Advance Journal of Food Science and Technology 9(8): 626-632, 2015
DOI: 10.19026/ajfst.9.1977
ISSN: 2042-4868; e-ISSN: 2042-4876

Submitted: April 14, 2015 Accepted: May 10, 2015 Published: September 10, 2015

Research Article

Compare Two Contrasting Breeds of Pigs Postmortem for Differential Protein Expression in Relation to Meat Quality

[1]Daofeng Qu, [1]Jianzhong Han, [1]Kaili Jia, [1]Luoting Ye, [2]Xinjie Wang, [3]Xiaoning Liu, [1]Yang Shen and [1]Keke Ye
[1]Food Science and Engineering the Most Important Discipline of Zhejiang Province, School of Food Science and Biotechnology, Zhejiang Gongshang University, Hangzhou 310035,
[2]Jinhua Polytechnic, No.1188 Wuzhou Street, Jinhua, Zhejiang,
[3]Logistics Center Quarantine Station of Yuhang District Agricultural Bureau, Hangzhou 310029, P.R. China

Abstract: The aim of this study was to assess the postmortem changes of muscle proteins and their relationships between these changes and meat quality traits. Six Jinhua and 6 crossbred (Duroc×Landrace×Yorkshire) male pigs at 180 d were used to determine the post-mortem changes of meat during the storage. Differential proteins between Jinhua pigs and crossbred pigs were profiled and identified by the application of two-dimensional electrophoresis and Mass Spectrometry. Two-dimensional electrophoresis results showed that the expression level of 27 proteins from different breeds of pigs varied during postmortem time (6, 24, 48 and 72 h) and 16 of them was up-regulated in Jinhua pigs ($p<0.05$). Furthermore, the structure and function of 12 proteins were identified by the application of Mass Spectrometry and referring to the protein database. Analysis showed that heat shock protein 27, desmin, β-enolase and pyruvate kinase were highly correlated with the integrity of meat tenderness, water-holding capacity as well as shelf life.

Keywords: Differential proteomics, meat quality, pig, postmortem changes, storage

INTRODUCTION

The quality of fresh pork is affected by many factors such as genetic, breeding management, transportation, slaughtering and so on (Beaulieu *et al*., 2010). While, almost all of the meat quality traits are associated with complex genes and proteins (Bendixen, 2005). Two-dimensional electrophoresis and mass spectrometry-based proteomics provide a classic method for the the research of meat quality. Proteomics is the large-scale study of proteins, the product of gene expression, which can directly reflect the expression and activity status of proteins (Hollung *et al*., 2007; Zheng *et al*., 2008). Thus, the study on the quality of fresh meat by the method of differential proteomics is valuable for finding the important proteins, controlling the quality of meat, rating and marketing the meat.

In recent years, efforts have been made to analyze the post-mortem changes of meat, the feasibility of which was suggested by the novel proteins found by the method of differential proteomics (Lametsch *et al*., 2003; Zellner *et al*., 2011). The researchers attempted to look for specific protein markers which is related to the meat quality (tenderness, water holding capacity, color, etc.) from the post-mortem changes of muscle protein and have made a series of fruitful progress (Lametsch and Bendixen, 2001; Lametsch *et al*., 2003, 2004). However, the research on the post-mortem changes of meat from the Chinese local pigs has been little reported.

There are plenty of swine germplasm resources in China. The meat quality of some local pigs (Jinhua pig) has advantages compared with the meat quality of crossbred pigs (DLY-Duroc×Landrace×Yorkshire). Jinhua pig is also called panda pig, the reason of which is that the head and buttock of Jinhua pig are black (Guo *et al*., 2011; Miao *et al*., 2009). In this study, differential proteomics of fresh pork from Jinhua and DLY pigs after different storage time were analyzed. Some protein markers were found and identified by mass spectrometry. Further analysis of these data provides useful information for the research of meat quality.

Corresponding Author: Jianzhong Han, Food Science and Engineering the Most Important Discipline of Zhejiang Province, School of Food Science and Biotechnology, Zhejiang Gongshang University, Hangzhou 310035, P.R. China, Tel.: +8613758032556; Fax: +86057188071024

MATERIALS AND METHODS

All of the experiments were carried out according to the guidelines for animal experiments at the National Institute of Animal Health.

Animals and sample collection: Six Jinhua and 6 crossbred (Duroc× Landrace×Yorkshire) male pigs at 180 d were used to determine the post-mortem changes of meat during the storage. Muscle samples were taken from the Longissimus dorsi muscle, at the position of the last rib and stored at 4°C in refrigerator. The samples were taken from the muscles in the refrigerator immediately after 6, 24, 48 and 72 h post-mortem and kept at -80°C until the time of protein extraction.

Extraction of muscle proteins: The method was adapted from Lametsch *et al*. (2002). The frozen muscle tissue (100 mg) was homogenized in 1 mL of 8 M urea, 2 M thiourea, 65 mM DTT, 2% CHAPS-O and 1% carrier ampholytes (Ampholyte 3-10, Pharmacia, Uppsala, Sweden), in a handheld glass homogenizer. Crude extracts were transferred to an Eppendorf tube and vigorously shaken for 2 h at room temperature, followed by a 30 min centrifugation step at 10000 g to remove unextracted cellular components, high molecular weight protein complexes and insoluble proteins. The protein concentration was determined by the RC-DC assay (Bio-Rad).

Two-dimensional electrophoresis: The preparation and running of the Two-Dimensional Electrophoresis (2 DE) analysis were done according to Theron *et al*. (2011). First 1 mg of proteins was incorporated in a buffer containing 7 M urea, 2 M thiourea, 2% CHAPS (w/v), 0.4% carrier ampholyte (v/v), 1% DTT (w/v) and bromophenol blue. Samples were loaded onto immobilized pH gradient strips (pH 3-10 NL, 17 cm, Bio-Rad) and isoelectric focusing was performed using a Protean IEF cell system (Bio-Rad). Gels were passively rehydrated for 16 h. Rapid voltage ramping was subsequently applied to reach a total of 86 kVh. After strip equilibration, proteins were resolved on 12% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) gels using a Protean II XL system (Bio-Rad) for the second dimension. Gels were stained with Coomassie Blue (colloidal blue). Three gels were produced per sample, giving 24 gels in all.

Image analysis: Gels were visualized and analysed using the two-dimensional electrophoresis image analysis software (Bio-Rad). First, all spot positions were recognized and relative integrated spot intensities in the individual gels were estimated. Then, the 2 DE images were matched by comparing the relative positions and integrated intensities of the individual spots on each gel. For comparative image analysis, the images were grouped, after which the relative levels of expression of individual spots were analyzed and compared within and between the image groups. The matches suggested by automated image analysis were finally individually inspected and confirmed. The gel images were normalized according to the total quantity in the analysis set. Relative comparison of intensity abundance among different groups at three time points (three replicate samples for each group) was performed using Student's t test. Expression intensity ratio values larger than 2.0 ($p<0.05$) or smaller than 0.5 ($p<0.05$) were set as a threshold indicating significant changes.

In-gel digestion of 2 D gel electrophoresis separated proteins: The protein spots were manually excised from the silver-stained gels and then transferred to V-bottom 96-well microplates loaded with 100 μL of 50% ACN, 25 mM ammonium bicarbonate solution/well. After being destained for 1 h, gel plugs were dehydrated with 100 μL of 100% ACN for 20 min and then thoroughly dried in a SpeedVac concentrator (Thermo Savant) for 30 min. The dried gel particles were rehydrated at 4°C for 45 min with 2 μL/well trypsin (Promega, Madison, WI) in 25 mM ammonium bicarbonate and then incubated at 37°C for 12 h. After trypsin digestion, the peptide mixtures were extracted with 8 μL of extraction solution (50% ACN, 0.5% TFA) /well at 37°C for 1 h. Finally the extracts were dried under the protection of N_2.

Protein Identification by Mass Spectrometry: The peptide mixtures were redissolved in 0.8 μL of matrix solution (α-cyano-4-hydroxycinnamic acid (Sigma) in 0.1% TFA, 50% ACN) and then spotted on the MALDI plate. Samples were allowed to air dry and analyzed by a 4700 MALDI-TOF/TOF Proteomics Analyzer (Applied Biosystems, Foster City, CA). Trypsin-digested peptides of myoglobin were added to the six calibration spots on the MALDI plate to calibrate the mass instrument with internal calibration mode. The UV laser was operated at a 200-Hz repetition rate with wavelength of 355 nm. The accelerated voltage was operated at 20 kV. All acquired spectra of samples were processed using 4700 ExploreTM software (Applied Biosystems) in a default mode. Parent mass peaks with mass range of 700-3200 Da and minimum signal to noise ratio of 20 were picked out for tandem TOF/TOF analysis. Combined MS and MS/MS spectra were submitted to MASCOT (Version 2.1, Matrix Science, London, UK) by GPS Explorer software (Version 3.6, Applied Biosystems) and searched with the following parameters: National Center for Biotechnology Information non-redundant (NCBInr) database, taxonomy of bony vertebrates or viruses, trypsin digest with one missing cleavage, no fixed modifications, MS tolerance of 0.2 Da, MS/MS tolerance of 0.6 Da and possible oxidation of methionine. Known contaminant ions (human keratin and tryptic autodigest peptides) were excluded. A total of 4,736,044 sequences and 1,634,373,987 residues in the database were actually searched. MASCOT protein scores (based on combined MS and MS/MS spectra) of greater than 72 were considered statistically significant ($p\leq0.05$). The

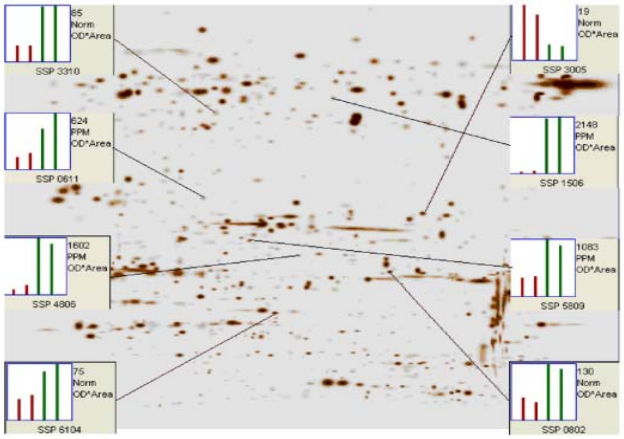
Table 1: Primer sequences and amplicon lengths of quantitative real-time PCR products of target genes

Target genes	Primers (5' to 3')	Amplicon length (bp)
HSP 27	FP: TCCCTGGACGTCAACCACTTC	143
	RP: GGCAGCGTGTATTTTCGAGTG	
Desmin	FP: TCAATGTCAAGATGGCCCTG	150
	RP: TATGGACCTCAGAACCCCTT	
Enolase	FP: GGCTTCCACGGGTATCTAT	131
	RP: GTTTCTTTTCCAGCAGCGC	
Pyruvate kinase	FP: TTCGCATCTTTCATCCGTAA	227
	RP: CGCCCAATCATCATCTTCT	
GAPDH	FP: TGAGACACGATGGTGAAGGT	163
	RP: CGTGGGTGGAATCATACTG	

FP: Forward primer; RP: Reverse primer

individual MS/MS spectrum with a statistically significant (confidence interval ≥95%) ion score (based on MS/MS spectra) were accepted.

Real Time RT-PCR verification: Specific primers Table 1 suited to simultaneously amplify various target genes were designed according to the corresponding gene sequences of MS/MS-identified proteins and the available gene information deposited in the GenBank™ library by using the Lasergene sequence analysis software (DNAStar, Inc., Madison, WI). Total muscle RNA was extracted using the RNeasy minikit (Qiagen, GmbH, Hilden, Germany) according to the manufacturer's protocol. RNA concentrations were measured using a spectrophotometer (260/280 nm). After heating at 65°C for 5 min to denature RNA and to inactivate RNases, 1 μg of total RNA was subjected to reverse transcription using 200 units of SuperScript III reverse transcriptase (Invitrogen), 40 units of RNaseOUT recombinant RNase inhibitor (Invitrogen), 200 ng of random hexamer primers (TaKaRa), 0.5 mM (each) dNTPs (TaKaRa), 4 μL of 5×First-Strand Buffer (Invitrogen) and 1 μL of 0.1 M DTT (Invitrogen) in a total volume of 20 μL at 25°C for 5 min and then incubated at 50°C for 1 h. The reaction was terminated by heating at 70°C for 15 min. The real time RT-PCR was performed by using the 7500 Real-Time PCR System (Applied Biosystems) in a total volume of 20 μL containing 100 ng of cDNA template, 1×SYBR Premix Ex Taq (Perfect Real Time, TaKaRa) and a 200 nM concentration of each primer. After initial denaturation at 95°C for 30 s, the amplification was carried out through 40 cycles, each consisting of denaturation at 95°C for 15 s, primer annealing at 58°C for 15 s and DNA extension at 72°C for 40 s. Melting curves were obtained and quantitative analysis of the data was performed using the 7500 System SDS software Version 1.3.1 in a relative quantification(ddCt) study model (Applied Biosystems). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a housekeeping gene for the tests.



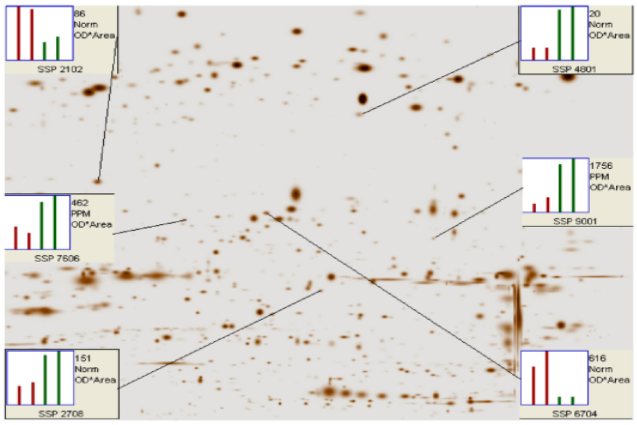
Analysis Set	Description	Type	Count	Created	Method	Operand A	Operand B
Union of all auto sets		BoolN	582	2009-03-02/17:37:03	Union	Quant 0d--200902 vs 1d--20090	T-Test 0d--200902 vs 1d--20090
Intersection of all auto sets		BoolN	76	2009-03-02/17:37:03	Intersection	Quant 0d--200902 vs 1d--20090	T-Test 0d--200902 vs 1d--20090
Quant 0d--200902 vs 1d--20090		Quant	449	2009-03-02/17:37:03	Outside limits	0d--2009022	1d--20090
T-Test 0d--200902 vs 1d--20090		Stati	209	2009-03-02/17:37:03	Student's t-test	0d--2009022	1d--20090
selected spot	postmortem 6h	Arbit	8	2009-08-29/20:46:27	No spots		

Fig. 1: Two-dimensional model electrophoresis protein pattern of pork muscles (6 h). 2-DE of skeletal muscles from Jinhua and DLY pigs at postmortem 6 h were compared

RESULTS

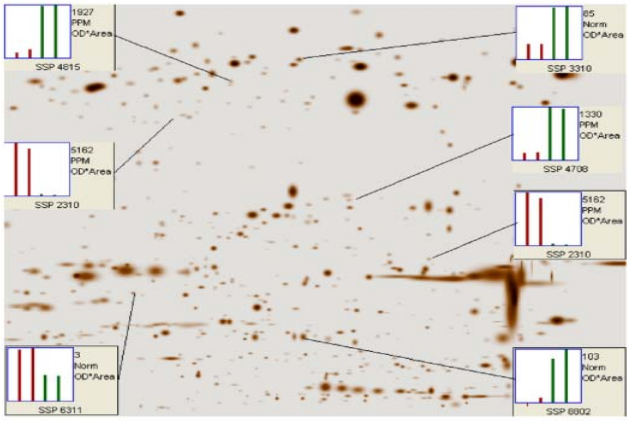
D Gel electrophoresis and analysis: Figure 1 to 4 respectively show two dimensional gel electrophoresis of skeletal muscles from different breed of pigs during postmortem time (6, 24, 48 and, 72 h, respectively). As shown in the figures, the molecular weights of proteins of porcine muscles at different time are mainly at the range of 10 kDa~90 kDa. With the help of PDQuest 2-D analysis software, 27 different protein spots were found, of which 16 proteins were over-expressed in Jinhua pork compared with DLY pork. 12 protein spots which were statistically significant ($p<0.05$) were selected for the subsequent mass spectrometry analysis.

Identification of the differentially expressed proteins: The proteins in the gel plugs were digested with trypsin, which cleaves peptide bonds only on the



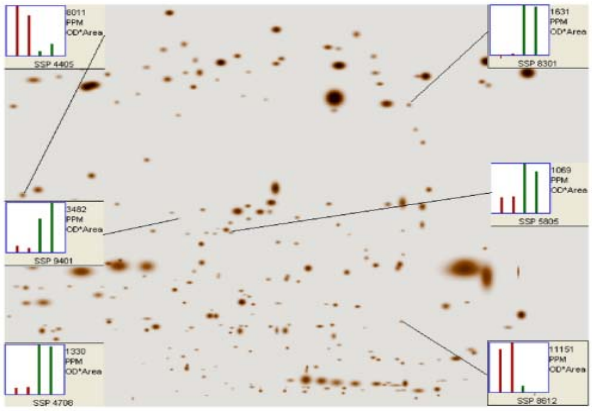
Analysis Set	Description	Type	Count	Created	Method	Operand A	Operand B
Union of all auto sets		Booln	256	2009-08-27/10:19:26	Union	Quant 0d---20090 vs 8d---20090	T-Test 0d---20090 vs 8d---20090
Intersection of all auto sets		Booln	73	2009-08-27/10:19:26	Intersection	Quant 0d---20090 vs 8d---20090	T-Test 0d---20090 vs 8d---20090
Quant 0d---20090 vs 8d---20090		Quant	173	2009-08-27/10:19:26	Outside limits	0d---20090	8d---20090400
T-Test 0d---20090 vs 8d---20090		Stati	156	2009-08-27/10:19:26	Student's t-test	0d---20090	8d---20090400
jinhua vs du	postmortem 24h	Arbit	5	2009-08-29/20:52:48	No spots		

Fig. 2: Two-dimensional model electrophoresis protein pattern of pork muscles (24 h). 2-DE of skeletal muscles from Jinhua and DLY pigs at postmortem 24 h were compared



Analysis Set	Description	Type	Count	Created	Method	Operand A	Operand B
Union of all auto sets		Booln	256	2009-08-27/10:19:26	Union	Quant 0d---20090 vs 8d---20090	T-Test 0d---20090 vs 8d---20090
Intersection of all auto sets		Booln	73	2009-08-27/10:19:26	Intersection	Quant 0d---20090 vs 8d---20090	T-Test 0d---20090 vs 8d---20090
Quant 0d---20090 vs 8d---20090		Quant	173	2009-08-27/10:19:26	Outside limits	0d---20090	8d---20090400
T-Test 0d---20090 vs 8d---20090		Stati	156	2009-08-27/10:19:26	Student's t-test	0d---20090	8d---20090400
postmortem 48h	postmortem 48h	Arbit	7	2009-08-30/12:17:01	No spots		

Fig. 3: Two-dimensional model electrophoresis protein pattern of pork muscles (48 h). 2-DE of skeletal muscles from Jinhua and DLY pigs at postmortem 48 h were compared



Analysis Set	Description	Type	Count	Created	Method	Operand A	Operand B
Union of all auto sets		Booln	446	2009-08-27/10:31:31	Union	Quant 0d- vs 1d-	T-Test 0d- vs 1d-
Intersection of all auto sets		Booln	71	2009-08-27/10:31:31	Intersection	Quant 0d- vs 1d-	T-Test 0d- vs 1d-
Quant 0d- vs 1d-		Quant	314	2009-08-27/10:31:31	Outside limits	0d-	1d-
T-Test 0d- vs 1d-		Stati	203	2009-08-27/10:31:31	Student's t-test	0d-	1d-
duchanda vs jinhua	huang	Arbit	6	2009-08-29/20:58:05	No spots		

Fig. 4: Two-dimensional model electrophoresis protein pattern of pork muscles (72 h). 2-DE of skeletal muscles from Jinhua and DLY pigs at postmortem 72 h were compared

Table 2: PMF database searching results of protein spots between DLY pig and jinhua pig

Types of protein	Protein score	Reference species	Mw	Intensity matched
6 h post-mortem				
Sarcolumenin	52	Rat	17974.7	10.678
Leucine rich repeat	88	Bovine	20265.6	20.816
Heat shock protein(27KD)	141	Pig	14211.2	44.636
24 h post-mortem				
Adenylate kinase	96	Pig	21621.3	17.583
26S protease regulatory subunit	110	Rat	48413.0	27.512
ATP phosphoribosyltransferase	67	Human	23027.1	6.709
48 h post-mortem				
desmin	105	Pig	53465.2	17.187
Zinc fingering protein	62	Mouse	39829.2	21.181
β-enolase	74	Pig	46864	24.498
72 h post-mortem				
Pyruvate kinase	158	Pig	57844.9	29.392
Glycogen phosphorylase	111	Rabbit	97096.8	34.127
NADH-ubiquinone oxidoreductase	86	Bovine	49142.6	27.162

N-terminal side of lysine and arginine residues. The resulting peptide mixtures were eluted from the gel plugs and the peptide masses were measured by MALDITOF MS. The information of the specific peptide masses was used for searching alignments to proteins found in theoretic digest databases in order to identify the 12 proteins and peptides. Porcine as well as other mammalian sequence databases were used in these searches. This approach allowed the identification of 12 of the 16 selected spots. The 12 proteins identified were differentially expressed at 6, 24, 48, 72 h post-mortem Table 2. Four of 12 proteins are heat shock protein, desmin, enolase and pyruvate kinase respectively which come from the pork protein group. While the other 8 proteins are from the groups of human, rat and other animals, the reason of which may be the incomplete of the pork proteomics database. Whether the function of the 8 proteins in the pig is consistent with it in other animal species remains unknown and needs further research.

Quantitative real-time PCR verification of differentially expressed proteins: Four genes corresponding to the protein spots, heat shock protein, desmin, enolase and pyruvate kinase were chosen for quantitative real-time PCR analysis to quantify their transcript levels. The real time PCR results were consistent with those of the 2-DE studies and suggested that these proteins identified as differentially expressed were regulated at transcriptional level.

DISCUSSION

In the present study, we have compared the differential proteins between Jinhua pigs and DLY pigs during postmortem time (6, 24, 48 and 72 h,

respectively). From the results, the relationship between meat quality and muscle protein degradation during postmortem storage can be analyzed, which can provide valuable information for understanding the molecular mechanism responsible for breed specific differences in meat quality.

Heat shock protein (27 kDa, HSP 27) is highly expressed in the muscle of Jinhua pig at 6 h postmortem. The functions of HSP27 are mainly including stabling the signal transduction of actin filaments and cytokine, as well as enhancing the resistance to the various stresses as molecular chaperones (Bao *et al*., 2009; Zhang *et al*., 2011). The high expression of HSP27 can provide Jinhua pigs' tissues and cells effective resistance to the stress responses. At the beginning of the protein denaturation, HSP combines with myofibrillar protein which plays a role in protection and preventing the enzymes to decompose the myofibrillar. Pulford reported that the expression amount of heat shock protein reached its peak value at 3 h postmortem. He also studied the correlation between the protein decomposition and the amount of HSP, finding that small molecular weight heat shock protein effectively inhibits the decomposition of desmin (Pulford *et al*., 2008a, b). Some researchers observed HSP27 expressed differently in the different quality grades of beef (Kim *et al*., 2008). Others have shown that the contents of HSP in the fresh pork is highly correlated with its sensory and quality (Morzel *et al*., 2008). The heat shock protein of small molecular weight can effectively delay the rupture of membrane, thereby preventing the release of endogenous proteases, reducing the water loss rate of muscles, slowing down the release of flavor substances and thus improving the tenderness of pork. With the extension of post-mortem storage time, the content of heat shock protein decreased resulting in unable to maintain the cells integrity and the repair function of certain proteins, such as desmin, myoplasm and myofibril.

The content of desmin in Jinhua pigs was higher than that in DLY pigs ($p<0.05$). Desmin is distributed around Z line, connecting the neighboring filaments to be arranged in a structure of highly sophisticated, which plays an important role in maintaining muscle cell skeleton integrity. During the post-mortem conditioning, the degradation of desmin and troponin T is the typical characteristic of protein changes. The degradation of desmin is highly correlated with the tenderness and water-holding capacity of post-mortem muscle (Van de Wiel and Zhang, 2007). The water-holding capacity, shelf-life, antioxidant capacity of post-mortem meat in Jinhua pigs were better than those in DLY pigs ($p<0.05$) which may be correlated with the high expression of HSP and desmin.

Enolase is a kind of rate-limiting enzyme, which not only can catalyze the conversion of 2-phosphate-D-glycerate (PGA) to phosphoric acid-pyruvate (PEP) during glycolysis but also can catalyze the reverse reaction in the process of glycogen synthesis (Bolten *et al*., 2008). Pyruvate Kinase (PK) is one of the three key enzymes in the process of anaerobic glycolysis which can catalyze the conversion of phosphoric acid-pyruvate (PEP) to pyruvicacid (Lametsch *et al*., 2002). The pyruvicacid generated can be catalyzed to lactic acid by lactic dehydrogenase (LDH), at the same time NADH is oxidated to NAD+ (Picariello *et al*., 2006). The expression level of enolase in Jinhua pigs was higher than that in DLY pigs ($p<0.05$), while pyruvate kinase in DLY pigs was higher ($p<0.05$), the results of which were that anaerobic metabolism was accelerated and muscle glycogen was mostly hydrolyzed in post-mortem pork of DLY pigs compared with Jinhua pigs.

Glycogen phosphorylase is a kind of complex allosteric enzyme which can catalyze the degradation of glycogen (Guo *et al*., 2011; Lametsch *et al*., 2003). Adenylate kinase is a kind of monomeric enzyme which indirectly involved in cellular activities as well as in the process of apoptosis through regulating the value of ATP, GTP and ADP in vivo (Theron *et al*., 2011). 26S proteasome is part of the ubiquitin system (UPS) which is closely related with variety of cellular activities. 26S proteasome plays an important role in the process of tenderization of the post-mortem muscles. It is reported that the Z-disk and I-line of beef fiber were rapidly destroyed when 26S proteasome was added (Dutaud *et al*., 2006). After 24 h debris and residue were produced and α-actin was dissolved (Robert *et al*., 1999). Arcolumenin is a kind of sarcoplasmic reticulum protein which plays a role in the maintenance of the structure of calcium channels in the membrane (Fan *et al*., 2008). Zinc finger protein plays an important role in the regulation of related genes (Ren *et al*., 2012).

CONCLUSION

This study showed that some proteins (HSP 27, Desmin, Enolase, Pyruvate kinase and so on) were expressed differentially in post-mortem pork of Jinhua pigs compared with DLY pigs ($p<0.05$), which maybe highly associated with meat quality and needs further research. Our findings may help future meat science projects focus on novel post-mortem processes that maybe relevant to meat quality.

ACKNOWLEDGMENT

This study was financially supported by the program of National innovation project (GJ20141007), Zhejiang Xinmiao project (2014R408023).

REFERENCES

Bao, E., K.R. Sultan, N. Bernhard and J. Hartung, 2009. Expression of heat shock proteins in tissues from young pigs exposed to transport stress. Deut. Tierarztl. Woch. , 116: 321-325.

Beaulieu, A.D., J.L. Aalhus, N.H. Williams and J.F. Patience, 2010. Impact of piglet birth weight, birth order and litter size on subsequent growth performance, carcass quality, muscle composition and eating quality of pork. J. Anim. Sci., 88: 2767-2778.

Bendixen, E., 2005. The use of proteomics in meat science. Meat Sci., 71: 138-149.

Bolten, K.E., A.E. Marsh, S.M. Reed, J.P. Dubey, R.E. Toribio and W.J. Saville, 2008. Sarcocystis neurona: Molecular characterization of enolase domain I region and a comparison to other protozoa. Exp. Parasitol., 120: 108-112.

Dutaud, D., L. Aubry, M.A. Sentandreu and A. Ouali, 2006. Bovine muscle 20S proteasome: I. Simple purification procedure and enzymatic characterization in relation with postmortem conditions. Meat Sci., 74: 327-336.

Fan, G.C., Q. Yuan and E.G. Kranias, 2008. Regulatory roles of junctin in sarcoplasmic reticulum calcium cycling and myocardial function. Trends Cardiovas. Med., 18: 1-5.

Guo, J., T. Shan, T. Wu, L.N. Zhu, Y. Ren, S. An and Y. Wang, 2011. Comparisons of different muscle metabolic enzymes and muscle fiber types in Jinhua and Landrace pigs. J. Anim. Sci., 89: 185-191.

Hollung, K., E. Veiseth, X. Jia, E.M. Faergestad and K.I. Hildrum, 2007. Application of proteomics to understand the molecular mechanisms behind meat quality. Meat Sci., 77: 97-104.

Kim, N.K., S. Cho, S.H. Lee, H.R. Park, C.S. Lee, Y.M. Cho, Y.H. Choy, D. Yoon, S.K. Im and E.W. Park, 2008. Proteins in longissimus muscle of Korean native cattle and their relationship to meat quality. Meat Sci., 80: 1068-1073.

Lametsch, R. and E. Bendixen, 2001. Proteome analysis applied to meat science: Characterizing postmortem changes in porcine muscle. J. Agr. Food Chem., 49: 4531-4537.

Lametsch, R., P. Roepstorff and E. Bendixen, 2002. Identification of protein degradation during post-mortem storage of pig meat. J. Agr. Food Chem., 50: 5508-5512.

Lametsch, R., A. Karlsson, K. Rosenvold, H.J. Andersen, P. Roepstorff and E. Bendixen, 2003. Postmortem proteome changes of porcine muscle related to tenderness. J. Agr. Food Chem., 51: 6992-6997.

Lametsch, R., P. Roepstorff, H.S. Moller and E. Bendixen, 2004. Identification of myofibrillar substrates for mu-calpain. Meat Sci., 68: 515-521.

Miao, Z.G., L.J. Wang, Z.R. Xu, J.F. Huang and Y.R. Wang, 2009. Developmental changes of carcass composition, meat quality and organs in the Jinhua pig and Landrace. Animal, 3: 468-473.

Morzel, M., C. Terlouw, C. Chambon, D. Micol and B. Picard, 2008. Muscle proteome and meat eating qualities of Longissimus thoracis of "Blonde d'Aquitaine" young bulls: A central role of HSP27 isoforms. Meat Sci., 78: 297-304.

Picariello, G., A. De Martino, G. Mamone, P. Ferranti, F. Addeo, M. Faccia, S. Spagnamusso and A. Di Luccia, 2006. Proteomic study of muscle sarcoplasmic proteins using AUT-PAGE/SDS-PAGE as two-dimensional gel electrophoresis. J. Chromatogr. B, 833: 101-108.

Pulford, D.J., P. Dobbie, S. Fraga Vazquez, E. Fraser-Smith, D.A. Frost and C.A. Morris, 2008a. Variation in bull beef quality due to ultimate muscle pH is correlated to endopeptidase and small heat shock protein levels. Meat Sci., 83: 1-9.

Pulford, D.J., S. Fraga Vazquez, D.F. Frost, E. Fraser-Smith, P. Dobbie and K. Rosenvold, 2008b. The intracellular distribution of small heat shock proteins in post-mortem beef is determined by ultimate pH. Meat Sci., 79: 623-630.

Ren, A., H. Zhang, Z. Xie, X. Ma, W. Ji, Z. He *et al*., 2012. Regulation of hippocampus-dependent memory by the zinc finger protein Zbtb20 in mature CA1 neurons. J. Physiol., 590: 4917-32.

Robert, N., M. Briand, R. Taylor and Y. Briand, 1999. The effect of proteasome on myofibrillar structures in bovine skeletal muscle. Meat Sci., 51: 149-153.

Theron, L., T. Sayd, J. Pinguet, C. Chambon, N. Robert and V. Sante-Lhoutellier, 2011. Proteomic analysis of semimembranosus and biceps femoris muscles from Bayonne dry-cured ham. Meat Sci., 88: 82-90.

Van de Wiel, D.F. and W.L. Zhang, 2007. Identification of pork quality parameters by proteomics. Meat Sci., 77: 46-54.

Zellner, M., R. Babeluk, L.H. Jakobsen, C. Gerner, E. Umlauf, I. Volf, E. Roth and J. Kondrup, 2011. A proteomics study reveals a predominant change in MaoB expression in platelets of healthy volunteers after high protein meat diet: Relationship to the methylation cycle. J. Neural Transm., 118: 653-662.

Zhang, M., Y. Lv, Z. Yue, A. Islam, B. Rehana, E. Bao and J. Hartung, 2011. Effects of transportation on expression of Hsp90, Hsp70, Hsp27 and alphaB-crystallin in the pig stomach. Vet. Rec., 169: 312.

Zheng, X., L. Hong, L. Shi, J. Guo, Z. Sun and J. Zhou, 2008. Proteomics analysis of host cells infected with infectious bursal disease virus. Mol. Cell. Proteomics, 7: 612-625.