PE1.05 Changes during the early postmortem storage period of structural proteins in bovine longissimus thoracis muscle 74.00

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***Abstract*— The changes of structural proteins in bovine *longissimus thoracis* muscle during the first 48 h *postmortem* were studied by proteomics. A total of 35 proteins were found to change from 1 h to 48 h *postmortem*. The 30 identified proteins could be classified into three different classes; metabolic enzymes, cellular defense/stress proteins and cell structure proteins.**

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***Index Terms*—MALDI-TOF MS, muscle proteins, postmortem, proteomics.**

I. INTRODUCTION

Tenderness and juiciness are important product qualities for the meat industry. The protein composition of raw materials can vary, and this will have an effect on functional properties and final product quality. To be able to control this variation, more knowledge is needed about the mechanisms underlying the variation and the possibilities of controlling the variation.

The aim of this study was to map and identify changes in structural proteins in bovine *longissimus thoracis* muscle during *postmortem* storage. Proteome changes of water soluble proteins have previously been analysed for the same samples [1]. Ultimately, the changes will be compared to the quality of the final product. Hence the goal is to find biomarkers that can be related to meat tenderness and juiciness.

II. MATERIALS AND METHODS

a. Animals and Sampling

The experiment included two sampling times after slaughter, 1 h and 48 h *postmortem*, from a total of eight NRF (Norwegian Red) young bulls. The hot boned M. *longissimus thoracis* (LT) were packed and kept at 12 °C for the first 10 h *postmortem* and at 4 °C for the rest of the storage period. A piece of muscle tissue was taken at 1 and 48 h *postmortem*, snap frozen in liquid nitrogen, and stored at -80 °C until further analysis.

b. Two-Dimensional Gel Electrophoresis (2-DE)

Proteins were extracted from the LT muscle and separated by 2-DE. In order to remove all the soluble muscle proteins, frozen muscle tissue was dissolved and washed in TES-buffer (10 mM Tris, pH 7.6, 1 mM EDTA and 0.25 M sucrose). The resulting pellet was then dissolved in UREA-buffer (7 M urea, 2 M thiourea, 2 % CHAPS and 1 % DTT). The 2-DE was performed according to methods described previously [2].

Protein separation in the first dimension was performed on immobilized pH gradient (IPG) strips, 24 cm, spanning the pH region 5-8. For analytical 2-DE, 150 µg proteins were loaded onto each IPG strip by in-gel rehydration overnight at room temperature. For preparative 2-DE, 500-1000 µg proteins were loaded onto the IPG strips. In the second dimension proteins were separated on 12.5% SDS-PAGE. Analytical gels were stained with Krypton staining, while preparative gels were silver stained according to previously described methods [3]. For repeated identification of the proteins several preparative gels were made.

c. Image Analysis and Data Analysis

Comparative image analysis by Progenesis SameSpot (version 3.1) included the proteins in the molecular mass region of 10-75 kDa, and the pH range 5-8. The expression patterns of samples taken at 1h and 48 h *postmortem* were compared.

The data set was imported into 50-50 MANOVA in order to run significance test for each spot, where matching spots and time points were defined as x- and y-variables, respectively. Rotation test was used for calculation of p-values, where spots having $p \leq 0.05$ were considered as significantly changing over the first 48 h *postmortem*.

d. Identification of Proteins

Protein spots changing significantly over time were excised from silver stained preparative gels and extracted from the gels according to previously described methods [4]. After purification and

concentration of the digested proteins, they were analyzed by an Ultraflex MALDI-TOF/TOF mass spectrometer followed by identification searching the National Center for Biotechnology Information nonredundant database (NCBInr) using the search engine MASCOT.

III. RESULTS AND DISCUSSION

Figure 1 shows a representative 2-DE pattern of the urea-soluble protein fractions extracted from the LT muscle. Image analysis allowed matching and relative quantification of 300 spots across all 32 gels (2 sampling times × 8 biological replicates (animals) × 2 technical replicates).

A total of 35 protein spots were found to change significantly with time, and the majority of them were increased in intensity at 48 h postmortem. 30 of these 35 spots have been identified by MS. The identified proteins could be classified into three different classes; metabolic enzymes, cellular defense/stress proteins and structural proteins. Proteins identified by MS are listed in Table 1.

Eight metabolic enzymes were identified; five of them having higher intensity after 48 h *postmortem* and three showing lower intensity. Four of the five proteins showing higher expression at 48 h *postmortem*, creatine kinase and adenylate kinase 1, are involved in the energy metabolism of the cell. Creatine kinase (fragment) and adenylate kinase 1 in the soluble fraction from these samples have previously been found to increase in abundance during *postmortem* [1]. Moreover, adenylate kinase 1 has been related to meat quality traits in pig meat [5].

All of the 12 cellular defense/stress proteins identified are members of the small heat shock protein family, majority of them being heat shock 27 kDa proteins. Six of the identified proteins in this class showed higher expression after 48 h *postmortem*, while the other six had lower intensity. It is believed that crystallin and Hsp27 are involved in regulation and stabilization of the myofibrillar proteins, as well as the protection of actin filaments and other cytoskeletal proteins from fragmentation caused by stress conditions [6, 7]. The observation of higher expression of these proteins during *postmortem* storage of bovine muscle might therefore indicate that changes are taking place in the myofibrillar proteins.

From the group of structural proteins a total of ten proteins were identified. Three of them had higher intensity after 48 h *postmortem*, while the other proteins in this group showed lower expression. Four of the actin proteins seem to be fragments, and are probably a result of proteolysis occurring during the tenderisation phase.

Some of the proteins identified by MS have large differences between their experimental and theoretical pI and MW values. This indicates that the protein has undergone some kind of modification affecting its theoretical pI and MW values. This is the case for three of the heat shock 27 kDa proteins, as well as one of the actin proteins. Differences in experimental and theoretical MW value can also indicate that the identified protein is a fragment, which can be confirmed by looking at the peptide sequence from MS identification. This applies for several of the actin proteins.

IV. CONCLUSION

The majority of proteins changing during the early *postmortem* period are involved in the energy metabolism of the cell or in the regulation and stabilisation of cytoskeleton proteins. These findings indicate that the highly stressing conditions occurring during the early *postmortem* period initiates energy pathways and protective mechanisms in order for the cell to maintain homeostasis. The majority of the structural proteins changing over this time period were identified as actin. The changes observed in structural proteins may reflect proteolysis taking place during the tenderization phase.

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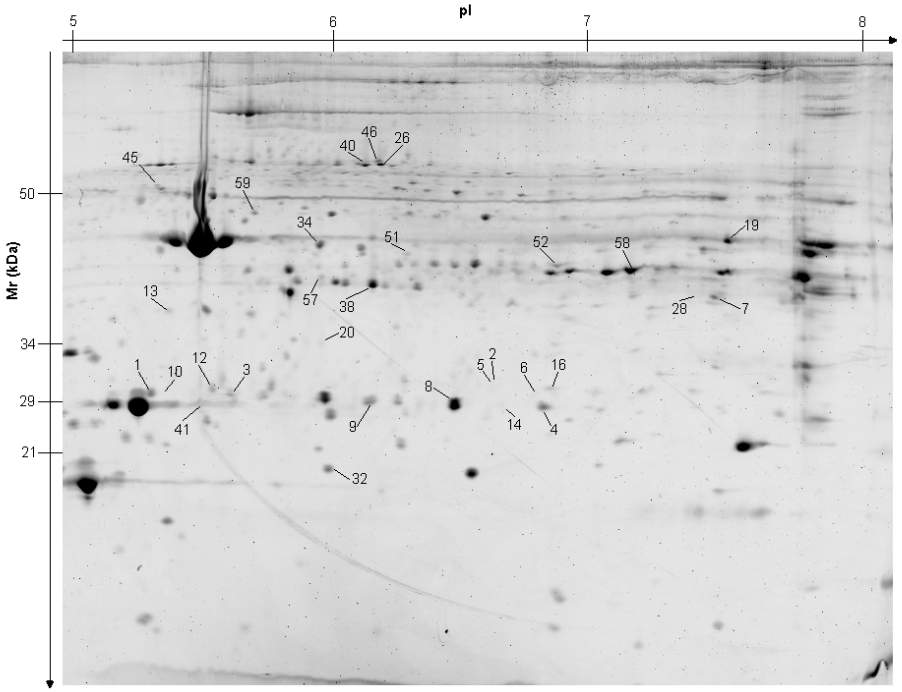

Figure 1. Representative 2-DE pattern of the urea-soluble protein fractions extracted from the *longissimus thoracis* muscle. Proteins changing significantly over the first 48 h *postmortem* and identified by MALDI-TOF/TOF MS are marked on the figure.

Table 1. Identified proteins from the *longissimus thoracis* muscle changing significantly over the first 48 h *postmortem*. Proteins showing higher expression after 48 h are marked with grey.

Spots	Identified proteins	Experimental pI/Mr	Matched peptides/ % sequence coverage	NCBI accession no. (source)	Theoretical pI/Mr
		Metabolic Enzymes			
6	adenylate kinase 1	7.00/25700	8/45	gi|61888850 (bovine)	8.40/21764
7	creatine kinase M chain	7.40/42800	11/30	gi|4838363 (bovine)	6.63/43172
16	creatine kinase M chain	7.10/27200	8/23	gi|4838363 (bovine)	6.63/43172
19	creatine kinase, muscle	7.40/50500	37/77	gi|60097925 (bovine)	6.63/43190
20	NADH dehydrogenase (ubiquinone) Fe-S protein 3, 30 kDa	6.20/28900	9/31	gi|27807353 (bovine)	6.25/30378
52	glycerol-3-phosphate dehydrogenase 1 (soluble)	7.00/45700	10/27	gi|78365297 (bovine)	6.42/38236
58	glycerol-3-phosphate dehydrogenase 1 (soluble)	7.20/46100	13/36	gi|78365297 (bovine)	6.42/38236
2	2,3-bisphosphoglycerate mutase	6.70/28400	9/37	gi|78369370 (bovine)	6.03/30271
		Cellular Defense/Stress Proteins			
4	crystallin, alpha B	7.20/24800	15/72	gi|27805849 (bovine)	6.76/20024
1	heat shock 27 kDa protein 1	5.60/26300	4/27	gi|71037405 (bovine)	5.77/22722
3	heat shock 27 kDa protein 1	5.90/26300	13/53	gi|71037405 (bovine)	5.77/22722
8	heat shock 27 kDa protein 1	6.60/26300	12/58	gi|71037405 (bovine)	5.77/22722
9	heat shock 27 kDa protein 1	6.30/26100	7/39	gi|71037405 (bovine)	5.77/22722
10	heat shock 27kDa protein 1	5.70/27000	9/48	gi|71037405 (bovine)	5.77/22722
12	heat shock 27 kDa protein 1	5.80/26300	11/49	gi|71037405 (bovine)	5.77/22722
14	heat shock 27kDa protein 1	6.70/26500	9/48	gi|71037405 (bovine)	5.77/22722
32	heat shock protein, alpha-crystallin-related, B6	6.20/19700	7/60	gi|119224088 (bovine)	5.95/17525
26	heat shock 70 kDa protein 1A	6.30/68300	27/46	gi|73853769 (bovine)	5.68/70530
40	heat shock 70 kDa protein 1A	6.30/68300	10/22	gi|73853769 (bovine)	5.68/70530
46	heat shock 70 kDa protein 1A	6.30/70000	27/44	gi|40254806 (bovine)	5.55/70492
		Cell Structure Proteins			
13	F-actin-capping protein subunit beta (CapZ beta)	5.60/36200	11/40	gi|13124696 (bovine)	5.36/31616
45	capping protein (actin filament) muscle z-line, beta	5.40/56400	14/42	gi|28603770 (bovine)	6.02/34176
57	capping protein (actin filament) muscle z-line, alpha 1	6.20/41000	10/54	gi|134085807 (bovine)	5.53/33082
5	actin, alpha 1, skeletal muscle	6.70/24900	14/60	gi|134024776 (bovine)	5.23/42338
34	actin, alpha 1, skeletal muscle	6.10/51400	7/19	gi|27819614 (bovine)	5.31/42451
41*	actin, alpha 1, skeletal muscle	5.80/26800	13/29	gi|27819614 (bovine)	5.31/42451
51	actin, alpha 1, skeletal muscle	6.40/46100	7/22	gi|134024776 (bovine)	5.23/42338
59	actin, alpha 1, skeletal muscle	5.90/53600	9/42	gi|134024776 (bovine)	5.23/42338
28	Troponin T fast skeletal muscle type	7.40/42800	11/35	gi|21039002 (bovine)	8.70/30684
38	Troponin T1, skeletal slow	5.70/40300	13/30	gi|41386697 (bovine)	5.71/31265

*possible C-terminal fragment