ANALYSIS OF POST MORTEM PRODUCTS OF PROTEOLYSIS IN PORCINE TCA SOLUBLE EXTRACTS AND THEIR ASSOCIATION WITH TENDERNESS

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Background

Proteolytic degradation of myofibrillar proteins has been shown to contribute to *post mortem* tenderisation with the rate and extent of proteolysis being associated with meat tenderness (Troy and Tarrant., 1987; Sentandreu et al. 2002, & Sawdy et al., 2004). Analysis of the products of proteolysis has held much interest in terms of developing diagnostic tests for meat tenderness. In the past a lot of focus has been directed to the study of products of *post mortem* proteolysis in the myofibrillar fraction of muscle. In more recent years, however, the analysis of more soluble proteolytic fragments has held much interest (Nakai et al., 1995; Mullen et al., 1998; Stoeva et al., 2000). One dimensional gel electrophoresis (1DGE) allows visualising products on the basis of molecular weight while two dimensional gel electrophoresis (2DGE) separates proteins according to their isoelectric point and molecular weight. In this manner 2DGE provides a very high resolution separation. Application of these techniques may add further definition to the pattern of proteolysis over the *post mortem* ageing process and its association with pork tenderness.

Objectives

The objective was to apply 1-DGE and 2-DGE to track *post mortem* proteolytic events and their associations with tenderness, in Trichloroacetic acid (TCA) soluble extracts from porcine *M.longissimus dorsi*.

Materials and methods

Forty pigs were slaughtered and carcasses treated conventionally in a commercial abbatoir. A sample was excised from the *M.longissimus dorsi* (striploin, LD) at 1d, 3d and 7d *post mortem*. These samples were snap frozen in liquid nitrogen and stored at –80^{o}C. The LD was excised at 1d postmortem and chops were taken for analysis of eating quality. Tenderness was measured by Warner Bratzler shear force (Shackelford et al., 1991). Compositional analysis was conducted to determine any variation in intramuscular fat, protein and moisture levels. Sarcomere lengths were determined according to the laser diffraction method (Cross *et al.,* 1980). Other quality measurements included drip loss (Honikel and Hamm, 1994), cook loss, pH rate of decline and temperature profile up to 1d postmortem.

Based on the quality analysis, carcasses which displayed extreme values of tenderness were selected while controlling for extreme values of sarcomere length and intramuscular fat. Samples were alloted to three catagories, namely tender (Class I), tough becoming tender (Class II) and tough (Class III). A representative sample from each category was selected and TCA soluble extracts were prepared for both 1DGE and 2DGE analysis. 2-D gels were produced in triplicate for each sample, according to the method described in Morzel et al. (2004) with slight modifications.
Spots were detected and quantified by the image analysis software PDQuest. Spots of interest, i.e. those more associated with the "tender" class, were defined as spots that were either specific to the class I sample, or spots that were over-expressed at least three-fold in the class I (compared to class III) on day 7.

Results and discussion

Initially 1DGE analysis was conducted on extracts (1d, 7d, 14d) from each of the classes where bands of various molecular weights were seen to increase and decrease over the ageing period (Figure 1). In the tender sample increases were observed in lower molecular weight bands (Areas 3 & 4) which were not so obvious in the tough sample. These bands may be the product of proteolysis of myofibrillar or sarcoplasmic proteins over the postmortem ageing period. Two other areas of interest were noted (Areas 1 & 2). The tough sample appeared to have a higher level of the higher molecular weight band in Area 1, compared to the

tender sample. In addition in Area 3 the bands seem to appear sooner in the tough sample. Each lane received similar protein loading, however in some instances some of the changes observed may be due to larger proteins being held back in the gel matrix. However, repeated analysis of these gels have revealed similar banding patterns.

A representative 2DGE image is presented in Figure 2. Approximately 120-160 spots were successfully detected and quantified on all gels. Spots specific to class I are squared in blue, whereas spots over-expressed in class I are squared in red. Spots are designated by their unique number attributed by PDQuest. As an example, figure 3 shows the quantity (ppm) of spot 2504 in the three samples, as affected by *post mortem* time. It is very likely that most of those potential biomarkers are protein fragments, generated by *post mortem* proteolysis.

Both 1DGE and 2DGE will be repeated on further samples. Comparisons between gels will be invaluable in the interpretation of 1D gels. Proteins of interest in both gel formats are being identified by MALDI-ToF Mass Spectroscopsy.

Conclusions

Changes in banding protein patterns were evident in 1DGE gels. 2DGE electrophoresis allowed us to propose a list of potential biomarkers of tenderness in pork meat. In order to confirm their relevance, these need to be identified by MALDI-ToF Mass Spectroscopy and quantified in a larger set of samples.

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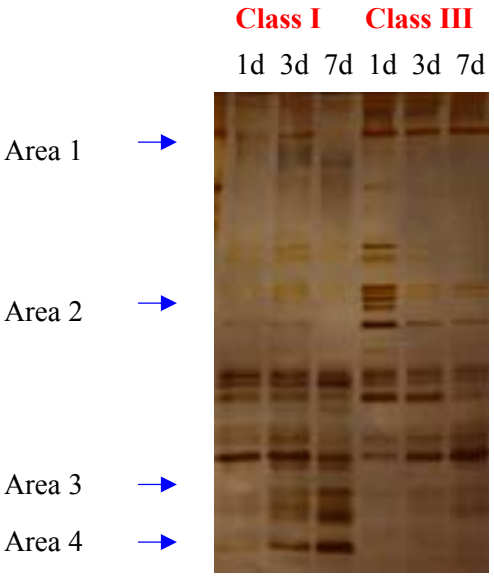

Figure 1. 1-D SDS PAGE of Class I and III TCA soluble protein extracts aged to 1d, 3d and 7d *postmortem*. Blue arrows mark areas of interest.

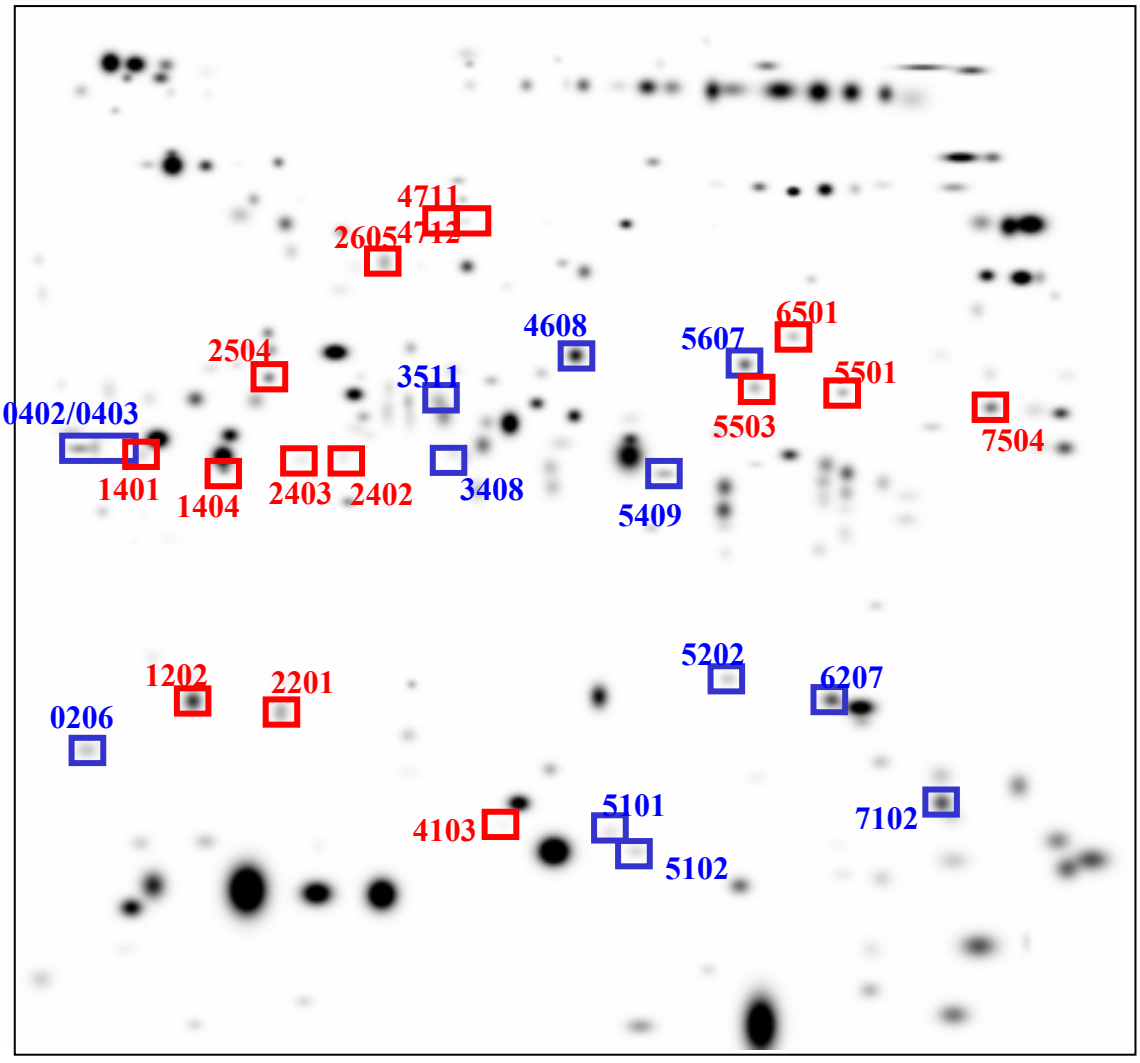

Figure 2. 2DGE: Synthetic representation TCA-soluble proteins, separated according to their pI (3-10 left to right) and MW (top to bottom). Potential biomarkers of pork meat tenderness are squared

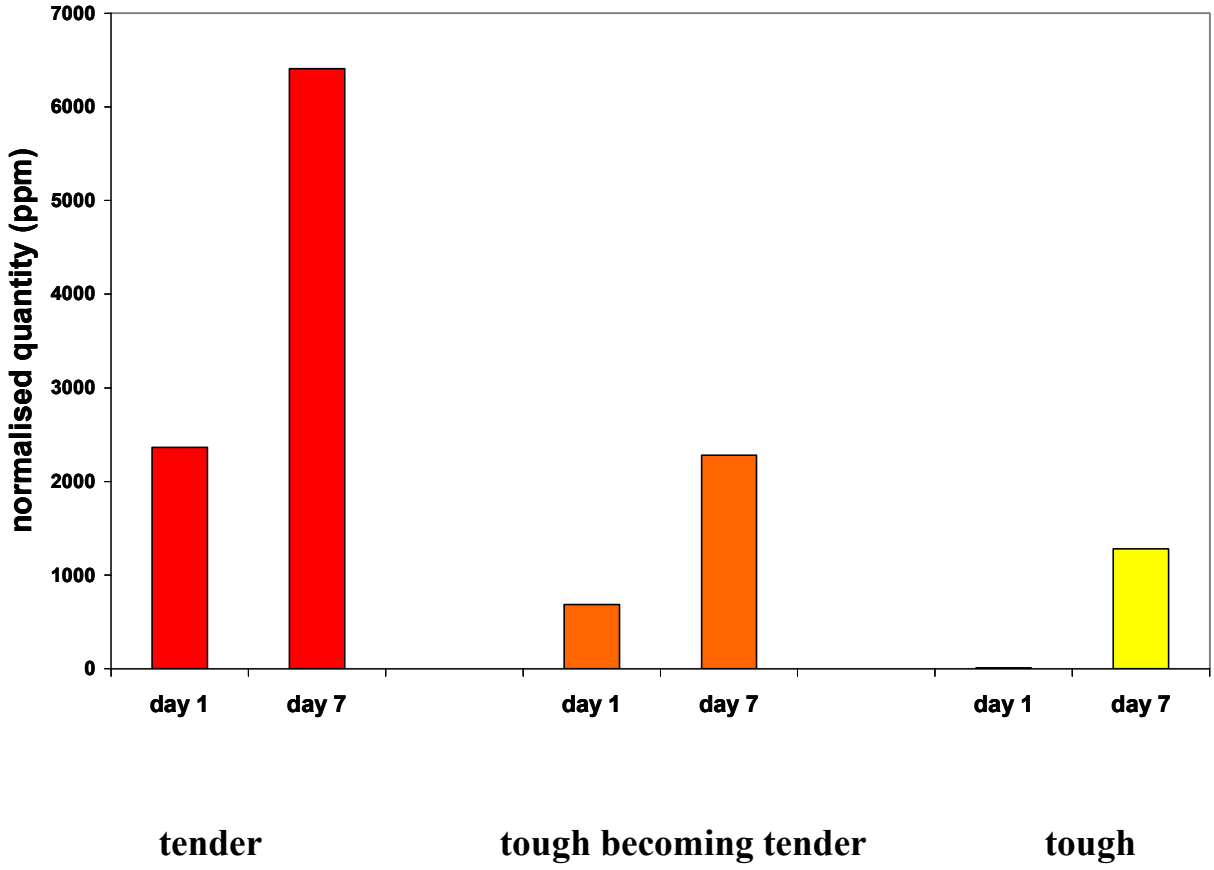

Figure 3. Normalised quantity (ppm) of spot 2504 in the three samples, as affected by *post mortem* time