The role, at the molecular level, of collagen in meat texture

LIGHT, N.D. and BAILEY, A.J.

ARC Meat Research Institute, Langford, Bristol, UK

INTRODUCTION

It is generally agreed that collagen plays a significant part in determining the texture of meat, but the precise mechanisms involved have not been elucidated owing to the complexity of the role of the fibre organisation, collagen types and nature of the cross-linking in determining the physical properties of the collagenous matrix.

The collagenous connective tissue in striated muscle has long been classified on anatomical and histological grounds as epimysium, perimysium and endomysium. These connective tissues are clearly physically and functionally different, particularly the endomysium which consists primarily of an amorphous basement membrane. A study of the collagen cross-links revealed that the peri- and endomysium possess a higher proportion of thermally stable keto-imine cross-links than the labile aldimine cross-links of the perimysium (Shimokomaki, 1972). With increasing age of the animal both types of cross-link are further stabilised by conversion to unknown multivalent cross-links (Light and Bailey, 1980). The higher proportion of thermally stable cross-links is clearly important in determining the texture of meat following denaturation and shrinkage of collagen during cooking. The tension generated during thermal denaturation results in a compression of the muscle and loss of fluid, the greater the tension the greater the compression and hence toughness of the meat. The muscles producing the toughest meat might be expected to possess a higher proportion of the thermally stable cross-links.

A later study using collagen type specific antibodies revealed that the epimysium contains predominantly Type I collagen, the perimysium a high proportion of Type III collagen, and the endomysium predominantly Type IV with some Type V collagen (Bailey et al., 1979). The importance of the relative proportions of the different types of collagen in different muscles has not been determined.

In this paper we report the relationship of Type I/Type III ratios and intermolecular cross-links for muscles of varying toughness.

MATERIALS AND METHODS

The psoas major, longissimus dorsi, semitendenosis, pectoris profundis, gastrocnemius and sternomandibularis were chosen as muscles typical of increasing toughness and were obtained from a freshly slaughtered 2 year old steer. Epimysium was carefully dissected from each muscle and then 50 g wet weight of each muscle was chopped into 1 cm^3 cubes. Perimysium and endomysial sheaths were prepared by the method of McCollester (1962) with slight modifications.

Briefly, muscle was homogenised in cold mM $CaCl_2$ in a Waring Blendor. Filtering through fine gauze collected the perimysium in the retentate and allowed passage of the finely homogenised muscle fibres through into the filtrate. The perimysium of each muscle was washed exhaustively with water and then blot dried and weighed. Ten 20 mg portions of each perimysium were accurately weighed, dried and re-weighed. In this way the total dry weight of perimysium from each muscle was obtained.

Empty endomysial sheaths were obtained by lysis of the muscle proteins with sequential washes in 25 mM NaCl pH 7.4 at 37°C and cold water. These endomysial preparations were heated through a linear temperature gradient on a microscope stage and the shrinkage temperature was determined.

Determination of collagen cross-links

A portion of each perimysial preparation was homogenised briefly in 2% (w/v) sodium dodecylsulphate (SDS), stirred at room temperature for 30 m and then collected by centrifugation. The process was repeated and then the perimysial samples and epimysial samples were washed in phosphate buffered saline (PBS) pH 7.4. After suspension in the same buffer samples were reduced with tritiated KBH_4, washed and hydrolysed in 6 M HCl at 110°C as previously described (Robins et al., 1973). Collagen cross-links were analysed by ion-exchange chromatography on Zeolit 225 in pyridine-formate buffers as described (Light and Bailey, 1982) and the relative proportions of the tritiated cross-links were calculated.

Quantification of the ratio of Type I to Type III collagen

SDS washed perimysial and epimysial samples were digested with an equal weight of CNBr in 70% (v/v) formic acid at a final collagen concentration of 10-20 mg/ml for 4 h at 30°C. Aliquots of the dried digests were then subjected to SDS-polyacrylamide gel electrophoresis and the protein bands were visualised by staining with Coomassie brilliant blue. Type I and Type III collagen ratios in each sample were estimated by quantifying specific peptide bands as previously described (Light, 1982).

RESULTS AND DISCUSSION

The six bovine muscles studied in this survey ranged in relative toughness from psoas major (rated 1) to the toughest, sternomandibularis (rated 6). Over this range of toughness the total amount of perimysium in each muscle increased from 20 mgm/gm to about 60 mgm/gm. Also, the % collagen in each dry perimysium increased from 6.4% in psoas major to 16.1% in sternomandibularis. However, as we have previously demonstrated, it is not merely the quantity buy the quality, as assessed by cross-linking and collagen type, that is the major determining factor in the toughness of meat.

Texture has long been known to be related to the age of the animal. This is due to the fact that with increasing age there is a conversion of the divalent reducible cross-links to stable multivalent cross-links. The nature of these cross-links has not yet been elucidated, therefore it is not possible to determine their concentration in the collagenous tissue. We have therefore confined this study to muscles from the same animal on the assumption that ageing of the collagen in different muscles occurs at the same rate.

The total amount of cross-links, based on the tritium incorporated, was, as might be expected, constant for muscles from the same animal. However, there was a significant change in the ratio of the stable keto-imine (B') to the labile aldimine cross-link (B). The psoas major possessed a ratio of B'/B of 1:3 whilst the sternomandibularis possessed a ratio of 2:5. This increased proportion of the thermally stable cross-link with increasing toughness over the range of muscles studied is consistent with our previously stated proposal that the toughness due to collagen is determined by the tension generated against the muscle fibre during thermal contraction of the collagen. The extent of this contraction is directly related to the proportion of thermally stable cross-links. Furthermore, the residual strength of the denatured gelatinous fibre is also higher and since these fibres bind the muscle fibres together, there is greater adhesion, hence toughness of the cooked meat. The contribution of the 'mature' multivalent cross-links cannot as yet be assessed.

The proportion of Type III to Type I was found to be about 1:2 for all muscles investigated except longissimus dorsi which only showed a ratio of 1:5. The presence of Type III in perimysium has added a further parameter that has to be taken into account in propounding a molecular mechanism for the role of collagen in meat texture. The Type III collagen fibres possess the same intermolecular cross-links as the Type I collagen fibres but determination of their contribution to toughness is difficult to assess since this collagen is also stabilised by disulphide bonds. However, our data indicate that the relative proportions of the major genetic forms of collagen in the perimysium have little effect on toughness.

The role of the endomysium has received little attention primarily due to the difficulty of isolating and characterising the basement membrane collagen. The organisation of the Type IV molecules in the basement membrane has recently been proposed as a chicken-wire mesh and we have demonstrated that the molecules within that matrix are stabilised by the thermally stable keto-imine cross-link (Bailey et al., in preparation). Like

collagen fibres, the basement membrane collagen denatures and shrinks on heating, but owing to its less crystalline organisation, this occurs over a wider temperature range. Our preliminary studies, using the heating stage of a standard light microscope, indicate continuous shrinkage of endomysial sheaths from 55°C through to 80°C. Despite the stability of its cross-links, we cannot yet compute the extent of compression caused by endomysial shrinkage as its structure has not yet been determined. It is likely, however, that the tension generated is sufficient to cause further loss of water from the cooking meat.

Although the precise details need to be worked out, there is now a clear rationale for the role of collagen in the texture of meat. During cooking the actomyosin denatures at 40-50°C such that the muscle fibres shrink within the endomysium. This stiffening of the actomyosin gel leads to an increase in texture of the meat. A second more dramatic increase in texture, as determined by shear value, occurs between 65-70°C when the collagen denatures, and if restrained, for example by the muscle fibres, generates a tension. Shrinkage of both the perimysial collagen fibres and the collagen network of the endomysium therefore results in a compression of the intracellular space. This force is certainly sufficient to result in loss of fluid from the muscle fibres and a pushing together of the denatured muscle fibres. In extreme cases, higher temperature or highly cross-linked old collagen, compression of the denatured muscle fibres themselves may even occur.

In short, collagen, although only constituting about 2% of the total protein of meat, plays a significant role in determining the quality of the sensation of toughness of meat.

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Table 1

Total content of perimysial collagen, its cross-links and major genetic types in muscles of different texture

Muscle	Texture Score[1]	Wet Weight per gm wet meat (mg)	Dry Weight per gm wet meat (mg)	Total Collagen per gm wet meat (mg)	% Collagen in Dry Perimysium	Cross-links per gm collagen	B'/B	% Type III $\frac{\text{Type III} \times 100}{\text{Type I + Type III}}$
Psoas major	1	85	27	1.7	6.4	144	1.3	33.0
Longissimus dorsi	2	220	63	4.1	6.5	nd	nd	17.5
Semitendenosus	3	122	46	3.1	6.7	380	2.3	30.5
Pectoralis profundis	4	54	23	1.6	6.9	194	2.5	29.5
Gastrocnemius	5	180	60	9.2	15.4	158	2.3	27.0
Sternomandibularis	6	241	67	10.8	16.1	174	2.6	29.5

[1]Texture score - increasing score equals increasing toughness