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A COMPARATIVE STUDY OF *SUS SCROFA M. LONGISSIMUS DORSI* WITH DIFFERENT CHANGES IN QUALITY

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ABSTRACT

The comparative study of *Sus scrofa* muscle tissue with different defects of quality was carried out. Such analysis methods as determination of water-binding capacity (WBC) and pH values, microstructural studies, proteome methods, mass spectrometric methods, high performance liquid chromatography (HPLC) and determine the concentration of cathepsin D, calpain 3 and myoglobin were applied in current study. DFD meat was characterized by high pH (6.2) and WBC (93.18%), while PSE – low pH (5.5) and WBC (79.19%). pH and WBC values in NOR pork was medium and averaged 5.9 and 92.05%, respectively. Microstructural studies revealed that fiber in exudative pork was the least and averaged 39.7 μm, normal pork was characterized by diameter of muscle of 45.5 μm, while DFD pork - 48.3 μm. Protein composition of *Sus scrofa* muscle tissues were also investigated by one-dimensional and two-dimensional electrophoresis. 6 protein fractions were identified by mass spectrometry and confirmed as potential biomarkers of pork quality defects in meat and processes meat product. It also has been shown that PSE pork contains 8 characteristic peptides, NOR – 14, DFD – 18. Cathepsin D, calpain 3 and myoglobin content were measured in pork of different categories. The highest content of cathepsin D, calpain 3 and myoglobin was noticed in PSE pork and averaged 82.16 ±3.30 $ng.mL^{-1}$; 1.280 ±0.082 $ng.mL^{-1}$; 3.973 ±0.506 $ng.mL^{-1}$, respectively. Since the study was carried out on samples of meat 24 hours after slaughter, this work had the prospect to further study of meat with different defects of quality during long-term autolysis.

Keywords: muscle proteins; pork; PSE; DFD; electrophoresis

INTRODUCTION

Proteomic studies of *Sus scrofa* muscle tissues composition are of considerable interest for many reasons. The most important reason is pork predominant use in the production of meat and meat products in many countries **(De Almeida and Bendixen, 2012)**. Moreover, this species is a quite good object for biomedical problems science studies. Therefore, proteomics of muscle tissue is important for determining the quality of raw materials **(Kovalyov et al., 2013)**. To date, there are three main quality defect of meat: normal meat (NOR), meat with high ultimate pH (DFD – dark, firm, dry) and exudative meat (PSE – Pale, Soft, Exudative) with low pH values.

Special attention is paid to exudative meat. Currently, exudative pork is spread in all countries and ranged from 5 to 40% of the total volume of pork. The main causes of exudative pork (PSE meat) are reduction in organism adaptation to increased load, nervous excitement of animals in pre-slaughter period as well as pigs overcrowding in the household **(Kopeikina and Chudzickaya, 2005)**. *M. longissimus dorsi* are the most prone to exudative changes. It's changed in color and structure faster than other; therefore samples of *M. longissimus dorsi* were object of the study.

Scientific hypothesis

The aim of the study was to investigate different meat types (PSE, DFD and NOR) in order to identify potential protein markers of quality defects as pork quality control parameters.

MATERIAL AND METHODOLOGY

The objects of study were the samples of *Sus scrofa M. longissimus dorsi* (OOO «Wepoz – torgovyi dom», Rostov, Russiawith a various defects of quality (NOR, PSE, DFD). The samples were stored at temperature 2 ±2 °C in industrial refrigerator for 24 h before experiments.

The value of pH was measured by potentiometric method on pH-ionometer "Expert" in accordance with GOST R 51478-99 (ISO 2917-74) "Test method for the determination of hydrogen ion concentration (pH)".

The value of WBC was measured by Grau-Hamm method (press techniques). The moisture in the raw meat were determined by drying a sample with sand till constant weight at a temperature of (103 ±2) °C in accordance with

GOST R 51479-99 (ISO 1442-97) "Meat and meat products. The method of determination of moisture mass content".

Microstructural studies were carried out according to GOST 19496-1993 "Meat. The method of histological study". Samples were fixed in 15% neutral formalin solution for 48 h. After fixation samples were washed in running water, slices were prepared on freezing microtome-cryostat with thickness of 20 μm. Slices were stained with Ehrlich's hematoxylin followed by staining by 1% eosin solution. The examination of slises was carried out using a system of image analysis Motik (China) with the increase in 360 times.

One-dimensional (1D) electrophoresis was performed according to the method of Laemmli **(Laemmli, 1970)** under denaturing conditions in 12.5% polyacrylamide gel with the presence of SDS. The marker was used comprising of eleven standards (recombinant proteins) "Thremo" (USA).

Two-dimensional (2D) electrophoresis was performed according to the method of O'Farrell **(O'Farrell, 1975)** with isoelectric focusing in ampholine pH gradient (IEF-PAGE). The subsequent detection of the proteins was carried out by staining with Coomassie Brilliant Blue R-250. Identification of protein fractions was performed on DE after trypsinolysis by MALDI-TOF/MS and MS/MS mass spectrometry on Ultraflex MALDI-TOF mass spectrometer (Bruker, Germany) with UV laser (336 nm) in the positive ion mode in molecular weight range of 500-8000 Da with calibration according to known peaks of trypsin autolysis. Analysis of obtained tryptic peptides mass spectra was performed using Peptide Fingerprint option in Mascot software (Matrix Science, USA) with MH+ mass determination accuracy of 0.01%; search was performed in databases of the National Center for Biotechnology Information, USA (NCBI).

Analysis of the peptide profile was carried out on a system of high performance liquid chromatography (HPLC) with t mass spectrometer (liquid chromatograph AGILENT 1200 C with a mass selective detector, AGILENT 6410, USA).

The concentration of cathepsin D, calpain 3 and myoglobin were measured by enzyme-linked immunosorbent assay (ELISA) on a microplate reader, ImmunoChem 2100 (High Technology INC, Walpole, MA USA) using a microplate, Thermo-Shaker Immunochem-2200 (High Technology INC, Walpole, MA USA) using commercial Elabscience Biotechnology Co., Ltd (China, Guandong Science and Technology).

Statisic analysis

STATISTICA 10.0 software was used in this study for the statistical analyses. Significant differences were tested by using two-way analysis of variance (ANOVA), followed by Duncan's test. Differences with *p*-values less than 0.05 were considered as statistically significant.

RESULTS AND DISCUSSION

Samples of *Sus scrofa M. longissimus dorsi* were selected according to pH values, as this value largely reflected on mechanism of quality (PSE, DFD and NOR) defects

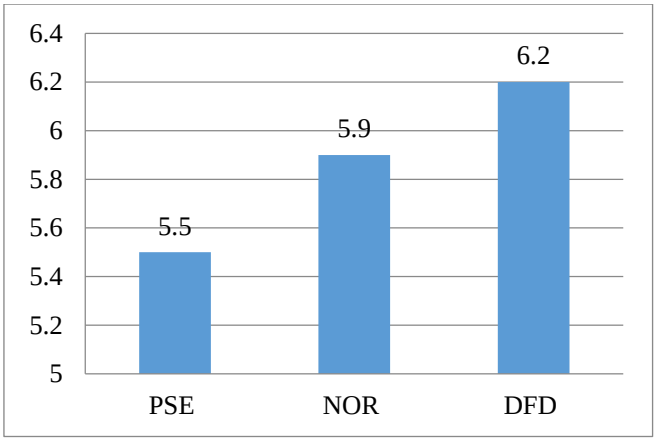

Figure 1 The values of pH in pork *M. longissimus. dorsi* (PSE, NOR, DFD).

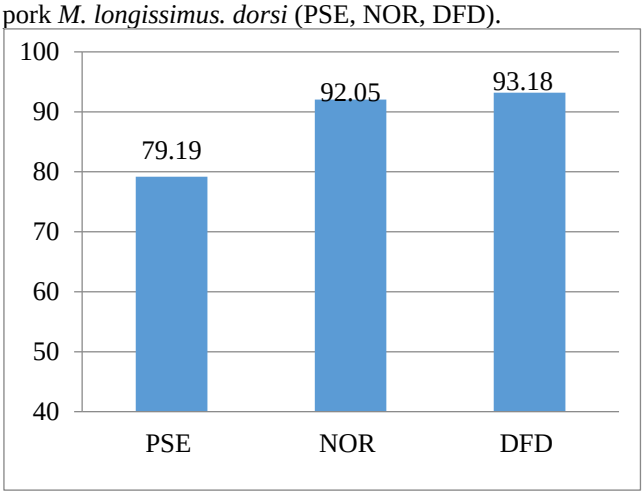

Figure 2 WBC, % to total humidity in pork *M. longissimus. dorsi* (PSE, NOR, DFD).

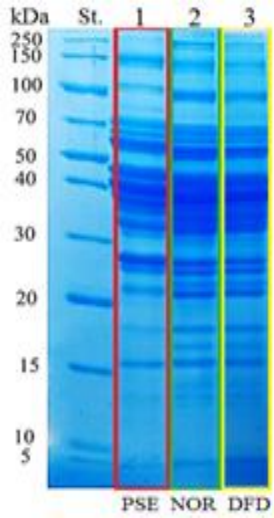

Figure 3 1D electrophoresis of pork *M. longissimus. dorsi* (PSE, NOR, DFD).

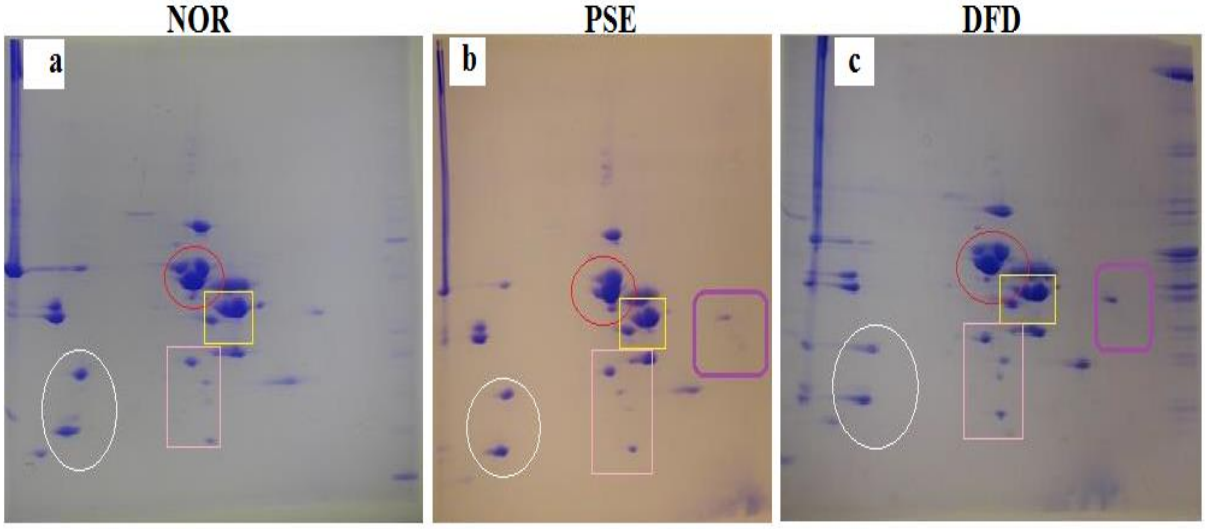

Figure 4 2D electrophoresis of pork *M. longissimus. dorsi* (PSE, NOR, DFD).

formation **(Bazhov et al., 2013)**. Significant differences in pH values were determined between pork samples (Figure 1).

Nevertheless, 24 h after slaughter the pH value of meat samples corresponded to the norms for PSE-, NOR-, DFD-pork categories. These results were also correlated with means described in work **Adzitey and Nurul (2011)**.

Water binding capacity (WBC) is parameter useful for characterization of meat quality. WBC was higher in DFD pork than in normal and exudative pork samples (Figure 2). Low WBC in PSE pork and high WBC in DFD pork were noticed in study of **Poznyakovskiya et al. (2015)**.

According to microstructural studies, the smallest diameter of muscle fibers was noticed in exudative pork sample – a 39.7 μm. Obtained result corresponded to low WBC value. Diameter of muscle fibers in normal pork was 45.5 μm, while in DFD – 48.3 μm.

Proteomic study (1D electrophoresis) revealed noticeable differences in fractional composition of meat with various qualities, mainly in the range of from 15 kDa to 40 kDa. Thus, fractions intensity of meat with PSE characteristics was relatively less than in DFD and NOR meat (Figure 3). Revealed results correlated with the data observed **Poznyakovskiya at el. (2015)** and **Shipulin (2009)**, but noticed differences in treks were detected till 50kDa.

Proteomic analysis of *Sus scrofa* muscle tissue showed the presence of tissue-specific proteins nature (Figure 4, Table 1). Intensity of pyruvatekinase (58.0 kDa) reduced in meat with PSE characteristics compared with NOR, while in DFD meat this fraction was almost absent. Intensity of creatine kinase M-type (41.0 kDa) and glyceraldehyde-3-phosphate dehydrogenase (34.0 kDa) uniformly decreased from NOR till DFD. Adenylate kinase isoenzyme 1 isoform X2 (21,0 kDa) was most noticeable in PSE meat compared with NOR and DFD meat, while intensity of myosin regulatory light chain 2 and skeletal muscle isoform (15,0 kDa), on the contrary, was less. **Luca el al. (2013)** also identified such pork muscle proteins as creatine kinase M-type, adenylate kinase and alpha-enolase, but did not describe its intensity according to type of autolysis in pork.

The effect of autolysis on cathepsin D, calpain 3 and myoglobin levels in pork with different quality defects was also evaluated. It was shown that 24 h after the slaughter the highest content of cathepsin D was observed in PSE pork (82.16 ±3.30 ng.mL^{-1}) and was higher NOR and DFD by 22.2% (p <0.05) and 41.4% (p <0.05), respectively. Cathepsin D level in DFD pork was lower than in normal

Table 1 The results of mass spectrometric identification (MALDI-TOF/MS and MS/MS) of protein fractions in *Sus scrofa* samples with different post-mortem autolytic changes.

No.	Protein name; (Gene symbol)	S/M/C*	mM.pI** (experiment)	mM/pI** (calculation)
1	pyruvate kinase PKM isoform X6 (PKM2)	242/92/82	58.0/6.80	58.0/7.62
2	alpha-enolase (ATP5A1)	368/51/85	52.0/5.80	47.0/6.44
3	creatine kinase M-type (CKM)	234/66/	41.0/6.60	43.0/6.61
4	glyceraldehyde-3-phosphate dehydrogenase (GPDH)	399/60/82	34.0/7.30	35.8/8.51
5	adenylate kinase isoenzyme 1 isoform X2 (AK1)	264/32/83	21.0/6.70	21.6/8.38
6	myosin regulatory light chain 2, skeletal muscle isoform (HUMMLC2B)	452/47/88	15.0/4.65	19.0/4.90

Note:
*S/M/C – traditional identification indicators adopted in the English literature: Score – indicator of conformity or "scorecard"; Match peptides – the number of matched peptides; Coverage – % coverage of the entire amino acid sequence of the protein by identified peptides.
** mM/pI (experiment) – scores obtained as a result of electrophoretic mobility on the DE and mM/pI (calculation) – estimates made based on amino acid sequence data with consideration of signal peptide removal, but with no consideration of other post-synthetic modifications using the ExPASy Compute pI/Mw tool software.

Table 2 Cathepsin D, calpain 3 and myoglobin levels in pork *M. longissimus. dorsi* (PSE, NOR, DFD).

	Cathepsin D, pg.mL^{-1}	Calpain 3, ng.mL^{-1}	Myoglobin, ng.mL^{-1}
PSE	82.16 ±3.30*,+	1.280 ±0.082+	3.973 ±0.506*
NOR	67.24 ±1.61+,#	1.135 ±0.042	2.306 ±0.152+,#
DFD	58.12 ±3.62*,#	1.014 ±0.059#	3.672 ±0.528*

Note:

* – Significant difference when compared with NOR meat (p <0.05); # – Significant difference when compared with PSE meat (p <0.05); + – Significant difference when compared with DFD meat (p <0.05).

meat by 13.6% (p <0.05) (Table 2). **Flores and Toldra (2014)** in their work published results concerning cathepsins and calpains activity. They observed that the activity of autolytic enzymes depended on pork type.

Analysis of peptide composition of PSE-, NOR-, DFD-pork categories showed that the least number of peptides was noticed in PSE pork, while the greatest number of low molecular weight compounds was revealed in DFD pork. Presumably, observed data is a result of high activity of proteolytic enzymes in PSE meat, which maintain a dynamic balance. The main differences between the types of meat were observed in areas of more than 1000 Da, in ranges 600-700Da and 900- 999Da. Moreover, PSE pork was characterized by absence of peptides with molecular weight more 1000Da. In all pork categories 28 constant peptides were found. By the way, 8 specific peptides were detected in exudative pork. 14 – in normal and 18 – in DFD.

CONCLUSION

The comparative study of *Sus scrofa* muscle tissue with different defects of quality was carried out. It was revealed that pH, water-binding capacity and microstructural studies were correlated. Thus, low pH value as well as low WBS corresponded to smaller muscle fiber diameter.

Proteins of *Sus scrofa* muscle tissue for all quality defects were separated and characterized, 6 fractions of them were identified. Moreover, 8 specific peptides were detected in PSE pork, 14 – in NOR and 18 – in DFD. It was shown that the highest content of cathepsin D was observed in PSE pork (82.16 ±3.30 ng.mL^{-1}) and was higher NOR and DFD by 22.2% (p <0.05) and 41.4% (p <0.05), respectively. Since the study was carried out on samples of meat 24 hours after slaughter, this work had the prospect to further study of meat with different defects of quality during long-term autolysis.

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