Title - IDENTIFICATION OF BOVINE PROTEIN FRAGMENTS PRODUCED DURING MEAT AGEING.

Authors - [1]Mullen, A.M., [2]Stoeva, S., [2]Voelter, W. & [1]Troy, D.J.
Institution - [1]The National Food Centre, Teagasc, Castleknock, Dublin 15, Ireland.
[2]University of Tuebingen, Germany.

Background - One of the main problems facing the meat industry today is the large and unpredictable variation in the consistency of meat quality. Many reports have acknowledged the involvement of proteolytic enzymes, in particular the calpain system and myofibrillar proteins in the ageing process. We have previously identified myofibrillar proteolytic fragments and their parent compounds in our efforts to identify the precise proteins involved in the *postmortem* tenderisation process (Tsitsilonis *et al.*, & 1996 Troy *et al.*, 1997). Degradation of myofibrillar proteins can also lead to soluble proteolytic fragments which may be of importance in understanding the tenderisation process. In the current paper we present data on both the isolation and identification of soluble components of meat, which are altered during *postmortem* ageing.

Objectives - Isolation and identification of soluble fragments produced during *postmortem* ageing in bovine M. *longissimus dorsi* (LD).

Methods - Three Hereford cross heifers were slaughtered conventionally. The LD was excised from the carcass at 1hr *postmortem* and stored at 4°C for 14 days. At 1h, 1d, 3d and 14d *postmortem* samples of approximately 40g weight were excised from each of eight locations along the LD. Extractions were carried on these samples immediately after slaughter using a procedure adapted from Nakai *et al.*., (1995). This involved homgenisation of tissue in H_2O, after which TCA was added to a final concentration of 5%. Supernatants were collected after centrifugation at 3,600rpm for 30min. RP-HPLC analysis was carried out on the supernatants using a LiChropsher 100 RP-18 (5µm) 250x4mm column with a TFA/CH_3CN gradient system. Peaks were detected and fractions collected for mass spectrometric analysis (MALDI I, KRATOS, Shimadzu, Europa) and Edman degradation (ABI 473A pulsed liquid protein sequencer). Comparisons of the sequences obtained were carried out with the aid of the European Molecular Biology Laboratory (EMBL) via an Internet connection.

Results and Discussion - As a result of HPLC analysis of TCA soluble components of bovine M. *longissimus dorsi* three components were isolated which increase over the ageing process. These components have been assessed be mass spectrometry and amino acid sequencing to reveal their identity. From the results obtained (Table 1) these components seem to be both sarcoplasmic and myofibrillar in origin.
The higher molecular weight fraction, 5572Da (Fraction 3 - retention time (RT) 27min) demonstrated 100% homology with creatine kinase. Creatine kinase (43kDa) is a myofibrillar protein which is localised at the M-line and appears to be bound to M-protein as a 1:1 complex (Turner *et al.*, 1973).
Comparison of the 1722.9Da component (Fraction 2, RT 42 min) with the known troponin T structures revealed an average identity score of 50% including isofunctional amino acid residues. This is in agreement with results obtained by other authors, (Nakai *et al.*, 1995). However, following our analysis the presence of one additional histidine residue at the C-terminus was detected.
The 1283.5Da fragment eluting at 39 minutes (Fraction 1) revealed an 87.5% homology with glyceraldehyde-3-phosphate dehydrogenase in 8 overlapping amino acid sequences.
It is interesting to note that the parent compounds (troponin T, glyceraldehyde-3-phosphate dehydrogenase and creatine kinase) of three of our fractions have previously been identified as the parent compounds of fragments which increase over the ageing process on SDS-PAGE gels (Troy *et al.*, 1997). While the precise role of these three proteins in the ageing process is not clear, they may be indicative of the conditioning process of ageing muscle. Based on these results we propose that HPLC analysis of soluble proteolytic fragments from bovine muscle, may provide a rapid method for indicating and monitoring the ageing process in the muscle.

Conclusions - Fragments produced during the ageing process can be isolated and identified using HPLC analysis. Three of these fragments originate from a similar parent compound as previously identified fragments on SDS-PAGE gels from aged muscle.

Literature

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Table 1: Comparison of sequenced primary structure segments of bovine proteins with known proteins.

Molecular weight soluble fragment	Identified Sequence	Parent Protein	% homology
Fraction 1 - 1283.5 Da	**KVVKQASEGPLK**	**Glyceraldehyde-3-phosphate dehydrogenase**	
		(258-265) rabbit, mouse, pig	87.5%
		(260-267) human	87.5%
Fraction 2 - 1735 Da	**APPPPAEVPEVHEEVH**	**Troponin T**	
		(29-42) quail and chicken	56.6%
		(39-54) rat	37.5%
Fraction 3 - 5570 Da	**DPIIQDRHGGFKPTKKHKTD LNHENLKGGDDLDPNYVLS**	**Creatine Kinase**	
		(4-42) rat muscle	100%
		(90-128) rat skeletal muscle, rabbit M chain	100%
		(90-128) human M chain, chicken muscle, T.calfornica, T.marmorata, trout, G.gallus, dog brain, rabbit brain, mouse.	97%

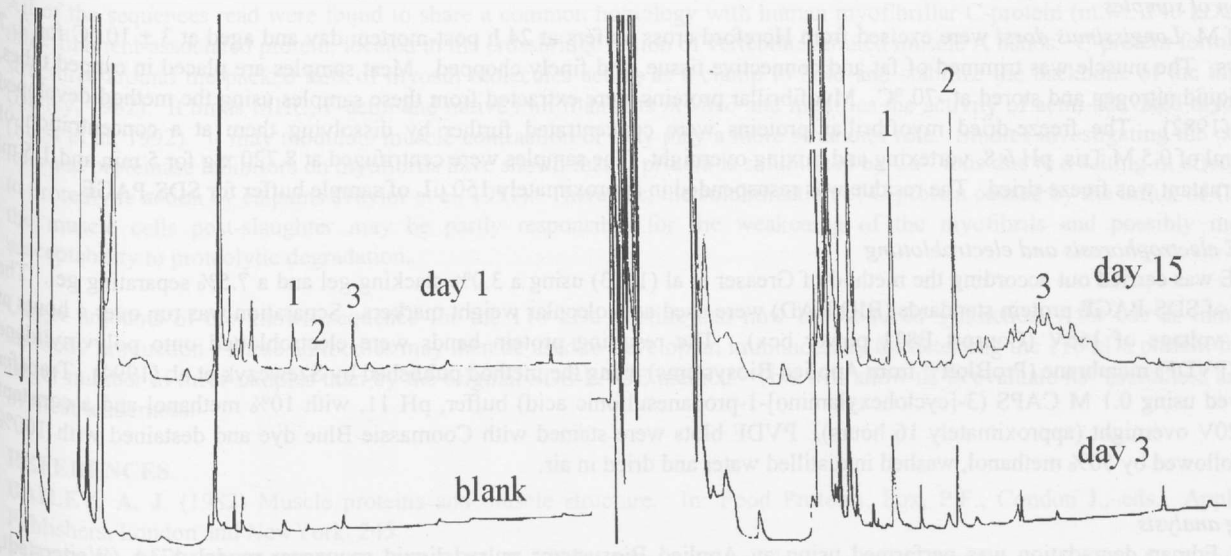

Figure 1. HPLC profiles of soluble components extracted from bovine M.*longissimus dorsi*, during ageing for 15 days. Peaks labelled 1-3 were collected for further identification.