SDS-PAGE ANALYSIS OF TCA SOLUBLE BOVINE MUSCLE EXTRACTS OVER THE POSTMORTEM AGEING PERIOD

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Background

Muscle proteins fall into three divisions according to their structure, function and/or degree of solubility (Ito *et al.,* 2003) and consists of; myofibrillar (salt-soluble), stomal or connective tissue (acid-soluble) and sarcoplasmic (water-soluble). In most publications dealing with meat proteins and/or polypeptides, the investigated fraction is usually determined by the extraction and separation methods applied (Claeys *et al.,* 2004). Much work concerning the proteolysis of ageing beef has focused on the myofibrillar fraction and the emergence of a 30 kDa fragment from Troponin T (Huff-Lonergan *et al.,* 1995 and Wheeler and Koohmaraie, 1999). However as fragments which are cleaved from myofibrillar proteins may themselves be soluble, analysis of the myofibrillar fraction only may not provide the researcher with a comprehensive overview of the proteolytic profile during postmortem ageing. Recently proteolytic fragments of troponin T were observed in a tricholoracetic acid (TCA) extract of beef (Nakai *et al*., 1995 and Stoeva *et al.,* 2000). Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) analysis can provide a clear profile of postmortem proteolytic pattern muscle extracts and maybe useful to view changes in a broad range of proteins/peptides over the ageing process. Altering the concentration of TCA used to obtain these extracts may be useful to optimise this visualisation technique.

Objectives

The purpose of the study was to monitor the changes in protein patterns in TCA extracts of bovine *M. longissimus dorsi* (loin, LD), over a fourteen-day ageing period, using SDS-PAGE and varying concentrations of TCA.

Materials and Methods

Sample collection Bovine *M. longissimus dorsi* (loin, LD) samples were collected at 1h, 3h, 2d, 7d and 14d post mortem, snap frozen in liquid nitrogen and stored at –80°C.

Protein Extraction Soluble protein extracts were prepared from two LDs at 1h, 3h, 2d, 7d and 14d using increasing concentrations of TCA, (0.5, 1.0, 5.0, 10.0 and 20,0%, w/v) following a modification of the method of Stoeva *et al.,* (2000).

Protein Concentration was determined using the Biuret method (Gornall *et al.,* 1949).
SDS-PAGE extracts were prepared by incubation in SDS-reducing buffer for 15 minutes at 50°C then stored at –20°C. Electrophoresis was performed according to the method of Laemmli (1970). Samples were applied to 12 % acrylamide 2mm dual vertical slab gels (BioRad) for a total of 3,500V/h then stained with either Coomassie Blue G-250 or Silver. Gels were then scanned using a colour image scanner (Epson Perfection 3200) for the visualisation of bands.

Repeatability study In order to determine the validity of the extraction method and its ability to return consistently similar degradation patterns, a repeatability study was carried out. To this end a single 2d muscle sample was independently extracted five times and run on an SDS-PAGE gel to determine if a quantitatively similar profile was obtained in each lane.

Results and Discussion

Results obtained show that increasing TCA concentrations have a negative effect on the extent of protein retrieval. The lower (0.5 and 1.0% TCA concentrations) gels have a higher degree of protein extractability,

compared to that of 5.0% (Figure 1). Higher TCA concentrations examined (10.0%) resulted in remarkably less protein extraction, with an absence of protein at 20.0% (results not shown).

The lower percentages of TCA (0.5 and 1.0%) provided extracts which were easily visualised on SDS-PAGE gels. As shown in Figure 1., clear banding patterns were observed from below 20kDa up to 205kDa. The most obvious changes over the ageing process were the increased intensities of bands appearing in the 20kDa to 36kDa region and the higher molecular weight 116kDa to 205kDa region. In terms of actual protein recovery, differences are visible between the two animals. Animal 2 has an absence of lower (< 29kDa) molecular weight proteins at lower TCA concentrations, with sharper more pronounced bands in animal 1 for the same concentration. Further characterisation of the individual animals will rule out if this is due to other factors such as, phenotypic differences.

A repeatability study was conducted on a single 2d sample. In this way the efficiency of the assay procedure to consistently isolate proteins in a reproducible manner was assessed. The results for the gel that was silver stained for greater sensitivity, show that the extraction procedure is indeed reproducible qualitatively, as the banding pattern of all five profiles are similar.

Conclusions

- SDS-PAGE separation techniques are useful in tracking changes in bovine TCA soluble protein extracts, over the post mortem ageing period.
- TCA concentration is a major factor in the extent of protein retrieval.
- Further work will be necessary in order to quantitatively categorise the specific changes observed.

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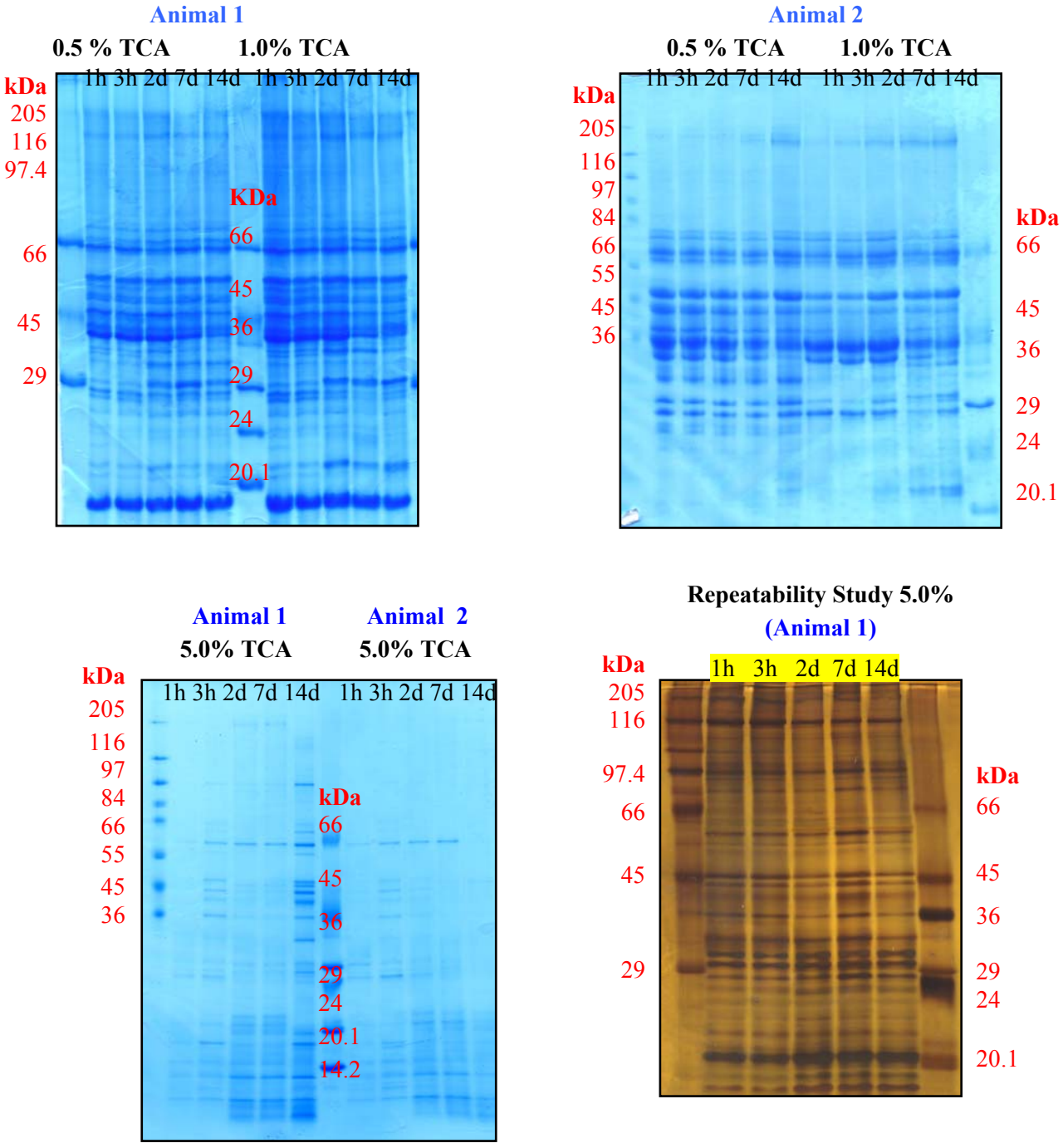

Figure 1. SDS-PAGE gels of two bovine LD samples (Animal 1 and 2) extracted into various concentrations of TCA (0.5, 1.0 and 5.0%, w/v) and stained with Coomassie Blue and a repeatability study showing a 2d sample (Animal 1) extracted into 5% TCA and Silver stained.