Relationship of collagen content, type and cross-linking with texture of different muscles

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Summary

Perimysium and endomysium were purified by a new method from six bovine muscles of varying toughness. The mean perimysial collagen fibre diameter and the perimysial collagen cross-link content were measured. Correlations were found with meat toughness for both parameters. Purified endomysial 'ghosts' were shown to shrink on heating to at least half their native resting volume between 57°C and 65°C. A theory for the involvement of collagen in determining meat toughness and texture at the molecular level is proposed.

Introduction

Collagen is the major connective tissue protein and accounts for 55-95% of the total dry weight of insoluble intramuscular connective tissue (Light et al., 1984). It is found in the three hierarchical structural domains of muscle connective tissue (Bailey et al., 1979); the epimysium, which forms the muscle sheath, the perimysium, which ensheaths the muscles fibre bundles, and the endomysium, the sheath surrounding each muscle fibre (Ham, 1969).

Collagen is a long, rod-like molecule formed as a triple-helix from three polypeptide chains. The molecules aggregate to form either extended fibres (epimysium and perimysium) or a structural matrix (endomysium). When heated above their denaturation temperature, the molecules shrink to one quarter their native length and melt. If the molecules are linked by intermolecular bridges (cross-links) then (providing these bonds are heat-stable), instead of melting to form gelatin, the fibres and matrices shrink, remaining insoluble, and force is developed. It can be postulated that the number of heat-stable cross-links must be related to the force developed and early experiments showed this hypothesis to be accurate (Shimokomaki et al., 1972; Allain et al., 1978). It should be noted, however, that collagen, when newly laid down, contains both heat-stable and heat-labile cross-links and that, as it matures, these are converted to more complex, more stable bonds (Light & Bailey, 1980).

Shrinkage of cross-linked collagen fibres and matrices may be expected to influence the toughness and texture of cooked meat in at least two ways. Firstly, the shrinkage of large tracts of perimysium and endomysium may initiate and accelerate water loss. Secondly, as Purslow (1984) has shown that the preferential site of fracture in cooked meat is through the perimysium, then the extent of heat-stable cross-linking in this connective tissue must determine the ease with which fracture is induced.

In the case of cooked meat the situation is complex and the great variations in toughness and texture between muscles from the same animal cannot simply be attributed to differences in collagen cross-link content. Many other factors must influence quality. However, our recent studies have illustrated good correlations between collagen cross-linking and known textural variations between different muscles (Light & Bailey, 1983; Light et al., 1984).

In this paper we investigate the differences in perimysial collagen fibre diameter and cross-linking in a range of muscles of differing toughness. We have also studied the extent of shrinkage of the endomysium during cooking in a further attempt to understand the role, at the molecular level, of collagen in determining texture.

Materials and Methods

Psoas major (PM), longissimus dorsi (LD), semitendinosus (ST), pectoralis profundis (PP), gastrocnemius (G) and sternomandibularis (SMD), chosen to represent a set of samples with widely varying texture and toughness, were obtained whole by careful dissection from a normal, 18 m steer after slaughter and were treated immediately. Tritiated KBH_4 was obtained from Amersham International, Bucks, U.K.

The perimysium and endomysium from each muscle were prepared by a modification of McCollester's method (1962) as previously described (Light and Champion, 1984). The process involved homogenisation, filtering and finally, extensive washing with sodium dodecyl sulphate (SDS) buffered at pH 7.4 with Tris-Cl. Washed perimysial preparations were treated and positively stained as previously described (Light and Champion, 1984) prior to embedding in resin blocks and sectioning and viewing by transmission electron microscopy.

SDS-extracted perimysium and endomysium were resuspended, after milling in liquid N_2, in phosphate-buffered saline (0.15M NaCl, 0.02M sodium phosphate buffer pH 7.2) and were reduced with tritiated KBH_4 as described by Robins et al. (1973). The reduced material was washed, hydrolysed in acid and subjected to ion-exchange chromatography by which process tritiated cross-links were separated and quantified (Light & Bailey, 1982).

Purified endomysial 'ghosts' which had not been subjected to SDS washing were heated on the stage of a standard light microscope adapted for the experiment. A gradient from 30°C to 80°C was applied in steps of 2°C or 5°C. At each temperature point a time of 2 min was allowed for equilibration of the system. The shrinkage temperature range for endomysium was thus calculated.

Results and Discussion

Fig. 1 shows electron micrographs of transferse sections of perimysial collagen fibres from the six muscles investigated. Measurement of the mean collagen fibre diameter in these perimysial samples showed a division into two groups. The two toughest muscles, sternomandibularis and gastrocnemius, had fibre diameters of 75 ± 12nm and 92 ± 24nm respectively. The other four muscles had perimysial collagen fibres of approximately 50 nm (49-54, mean 51.2 ± 11 nm). The variability in fibre diameter (i.e. the distribution of sizes in any one muscle) was roughly similar in all muscles except in gastrocnemius where the standard deviation was double that seen in the other five muscles. This grouping is interesting and suggests that collagen fibre size may have some contribution to differences in gross quality. However, fibre size alone cannot account for the differences in eating quality of muscles such as semitendinosus and pectoralis profundis. Other causative factors must be sought.

When the quantity of heat-stable cross-links in the perimysium was plotted as a function of the total compressive force obtained with each muscle after cooking at 75°C for 1 hr (Dransfield, 1977), it was seen that an approximately linear relationship existed (Fig. 2). Such a clear cut result could not be demonstrated for endomysium although the general trend was similar.

In our earlier study (Light et al., 1984) we showed that differences in the extent and type of cross-linking in epimysial, perimysial and endomysial collagen correlated well with taste panel toughness ratings for different bovine muscles. This finding corroborated an earlier report (Shimokomaki et al., 1972) which suggested that the higher levels of heat-stable cross-link lead to greater tension in the connective tissue during cooking. The differences we found (Light et al., 1984) were greatest in the epimysium. Calculation of the ratio of heat-stable to heat-labile cross-links as an indicator of toughness showed a four fold higher ratio in tough muscles compared to good quality muscles. The same trend was followed in the perimysium and endomysium where the maximal difference in the ratio was shown to be 2-2.5 fold.

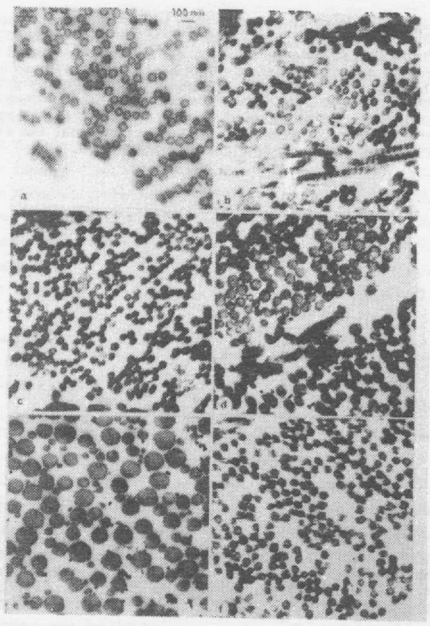

Fig. 1 Electron micrographs of perimysial preparations from the six muscles studied in this investigation. (a) Psoas major (b) longissimus dorsi (c) pectoralis profundis (d) sternomandibularis (e) gastrocnemius and (f) semitendinosis. The bar shown represents 100 nm.

In the present study we have correlated the total quantity of heat-stable cross-links in the perimysia studied with the compressive force measured for each muscle after cooking at 75°C for 1 hr. It is clear that a linear relationship exists (Fig. 2). This result shows that the type and quantity of the collagen cross-links plays a key role in contributing to the characteristics of tenderness or toughness. The combined effect of cross-link type, the differences in total collagen content (Light et al., 1984) and the variation in fibre diameter are all likely to contribute greatly to the known variation in quality of the six muscles examined. An important point to note is that the perimysium is highlighted by our results and those of Purslow (1984) as a key structure in determining texture.

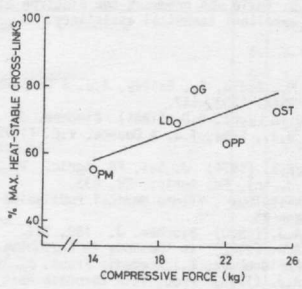

Fig. 2 Plot of total heat-stable (keto-imine) cross-links in six muscle perimysia vs. compressive force estimated after cooking the muscles for 1 hr at 75°C (data taken from Dransfield, 1977). For key to abbreviations see Materials and Methods.

We can predict the effect at the molecular level of the differences we have observed. Muscles with low levels of heat-stable cross-links in their perimysia will be more sensitive to mechanical disruption. Such perimysia will provide good routes for fracture during eating. On the other hand, perimysial collagen which contains high quantities of the heat-stable cross-links will be far less likely to shear on mechanical challenge leading to the sensation of 'tough meat'. Furthermore, endomysial collagen which is linked to perimysium by high quantities of heat-stable cross-links may be expected to be similarly less likely to break away from perimysium during mastication than interfaces containing low levels of heat-stable cross-links.

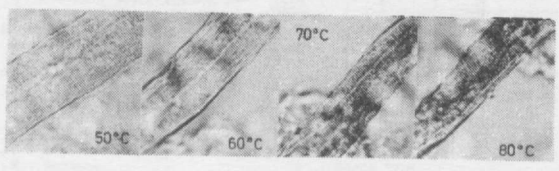

Fig. 3 Micrographs of an endomysial 'ghost' preparation at 50°C, 60°C, 70°C and 80°C.

Our preparations of endomysial 'ghosts' were heated on the microscope stage through a controlled temperature gradient. Heating was suspended at every 2°C rise in temperature to allow equilibration of the system. Fig. 3 shows that endomysial 'ghosts' readily shrink to about half their native volume at temperatures between 50°C and 80°C. By careful measurement of enlarged micrographs it was possible to obtain accurate measurements of endomysial diameters at each temperature. The graph shown in Fig. 4 shows that the main shrinkage of the 'ghosts' occurred between 51°C and 65°C. Note that two size population of endomysial 'ghost' were observed in our preparations.

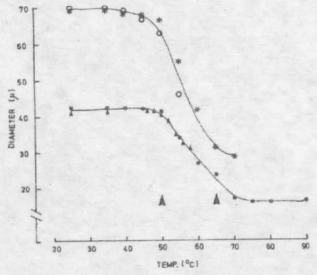

Fig. 4 Plot of volume of endomysial 'ghost' vs. temperature. Each set of symbols represents the results obtained for a different endomysial 'ghost'. The arrow-heads indicate the start and finish of the major shrinkage.

We showed that the type IV collagen of basement membrane (endomysium) contained exclusively heat-stable cross-links (Bailey et al., 1984) so we can assume that the shrunken endomysium will be particularly stable and retain its integrity and strength at high temperatures (i.e. at least 90°C - see Allain et al., 1978). What we do not yet know is the extent of the compressive force which can be developed by the endomysium on shrinking. In a recent study Offer et al. (1984) showed that the denaturation of the muscle fibres within the endomysial sheat at 40-50°C leads to their shrinkage. This is associated with a corresponding change in texture of the meat (Davey & Gilbert, 1974). At the same time, fluid is left filling the space left by the shrunken muscle fibres. Once the endomysial sheath begins to contract at 51°C the fluid may be squeezed out of the cut ends of the meat. Thus, the extent of shrinkage of the endomysium must directly influence the final water content of the cooked meat.

Secondly, as the integrity of the collagen matrix in the endomysium is likely to be retained at high temperatures, it must be considered as an equally important barrier to transverse fracture as is the muscle fibre . Indeed, no firm evidence e..sts to show that either structural unit is more important than the other in this respect. It is possible that it is the endomysium which most strongly resists transverse shear in cooked meat. Only further, detailed analysis can resolve this important point.

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