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PROTEOLYTIC FRAGMENTS IN BOVINE EXUDATE AS POTENTIAL TENDERISATION MARKERS

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Introduction

The variability in meat quality, particularly in tenderness has become a significant consumer concern and is becoming an increasing problem for the meat industry. However, even with the best available controls during the breeding, slaughtering and storing, the tenderness of meat still varies, preventing meat producers from marketing their produce on the basis of consistent quality (Maher *et al*., 2004). These difficulties could be reduced if carcasses showing inferior quality could be identified on the slaughterline, thus enabling them to be handled and marketed separately from high quality carcasses.

The appearance of proteolytic fragments is related to the rate and extent of tenderness in beef (Ouali, 1990; Koohmaraie., *et al* 1991). Much work concerning the proteolysis of ageing beef has focused on degradation as observed within the myofibrillar fraction (Hopkins & Thompson, 2002). It is likely that products of myofibrillar proteolysis may appear in other muscle fractions. Similarly it may be that proteolysis of sarcoplasmic proteins may provide some information regarding the rate or extent of proteolysis with in a muscle. Previous research at The National Food Centre has focused on TCA soluble extracts (O'Reilly *et al*., 2004). This research focuses bovine exudate and investigates if information regarding postmortem proteolysis can be obtained through 1D gel electrophoresis studies of this exudate over the postmortem ageing period.

Objectives

The first objective of this study was to optimise methods to study products of proteolysis in bovine exudate. The second objective was to determine if potential markers of beef tenderness were present in the exudate.

Methodology

Forty steers were slaughtered according to standard commercial procedures in an Irish abattoir. pH, conductivity and temperature were recorded up to 24hrs postmortem in the factory and thereafter at The National Food Centre. M. *longissimus dorsi* (LD) was removed and steaks (2.54 cm thick) prepared for quality analysis over the 14 days post-

mortem ageing period. In order to minimize variation, each steak was taken from the LD at the same area on each carcass. Attributes measured included sarcomere length (day 2 post-mortem), colour (day 14 post-mortem), warner-bratzler shear force (day 2, 7 and 14 post-mortem), water holding capacity, intramuscular fat and moisture. Data were analysed to categorised the samples on the basis of toughness/tenderness. Potentially confounding factors such as extreme values of sarcomere length and intermuscular fat (IMF) were considered when catagorising samples.

Different methods for the collection of exudate were evaluated: (1) collection directly from the carcass shortly after slaughter, (2) collection at 2, 7 and 14 days using the protocol of Honickel (1987) (gravitational) and (3) collection after centrifugation of a 9g core at 37000g (4ºC) for 20 minutes (centrifugal method). Various sample preparation methods were also considered. The optimal collection/preparation procedure was shown to be a simple centrifugation step combined with dissolving the sample in SDS sample buffer. SDS-PAGE (Laemmeli *et al*., 1970) was conducted using different acrylamide concentrations (7%, 12% and gradient (8-18%) acrylamide gels). Based on visual assessment bands of interest were identified based on differences between tough and tender meat. Repeatability studies (n=10) were carried out to ensure reproducibility of these results. Gels were scanned using a colour image scanner (Epson perfection 3200) and optical density (OD) values obtained using Labworks 4 proteolysis analyser package. Coefficient of variation (CV) was calculated on these values (expressed as 'OD of band of interest' and 'OD relative to OD for total lane') to aid future semi-quantitative analysis of results. Bands of interest were selected and qualitative analysis carried out by MALDI-ToF following typsin digest.

Results & Discussion

From the comparison of the three different methods for retrieving drip loss it was found that the optimal method was the centrifugal method (Figure 1). The gravitational one produced similar qualitative results but was more time consuming and did not always provide sufficient volume of exudate.

Two samples from the tough and tender groups were selected for analysis by SDS-PAGE. Visual assessment of these gels indicated some differences in banding patterns between the two. In particular a fragment around the 37 kDa region was present in both groups early postmortem but disappeared totally in the tender group (Figure 2). Repeatability studies were successful as CVs of approximately 2-6% were obtained for 'OD of band of interest' and 'OD relative to OD for total lane'.

In order to characterise the protein fragments that could be isolated from the exudate by 1D SDS-gels a MALDI-ToF was made finding that all the proteolytic fragments identified were products of regulatory enzymes from the muscle metabolism such as amylo-1,6-glucosidase, pyruvate kinase, glyceraldehyde 3-phosphate dehydrogenase, lactate dehydrogenase, carbonic anhydrase and phosphoglycerate mutase (Figure 3). The lactate dehydrogenase fragment corresponds to the 36.9kDa. Previous studies (Stoeva *et al*., 2000, Nakai *et al*., 1995) on TCA soluble fragments from beef and in drip loss from pork (Lametch. *et al*, 2003) had reported some peptides originated from degradation of sarcoplasmic and myofibrillar proteins.

Initial semi-quantitative analysis has been carried out on a larger number of samples and results indicate that the density of this band may be a useful indicator of tenderness in conjunction with other measurements. Further research is on-going to verify this.

Conclusions

A method was developed to enable visualisation of products of proteolysis in bovine muscle exudate. Bands of interest were analysed qualitatively by MS MALDI ToF. Disappearance of lactate dehydrogenase in the bovine exudate may be indicative of the proteolysis of muscle proteins and hence may have potential as an indicator for tenderness.

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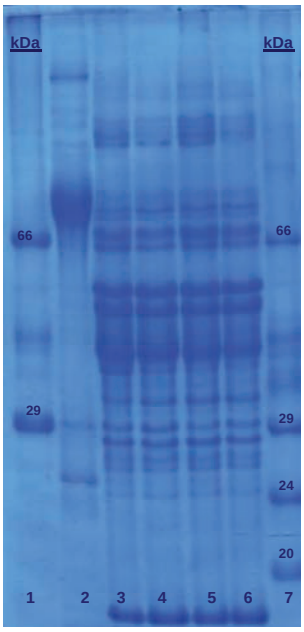

Figure 1: 12% acrylamide SDS-PAGE gel comparing three collection methods of drip loss. The drip collected directly from the carcass did not have a clear band pattern.
Lanes:

1. **High molecular standard marker (kDa)**
2. **0 days drip collected directly from the carcass**
3. **2 days gravitational**
4. **2 days centrifugal**
5. **7 days gravitational.**
6. **7days centrifugal**
7. **Low molecular standard (kDa)**

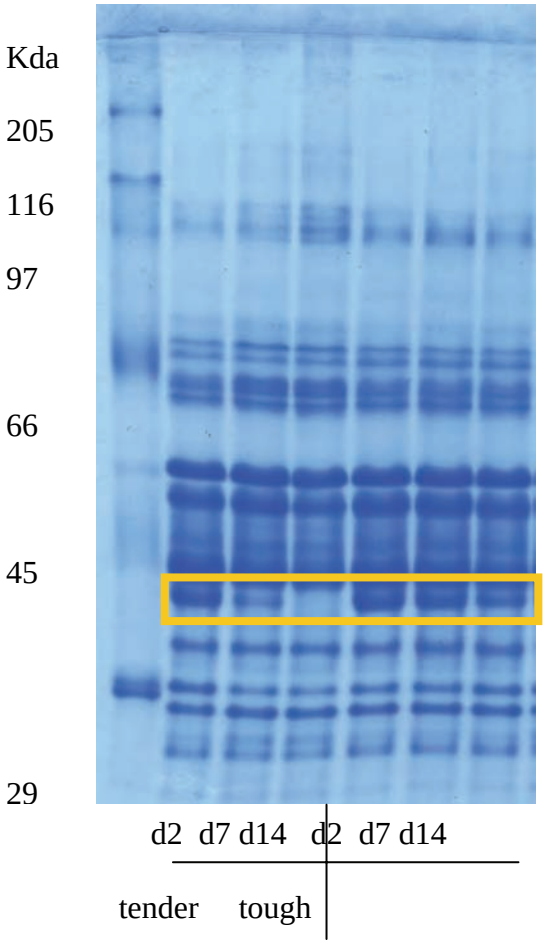

Figure 2: 12% acrylamide SDS-PAGE gel comparing centrifugal exudate from samples classified as tender and tough. We can see how the 36.9 kDa band is disappearing with time

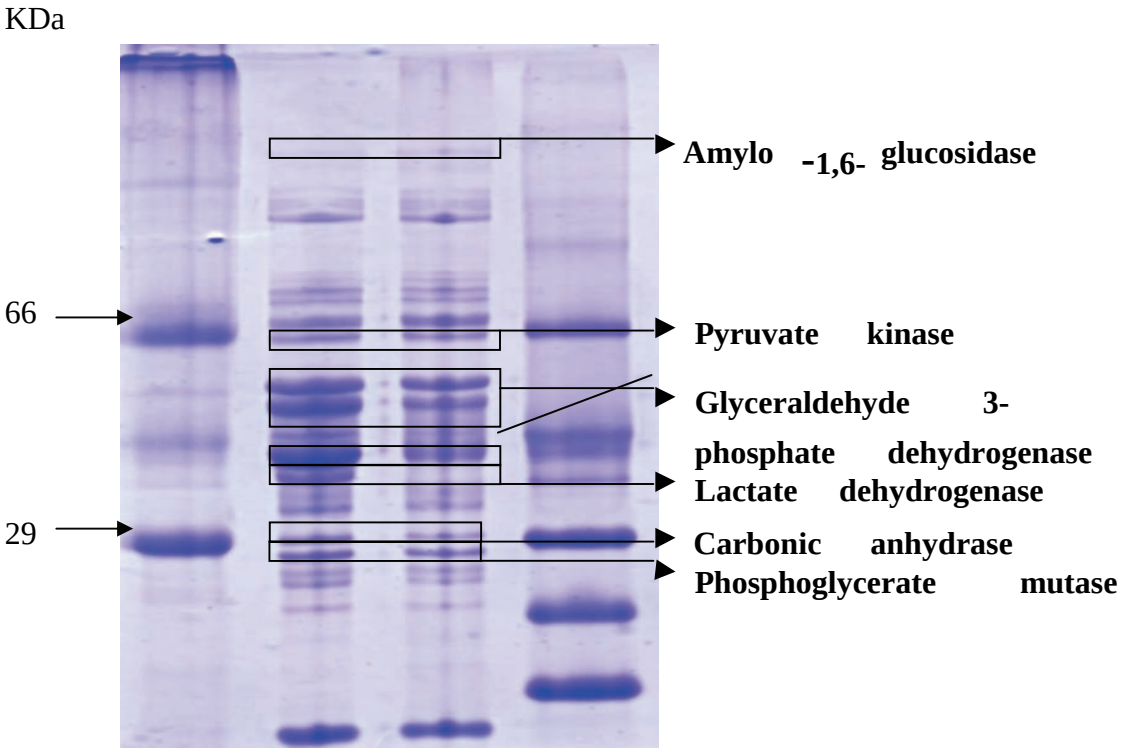

Figure 3: 12% acrylamide SDS-PAGE minigel. Lanes 1,4 : molecular weight markers 2 : tough sample and 3: tender sample. This minigel was used for isolating the samples for identification of fragments using MADI-TOF