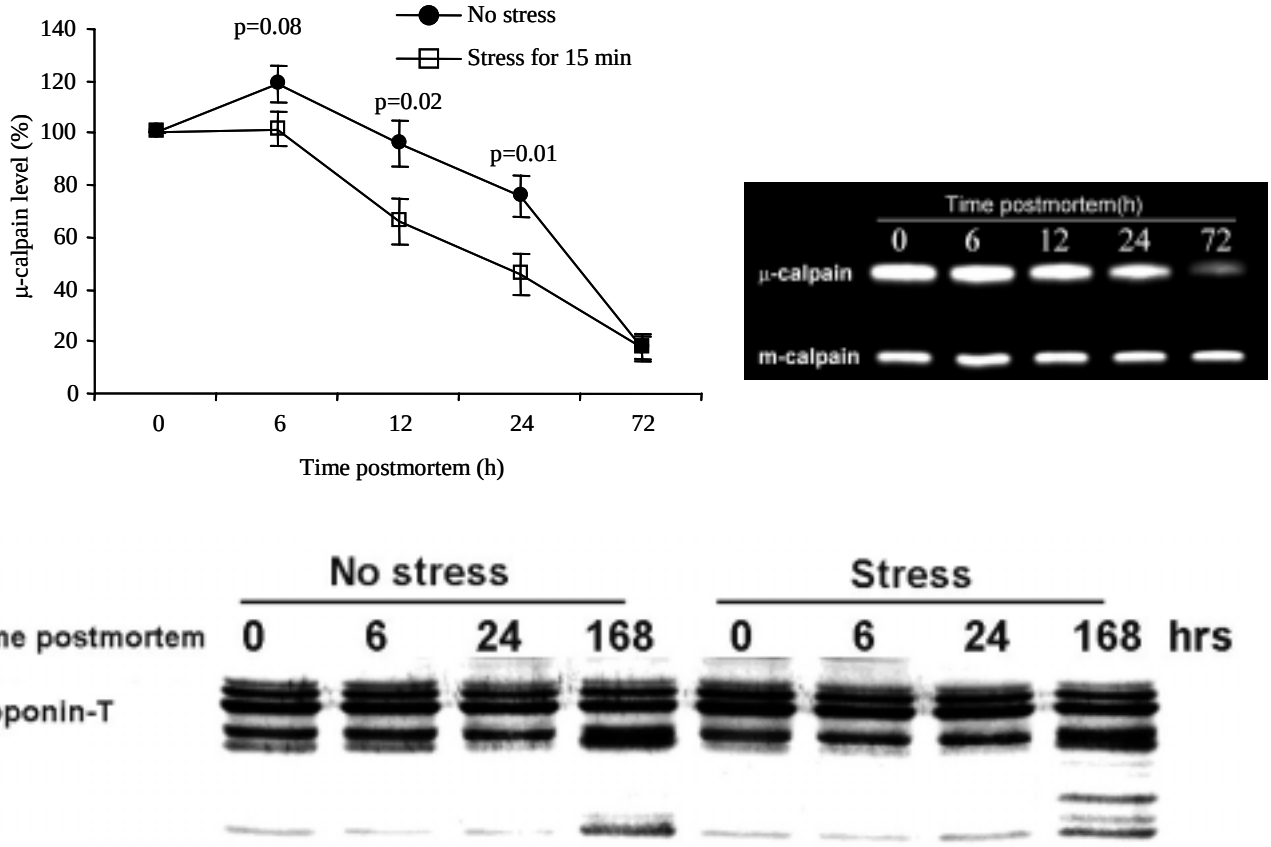

Figure 1. Changes in μ-calpain activity and troponin-T degradation during rigor development and ageing time as a function of pre-slaughter stress. An example of zymography for μ- and m-calpain activities is also presented.

the rate and extent of glycolysis were affected by the initial glycogen content, and that muscle with a higher glycogen had a faster rate of glycolysis during the onset of rigor. However, the confounding effect of high temperature at 30 min and 24 h for the fasted animals could not be dispelled in spite of the similar temperature at 1.5 h. Expectedly, meat from the fasted pigs showed greatly ($p<0.05$) reduced cooking loss and lower lightness. In several countries, 12-15 h pre-slaughter fasting is a common practice to retain appropriate muscle glycogen stores at the time of slaughter, and consequently to reduce PSE-incidence (Eikelenboom et al., 1991; Wittmann et al., 1994). In general, the current result was in agreement with that of previous studies.

There was a significant interaction ($p<0.05$) between the feeding treatment and chiller ageing on hunter a* value. Redness for the fed pigs was significantly ($p<0.05$) higher at both 3 and 7 d than at 1 and 14 d, while the fasted ones maintained a stable redness during 7 d of chiller ageing, followed by a significant ($p<0.05$) increase at 14 d. The result was consistent with that of Rees et al. (2003) who showed that a fast pH decline during rigor development accelerated increase in redness during ageing. On the other hand, Juncher et al. (2001) showed that a faster decline in pH increased redness at 1 d, but was reduced by chiller ageing. Pearson and Dutson (1985) noted that free water at the tissue surface resulted in increased reflectance and consequently lighter appearance. In this study, lightness was constant for 7 d, which corresponded with stable cooking loss. Collectively, the results suggest that color and water-holding capacity for KNP was stable for 7 d of chiller ageing.

Sarcomere length (i.e., ca. 1.85 μm, Table 1) indicated that there was no muscle shortening. None of the pre-slaughter treatments affected WB-shear force, which had a significantly ($p<0.05$) linear reduction from 1 to 7 d, indicating that a significant tenderization was completed by 7 d. Watanabe et al. (1996) showed a coverlinear relationship between ultimate pH and tenderization where, pH approximately 6.0 had the highest tenderization. However, in spite of the significant difference in ultimate pH between the treatment groups, there was no interaction between feeding and ageing. van Laack et al. (2001) reported that the relationship was dependent on breed line (Duroc, quadratic; Hampshire, linear; Berkshire, no relationship). Hwang et al. (2001a) reported that the time of μ-calpain activation during early postmortem was related to the rate and extent of tenderization. As discussed previously,

Table 2. List of tentatively identified proteins and their characteristics. Longissimus tissue at 1 d postmortem was separated by 2DE, stained with Coomassie Brilliant Blue G-250, and average spot densities of triplicate gels were compared by using a 2DE image analysis software

Spot No.	Consensus protein identity	NCBI gi number	SC (%)[1]	MI[2]	p*I*/MW ($\times 10^3$ Da)
Spots that differed greater than two-folds					
2202	Chain A, deoxyribonuclease I complex with actin, rabbit	gi|229690	36	GM	5.09/41.8
2302	Chain A, deoxyribonuclease I complex with actin, rabbit	gi|229690	39	GM	5.09/41.8
3102	Heat shock 27 kDa protein, dog	gi|1170366	11	QT	6.23/22.9
3409	unidentified				
3504	Chain A, aldehyde dehydrogenase, bovine mitochondria	gi|2624886	18	GM	6.05/54.9
4104	Similar to cardiac Ca^{2+} release channel [Rattus norvegicus]	gi|34876273	12	MT	6.18/392
4205	Glycerol-3-phosphate dehydrogenase, human	gi|33695088	18	GM	5.81/38.2
5101	unidentified				
5108	Myosin heavy chain 2×[Sus scrofa]	gi|5360750	14	GM	5.6/223.9
5306	unidentified				
6108	unidentified				
8001	Hemoglobin alpha chain, pig	gi|122465	42	GM	8.76/15.1
8102	unidentified				
8211	unidentified				
9103	unidentified				
Spots that did not differ					
4	Myosin light chain [Sus scrofa]	gi|5834684	39	MT	4.63/16.8
10	Myosin light chain 3, [Rattus norvegicus]	gi|6981240	38	MT	5.30/22.3
3302	NAD^+-isocitrate dehydrogenase, [Macaca fascicularis]	gi|1182011	22	MT	5.72/37.2
3414	Actin, fetal skeletal, mouse (fragment),	gi|90263	20	MT	5.83/39.5
5109	Heat shock 27 kDa protein, cat	gi|1170366	12	QT	6.23/22.9
5605	Transferrin [Sus scrofa]	gi|5360750	14	MT	6.73/78.95
6001	Basic cytosolic protein, 21 K-bovine protein, 21 K-bovine	gi|89407	10	QT	7.38/20.95
6505	Pyruvate kinase, M1 isozyme, cat	gi|125598	4	QT	7.23/58.4
9001	Hemoglobin alpha chain, pig alpha chain, pig	gi|122465	38	MT	8.76/15.1
9503	Pyruvate kinase, M1 isozyme, cat,	gi|125598	28	MT	7.23/58.4

[1] Sequence coverage rate (SC, %).
[2] Method of identification (MT: MALDI-ToF, QT: EST-MS/MS, GM: Gel match).

the two feeding treatments resulted in significantly different profiles in pH decline in terms of rate and extent. However, in the current μ-calpain assay, no difference was detected. Here, we faced with a question: Why was the stress treatment had a significant effect on μ-alpain in spite of a similar rate of postmortem glycolysis? While one possible factor was significantly difference in muscle temperature at 1.5 (during rigor development), the more fundamental question remained unclear.

Taken together, the current result showed that meat color and cooking loss were relatively stable for 7 d, by which time significant tenderization was achieved. This also suggests that a 7 d chiller ageing would result in the most desirable KNP quality. Given the fact that Korean consumers prefer brighter and red pork, as well as tender meat, feeding until the morning of slaughter appeared to be more favorable. However, the treatment may reduce palatability due to increased cooking loss.

Meat colour and proteome properties

Gene products go through more than 400 possible chemical modifications prior to their functions largely in a manner of interactions (Pennington and Dunn, 2001). The fact suggests that a concomitant analysis of muscle proteins is central to understanding their relations to meat quality. Recently, proteome analysis technique was applied to evaluate postmortem proteolysis and its relation to Danish pork tenderness (Lametsch, 2003) and landrace (Hwang et al., 2004a) during chiller ageing. These studies demonstrated that the approach was a feasible tool to identify muscle proteins related to meat quality. As a model study, the current analysis was attempted to examine the difference in protein density between low (two animals with hunter L*=28, group A) and high hunter L* (two animals with hunter L*=40, group B) animals. As discussed previously, pigs for the low and high lightness values were selected from the fasted and fed groups, respectively. Figure 2 shows representative 2DE images for each group. Spot

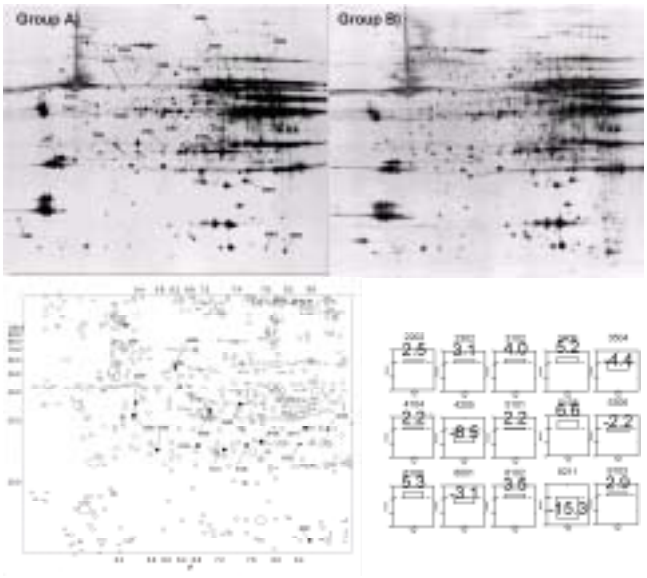

Figure 2. Representative image for groups A (lower lightness) and B (high lightness), and selected spots by a threshold analysis for two-fold difference between the two groups.

density of semi-quantitative triplicate gels for each sample was averaged, and the difference in density between two groups was analyzed by a threshold analysis using a commercial 2DE-image analyzer. Spot density of group A was used as a reference level, and spots with higher or lower than two-folds for group B were selected. Figure 2 presents the scanned images of selected spots and the difference in density between the two groups. The result showed that group A had a higher density for ten spots including chain A, deoxyribounclease I complex with actin, heat shock protein 27 kDa, a protein similar to cardiac Ca^{2+} release channel, and myosin heavy chain, while it had a lower density for five spots including chain A, aldehyde dehydrogenase, glycerol-3 phosphate dehydrogenase, and hemoglobin alpha chain. Table 2 summarizes the electrophoretic characteristics of the selected protein spots and some other identified proteins without difference in density. These are also numbered in Figure 1.

Due to the inherent limitations of gel-based proteome analysis in terms of risk of co-migration of intact proteins and/or intermediate degradation products, particular caution needs to be taken to compare postmortem muscles. In one example of yeast cell extract (Gygi et al., 2000), the products of six genes migrated to the same spot on a silver-stained gel. Particularly for postmortem muscle, there is a greater risk of co-migration for intermediate degradation peptides. This aspect was also reported by Lametsch and Bendixen (2001) who identified 15 spots that changed from slaughter for 48 h, where the density of 11 spots increased while those of 4 spots decreased. In the current study, myosin heavy chain (spot 5108) and a protein similar to calcium release channel (spot 4104) were identified, and density differed between the two groups. Molecular weights of these proteins are approximately 224 and 392 kDa in intact forms, respectively. Taking the resolution range of 12.5% second-dimension gel used in this study, and geological point on the 2DE (Figure 2), these proteins were likely degradation products or intermediate peptides of protein metabolism.

A coincidence of the difference in spot density and meat quality traits between the two groups did not mean a direct linkage between proteins and meat quality traits, because the levels at 1 d postmortem could be confounded by expression levels caused by the feeding treatment and their changes during conversion to meat. However, the spot densities were ultimate levels of the muscle samples for different color properties, suggesting that meat lightness at 24 h was related to the status of various proteins including contractile, enzyme, and housekeeping proteins. To segregate the confounding effects of pre- and post-slaughter factors, biopsy samples (taken during bleeding) should be analyzed, but further analysis could not be performed due to a limited amount of reserved sample. Dark, firm and dry meat was related to high ultimate pH and that had high water-holding capacity (Bendall and Swatland, 1988). On the other hand, drip was formed by denaturation of contractile proteins and shrinkage of myofibrils, which increased meat lightness (Pearson and Dutson, 1985; Bertram et al., 2004). In addition, the most possible endogenous protease responsible for postmortem proteolysis (i.e., μ-calpain) was significantly affected by pH and temperature profiles during rigor development (Claeys et al., 2001). These studies indicate that the effect of biological process during rigor development on meat quality traits such as tenderness, color and water-holding capacity are closely associated with its effect on rate and extent of endogenous proteolysis and protein denaturation (Hwang and Thompson, 2001b; 2003).

In this regard, the selected proteins also might be related to other meat quality traits in part (if not all), because the group which was subjected to fasting had higher ultimate pH, lower drip loss, and darker color than those of the fed group. Lametsch et al. (2003) reported that proteins related to pork tenderness included actin, endolase 3, pyruvate dehydrogenase, myosin light chain, and myosin heavy chain. The current result was in general consistent with the previous observation, where there was no significant difference in actin, myosin light chain, and pyruvate kinase. In this study, one significant observation was that the spot density for one (1) isoforms of heat shock 27 kDa (spot 3102) and hemoglobin alpha chain (spot 8001) protein differed between the two meat quality groups, while the other isoforms for the same proteins (spots 5109 and 9001) were identical. This suggests that the rate and extent of degradation and/or expression for the isoforms of some proteins varies.

IMPLICATIONS

Based on objective meat quality measurements, feeding until the morning of slaughter and a 7 d chiller ageing appeared to be desirable for a more favorable meat color and tenderness of Korean native pork. However, the treatment likely has an adverse effect on palatability due to increased cooking loss. This aspect has yet to be answered. Proteome analysis selected 15 candidate proteins which differed in spot density between animals for low and high hunter L* values at 1 d. These included contractile, housekeeping, and enzyme proteins, suggesting that various proteins were concomitantly involved in the determination of final meat color. In addition, the current results also suggest that the rate and extent of degradation and/or expression for the isoforms of some proteins vary in different meat qualities. As the current proteome analysis was performed as a model approach using a limited number of animals, a larger scale experiment is required prior to industrial consideration.

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