Molecular analysis of tenderstretch on postmortem bovine skeletal muscle protein composition using 2-dimensional electrophoresis based proteomics

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Abstract

Using 2-dimensional electrophoresis based proteomics we have investigated the protein expression profile of bovine *M. longissimus thoracis et lumborum* at day 2 (n=3) and day 14 (n=3) in tenderstretched and conventionally hung carcasses. From 1248 protein features detected by image analysis, 191 manually curated spots were subjected to statistical analysis. From this initial analysis, at day 2 *postmortem*; 33 spots were up regulated and 10 were down regulated (P.<0.01); whilst at day 14 *postmortem*, 60 up regulated and 20 down regulated protein spots (P.<0.01) were found in the tenderstretched samples. Mass spectrometry (MALDI TOF-TOF and LTQ linear ion trap) will be used to identify some of these proteins and it is anticipated that this will lead to a greater understanding of the molecular mechanisms involved in tenderisation.

Introduction

'Tenderstretch' has been shown to help prevent sarcomere shortening in *postmortem* skeletal muscle and subsequently decrease shear force and improve meat tenderness in some commercially important muscles- striploin, topside, silverside and rump (Troy, 1999). Tenderstretch has also been reported to reduce variability in palatability scores in beef by up to 25% compared to conventionally hung samples (Sorheim *et al*., 2001; Thompson, 2002). However, little is known about the molecular mechanisms involved in the stretching of muscle *postmortem*. It has been suggested that 'tenderstretch' may affect the structural integrity of the sarcomere by aiding in the degradation of the Z-disk (Hopkins and Thompson, 2001). As well as the physical tearing of muscle fibres due to increased stretch in the muscle *postmortem*, other theories regarding the molecular contribution of 'tenderstretch' to increased meat tenderness include an increased rate of proteolysis due to the stretch induced exposure of potential proteolytic substrates within the muscle fibre (Hwang *et al.,* 2003) and a possible activation of calcium dependant proteases due to an elevation of intracellular Ca^{2+} levels caused by the stretching of the muscle (Armstrong *et al.,* 1993). Genetic studies have indicated that pre-rigor stretching causes a decline in both additive and phenotypic variance in shear force, while heritabilities were not changed, genotype differences were reduced. This suggests that more severe stretching of muscle pre-rigor may minimise genotype effects on tenderness (Burrow *et al*., 2006).

Materials and methods

Crossbred heifers (n=3) were captive bolt stunned and exanguinated conventionally at the Meat Industrial Development Unit, Ashtown Food Research Centre, Dublin 15. The carcasses were electrically stimulated at 60V for 20 seconds. Half of each carcasses (n=3) was conventionally hung while the other half (n=3) was 'tenderstretch' hung by the aitch bone. *M. longissimus thoracis et lumborum* samples were taken from the excised muscle for protein analysis at day 2 and day 14 *postmortem*. Samples were snap frozen in liquid nitrogen and stored at -80ºC until required. Both sarcomere length (day 7 *postmortem*) and Warner Bratzler shear force (day 14 *postmortem*) were measured according to the methods of Cross *et al.* (1980) and Shackelford *et al.* (1991).

Myofibrillar protein extraction: Approximately 110mg of powdered tissue was homogenised in 1ml lysis buffer containing 7M urea, 2M thiourea, 2% CHAPS, 1% DTT, and 0.8% Pharmalyte pH 4-7 non-linear according to the method of Hwang *et al.* (2005) with minor modifications.

Protein Quantification: The concentration of protein present in the samples was determined using a modified Bradford assay according to the method of Ramagli and Rodriguez (1985) using bovine serum albumin (BSA) in lysis buffer as standard reference.

2-dimensional electrophoresis: 100ug of solubilised myofibrillar protein was loaded onto a Immobiline™ DryStrip™, 24cm, pH 4-7 using an in-gel rehydration method as described by Rabilloud *et al.* (1994) and Sanchez *et al.* (1997). The first and second dimensions were carried out according to the protocol detailed in Focking *et al.* (2006). Iso-electric focussing (IEF) was carried out for 75kVh at 20ºC (Ettan

IPGphor3, GE Healthcare) and SDS-PAGE was performed at 1W/gel overnight using 12% separating polyacrylamide gels without a stacking gel using the Ettan DALT12 system (GE Healthcare).

Gel Staining: Gels were silver stained using a Daiichi silver staining kit (InsightBio).

Image analysis: The 2-DE gels were scanned using a GS-800 calibrated densitometer (Bio-rad) and analysed using Progenesis software (Nonlinear Dynamics). 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 expressions of the individual spots were analyzed and compared between groups.

Results and discussion

The sarcomere lengths and Warner Bratzler shear force values for conventional and tenderstretched carcasses were determined (Table 1). In all cases the SL of the tenderstretched side was found to be longer than it's conventionally hung counterpart showing that the treatment altered the structural unit of the muscle fibre. However, WBSF values do not appear to reveal any beneficial effect of tenderstretching compared to conventional hanging however this trend may alter by increasing the number of replicates within each treatment group.

Table 1: Sarcomere length and Warner Bratzler shear force measurements for tenderstretched and conventionally hung carcasses

Sample	Sarcomere Length (µm)	Warner Bratzler shear force (N)
Tenderstretch 1	1.91 ± 0.07	32.67
Tenderstretch 2	1.89 ± 0.15	24.56
Tenderstretch 3	1.66 ± 0.08	*
Conventional 1	1.68 ± 0.09	32.67
Conventional 2	1.61 ± 0.31	52.25
Conventional 3	1.49 ± 0.08	*

* analysis ongoing

This work focused on the separation of bovine skeletal muscle proteins by 2-dimensional electrophoresis to examine the effects of "tenderstretch" on low voltage electrically stimulated muscle. Proteins were extracted from bovine *M. longissimus thoracis et lumborum* and visualised after 2-dimensional electrophoresis with a silver staining procedure.

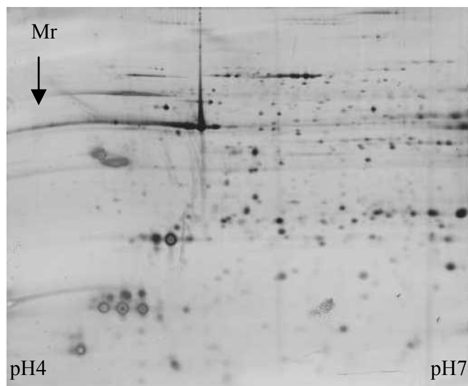

Figure 1: 2-DE of electrically stimulated, conventionally hung bovine *M. longissimus thoracis et lumborum* at day 14 post-mortem.

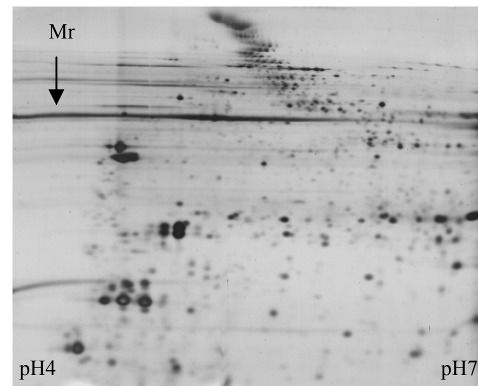

Figure 2: 2-DE of electrically stimulated, tenderstretched hung bovine *M. longissimus thoracis et lumborum* at day 14 post-mortem.

The proteolytic patterns of electrically stimulated bovine carcasses what were either conventionally hung or tenderstretched were monitored at both day 2 and day 14 *postmortem* as illustrated by representative gel images in figure 1 and figure 2. 1248 protein features were detected by image analysis software across the two treatment groups. 191 spots were visually selected and subjected to further statistical analysis. Initial analysis revealed that at day 2 *postmortem*: 33 spots were up regulated and 10 were down regulated

(P.<0.01) in tenderstretched muscle samples compared to conventionally hung carcasses. After aging to 14 days *postmortem*, 60 up regulated and 20 down regulated protein spots (P.<0.01) were detected in the tenderstretched samples. Spots are currently being identified by mass spectrometry analysis on a 4800 MALDI TOF-TOF and a LTQ linear ion trap.

Conclusions

In this study the initial results indicate that there are differences in the protein expression profiles of electrically stimulated tenderstretched carcasses than carcasses hung conventionally. Protein spots will be identified by mass spectrometry and verification of protein expression differences will be carried out.

Acknowledgments

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