IDENTIFICATION AND CLASSIFICATION OF PROTEINS RELATED TO HUNTER L* VALUE AND DRIP LOSS IN PORK LONGISSIMUS MUSCLE

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Introduction

Meat color and water-holding capacity (WHC) are the most important pork traits determining consumers' preference (Rosenvold et al. 2003) because their relation to pale, soft, and exudative (PSE) characteristicse (Offer et al., 1989). Until recent years, biological mechanisms related to PSE meat has been determined largely by one-dimension electrophoresis and/or western blotting for a particular protein (Warner et al., 1997), myofibril fragmentation index (Rees et al., 2002), and peptide and amino acid profiles (Moy et al., 2001). However, recent development of a commercial high throughput proteome analysis equipment opened the possibility to characterize whole muscle proteins simultaneously and determine their relation to PSE meat.

Objectives

As a model study, the current study was conducted to identify proteins related to hunter L* value and drip loss in pig *m. longissimus dorsi* during chiller ageing.

Methodology

Six male landraces (100 ± 11 kg) were sampled from the National Livestock Research Institute (NLRI) breeding program, and slaughtered conventionally at the NLRI abattoir. To generate various meat qualities during chiller ageing, both feeding and physical stress treatments were enforced prior to slaughter(Hwang et al., 2005). The day following slaughter, *m. longissimus dorsi* muscles (from the 7^{th} thoracic vertebrate to the last lumber vertebrate) were removed, cut into three potions, vacuum-packed, and randomly assigned to one of three ageing periods (1, 3, and 7 day) for the objective quality measurements of meat color and drip loss. The samples were held at 1^{o}C for the relevant ageing period. Hunter L* value (lightness) was determined by a Minolta Chromameter (CR300, Minolta, Marunouchi, Japan) other materials and methods including two-dimension electrophoresis and identification procedure were reported by Hwang et al. (2005). Discriminant analysis was performed by Systat (version 10.2) using a backward stepwise method with alpha to enter = 0.15.

Results & Discussion

To examine the relationship between changes in semi-quantitative spot density and meat quality traits, 2DE profiles were examined at 0, 1, 3, and 7 day postmortem in triplicates. Animal variations including state of health, growth path, and animal handling prior to slaughter were removed by calculating the levels of spot density at 1, 3, and 7 day postmortem as a percentage of 0 hr (i.e., biopsy tissue). Figure 1 illustrates a 2DE reference map and identified sports. There were more spots altered during ageing in terms of density and electrophoretic properties, but only those identified proteins were presented in this study. Density of each protein and its relationship with meat quality varied to a large extent depending on meat quality. However, based on average value, nine spots (spots, 11, 22, 27, 32, 34, 46, 69, 77, and 94) were consistently reduced, while five spots (spots 42, 73, 84, 88, and 96) fluctuated during ageing (Data not shown). The result was likely related to the limited number of animals in this study. In addition, this could be due to the inherent limitation of 2DE-based proteome analysis in terms of risk of co-migration of intact proteins and/or intermediate degradation products (Lametsch et al., 2001). Table 1 shows consensus protein identities and their electrophoretic properties and their relationship with objective meat quality. A large number of spots were significantly matched by other species such as human, rat, and bovine. This could be due to the limited number of public accessible porcine protein information. Twelve proteins were related to hunter L* value, which included contractile apparatus and related proteins such as alpha actin, myosin light chain 1, cofilin 2 and troponin T, and chaperone proteins of alpha-b crystalline. Four proteins (troponin T, adenylate kinase, ATP-dependent proteinase SP-22, and DJ-1 protein) were related to drip loss. Lametsch *et al*. (2003) found that 26 proteins were related to pork tenderness. These proteins included myosin heavy chain, titin, myosin light chain I, myosin light II, CapZ, and coflin. The current result was largely in agreement with the previous observation in a sense that most proteins were contractile proteins. This suggested that objective meat qualities of tenderness, meat color, and drip loss were closely associated with postmortem proteolysis.

It is true that objective assessment of pork quality is not a direct reflection of PSE meat, but in the current study Hunter L* value was used to classify pork quality based on Warner *et al.* (1997), who defined hunter L* value higher than 50 as PSE, between 50 and 42 as normal , and lower than 42 as dry, firm, and dark (DFD). Regardless of the fact, to examine whether these proteins were able to classify objective pork qualities (i.e., PSE, normal, DFD), a discriminant analysis was performed by a backward stepwise selection. As meat color and drip loss were concomitant phenomena, all proteins related to drip and hunter L* value were incorporated into the model. Table 2 and Fig. 1 show canonical discriminant functions and their scores on biplot. By the analysis, nine proteins were retained in the final model, in which 69% of samples were classified correctly (PSE: 100%, Normal: 83%, DFD: 33%, data not shown). The final model excluded myosin light chain 1, substrate protein proteinase SP-22, adenylate kinase, and actin fragments. The model classified 100% of PSE meat, while four out of six DFD samples were classified into PSE (two samples) and normal (two samples). Given the fact that PSE meat is the most notorious single factor causing economic loss for the pork industry, while DFD meat is rarely observed in practice, the current model meets the industrial needs. The cumulative proportion indicated that the first canonical variable accounted for 65.3% of the dispersion, where Chain A deoxyribonuclease I (SP77), Actin alpha 1

(SP27), and Troponin T (SP73) were main drivers to separate normal and PSE meats. The results implied that PSE meat was largely characterized by both lower densities of Actin alpha 1 and Troponen T, and high density of Chain A deoxyribonuclease I.

Conclusions

The current study demonstrated that the 2DE-based proteome analysis could be a feasible approach in exploring proteins related to postmortem meat quality traits. However, a larger scale study is necessary to ensure the current results, as the number of animals and identified proteins in this model study were very limited.

References

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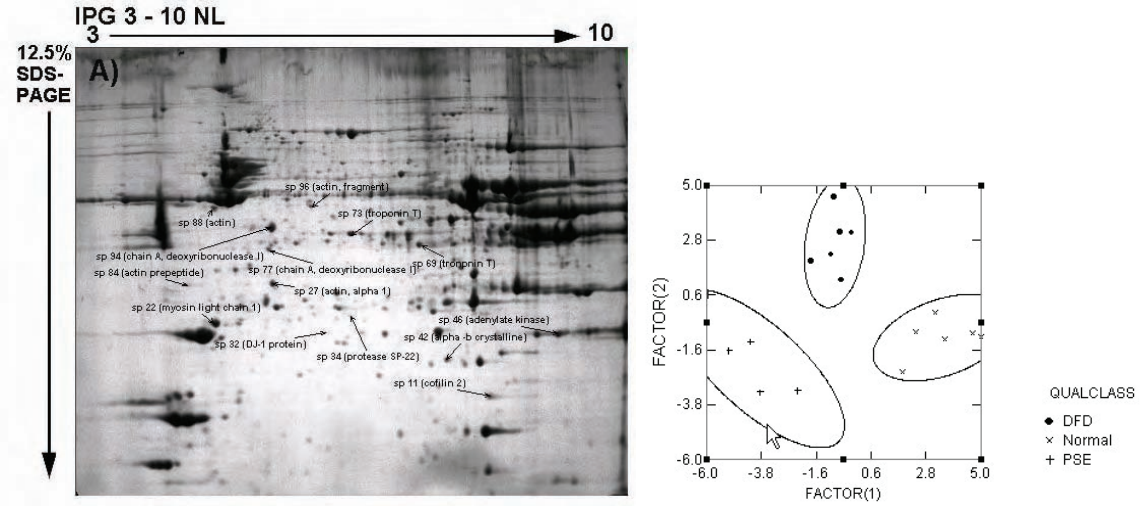

Fig. 1. Reference 2DE protein map and identified proteins related to Hunter L* and drip loss during ageing, and canonical score plot of discriminant analysis at Table 2(PSE: 100%, Normal: 83%, DFD: 33%).

Table 1. List of spot number (SP No.), consensus protein identity, NCBI gi number, method of identification (MI), theoretical pI and molecular($\times 10^3$ Da) on Fig. 1 gel

SP No.	Consensus protein identity	NCBI gi No.	MI[1)]	pI/MW	Correlation (r)	
					Drip loss	Hunter L*
SP11	Cofilin 2	gi|6671746	QT	7.66/18.8	NS[2)]	-0.49*
SP22	Myosin light chain 1	gi|127151	MT	5.0/22.2	NS	-0.64**
SP27	Actin, alpha 1	gi|27819614	MT	5.31/42.5	NS	-0.53*
SP32	DJ-1 protein	gi|7429593	QT	6.33/20.1	-0.54†	NS
SP34	Substrate of mitochondrial ATP-dependent proteinase SP-22	gi|627764	QT	5.73/21.7	-0.53†	-0.43†
SP42	Alpha-b crystalline	gi|7441290	MT	6.76/20.1	NS	-0.56*
SP46	Adenylate kinase	gi|230801	MT	8.37/21.9	-0.77**	NS
SP69	Troponin T slow type isoform sTnT2	gi|34393192	MT	6.41/30.0	NS	-0.61*
SP73	Troponin T slow type isoform sTnT1	gi|34393190	MT	5.92/31.2	-0.59*	-0.65**
SP77	Chain A, deoxyribonuclease I complex with actin	gi|229690	MT	5.09/41.8	NS	-0.62**
SP84	Actin prepeptide	gi|178067	MT	5.12/37.2	NS	-0.48*
SP88	Actin, fetal skeletal (fragment)	gi|90263	MT	5.83/39.5	NS	-049*
SP94	Chain A, deoxyribonuclease I complex with actin	gi|229690	MT	5.09/41.8	NS	-0.73***
SP96	Actin, fetal skeletal (fragment)	gi|90263	MT	5.83/39.5	NS	-0.50*

[1)] MT: MALDI-ToF peptide mass fingerprint, QT: ESI/MS/MS fragmentation sequencing

Table 3. Canonical discriminant functions of discriminant analysis, and canonical scores of group means determined by a backward stepwise method with alpha to enter = 0.15. The model included 14 spots having a significant correlation with hunter L* or drip loss

	Canonical discriminant function	
	Factor 1	Factor 2
Constant	-12.389	-6.158
Chain A, deoxyribonuclease I (SP77)	-0.228	0.001
Troponin T (SP69)	-0.081	-0.03
Alpha-b crystalline (SP42)	-0.025	0.007
Actin prepeptide (SP84)	-0.011	-0.055
DJ-1 protein (SP32)	0.064	0.033
Cofilin 2 (SP11)	0.074	0.026
Chain a, deoxyribonuclease I (SP94)	0.084	0.038
Troponin T (SP73)	0.132	0.066
Actin, alpha 1 (SP27)	0.192	0.008
Myosin light chain 1 (SP22)	.	.
Substrate protein proteinase SP-22 (SP34)	.	.
Adenylate dinase (SP46)	.	.
Actin, fragment (SP88)	.	.
Actin, fragment (SP96)	.	.
Cumulative proportion of total dispersion		
	0.653	1