Does the loss of Proteoglycan relate to the increased tenderness during conditioning of meat?

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INTRODUCTION

The texture of meat depends on the relative contributions of the myofibrillar proteins and the intramuscular connective tissue (IMCT). We have previously proposed that the IMCT exerts its effect through the presence of thermally stable intermolecular cross-links stabilising the collagen fibres. The presence of these cross-links increases the tension generated on thermal denaturation of the collagen during cooking, and provides strength to the denatured collagen fibres holding the denatured myofibrillar proteins together. In short, the loss of fluid due to shrinkage of the collagen, the resistance of the denatured myofibrils and the residual strength of the denatured IMCT collagen determine the texture of the cooked meat (Bailey 1989 ; Bailey and Light 1989) This concept applies to cooked meat whether conditioned for prolonged periods or not. However, many changes occur in both the myofibrils and the IMCT during conditioning which alter the overall level of tenderness and the relative contributions of these two components.

The changes occurring during the conditioning of beef involve enzymic degradation and affect both the native myofibrils and the intact IMCT. Although the amount of IMCT is small, usually about 1% of the myofibrillar content, its importance in determining texture has given rise to studies of the changes in its composition that occur during conditioning. The IMCT is comprised primarily of collagen fibres embedded in a very small amount of proteoglycans . Pedersen et al (1994) reported the presence of decorin and a large dermatan sulphate proteoglycan in bovine striated muscle, and further, that tough and tender muscles appeared to have different proportions of these PGs. Nishimuri et al (1996a;1997) have reported the presence of two PGs in the endomysium and one in the perimysium, and that the decorin of the perimysium was only detectable in bovine muscle after 6 months of age. Additional studies by this group demonstrated that the PGs of the perimysium are degraded during conditioning leading to a separation of collagen fibrils observed in the electron microscope, and they concluded that degradation of PGs appeared to be the main factor in a reduction of the mechanical strength of the IMCT and hence in the toughness of meat (Nishihara et al 1996b). Certainly one might expect the protein core of these PGs to be cleaved by enzymes but the observed effect on texture may have been due to an effect on the collagen. There is the possibility that the enzymes degrading the PGs are also capable of cleaving the telopeptide bonds, and this question was not investigated. The cleavage of only a few bonds along the fibre would reduce its strength but make chemical detection of the changes very difficult. Indeed, evidence for partial degradation of collagen during extensive conditioning has been reported using physical-chemical techniques, for example, slight increases in solubility (Stanton and Light 1990), a decrease in the thermal shrinkage temperature by differential scanning calorimetry. (Judge 1984), and a decrease in the isometric tension generated on heating (Etherington 1987).
In order to convincingly demonstrate whether PGs play a role in the mechanical strength of collagen and whether their loss during conditioning leads to a reduction in the tensile strength of the IMCT and consequently a decrease in toughness, it is necessary to demonstrate that the removal the PGs occurs without damaging the collagen fibre. In this paper we present preliminary evidence on the removal of PGs and the effect on the mechanical properties of the collagen fibres.

MATERIALS AND METHODS

Epimysium was carefully dissected from porcine extensor carpi radialis muscle of both left and right forelimb, approximately 1cm from the tendon insert, immediately following slaughter and a sample taken immediately for microscopy. The epimysium was washed in phosphate buffered saline (pH 7.5) and stored at -20 C until required for analysis.

1. Enzyme Incubation

The dissected epimysium was divided into three equal strips and one strip from each muscle placed in each of three vessels containing 20mls of 20mM ammonium carbonate, pH 8.0. **Sample 1**. The endogenous enzymes were inhibited by a cocktail consisting of 10 mM EDTA; 10mM N-ethyl maleimide ; 1mM PMSF; 0.05mM pepstatin. **Sample 2**. 25 ug sequence grade trypsin was added. **Sample 3**. No additions.
The three samples were incubated for 2 hours at room temperature, the fibres removed from the buffer, rinsed in 2x 20 ml washes of distilled water and stored at -20 C until required for analysis.

2. Electron microscopy

Samples were pre-treated with cuprolinic blue prior to normal preparation for transmission electron microscopy.

3. Proteoglycan assay

Aliquots of the 20mM ammonium carbonate incubation media was digested with papain at 60°C overnight and then assayed colourimetrically at 535nm after reaction with the metachromatic dye 1,9 dimethylmethylene blue according to the method of Farndale et al (1986)

4. Hydrothermal isometric tension. (HITT)

Replicate strips (2mm x epimysial thickness x 15mm) were cut from each piece of epimysium, using parallel blades, from each 2 hour incubation and mounted in an Emery paper (p400) template or "window" approximately 10mm long and secured in place using Loctite 406 instant adhesive. The whole template was then mounted in the jaws of the testing equipment and the window cut open to enable the sample to be tested over a temperature range from 40°C - 80°C using the method described by Bailey & Kent (1989).

5. Differential scanning calorimetry. (DSC) Small replicate aliquots of each sample (7 - 11.5mg wet weight) were accurately weighed into aluminium pans and sealed. The samples were then assayed using a Perkin Elmer DSC7 differential scanning calorimeter over the range 30°C to 80°C using an empty pan as a reference. From the original wet weights and the DSC thermograms the enthalpy value per unit mass of sample was determined. (Knott et al. 1995)

RESULTS and DISCUSSION

We have attempted to remove the proteoglycans associated with the collagen of a muscle epimysial sheath without affecting the collagen by employing a very short incubation with trypsin. This rapid digestion of the proteoglycans would not be expected to cleave the telopeptides of collagen since they do not contain the specific substrates of trypsin, i.e. lysine and arginine, but would readily digest the protein backbone of the proteoglycan.
The trypsin was found to be very effective in removing the proteoglycans as revealed by the electron microscopy (Fig 1 and 2) and the proteoglycan assays. However, analysis of the physical properties of the collagen by HITT in the absence of trypsin revealed considerable relaxation of the tension generated at the denaturation temperature of collagen when heating was continued to 80°C. This relaxation can only be attributed to absence of, or reduction in, the proportion of thermally stable intermolecular cross-links, due in this case to the cleavage of the telopeptides. The increased relaxation observed in the presence of trypsin is presumably due to the activation

of the naturally occuring endogenous enzymes capable of cleaving these telopeptides. The ability of these enzymes to cleave the telopeptides is confirmed by the inhibited samples, when little or no relaxation occurred (Fig 3). Incubation under these conditions in the presence of endogenous enzymes is analogous to prolonged conditioning at 5°C, and it is clear that the PGs are degraded within a few hours at this temperature. The effect of these enzymes on the collagen also is confirmed by the shoulder on the DSC thermogram peak, which indicates the presence of partially digested fibrils denaturing at a slightly lower temperature than the control incubated with totally inhibited enzymes (Fig 4).

Although preliminary these results suggest that the loss of PGs shown by electron microscopy cannot be directly related to the reduction in toughness of meat during conditioning since the collagen is also weakened.

Clearly further, more detailed, studies are required to demonstrate the role, if any, of the proteoglycans in the increased tenderness of meat during conditioning.

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Fig. 1

Zero time

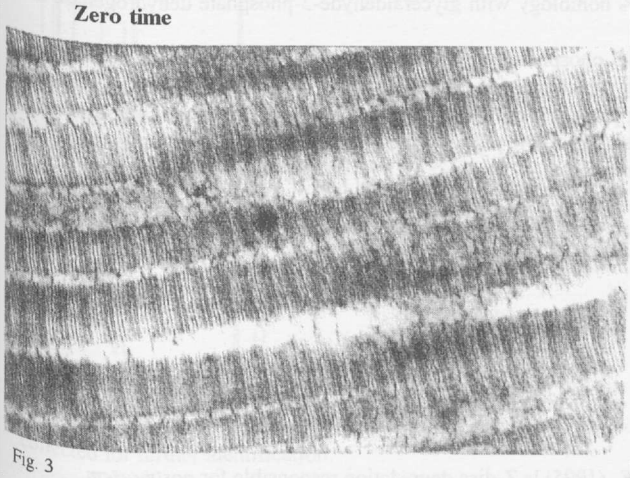

Fig. 2

2 Hours Trypsin digest

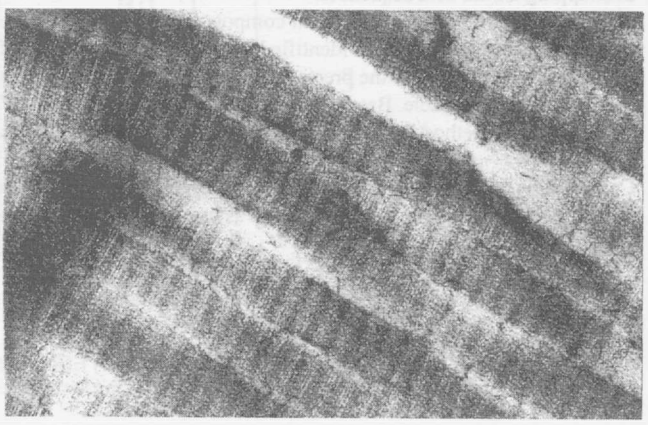

Fig. 3

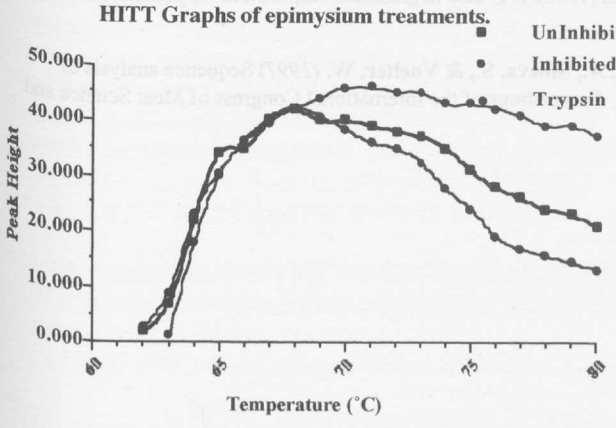

Fig. 4