Connective tissue: structure, function, and influence on meat quality

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To cite this version:

HAL Id: hal-02801237
https://hal.inrae.fr/hal-02801237
Submitted on 5 Jun 2020

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CONNECTIVE TISSUE: STRUCTURE, FUNCTION, AND INFLUENCE ON MEAT QUALITY

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This article is a revision of the previous edition article by RG Taylor, volume 1, pp 306–314, © 2004 Elsevier Ltd.

Glossary

Collagen It is the main component of connective tissue. There are many types of collagen, of which the main ones in muscle are type I and type III, which are the focus of this article. In muscle, collagen forms the endomysium, perimysium, and the epimysium.

Collagen content With approximately 25–35% of the whole body protein content, collagen is the most abundant protein in mammals. It generally constitutes 1–2% of muscle tissue but may represent up to 6% of the weight in high tendinous muscles.

Collagen cross-linking The individual protein subunits of collagen are assembled together by covalent intermolecular cross-links, which increase the tensile strength of collagen. The number of cross-links generally increases with the age of the animals.

Collagen solubility While heating, molecular chains of collagen separate and solubilizes to gelatin. The solubilization of collagen decrease when the number of cross-links increase.

Proteoglycans These are heavily glycosolated proteins that are a filler substance between cells that regulate movement of molecules through the matrix and affect activity and stability of proteins and signaling molecules.

Introduction

Connective tissue is produced by fibroblasts and is the most abundant protein in mammals, making up approximately 30% of the total protein content. When mineralized, it is found in skeletal tissues including bone, teeth, and cartilage. The non-mineralized soft connective tissue is found in skin, tendon, ligament, adipose tissue, blood and lymphatic tissue, and the connective fiber framework in muscles. It contains a mixture of polysaccharides and fibrous proteins. The most abundant protein in connective tissue is collagen, but it also contains various other proteins (such as elastin, fibronectin, and laminin) and proteoglycans in varying proportions, depending on the organ. Connective tissue represents a small proportion of skeletal muscle and is mainly found in extracellular matrix of muscle fibers. However, the meat is the result of transformation of the muscle after slaughter of the livestock animal. Connective tissue content and composition significantly affects the quality of meat and meat products, particularly texture and water-holding capacity. For this reason, numerous scientific studies have been led to characterize its structure, organization, and composition in order to understand its role in determining meat quality.

Distribution, Composition, and Structure of Muscle Connective Tissue

Muscle connective tissue content varies from 1.5 to 15% of dry matter depending on muscle and muscle function. Intramuscular connective tissue is principally comprised of collagen and elastin proteins within a proteoglycan matrix. Muscle elastin content is approximately 0.1–0.2% of dry matter, except in semitendinosus and latissimus dorsi muscles, where it reaches approximately 2%. The vast majority of this connective tissue is composed of collagen in amounts ranging from 1.5% to approximately 10% of dry weight. Collagen I and III are the predominant types in muscle, but collagens IV, V, VI, XII, XIV, XV, and XIX are also present in minor quantities (Figure 1).

Collagen is composed of three polypeptide alpha chains with a simple repeating primary structure Gly–X–Y, where X is often proline and Y is often hydroxyproline. The three chains are assembled together to form a triple helix of tropocollagen. The tropocollagen molecules are assembled together to form fibrils that are then assembled to form collagen fibers (Figure 2).

The fibrous collagen is cross-linked by a mechanism based on aldehyde formation from lysine or hydroxylysine side chains. Fibrils and fibers are stabilized by intermolecular lysine-derived cross-links formed between lysine aldehyde or hydroxylysine aldehyde and hydroxylysine to form an aldimine or oxo-imine bond. These bonds are replaced during aging by stable multivalent mature cross-links, which are thought to link microfibrils and increase matrix stability. Thus, higher collagen cross-linking translates into higher mechanical strength.

From a structural standpoint, intramuscular connective tissue is divided into three main structures (see Figure 3).

Epimysium is a layer of dense irregular connective tissue that surrounds whole skeletal muscle. It protects muscles from
friction against other muscles and bones. The epimysium is generally separated from the body of the muscle and need not be considered as a factor in meat texture, except in some meat products such as dry and cooked ham where muscles are intact.

Perimysium is a layer that separates bundles of muscle fibers within the muscle. It represents approximately 90% of total muscle connective tissue, where it is organized as a network of interconnected segments that vary extensively according to muscle type, species, age, and region in the muscle.


Figure 2  Collagen fibers (bovine semitendinosus muscle). (a) Collagen fibers seen in perimysium. (b) Longitudinal section of collagen fibers. Myo, myofibrils; Pm, perimysium.
muscle. The perimysium is usually arranged in large circular or pentagon-shaped fascicles several millimeters in diameter, with the smallest bundles, or primary perimysium, grouped within larger bundles of thicker perimysium, the secondary fascicles, which may be further organized by tertiary fascicles. Perimysium composition is highly variable and can change with nutrition and exercise. Strength muscles tend to have more collagen than postural muscles have, and collagen from old animals has significantly more cross-links than collagen from young ones.

Endomysium surrounds each muscle fiber. It is composed of basal lamina proteins, proteoglycans, and laminin, plus collagens I, III, and IV, and is very similar in all muscle types and even across species.

‘In vivo’ Function of Connective Tissue

Skeletal muscle connective tissue forms a network that plays a dynamic role in muscle differentiation and development. It serves as a supportive structure in skeletal muscle.

Connective tissue adheres to myofibers and fiber bundles, providing a scaffold that maintains muscle structure during contraction, and acts as an interface allowing fiber bundle sliding. Connective tissue provides the cell-to-cell connections both between individual muscle cells and between muscle cells and neighboring small blood vessels and nerves. It gives coherence and mechanical strength, functions as an elastic, stress-tolerant system, and distributes the forces of muscular contractions in both muscle and tendon. The muscle connective tissue participates in cell growth and tissue regeneration after damage.

The organization of muscle into fascicles separated by the perimysium reflects the need to accommodate shape changes as the muscle contracts, which is achieved by allowing fascicles to slide past each other. Functionally, different muscles have very different requirements in terms of accommodating the shear strains that necessarily occur as the muscle contracts and changes shape, which explains why the amounts and distribution of perimysial connective tissue varies so widely between functionally different muscles.

Connective Tissue Properties Related to Meat Texture

The content, nature, and heat solubility of collagen are key factor of meat tenderness.

Technological processes help to reduce the impact of connective tissue on the texture of meat products. Mincing, for example, can decrease the sensation of toughness in meat, whereas cooking, by solubilizing collagen, significantly reduces the mechanical strength of the connective tissue. Acidic marinating can also partially degrade the connective tissue, which may tenderize meat. However, some meats, like roasts and grilled meat, are consumed after maturation and short-term cooking, in which case cooking temperature at core of the meat piece remains too low to solubilize the collagen. As collagen content is relatively unaffected by the action of proteases during maturation, it ultimately defines the basal hardness of raw or undercooked meat. Thus, the less tender muscles, like pectoralis profundus, generally have high collagen concentrations and a high number of cross-links, whereas a tender muscle like Longissimus thoracis has a lower collagen concentration (1.86%) and fewer cross-links. It is assumed that collagen concentration and number of cross-links have a cumulative effect, leading to a negative impact on meat tenderness. Collagen type also plays a role in meat tenderness. Particularly, type-III collagen, which is more susceptible to proteases, is associated with tenderness, whereas type-XII and type-XIV collagen are thought to decrease total collagen solubility and therefore overall meat tenderness.

Method of Measuring Meat Texture

An important consideration when determining the connective tissue properties tied to texture is the texture measurement method used. In evaluating overall texture quality, and especially the contribution made by connective tissue, the best tests include adhesion force between fibers, peak shear force minus initial yield, and compression, which correlates well to sensory chewiness and texture. For example, old animals show increased adhesion force, decreased collagen solubility, and tougher meat. Understanding the mechanical properties of meat requires an assessment of large amounts of information about sample muscle fiber orientation, cooking temperature, fiber size, and the amount of connective tissue. Compression tests at 20–80% of the initial height of the sample with fibers perpendicular and parallel to the test tool can be used to estimate the relative contribution of each myofiber and connective tissue to the texture. These types of tests have shown that amount of connective tissue goes a long way toward explaining the between-breed and between-muscle differences in texture.

Sensory evaluation of the contribution of connective tissue to texture is not only the most sensitive and accurate but also the most laborious method. Results using this method follow the same trend as mechanical measures, indicating that collagen quantity is not a major factor in animals of the same age when comparing the same muscle type. Collagen content does explain some of the differences in sensory texture between different types of beef muscles, with the psosas and longissimus having low collagen content and tender texture, whereas biceps and pectoralis having high collagen content and tough texture. In
general, pork has similar connective tissue properties to beef, with a weak but significant relationship between hydroxyproline content and sensory tenderness, connective tissue, and flavor.

**Biological Factors, Connective Tissue, and Meat Tenderness**

In general, the content or quality of the connective tissue varies with biological factors.

**Species**

Structural characteristics are similar from one species to another. Longissimus muscle from young cattle, sheep, and pigs do not differ in collagen content and solubility, indicating that longissimus has similar collagen properties across species.

**Breed**

Textural differences can be related to connective tissue properties as well as aging potential. For example, *Bos indicus* breeds have a high collagen content, low collagen solubility, and a tough texture. However, a comparison between *B. indicus* and *B. taurus* crosses did not find significant differences in collagen content or solubility, which may suggest that connective tissue only makes a significant contribution to texture in purebred *B. indicus* lines. Similarly, a comparison of six European and African breeds found little difference in collagen content and solubility. Compared with normal animals, double-muscled cattle have less collagen, higher collagen solubility, and low raw meat texture scores but similar cooked texture scores. *Bos taurus* breeds that mature late have high collagen solubility, and the rheology of the connective tissue is variable in raw samples but not in cooked ones. In conclusion, it seems that no consistent differences amongst breeds for texture-related connective tissue properties are evidenced.

**Sex**

The sex of animal could have an effect on the connective tissue content and quality. Comparisons on animals at the same age beyond puberty show that meat from uncastrated males is less tender than meat from castrated males, which, in turn, is less tender than meat from females. These variations can be partly explained by differences in collagen solubility. Thus, the lower collagen content in muscle from heifers at 13 and 24 months compared with young bulls is a key determinant of differences in tenderness between the two types of animals. Moreover, the meat of females is softer than that of bulls, as it has a lower collagen content and higher collagen solubility.

**Age**

Tenderness is highest in meat from very young animals but subsequently declines with age and physical activity, along with an increase in the amount of collagen and its degree of complexity. The increase in toughness is more linked to a reduction in collagen solubility than an increase in collagen content. The water solubility of collagen under the action of heat decreases gradually with increasing age, with the result that the meat progressively becomes less tender. These changes are usually attributed to a gradual increase in the number of stable intermolecular cross-links.

Many studies have shown a progressive age-related decrease in muscle collagen solubility and tenderness, with little or no change in total collagen content. Although most of these studies were done on ruminants, the pattern is a general feature of connective tissue. The change in solubility results in increased meat toughness in old animals and is the major factor shaping the contribution of connective tissue to meat quality. Very young animals have more collagen and elastin than mature animals have, but veal and lamb have a very soluble collagen and consequently a very tender meat. There is no evidence that elastin per se is associated with texture, despite the fact that its content varies.

Several studies on the effect of animal age on connective tissue-related meat texture showed that from 1 to 60 months, the textural properties of meat were significantly related to collagen content and its solubility. In the same studies, comparisons of muscle types showed that all 12 types were acceptably tender in animals of approximately 24 months of age or less. Myofiber toughness did not change markedly after 24 months of age, but the connective tissue toughness increased markedly in 10 of the 12 meat types in animals aged 3 years upward. In addition, an increased cross-sectional area of perimsym is observed with age. Perimsym becomes either thicker or has more branched bundles, provided no change in content occurs. Increasing age also results in firmer fiber adhesion by connective tissue. Furthermore, fiber adhesiveness is lost at 60°C in meat from young animals but not old animals. This may be the property linking collagen solubility to cooked toughness, as it is also related to sensory chewiness.

**Muscle Type and Connective Tissue Organization**

Collagen and elastin contents differ severalfold between different muscle types. In general, the force muscles such as the biceps have more collagen than postural muscles such as the psoas have. In addition, perimsym volume and organization vary greatly between muscle types (see Figure 4). In the 1930s and 1940s, structural classification of muscle suggested that muscle type differences in texture were correlated to connective tissue content and organization. Muscle grain and muscle fascicles are known to vary significantly between muscle types. Fascicles vary in size, from approximately 1–10 mm, and shape and are hard to visualize and measure. A larger bundle size of fibers ensheathed in primary perimsym would presumably translate into a firmer meat texture, especially when the perimsym is thick and has extensive collagen cross-links that will not melt at cooking temperatures. Fascicle size is correlated to sensory tenderness and shear force texture. Thickness of the perimsym has been shown to be correlated to shear force in chicken and pork. Thicker perimsym melts more slowly and thicker regions have a different composition such as more type-I collagen, more heat-stable elastin, or more cross-links. However, veal also has thicker perimsym than that from mature animals, but the meat stays tender due to its high collagen solubility and small fiber size, which rules out a
systematic direct relationship between perimysium thickness and texture. As stated above, collagen can only influence shear force if it has more cross-links and is in greater quantity.

There have been attempts to link perimysium organization to meat quality, and a relationship has been found. Several research teams have used a qualitative grading system to show that perimysium organization varies with muscle type, species, and age. Semi-quantification by image analysis on digital images coming from magnetic resonance imaging and histology techniques show that tough muscles have smaller fiber bundles.

The last property to discuss in this section is myofiber adhesion to perimysium and fiber–fiber adhesions. These are the most fragile structures in cooked meat. The initial fracture plane is usually at the endomysium–perimysium junction. When stress is applied parallel to the fiber plane, the fracture occurs in the endomysium, whereas when stress is applied perpendicular to the fiber plane, the fracture occurs at the perimysium–endomysium junction.

**Postmortem Changes in Connective Tissue**

There are changes in connective tissue that could play a role in tenderness and thus require further study.

**Endomysium**

It has been shown that the endomysium starts to detach from fibers as early as 6 h postmortem, and at least half of the endomysium is detached within 24 h.

The detachment of the endomysium may be related to the very rapid drop in shear force observed soon after rigor and may be a general aspect shaping the development of texture. The detachment of endomysium in callipyge sheep, even though the meat is very tough, demonstrates that it is marginally involved in meat texture properties. Nevertheless, endomysium detachment from sarcomeres remains a necessary first step for texture development. However, mechanical measures show that lateral adhesion of fibers by endomysium is stable postmortem and accounts for approximately a tenth of the longitudinal force of fibers. The contribution of endomysium to meat texture is, therefore, stable postmortem and very minor compared with the contribution of myofibers.

**Perimysium**

Perimysial collagen is degraded during storage. Transmission and scanning electron microscopy studies show that the structural integrity of the intramuscular connective tissue decreases during postmortem aging. The major postmortem change in perimysium is that myofibers separate from perimysium within 6 h. Perimysium seems to be the structure most vulnerable to meat shearing action. It has also been shown that the isometric tension of intramuscular collagen decreases at 21 days postmortem in beef. Furthermore, the breaking strength of the perimysial connective tissue in raw beef decreases during postmortem aging. The thermal shrinkage temperature of bovine intramuscular collagen decreases by 7–8 °C within 7 days postmortem. These structural changes are strongly related to the mechanical strength of meat, as demonstrated by shear measurements on raw muscle or uncooked intramuscular connective tissue structures. Proteoglycan degradation occurs at the same time frame as structural changes in connective tissue (Figure 5), but further studies are required to better understand the role of proteoglycans in texture.

**Changes in Connective Tissue with Cooking**

Elastin is very heat stable, and its properties are generally unaffected by cooking. However, elastin content in the
Figure 5  Proteoglycan involvement on intramuscular connective tissue changes during postmortem ageing. IMCT, Intramuscular connective tissue; PGs, Proteoglycans. Reproduced from Nishimura, T., 2010. The role of intramuscular connective tissue in meat texture. Animal Science Journal 81, 21–27, with permission from Japanese Society of Animal Science, John Wiley & Sons Ltd.

perimysium is generally low (0.1–0.2% of the total connective tissue) and its impact on meat texture is, therefore, considered as not significant. However, elastin may contribute to resistance to shear in the semitendinosus muscle, where elastin accounts for up to 40% of total connective tissue.

In contrast, collagens and proteoglycans are unstable to heat, and their properties are affected by cooking. The thermal denaturation of proteoglycans does not play a direct structural role in mechanical terms, but it can play a role in the thermal stability of collagen due to molecular interactions with collagen fibers.

Although, connective tissue makes the largest contribution to texture in raw or undercooked meat, myofibers make the largest contribution to texture in cooked meat. Cooking at temperatures of 60–70 °C causes gelation of most of the perimysium. The temperature for structural changes tends to vary between studies: Some authors report beef perimysium gelation at 70 °C and above, whereas others cite 60–63 °C. Using isolated fibers and small muscle samples it was demonstrated that perimysium changes its thermal mechanical property at 50–60 °C, before the myofibers toughen. These changes are mostly due to collagen melting. The contribution of connective tissue to meat texture declines as temperature increases.

Although thermal stability is well established as the major property determining the role of connective tissue in cooked meat texture, it is less clear how structure and composition variability affect the structural changes involved. Perimysium attributes like thickness are highly variable in cooked meat. Furthermore, elastin distribution as well as collagen type varies greatly within small regions of perimysium. The relationship of connective tissue structure and composition to thermal and mechanical properties of meat requires further study in order to better evaluate the specific influence of collagen type, elastin, or perimysium thickness.

When heated at more than 60–65 °C, the collagen molecule changes from its native helical ordered state to a randomly coiled structure, and fibrinous collagen is converted from a fibrillar to a rubber-like amorphous structure. The destruction of hydrogen bonds in the molecule is accompanied by shrinkage of the collagen fibers. If collagen is free to shorten, it shrinks to one-quarter of its original length. The contraction of collagenous networks during cooking is a major mechanism by which collagen influences meat texture. The thermal contraction of collagenous fibers and fibrils starts with a free contraction, after which there is a forced contraction where collagen fibers and fibrils apply pressure on muscles fibers and muscle fiber bundles. The more the collagen fiber contracts during heating, the lower its mechanical strength.

As the thermal contraction of meat collagen fibers is limited by muscle fibers, the resistance of muscle fibers or muscle fiber bundles influences the ultimate elastic modulus of the collagen fibers. Moreover, the nature of the cross-links determines contraction strength, contraction amplitude, collagen solubilization, and the final mechanical properties of the connective tissue after heating. The thermal stability of collagen is related to the presence of heat-stable mature cross-links, which increase with age and contribute to toughness. At 70 °C, 42% of veal collagen is soluble compared with 2% solubility in beef from 10-year-old animals, and the thermal shrinkage temperature is 55 °C for veal and 70 °C for beef from 10-year-old animals. Collagenase digests 21% of the collagen in veal, whereas the percentage is approximately 10% in 10-year-old animals. These results indicate that the age-related toughness of cooked meat is directly associated with the change in collagen thermal stability.

The role of cross-links in meat quality is less clear in same-age animals. Stable cross-links do vary significantly by muscle type, with sheep longissimus having few and biceps many. But the tenderest muscle, the psoas, has a high number of
cross-links, yet it remains tender even at normal sarcomere lengths. These results suggest that both high collagen content and cross-link formation are needed for connective tissue in order to be able to influence toughness, as is the case in biceps muscle. Between-muscle differences in cross-link quantity have also been reported for beef, sheep, and goat. Several studies have failed to demonstrate a relationship between number of cross-links and collagen solubility. In addition, numerous studies have failed to find a relationship between cross-links and shear force in same-age animals, including two studies that measured five different types of cross-links. Consequently, a relationship can be ruled out between these cross-link types and shear force, especially as meat from callipyge sheep has relatively few cross-links but is still extremely tough. Therefore, it is not yet clear which cross-links are responsible for the age-related change in meat texture.

One hypothesis is that heat stability is the result of collagen interaction with decorin, which is found at every D-line (every 64 nm) along the collagen fibril, is heat stable, and cross-links collagen fibers. There is evidence that decorin is stable post-mortem, and there is little evidence that it has mechanical properties. However, decorin has not been yet sufficiently investigated to determine its importance in connective tissue stability at cooking temperatures, despite the fact that this may prove an important factor.

Conclusion

Skeletal muscle connective tissue is a supportive matrix composed of various fibrous proteins, primarily collagen. Many molecular interactions converge to stiffen the matrix, whose properties vary with the physiological and biological characteristics of source animals. The quantity, quality, and organization of this connective tissue depend on the muscle and its function in the body. Connective tissue changes as the animal matures, and the quantity and degree of cross-linking in muscle connective tissue are generally related to the basic toughness of animal meat. As it is impossible to fully control the characteristics of the muscle tissue, several strategies are employed in order to meet consumer expectations. The first is to sort the muscles according to their position on the carcass and their function, both of which are typically associated with connective tissue characteristics. Strength muscles are generally richer in cross-linked collagen and are oriented toward the production of minced meat or for long-term moist cooking processes to dissolve the collagen and tenderize the meats. Postural muscles are generally less collagen rich, and some are used as meat to grill. Therefore, it is possible to optimize meat tenderness by selecting breeds known for their low connective tissue content or by using young animals with lightly cross-linked collagen. Research to date has provided some insight to help elucidate the role and function of connective tissue in the muscle, to determine its composition and organization from the tissue level down to molecular level, and to assess its contribution to the organoleptic properties of the meat end product. Although the role of connective tissue in the mechanical properties of meat is generally understood, the properties of meat textures are complex and multifactorial, resulting from the behavior of connective tissue, muscle fibers, and interactions between these two muscle components. The future development of innovative technological processes that can degrade or even partially dissolve the connective tissue could improve the price and market value of those meat cuts known for their high mechanical strength.

Finally, food science research to date has mainly focused on connective tissue to understand its role in shaping meat texture, and it is only recently that the focus has shifted to also look at the nutritional qualities of meat. Although connective tissue by itself is not known for its nutritional value, its structural organization at different levels in whole tissue muscles may play a role in the distribution of small molecules of nutritional interest during various processes such as cooking, mincing, and cutting, thus resulting in a loss of micronutrients into exudates and/or cooking juices. The endomysium and perimysium envelopes are also more likely to limit the accessibility of digestive enzymes (pepsin, trypsin, and chymotrypsin) to the high nutritional value myofibrillar proteins. Such transformation processes, especially cooking, dissolve all or part of collagen depending on its degree of cross-linking and on the heating conditions. However, there is still a lack of data on the role played by the overall whole muscle tissue in the transfer of micronutrients and small molecules, and on the bioaccessibility of digestive enzymes in myofibrillar proteins.


Further Reading


Relevant Websites

http://www.ivy-rose.co.uk/HumanBody/Muscles/Muscle_Structure.php
http://en.wikipedia.org/wiki/Collagen
http://en.wikipedia.org/wiki/Connective_tissue_in_skeletal_muscle
http://en.wikipedia.org/wiki/Elastin
http://en.wikipedia.org/wiki/Proteoglycan
http://www.wisc-online.com/Objects/ViewObject.aspx?id=AP14504
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