# PE1.54 In search of protein markers discriminating meat tenderness in bovine longissimus muscles by a differential proteomic approach 345.00

*Jingshun Liu (1) jingshun.liu@teagasc.ie, Ruth Hamill( 1), Anne Maria Mullen (1)*
*(1)Ashtown Food Research Centre, Teagasc, Ireland*

***Abstract*—Tenderness is one of the most important quality attributes affecting consumer acceptance of beef. Two-dimensional electrophoresis was used to compare the proteomic components at 48hrs postmortem between two groups of bovine muscle classified by Warner-Bratzler shear force measured at 14 day post-mortem, to search for protein markers that discriminate final meat tenderness. By applying different statistical analyses based on normalized spot intensities and log-transformed data of these spot intensities, 6-14 protein spots were found to be differentially expressed between the two groups. Principle component analysis highlighted six spots common across methods which contribute to discriminate the final beef tenderness. The identifying of these spots is being achieved through mass spectrometry analysis.**

***Index Terms:* Beef, protein markers, tenderness, two-dimensional electrophoresis.**

†All the authors are with Meat Technology Department, Ashtown Food Research Centre, Teagasc, Ashtown, Dublin 15, Ireland.

Jingshun Liu (email: Jingshun.Liu@teagasc.ie)

Ruth Hamill (email: Ruth.Hamill@teagasc.ie)

Anne Maria Mullen (corresponding author, Tel.: +353 1 8059500, Fax: +353 1 8059550 email: Anne.Mullen@teagasc.ie)

## I. INTRODUCTION

TENDERNESS is one of the most important quality attributes affecting consumer acceptance of beef [1]. Large variation in this trait makes it difficult to market beef on the basis of tenderness. While many efforts have been made over the past decades to identify factors along the meat chain that determine tenderness in various species [2], the molecular mechanisms underlying the variability of tenderness are still not fully understood. Recently, two-dimensional electrophoresis (2-DE) based proteomics approach has demonstrated a great strength in studying metabolic alterations, post-mortem proteolysis, and changes induced by environmental and processing conditions in meat science research [3]. A number of studies have commenced in this area [4-5 for pork; 6-7 for beef] which are demonstrating the advantages and show the potential of this technology in understanding the molecular basis for variation in quality, however the relationships between individual proteins and meat tenderness are still poorly understood. Detection of the aging induced proteolytic products which contribute to meat tenderization provides a strong basis for predicting, controlling and optimising meat tenderness. Hence the aim of this study is to apply 2-DE to search for early postmortem protein markers for tenderness in bovine muscle.

## II. MATERIALS AND METHODS

The experiment involved a total of 60 Irish crossbred cattle with average body weight approximately 320 Kg. Cattle were slaughtered at a commercial meat abattoir in Ireland according to standard procedure. The pH and carcass temperature was monitored in the M. *longissimus thoracis* (LT) on the carcass at hourly intervals for 8 h and at 24 h, 48 h post-mortem. A piece of LT tissue was sampled at 48 h post-mortem between 12th and 13th for proteome analysis. Excision of LT muscles (up to 12th and/or 13th ribs) were carried out at 48 h postmortem. The muscles were individually vacuum-packed, and stored at 4 °C for 7 days and 14 days ageing for Warner Bratzler shear force (WBSF) measurement. Samples were taken at day 2 postmortem for sacromere length (SL) determination.

WBSF was measured on the steak samples (2.54 cm thick) after 7 days and 14 days ageing at 4 °C. Steaks were cooked to a core temperature of 70 °C monitored by Minitherm HI8751 temperature meter and probe (Hanna Instruments Ltd., UK) in a 72 °C water bath (Model Y38, Grant Instruments Ltd., Barrington, Cambridge CB2 IBR, UK), cooled to room temperature and tempered at 4 °C overnight. Six 1.25cm diameter cores per steak were cut parallel to the muscle fibre direction and sheared using the Warner Bratzler shear blade, attached to an Instron Universal testing machine (Model 5543 Instron Ltd., High Wycombe, UK), using a 500N load cell at a crosshead speed of 5 cm/min according to AMSA Guidelines 1995. Merlin series IX software software was used and results were expressed as load in Newtons (N) per 1.25 cm core. Warner Bratzler shear force value for each sample were calculated the average of 5 cores after elimination of the highest and lowest shear value of the seven cores, which were representative of the

sample.

SL was determined after two days ageing as described in [8].

The two groups of samples (Tender vs. Tough, n=4/each group) were chosen based on the contrasting level of WBSF value measured at day 14 postmortem. SL values were also considered to control for this potentially confounding factor. Total protein was extracted using high concentration urea-based lysis buffer (7 M Urea and 2 M Thiorea). 900 µg total proteins for each sample were loaded in a total volume of 450 µL rehydration buffer on precast Immobiline IPG (immobilized pH gradient) DryStrips strips (24 cm, 3-10 pH non linear, GE Healthcare) and subjected to passive rehydration overnight. Isoelectric focusing (IEF) was performed at 20 °C following the program: low voltage (150 V) applied initially for 1 h, followed by stepwise increase of voltage to 8000 V and finally 8000 V held for a total 70,000 Vh. After equilibration of the strips, the second dimension was performed with homemade 10% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) gels in a PROTEAN Plus Dodeca Cell Horizontal System (Bio-Rad). Eight gels were run at a time with equal number of samples from the tender and tough group. The running program was set at 0.25W/gel for 2 h and 1.5 W/gel until the dye running out of the edge of low molecular area. Analytic gels were made in triplicate for each biological sample, while two preparative gels were made by also loading 900 µg proteins for each gel, from the mixture of equal amounts of proteins from each biological samples (n=8). All the gels were stained by the sensitive colloidal coomassie blue (blue silver) method. GS-800 calibrated densitometer (BioRad) was used to scan the gels and the resulting TIFF images were imported into the Progenesis SameSpots software version 3.2 (Nonlinear Dynamics, Newcastle, UK) for image analysis. Intensity of each spot was normalized against the total intensity of all the spots on a gel and then multiplied by 100. After spot detection the dataset consisting of the spot intensities was exported as a Microsoft Excel file.

The group effect (tender vs. tough meat) was initially analyzed using classical student's T test with Progenesis software on each technical replicate of the two with highest quality assuming the equal variance between these two groups. For the exported spot intensity data, a Shapiro-Wilk test was performed and found nearly 90% of spots with their intensities following normal distribution across the gels in each technical replicate. To ensure the validity of statistic model, the spot intensities for each replicate and the average for both two replicates were firstly logarithmic transformed when analyzed by one-way analysis of variance (ANOVA) with SAS software (SAS Inst., Inc; Cary, NC). In all analyses, the spots with $p<0.05$ was considered for significance, and the fold change value ≥1.3 was considered biologically relevant, representing the expression ratio of the tender group to tough group. Because all the analyses were carried out on the detected features automatically by Progenesis software, the spots were carefully verified on 2-DE gels and saturated and poor defined spots or artifacts, were retarded. Finally, the spots obtained from both analyses (Progenesis vs. SAS) were compared and evaluated with Progenesis software built-in principal component analysis (PCA) and only the common spots between analysis of log-mean data and analysis of individual technical replicate were considered for further protein identification.

Protein spots of interest were manually excised using pipette tips. Protein digestion and mass spectrometry (MS) analysis were performed using MALDI-TOF/TOF mass spectrometer (Applied Biosystems, Framingham, MA, USA) in the positive ion reflector mode at Mass Spectrometry Resource Conway Institute in University College Dublin (UCD, Ireland).

## III. RESULTS AND DISCUSSION

Warner–Bratzler shear force (WBSF) was used to classify samples based on their tenderness values. Sarcomere length (SL) values were also taken into consideration to ensure SL values had little influence on the classification. As illustrated in Figure 1, the meat samples in tender group (n=4) had a mean value of WBSF 24.3 N (range 20.1-27.5 N) whereas those in tough group (n=4) had the mean of 64.7 N (range 58.0 -70.7 N). This selection was guided by using a cut off point of 50N, above which steaks are considered tough [9] and which is in alignment with the work of [10]. The sample grouping was also verified with WBSF values measured earlier, at day 7 post mortem: they were 23.3-32.7 N for tender group and 53.3-63.9 N for tough group. Because it is known that the extreme value of ultimate pH (at 24 h post-mortem) as well as pH decline profile postmortem can result in abnormal meat quality characteristic, all the samples was first checked in order to exclude the abnormal samples. The ultimate pH range for tender group and tough groups were 5.35-5.59 and 5.34-5.72, respectively, and thus were considered normal.

An average of 355 spots was detected across all the analytic gels in terms of the total number of alignment vectors. A typical 2-DE gel of muscles taken at 48 h post mortem is presented in Figure 2.

Classical student T test with Progenesis software revealed 12 and 13 spots with differential expression ($p<0.05$) between tender and tough group, based on normalized spot intensities in technical replicate 1 and 2, respectively (see Table 1). There were six spots in common between the technical replicates. Based on the logarithm-transformation of normalized spot intensities, one-way ANOVA analysis using SAS package revealed 13, 12 and 15 spots differentially expressed ($p<0.05$) between the two groups within technical replicate 1, replicate 2 and log-mean data of these two replicates, respectively. Six spots were in common between these analyses. They were the same spots with those obtained by student T test, as indicated in Figure 2 with circled spot ID number. In an attempt to reduce the false positive rate of the results, only spots showing differential expression in abundances between the analyses based on log-mean data and at least for one technical replicate were considered for further mass spectrometry identification. As indicated in bold character in Table 1, there were fourteen spots of biological interest in total. Spot 4 and 11 had fold change of -1.7 and -1.5, respectively, whereas other spots had fold changes ranging from +1.3 to +2.0.

Following PCA analysis performed with the fourteen spots, PC1 and PC2 were found to explain 57.31% and 11.36% of the total variance in the proteome data for discriminating the samples in the tender and tough group. PCA analysis on the six common spots returned PC1 and PC2 explaining 76.98% and 7.14%, respectively. These spots have been selected for identification by mass spectrometry.

## IV. CONCLUSION

The current study reveals up to 14 protein spots differentially expressed between tough and tender in bovine muscle at 48 h post-mortem. Different statistical methods and PCA analysis confirmed the six common protein spots might play a major part in discriminating the final meat tenderness. Identifying the (parent)protein for each spot will provide information regarding the biological processes in muscle which contribute to variation in tenderness.

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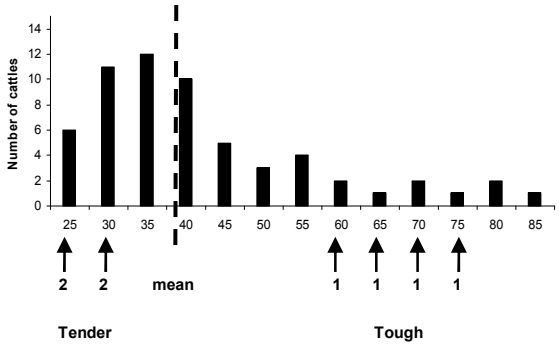


Figure 1 Distribution analysis of Warner-Brazler shear force measured at 14 day postmortem. Black arrows mean the number of cattle chosen for tender or tough meat group. The broken line stands for the mean of Warner-Brazler shear

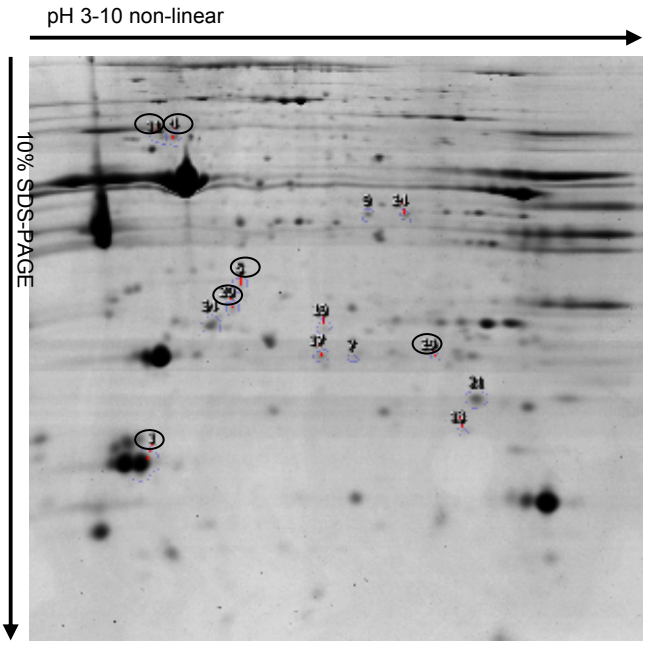


Figure 2 A typical 2-DE gel made on bovine longissimus muscle taken at 48 h postmortem. Fourteen spots were differentially expressed ($p<0.05$, absolute fold change$\geq 1.3$) between tender and tough group observed by both Progenesis and SAS analysis based on log-mean and at least one of technical replicate data; six of these spots showing differential expression in all the analyses based on log-mean and both two technical replicate data were circled for their spots ID.

Table 1 Comparison of differentially expressed spot lists by Progenesis and SAS analyses

| Spot ID | FC[a] (TE/TO) | Progenesis student T test | | SAS one-way ANOVA | | |
|---|---|---|---|---|---|---|
| | | Repl 1[b] | Repl 2[b] | Logrep l1[c] | Logrep l2[c] | LogMean[c] |
| **1** | **+2.0** | * | ** | * | ** | ** |
| **4** | **-1.7** | * | * | * | * | * |
| **5** | **+1.7** | ** | * | ** | * | *** |
| **7** | **+1.6** | | ** | | ** | ** |
| **9** | **+1.5** | * | | * | | * |
| 10 | -1.5 | | * | | * | |
| **11** | **-1.5** | * | * | * | * | * |
| **13** | **+1.5** | | | * | | * |
| 15 | +1.5 | * | | * | | |
| **17** | **+1.4** | ** | | ** | | ** |
| **19** | **+1.4** | * | | * | | * |
| **20** | **+1.4** | * | * | * | * | *** |
| **21** | **+1.4** | ** | | ** | | * |
| **24** | **+1.3** | | * | | * | * |
| 27 | +1.3 | | ** | | | |
| **29** | **+1.3** | * | * | * | * | ** |
| 33 | +1.3 | * | | * | | |
| **34** | **+1.3** | | * | | * | * |
| 35 | +1.3 | | ** | | ** | |
| 39 | +1.4 | | * | | * | |
| 72 | +1.3 | | | | | * |

a- Fold change representing the expression ratio of the normalized intensities of tender group to tough group.

b- Progenesis student T test for the group effect based on the normalized spot intensities either in technical replicate 1 or 2,

c- SAS one-ANOVA for the group effect based on the log-transformed data of normalized spot intensities either in technical replicate 1 or 2 and mean of these two replicates.

Significance levels were expressed as '*' for $0.01 \leq p$ value $\leq 0.05$, '**' for p value $\leq 0.01$.

Spots IDs in bold character were significant-differentially expressed between tender and tough group as identified by SAS analysis based on log-mean and at least one of technical replicate data.

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