THE STRUCTURAL BASIS OF MEAT TOUGHNESS: WHAT ROLE DOES THE COLLAGENOUS COMPONENT PLAY?

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SUMMARY

The current vogue in meat tenderness studies is weighted towards post-mortem enzymatic conditioning as a primary means of describing variations in toughness/tenderness. This paper steps back from proteolysis considerations and reviews our knowledge of the collagenous contribution to toughness. New studies show connective tissue to be the sole continuous element in many common muscles, and so a prime determinant of their longitudinal strength. Measurements of the tensile properties of single muscle fibres isolated from raw and cooked muscle are also described which imply a role for the endomysium in defining in part the yield point and the extensibility of muscle fibres before rupture.

INTRODUCTION

Historically our ideas on the structural basis of meat tenderness and research efforts to explain and control toughness have passed through phases when attention focused on either the myofibrillar component on the collagenous component of muscle. The current balance is currently weighted towards post-mortem enzymatic conditioning of myofibrillar structures as a primary means of describing variations in toughness/tenderness [Dransfield, 1994; Koomarie, 1994]. There are new insights and opportunities for improving meat tenderness from this approach, but in a system so complex as muscle tissue, it is perhaps wise to retain an appreciation of the relevant contributions of all possible sources of meat toughness. In the spirit of retaining a balanced view by giving other considerations equal time, this paper reviews our knowledge of the collagenous contribution to toughness. However, it must be stressed that a simple assignment of various factors affecting tenderness as either "myofibrillar" or "collagenous", to the point of exclusion of the other is likely to be a gross over-simplification. There is a possible mix of many types of structural mechanisms occurring side by side in the whole tissue, and the interactions between them.

CONNECTIVE TISSUE COMPOSITION AND MEAT TENDERNESS/ TOUGHNESS

Although there are arguments (Dransfield, 1992) that there are variations in the calpain content of different muscle fibre types and that older animals tend to have higher levels of calpain II (implying a decreased rate of post-mortem conditioning), the long-held and still generally accepted view is that the quantity, composition and distribution of intramuscular connective tissue (IMCT) are major causal factors in the variations in meat toughness seen between different muscles and increasing toughness with increasing animal age. As pointed out by McCormick (1994), IMCT is dynamically remodelled; its synthesis and turnover are regulated by a range of factors including exercise or immobilisation (reviewed by Purslow & Duance, 1990) and there is therefore a potential to manipulate IMCT so as to improve tenderness.

The overall amounts of IMCT in muscle change slightly with age in some muscles and not in others, but there is consistent evidence that IMCT composition alters with animal maturity. Allied with this is an increased thermal stability(Smith & Judge, 1991; Horgan et al., 1991). Much of the attention in recent years has focused on the role of mature collagen crosslinks in determining toughness. Although the number and identity of mature crosslinks are not fully known, the major mature crosslink of the hydroxylysine (ketoimine) pathway has been identified as hydroxypyridinium (HP) (Eyre, 1987). HP is present at high levels in tissues such as cartilage and intervertebral disc, but occurs at a much lower concentration (only one sixth or less of the levels in cartilage) in IMCT. Horgan et al. (1991) found that Erlich's Chromogen (EC), another putative mature crosslink of the ketoimine pathway, decreased with animal age in five muscles studied. However, the HP content of intramuscular collagen is known to increase with age, in line with increasing thermal stability of IMCT. McCormick (1994) summarises information in the literature on the variation in HP concentration with age in a wide range of muscles. In all cases the HP content of the intramuscular collagen increases with age. HP content may also be higher in slow-twitch muscles than in fast muscles (Palongas et al., 1992). However, the considerable variability in the HP content of muscles, the relatively low concentration of HP and EC in IMCT in comparison to other connective tissues and poor correlations between HP levels and sensory and Warner-Bratzler measurements of toughness (Young et al., 1994) all suggest that additional mature crosslinks may need to be quantified to establish convincing correlations between IMCT crosslinks and increasing toughness with animal maturity.

THE ROLE OF PERIMYSIAL CONNECTIVE TISSUE

Although methods for quantitation are rather imprecise, perimysium comprises the bulk of IMCT (Light et al, 1985) and is recognised as playing a major role in the connective tissue component of toughness (Carrol et al., 1987; Purslow, 1985, 1987; Totland et al. 1988; McCormick 1994). Lewis & Purslow (1990) demonstrated the high forces required to rupture perimysium relative to endomysial-perimysial separation, another mechanism involving IMCT that occurs in fracture of meat. Heat-induced alterations in the structure and properties of the perimysium are thought to contribute to development of toughness on cooking. Isometric tension experiments to study the effects of animal age on the shrinkage forces produced when collagen is heated have demonstrated fair agreement between the relaxation of the maximum force generated on heating and the thermal stability of covalent crosslinks between collagen molecules (Kopp and Bonnet, 1987). In isometric tests the collagen is heated whilst restrained at its original length, whereas the normal cooking losses of up to half of the original volume of the meat mean that significant shrinkage of the connective tissue component can and does occur. Lewis and Purslow (1989) point out that stiffness and strength measurements on perimysium isolated after normal *in situ* cooking are far more realistic than isometric tests. Their experiments on perimysium excised from cooked muscle allowed observation of its deformation and fracture properties by use of a small-scale mechanical testing apparatus fitted to a microscope stage. Their results showed the initial stiffness of perimysial material to increase with temperature, due to the crimped collagen fibres straightening out, and that the strength of perimysium increased up to 50°C, but monotonically decreased at higher temperatures up to 90°C. A fall in the intrinsic strength of the perimysial material above 50°C is in agreement with the fall in strength between 50°C and 60°C shown by the whole network of perimysial sheaths in a transverse tensile

specimen of cooked meat (Bouton & Harris, 1972). In contrast to the strength of the perimysial material, the transverse strength of whole meat rises again in most muscles tested by Bouton & Harris (1972), showing how results from this test combine the effects of temperature effects on the perimysial material together with the effect of cooking shrinkage, which increases the number of perimysial sheaths per unit cross-section of muscle. Subsequent studies on the strength of perimysium excised from meat before and after post-mortem conditioning (Lewis and Purslow 1991a) showed that conditioning reduces the strength of perimysium from raw meat, but that after cooking to 60°C and above, no effect of conditioning on perimysial strength is seen. Together with studies of the effect of low pH in decreasing the strength of perimysium isolated from cooked meat (Lewis and Purslow 1991b), this series of studies has clarified the changing properties ofthe perimysial connective tissue with cooking temperature under a variety of conditions.

MORPHOLOGICAL DISTRIBUTION OF IMCT; THE SERIES-FIBRED MUSCLES

Rupture of muscle fibre bundles (fascicles) whether during chewing, shear testing or in longitudinal tensile tests, involves the rupture of the endomysial connective tissue separating individual muscle fibres. A large number of muscles in mammalian and avian species, including common food animals, are series-fibred; ie muscle fibres do not run continuously along the entire length of the fascicle, but terminate intrafascicularly (The structure and occurrence of series-fibred muscles is reviewed by Trotter 1993). The endomysial connective tissue acts to link these short, discontinuous fibres and provide the means to transmit contractile force from them to the tendinous attachments. It has an obvious contribution to the mechanical integrity of the muscle because it is the only continuous structure along the length of a fascicle. The bovine sternomandibularis muscle has been a commonly used muscle in meat texture studies, and was the principal experimental material in the large series of papers by Davey and other workers at MIRINZ in the 1970's on the structural basis of toughness. This muscle has recently been the subject of a structural study by Purslow & Trotter (1994) in which it was shown that bovine sternomandibularis is a classic series-fibred muscle. The arrangement of fibres in a fascicle from this muscle is shown schematically in figure 1. Although the muscle fascicles that run from one end of the muscle to the other are typically about 35 cm long, individual muscle fibres are approximately 5 cm long, and end in fine tapering points within the body of the muscle, except for those ending at the muscle insertions. The pattern of innervation of the muscle cells by 18 -20 distinct motor end plate bands evenly spaced along the length of the muscle with inter-band distances in the order of 1.8 cm (Purslow & Trotter, 1994) implies that adjacent muscle cells are axially staggered by approx. 36 % of their length (ie neighbouring fibres overlap each other for approx. 64% of their length).

The interpretation that the effects of factors such as conditioning and cold-shortening on toughness are entirely myofibrillar in origin largely stems from the work of Davey et al. (1976) on this muscle, showing that the effects of these factors persist after very prolonged cooking at 90°C, which was assumed to solubilise the IMCT. The proportion of fibres that terminate within a specimen cut from the length of the muscle will depend on its length, the muscle fibre length and their degree of overlap. For a 5 cm long shear test specimen (such as that used by Davey & Gilbert, 1976) cut from the sternomandibularis muscle, the vast majority of the fibres will not run the length of this

block even in raw muscle. In a 5 cm length cut from cooked muscle, where there has been appreciable shrinkage of the length of the individual fibres, 100% of fibres will terminate within the specimen. In such a preparation from a series-fibred muscle, it is difficult to accept the assertion made by Davey & Winger (1979) that "prolonged cooking destroys the collagen of the connective tissue to the point that all adhesion between the muscle fibres is lost"; if this were true then the sample would just fall apart. In order for the short fibres to cohere together in a reasonably integral lump of tissue which has some measurable strength there must be a mechanically competent residuum of the intramuscular connective tissue network linking them together even after prolonged cooking. The possible contribution of endomysial connective tissue strength to variations in shear force with sarcomere length therefore exists. Davey & Winger (1979) do allow this as a possibility that cannot be disregarded.

Structurally, the endomysium is a somewhat disorganised planar feltwork of largely curvilinear collagen fibrils (Mauro & Adams,1961; Rowe, 1981; Trotter & Purslow, 1992). Although the collagen fibrils appear almost randomly oriented, changes in the distribution of fibril orientations in the endomysium of sternomandibularis muscle on changing muscle sarcomere length have been quantified by Purslow & Trotter (1994). The relationship between the mean fibril orientation and muscle length is shown in figure 2. Reorientation in the endomysial network is well-described by the model erected to describe themeasured reorientation measured by Purslow (1989) for perimysial collagen fibres in the same muscle.

The breaking strength of any fibrous network depends on the orientation of its fibres at rupture. In uncooked meat the crimped perimysial collagen fibres and curvilinear endomysial fibrils reorientate as the muscle is extended to breaking point; from any initial orientation (as determined by muscle sarcomere length) the network will reorientate and finally break in a longitudinally extended state. Longitudinal strength of the whole muscle therefore only depends on the number of IMCT sheaths per unit cross-sectional area, a parameter which increases linearly with sarcomere length. Cooking straightens out the collagen fibres and fibrils, which now cannot reorientate because the volume of the fibres and fascicles enveloped by straightened fibres would have to decrease for reorientation to occur. The strength of the heat-denatured IMCT networks now depends on their initial orientation, and so on sarcomere length. Fibrous composite theory (eg Harris, 1980; Hull, 1981) shows that the longitudinal strength (s_b) of a lamina containing fibres at an angle q to the longitudinal axis will be controlled by whichever is the weakest of the following three considerations;

$s_b = s_p/\cos^2 q$ for failure controlled by the tensile fracture of the fibres

$s_b = s_t/\sin^2 q$ for tensile failure normal to the fibres

$s_b = t/\cos q \sin q$ for failure controlled by shear between the fibres

where s_p= strength of composite parallel to the fibres, s_t= strength of composite transverse to the fibres and t= shear strength of composite parallel to the fibres.

All three failure mechanisms show an obvious dependence of strength on fibre orientation, q. Thus, as long as the assumption that straightening of collagen fibres on heating prevents reorientation is a fair one, it is reasonable to predict that the longitudinal strength of both endomysial and perimysial connective tissue components of cooked muscle may change with collagen fibre orientation, and hence sarcomere length. q effectively increases in both the perimysium and endomysium as the muscle length shortens. For failure controlled by the fracture of the collagen fibres or by shear between the fibres, this reorientation on muscle shortening leads to a higher strength of the network. (The contribution of the IMCT to the overall strength of the whole muscle will again be affected by the muscle length-dependent number of connective tissue sheaths per initial cross-sectional area, but even with this taken into account the strength of the IMCT components overall is still expected to rise with muscle shortening.) Lewis & Purslow (1989) observed fracture of perimysium to occur by shear failure, but this failure mode in strips excised from cooked meat (and so freed from the constraint of the muscle fibre bundles) may possibly be different to that of perimysium fracturing *in situ* with in whole meat. Moreover, the variation of IMCT strength with sarcomere length should be quite different between the raw case, where IMCT network reorientation can occur, and the cooked case, where reorientation is difficult. This corresponds to the observation that the variation in toughness with sarcomere length seen for cooked meat (Davey & Gilbert, 1975) is of a fundamentally different nature than that for raw muscle (Rhodes & Dransfield, 1974). No satisfactory explanation for this very different shape of relationship between toughness and sarcomere length in raw versus cooked meat has been proposed previously.

The above considerations do not necessarily mean that the strength of intramuscular connective tissue does indeed control the shortening toughness of cooked meat, but it does point to this mechanism as a possibility that should be taken into consideration. Rather than implying structural events responsible for toughness from modelling assumptions, we really need direct and unequivocal experimental tests of such models. One definitive test of the myofibrillar versus IMCT contribution would be provided by the measurement of the tensile strength of isolated single fibres or myofibrils from raw and cooked muscles. We have recently begun to establish such experiments.

SINGLE MUSCLE FIBRE STUDIES

Load-deformation curves and simultaneous light microscopy observations of single muscle fibres extended to breaking point have now been made on fibres from raw meat and from meat cooked to 80°C (Mutungi et al., 1994). This is the first reported case of load-deformation curves for individual fibres from cooked muscle, and shows strong evidence for endomysial involvement in the fracture properties of both raw and cooked fibres. For both raw and cooked fibres, the load-extension curve shows a high initial resistance to extension. After a yield point the fibres are more compliant, showing greater length extension as the load increases, until rupture finally occurs.

Structurally, the initial rising phase of the stress-strain curve is associated with very small but uniform extensions in sarcomere length within the fibre. At the yield point it is common to observe multiple transverse ruptures along the length of the endomysium surrounding the fibre. The post-yield region of the stress-strain curve is associated with non-uniform extension of sarcomeres; sarcomeric extension principally occurs at the site of endomysial rupture. The order of

magnitude increase in the extensibility of cooked versus raw fibres is principally due to the increased number of these extensible sites along the fibre in the cooked case. The role of the endomysium in determining the mechanical behaviour of single fibres has been demonstrated by comparing the properties of whole fibres to demembranated fibres. Whole fibres bear higher loads at low and intermediate extensions, but break at lower overall extensions.

CONCLUSIONS

The contribution of IMCT to variations in cooked meat tenderness/ toughness is but one element of many in a complex system. Changes in the interactions between IMCT and muscle fibre bundles on conditioning have been proposed previously (Purslow, 1991) and of course proteolytic events within the muscle fibres are important aspects which rightly deserve the attention they currently attract. Nevertheless, the role of IMCT in the overall picture should not be neglected in any balanced view of meat toughness.

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