Effect of low voltage electrical stimulation on changes in proteome of bovine longissimus muscle during postmortem aging

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Abstract - **The objective was to determine the effect of postmortem electrical stimulation (ES) on changes in insoluble and soluble protein fractions of bovine longissimus dorsi muscle (LD). Eight steers (498 kg) were harvested; carcasses were split. One side was a control (NS) and the other was ES (100 V, 60 Hz for 1 minute). Proteins from each muscle were separated into soluble (low ionic strength buffer) and insoluble fractions. The effect of ES on muscle protein profile was evaluated with 2D difference in gel electrophoresis using a pH 3-10 or pH 4-7 gradient in the first dimension and a 12.5 % acrylamide SDS-PAGE gel in the second dimension. ES resulted in an accelerated pH decline of LD during the 24 h postmortem chilling period. ES had an apparent effect on protein solubility; fructose-bisphosphate aldolase A, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase 1, pyruvate kinase isozyme M1 were all less soluble in response to ES. However, annexin, pyruvate dehydrogenase and creatine kinase were all more abundant in the soluble fraction in response to ES. At 1 d postmortem, ES had a notable affect on the abundance of two isoforms of myosin light chain 2 (MLC2). In the insoluble fraction, one MLC2 spot increased while a separate MLC2 spot decreased in response to ES. Results demonstrate a clear effect on the apparent solubility of proteins. These trends may be useful indicators of postmortem metabolism in beef.**

Keywords: Beef, electrical stimulation, proteome

I. INTRODUCTION

Protein degradation and denaturation during postmortem storage of beef are known to influence beef quality, especially tenderness of beef. The early postmortem environment in muscle can have a significant effect on the processes that dictate fresh meat quality. It is known that the rate and extent of pH decline influences protein denaturation as well as enzyme function. Small changes in enzyme activity or protein solubility have the capacity to alter the rate and extent of changes in muscle and meat ultrasturcture.

For many years it was understood that protein changes were not significant until after several days of postmortem storage. However it is likely that this observation is due to the sensitivity of methods used. New approaches to determine protein modifications (solubility, denaturation, degradation) have provided insight in the response - at the molecular and cellular level - to changes in the early postmortem environment. These techniques have been focused on directed experiments focused on the activity of singular enzymes, inhibitors and substrates. The development of technologies to monitor changes in the entire proteome provides the means for a global inquiry of the extent to which the early postmortem environment causes changes in protein profile. Alteration in the postmortem environment by early postmortem electrical stimulation of beef carcasses is known to increase protein degradation and the rate of tenderization during postmortem storage [1]. In previous experiments [2] we have documented the early postmortem electrical stimulation results in a more rapid pH decline, more rapid μ-calpain autolysis and more rapid tenderization in bovine top loin (longissimus dorsi) steaks. The objective of this study was to use Two Dimensional Difference In Gel Electrophoresis (2D-DIGE) to determine the effect of postmortem electrical stimulation (ES) on changes in insoluble and soluble protein fractions of bovine longissimus dorsi muscle (LD).

II. MATERIALS AND METHODS

Eight market weight beef steers (mean 498 kg market weight) were slaughtered at the Iowa State University

Meat Laboratory. Each carcass was split and one half if the carcass was electrically stimulated (**ES**; 100V, 60Hz for 1 min) within 90 min (average 81 min) of exsanguinations and the other half of the carcass was not-stimulated (**NES**) and was used as a control. At 24 h postmortem, *Longissimus dorsi* (LD) muscles (n=8, respectively) from each side of each carcass were removed. Steaks were cut perpendicular to the long axis of the muscle and individually vacuum packaged. Steaks were aged at 4°C for a total aging time of 1 day or 9 day postmortem. Because the LD is commonly used as a whole muscle retail cut and because it is known to respond to electrical stimulation the LD was used as the reference sample and served as a internal control for electrical stimulation for each side of each carcass. Decline of pH, as well as µ-calpain autolysis were determined [3]. Sensory tenderness was determined as previously described [4].

The soluble protein fraction was defined as the fraction soluble in 50 mM Tris-Cl pH 8.5, 1 mM EDTA. The insoluble protein fraction was defined as the pellet from the original separation. The pellet was washed 3 times with standard salt solution (100 mM KCl, 20 mM phosphate (K_2HPO_4 and KH_2PO_4), 2 mM $MgCl_2$, 2 mM EGTA, 1 mM NaN_3) and 3 times with 5 mM Tris HCl pH 8. After washing, 1 ml of myofibrillar extraction buffer (8.3 M Urea, 2 M Thiourea, 2% chaps; 1% DTT; pH 8.5) was added per 100 mg of pellet and rocked at 4°C for 30 min, then they were centrifuged (10,000 x *g* for 30 min at 4°C). Soluble (Lowry, 1951) and insoluble (2D Quant; GE Healthcare) protein concentrations were determined.

2D DIGE assays were designed to determine the protein profile differences in paired samples (from either side of the carcass) and were conducted as previously described [5]. The images were processed using the DeCyder 2D software 7.0 (GE Healthcare) and used for simultaneous comparison of abundance changes across all 8 carcasses and ES/NES treatment.

Preparative gels were loaded with unlabeled sample (300 µg of proteins per strip), electrophoretic conditions were as for 2-D DIGE. Proteins were visualized by colloidal coomassie to pick spots of interest. Excised gel spots were destained and after were digested with trypsin and identified with Electrospray Mass Spectrometry (ESI/MS) (Stewart, 1999). For the identification has been used a mass spectrometer Q-Star XL quadrupole-TOF (Applied Biosystems) equipped with ESI.

For confirmation of identifications spectra were also searched against by the program MASCOT (http://www.matrixscience.com/).

III. RESULTS AND DISCUSSION

The effects of ES on pH decline, µ-calpain autolysis, and sensory tenderness have been previously reported for the samples in this experiment [2]. Application of ES resulted in lower LD pH at 3.5, 5.5 and 8.5 h. No treatment differences were detected in ultimate pH. Application of ES resulted in greater autolysis of µ-calpain and greater sensory tenderness scores in LD steaks evaluated 1 d postmortem. No differences in sensory tenderness (at day 9) or proteolysis of desmin or troponin-T (at day 1 or day 9).

ES reduced the abundance of several enzymes in the soluble protein fraction of samples evaluated at d 1 and d 9 (Table 1) in experiments that utilized both pH 3-10 pH gradient strips as well as pH 4-7 pH gradient strips. Spots with homology to creatine kinase, annexin and pyruvate dehydrogenase were more soluble in response to ES.

Fewer differences were detected in the experiments designed to investigate the effect of ES on the insoluble fraction (Table 2). The corresponding loss of several enzymes (fructose bisphosphate aldolase, glyceraldehyde 3 –P dehydrogenase) in the soluble fraction resulted in an increase in those proteins in the insoluble fraction. This is consistent with some published observations [6]. ES resulted in a greater abundance of myosin light chain 2, tropomyosin beta chain and tropomyosin alpha chain in the insoluble fraction of steaks aged 9 days.

Table 1. Effect of ES on abundance of specific proteins in the soluble fraction of LD steaks at 1 and 9 d postmortem[a]

Day	Identified proteins	fold change[b]
	pH 3-10	
1	Fructose-bisphosphate aldolase A	+1.12
1	Glyceraldehyde-3-phosphate dehydrogenase	+1.13
1	Phosphoglycerate kinase 1	+1.08
1	Pyruvate kinase isozyme M1	+1.10
9	Glyceraldehyde-3-phosphate dehydrogenase	+1.20
9	Creatine kinase M-type	-1.08
	pH 4-7	
1	Glycerol-3 phosphate dehydrogenase	+1.20
1	Pyruvate dehydrogenase	-1.19
1	Annexin	-1.44
9	Glyceraldehyde-3-phosphate dehydrogenase	+1.30

[a] All differences reported are significant at the P<0.05 level.
[b] + is more abundant in NES than in ES and – less abundant in NES than in ES.

In this pilot study with 8 carcasses, differences in abundance of some of the key substrates to calpains (desmin, troponin-T) were not detected. However, the treatment did demonstrate that some proteins are less soluble in response to ES and a more rapid pH decline. In the current experiment, ES did result in a more rapid pH decline and more autolysis of μ-calpain. A slight improvement in sensory tenderness –detected at day 1 – was not maintained in steaks aged 9 days.

Table 2. Effect of ES on abundance of specific proteins in the insoluble fraction of LD steaks at 1 and 9 d postmortem[a]

Day	Identified proteins	fold change[b]
	pH 3-10	
9	Glyceraldehyde-3-phosphate dehydrogenase	-1.16
9	Fructose-bisphosphate aldolase A	-1.14
9	Fructose-bisphosphate aldolase A	-1.18
	pH 4-7	
1	Myosin regulatory Light Chain 2	-1.13
1	Tropomyosin Beta Chain	-1.14
1	Tropomyosin Alpha Chain	-1.17

[a] All differences reported are significant at the P<0.05 level.
[b] (–) less abundant in NES than in ES.

IV. LITERATURE CITED

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