PROTEOME CHANGES INDUCED BY ELECTRICAL STIMULATION AND POST MORTEM STORAGE IN THE INSOLUBLE PROTEIN FRACTION OF BOVINE LONGISSIMUS DORSI MUSCLE

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Abstract— **Changes in protein composition of the insoluble protein fraction of bovine *longissimus dorsi* muscle between electrical stimulated (ES) and non-electrical stimulated (NES) carcass sides were studied by proteomics. Changes in the protein composition during the first 24 h post mortem were also analysed for these samples. Most of the variation in the data set was related to protein changes occurring during post mortem storage, however electrical stimulation also contributes to variation in protein abundance. Interestingly, the ES treatment seems to induce more protein changes from 1 to 24 h post mortem than the NES treatment.**

Index Terms— **beef, ES, post mortem, proteomics.**

I. INTRODUCTION

The conversion of muscle into meat is a sequence of changes and events, where many of the pathways are poorly characterised. The application of proteomics in meat science has developed in recent years, and the aim is to achieve a better understanding of mechanisms behind meat product quality (Bendixen, 2005; Hollung, Veiseth, Jia, Faergestad & Hildrum, 2007). The protein composition of raw materials can vary, and this will have an effect on functional properties and final product quality. Electrical stimulation (ES) has been found to accelerate post mortem glycolysis, resulting in a rapid pH decline and earlier development of rigor mortis (Boles, Parrish, Huiatt & Robson, 1992; Laville et al., 2009). Moreover, studies have reported that ES enhances the proteolysis of myofibrillar and cytoskeletal proteins post mortem (Zapata, Zerby & Wick, 2009; Pulford, Vazquez, Frost, Fraser-Smith, Dobbie & Rosenvold, 2008).

The effect of ES on pH decline and WB shear force have previously been analysed for the samples in the present study, where low-voltage ES was found to accelerate pH decline and reduce WB shear force (Hollung, Veiseth, Froystein, Aass, Langsrud & Hildrum, 2007). The aim of this study was to map and identify changes in the insoluble protein fraction of bovine *longissimus dorsi* (LD) muscle as a result of ES and post mortem storage.

II. MATERIALS AND METHODS

A. Animals and Sampling

The experiment included two sampling times after slaughter, 1 h and 24 h post mortem, from a total of ten NRF (Norwegian Red) young bulls. The hot boned M. *longissimus dorsi* (LD) were packed and kept at 4 °C during the post mortem storage period. The sample preparation and the ES procedure have been described previously (Hollung et al., 2007). A piece of muscle tissue was taken at 1 and 24 h post mortem, 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 LD muscle and separated by 2-DE. In order to remove all the soluble muscle proteins, frozen muscle tissue was homogenised 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) and used for 2-DE.

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, 200 µg proteins were loaded onto each IPG strip by in-gel rehydration overnight at room temperature. In the second dimension proteins were separated on 12.5% SDS-PAGE and stained with

Krypton staining.

C. Image Analysis and Data Analysis

Comparative image analysis by Progenesis SameSpot (version 4.0) included the proteins in the molecular mass region of 10-75 kDa, and the pH range 5-8. The expression patterns of samples that were treated with ES were compared to samples that were non-electrical stimulated (NES). The expression patterns of samples taken at 1h and 24 h post mortem were also compared.

The data set from the SameSpot program was imported into Unscrambler version 9.8 (CAMO A/S, Norway) for validation of the data by regression analysis.

III. RESULTS AND DISCUSSION

The insoluble protein fraction from LD muscle of 10 NRF bulls, either ES or NES, were separated by 2-DE. Image analysis allowed matching and relative quantification of spots across all 40 gels (2 ES treatments × 2 sampling times × 10 biological replicates (animals)).

Overall, most of the variation in the data set seems to be related to post mortem storage, however ES also seems to contribute to the variation. Figure 1 and 2 illustrate the partial least square regression analyses of the ES-related changes. At both 1 and 24 h post mortem the ES and NES samples separate into two groups, illustrating the effect of ES on protein abundance at these time points. Comparison of the post mortem changes from 1 to 24 h in the ES and NES groups indicates that ES induces changes in more proteins during this time period compared to NES. Further investigations are currently in progress to assess whether these protein changes relate to the reported quality differences seen in these animals as a result of ES.

ACKNOWLEDGEMENT

This work was supported by grants from the Fund for Research Levy on Agricultural Products in Norway.

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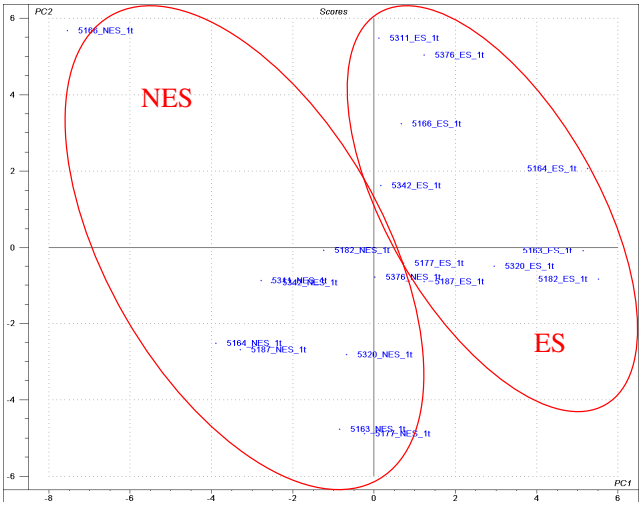

Figure 1. Score plot from partial least square regression analysis of samples taken at 1 h post mortem from *longissimus dorsi* (LD), showing the first two principal components. The ES and NES samples separate into two groups and illustrate the effect of ES on protein changes.

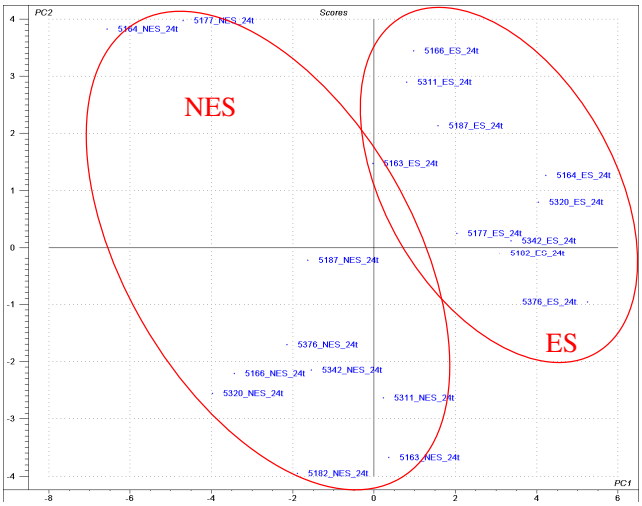

Figure 1. Score plot from partial least square regression analysis of samples taken at 24 h post mortem from *longissimus dorsi* (LD), showing the first two principal components. The ES and the NES samples separate into two groups, illustrating the effect of ES on protein changes.