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Nafiseh Soltanizadeh^a & Mahdi Kadivar^a

^a Department of Food Science and Technology , Isfahan University of Technology , Isfahan , 84156 , Iran

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Nanomechanical Characteristics of Meat and Its Constituents Postmortem: A Review

NAFISEH SOLTANIZADEH and MAHDI KADIVAR

Department of Food Science and Technology, Isfahan University of Technology, Isfahan 84156, Iran

Tenderness/toughness are two important factors in meat evaluation and can be well explained by their mechanical and nanomechanical characteristics, which in turn are highly affected by the complex arrangement of meat numerous constituents and the influence of endogenous enzymes on this biological structure. It has been also revealed that post-slaughter variations in muscle characters that make muscle to transform to meat are a consequence of changes in the nanomechanical properties of myofibrillar proteins through linkage with each other or even through their breakdown. However, background toughness that is related to the connective tissues exists just at the time of slaughter and does not change over the time. The most famous instruments that measure the mechanical characters of myofibrils in pico/nano scale are atomic force microscopy, also known as scanning force microscope, optical tweezer, and glass microneedle. In this regard, mechanical terms such as torsional strength, rotational Brownian motion, elastic modulus, viscoelasticity, flexibility, etc. are generally used to show different behavior of meat ultrastructure and the changes that usually occur in postmortem muscle. It is expected that the evaluation of nanomechanical properties of muscle cells and connective tissues, particularly when the muscle undergoes different changes, be useful in order to justify many features of meat postmortem.

Keywords Stiffness, tenderness, nanomechanical properties, enzyme, ultrastructural, atomic force microscopy, myofibrils

1. INTRODUCTION

Muscles, as an aggregate of compounds, are the best examples of systems whose arrangement and integrated interrelations form unified and harmonious structures. They undertake different tasks including, but not limited to, stance control, internal organ protection, assistance to respiration, peristaltic movement, and swallowing. To perform these and other life-sustaining tasks, muscles are formed from viscoelastic cells that increase stress and strain tolerance of muscles in live animals and maintain this characteristic over long periods. The viscoelasticity of cells has been the subject of many studies.

Living systems are essentially able to maintain their order over a long period of time, which can be an indicator of their complexity. This is according to the second law of thermodynamics which states “during any process in which an isolated system undergoes a transition between equilibrium states, the entropy will never decrease.” Therefore, the system may become disordered unless the energy is spent. In the case of mus-

cle cells, this involves consumption of energy to avoid stable rigidity (rigor mortis). It is widely accepted that the viscoelastic character of cells and their response to the mechanical environment are crucial determinants of their form, function, and vitality. Muscle performance is a typical physiological process in which cell viscoelasticity plays a key role. Indeed, skeletal muscle must rapidly and efficiently generate a large contractile force. Therefore, their cytoskeleton and motor proteins should be highly ordered and able to withstand large stresses and strains (Smith, 2004). The mechanical characteristics of a biological structure are usually understood as elastic or viscoelastic characters. The values which are most often determined for a muscle fiber are stiffness (analogous to the spring constant, N/m) and (Young's) modulus of elasticity (Pa), both longitudinal (against deformation along the main axis) and transverse (in the perpendicular direction). Obviously, the two parameters are interconnected, being integral components of fiber stiffness (Ogneva et al., 2010).

After slaughter of an animal, there is a generalized cell death which is a prestage to rigor mortis and/or during rigor development. Slaughtering involves removal of blood that leads to a generalized anoxia of the various cells of the body. This phenomenon is temporarily alleviated by a shift from aerobic to

Address correspondence to Mahdi Kadivar, Department of Food Science and Technology, Isfahan University of Technology, Isfahan 84156, Iran. E-mail: mak120@mail.usask.ca

anaerobic glycolysis in the cells to provide energy (Mohanty et al., 2010) and prevent disordering of cells. When ATP content decreases, muscle cells are not able to provide the energy required for keeping their integrity and are subjected to anarchy, a state which causes rigor mortis to start and the basic physiologic and defense processes of the body to become devoid of any inflammatory reaction. This induces cell death (apoptosis) (Kerr et al., 1972) to be ultimately followed by self-digestion. These processes result in muscle cells undergoing a series of nanomechanical alterations and the mechanical properties of meat to change. Indeed, these alterations occur in two independent stages. In the first stage, an irreversible link forms between actin and myosin, giving rise to rigor mortis to appear which is reflected in increased muscle resistance to external stresses. In fact, the elasticity of meat muscle enhances and consumers encounter difficulty in mastication. The second stage is accompanied by a decrease in muscle stiffness, which involves the digestion of muscle compounds by internal enzymes; hence, its reduced elasticity. Since mechanical properties of muscle rise from its proteins, the breakdown of proteins plays a key role in changes that occur during the second stage.

The mechanism of meat tenderness has been the subject of many studies over the years and several procedures and instruments have been employed to investigate the mechanism of changes in tenderness and the factors involved. The most recent methods are those that measure changes directly in myofibrils (Hopkins and Geesink, 2009). In this regard, five groups of biophysical methods including mechanical, optical and dielectrical methods, along with measurements with X-ray and nuclear magnetic resonance have been applied (Damez and Clerjon, 2008). The most famous instruments that measure the mechanical character of myofibrils in nano scale are atomic force microscopy (AFM), optical tweezer, and glass microneedle. AFM, also known as scanning force microscope, was developed approximately 25 years ago and is an instrument which probes the interaction forces between a sharp tip and the surface of a sample. The principle of AFM is simple as a sharp tip fixed at the end of a flexible cantilever is raster-scanned over the surface of a sample. As the tip interacts with the surface, the cantilever deflects and its deflections are monitored and used to reconstruct the topography and mechanical properties of the sample (Meyer, 1992). Surfaces can be scanned nondestructively because the interatomic spring constant of the sample is on the order of 10 N/m, compared to the typical contact mode AFM cantilevers which have spring constants in the range of 0.01–1 N/m (Kasas et al., 1997). Optical tweezer was first developed 23 years ago and its applications spread to molecular biology, biochemistry, and biophysics or in the study of individual molecules. Using this instrument, researchers are gaining essential new insights not only into the mechanical properties of biological macromolecules but also about the dynamics and mechanisms of molecular motors like myosin that employ chemical energy to perform mechanical tasks in the cell (Moffitt et al., 2008). In this technique, particles ranging in size between 25 nm and 25 μ m are trapped by the geometry and intensity gradient of a laser focus. Using two coaxial

counterpropagation laser beams, a small transport bead can be trapped with a force of ~ 100 pN. The force applied on the bead can be deduced from the movement of the trapping beam due to its reflection in the bead (Lavery et al., 2002). Typical force limit of optical tweezer is 50–100 pN. Glass microneedle measures the force on a fine glass probe as it is pushed against a cell surface. The probe is attached to a long flexible wire, positioned vertically with a piezoelectric motor. Force sensing is achieved by optical detection of tip position relative to the piezomotor with a force sensitivity in the range of 1–10 pN. Elasticity is determined qualitatively from the steepness of a force versus tip position plot and viscosity from the hysteresis (Smith, 2004).

Although meat textural quality is greatly affected by rigor mortis, background toughness, and tenderization followed by changes in its mechanical/nanomechanical characters, most studies have been conducted on enzyme activities and ultrastructural changes during postmortem storage. It has been revealed that postslaughter variations in muscle characters that transform muscle to meat are a consequence of changes in the nanomechanical properties of meat proteins through linkage with each other or even through their breakdown. While toughening and tenderization phases take place during the postmortem storage or aging period, background toughness exists at the time of slaughter and does not change during the time (Hopkins and Geesink, 2009).

This review outlines the tremendous strides made in recent years in the field of nanomechanical properties of muscle cells, particularly those pertaining to understanding the roles of proteins and enzymes in postmortem variations in muscle mechanical properties and will endeavor to investigate the relationship between changes in mechanical properties of muscle fibers and meat.

2. MYOFIBRIL STRUCTURE AND ITS NANOMECHANICAL PROPERTIES

Muscle is the tissue that moves the body around and conveys liquids within the body. There are two general types of striated and smooth muscles, the nomenclature derived from their appearance under microscope. The microscopic appearance of a skeletal muscle cell is primarily determined by patterns of its intracellular structure in dark and light bands. The cell involves several structural elements including the plasma membrane called the sarcolemma, highly developed intracellular membrane networks that include the T-tubules, and sarcoplasmic reticulum which originate from sarcolemma, as well as mitochondria, nuclei, and myofibrils. The myofibril is a string-like structure resulting from longitudinal repetition of fundamental constituent units of muscle contraction called sarcomeres. The boundaries of sarcomeres are determined by the α -actenin containing Z-lines serving as the anchoring structure for the actin that contains thin filaments. Myosin-containing thick filaments are in the center of the sarcomere that form the A-band by

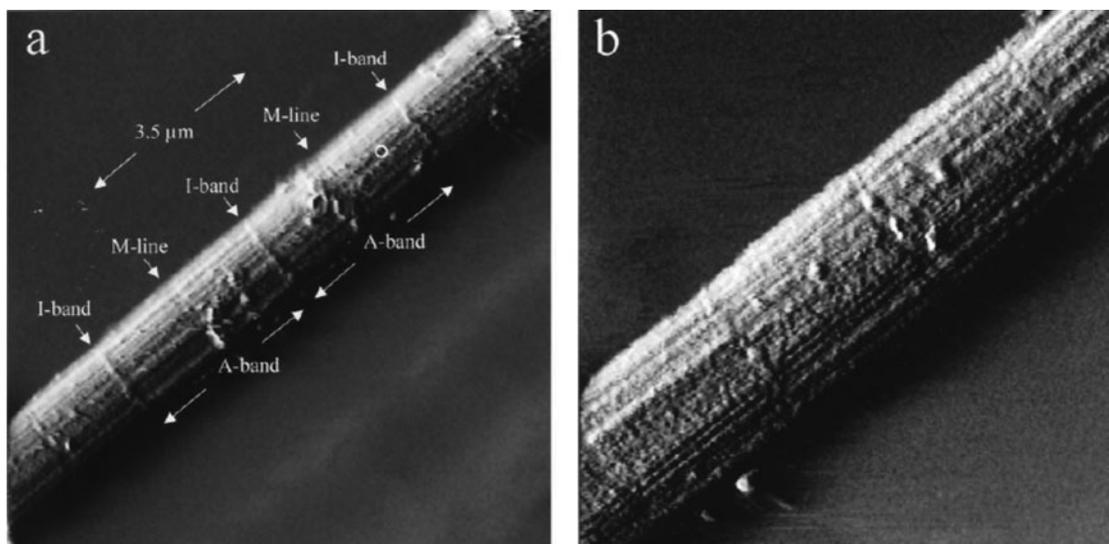


Figure 1 (a) A typical AFM image of a bundle of myofibrils in rigor solution ($10 \mu\text{m} \times 10 \mu\text{m}$). (b) A detailed AFM image of the surface of a myofibril ($5 \mu\text{m} \times 5 \mu\text{m}$) (Nyland and Maughan, 2000).

overlapping with thin filaments. In the center of the A-band is the M-line that is formed from the end of myosin tails and a series of structural proteins including myomesins and part of titin involved in the formation of the A-band and positioning of the thick filaments somewhere in the middle of the sarcomere. The I-band consists of the dark Z-line at its center and the light nonoverlap region of the thin filaments from the adjacent half sarcomeres on each side (Fig. 1). Classically, the sarcomere is described as having thin filament-containing I-bands flanking the thick filament-containing A-band but this does not convey the functional aspect of the overlap region. The proteins of the sarcomere are organized into a near-crystalline lattice and the form of these proteins and their interactions with themselves and other proteins result in a highly organized structure (Swartz et al., 2009).

Less is known, however, about the mechanical properties of muscle fibers and molecules affecting their transverse mechanical behavior. In general, the major mechanical property of skeletal myofibrils is viscoelasticity (Mathur et al., 2001) and titin filaments appear to be responsible for both elasticity and viscoelasticity of skeletal myofibrils (Minajeva et al., 2001). It has been estimated that the elastic modulus of the skeletal muscle cell surface is around 25 kPa (Mathur et al., 2001). The structure and transverse stiffness of the sarcolemma of fully differentiated muscle fibers under various conditions have also been reported. To obtain more instructive images without sarcolemmal damage by the cantilever, the structure has been scanned in the half-contact AFM mode. Defranchi et al. (2005) manifested sarcolemmal nubs and supposed that they exhibited a costameric protein at the Z disk. This was verified by comparing the optical and structural images after staining the α -actinin with fluorescent antibodies (500-nm spheres detectable by AFM). When they evaluated the mechanical characteristics of muscle cell in the contact mode, they found that the maximal tolerable force

via muscle cell was 1 nN, and in this condition the membrane indented from several hundred to a thousand nanometers (Mathur et al., 2001; Defranchi et al., 2005). The elasticity modulus was estimated to be almost 2.5 times higher than that reported for skeletal muscle by Mathur et al. (2001). This difference may be due to differences in the two studies in that Defranchi et al. (2005) worked with fully differentiated cells whereas Mathur et al. (2001) dealt with myoblasts, providing nonuniform ontogenetic dynamics of the expression of proteins that transverse stiffness is emanated from. Collinsworth et al. (2002) comprehensively resolved this controversy (Collinsworth et al., 2002). They found that during the conversion of undifferentiated myoblast to 8–10 days differentiated myoblast, the elasticity modulus of muscle fiber increased from 11.5 ± 1.3 to 45.3 ± 4.0 kPa, in which actin and myosin were the major contributors to alterations in the transverse elastic modulus. They also indicated that no change was observed in viscosity during the development of muscle cell that could be measured by hysteresis between the direct and reverse movements of the cantilever as reflected in recordings of the force curves (Ogneva et al., 2009). Yoshikawa et al. (1999) measured transverse stiffness of myofibrils at various points along the sarcomere by AFM and estimated rigidity of the Z-line, I-band, overlap region, and M-line of myofibrils at relax state to be 1.2 ± 0.2 , 0.8 ± 0.2 , 1.0 ± 0.2 , and 0.7 ± 0.2 pN/nm, respectively. It was evident that Z-line was the most and I-band and M-line were the least rigid parts of myofibrils. Indeed, Z-line acts as a mechanical wall in the sarcomere structure. They concluded that the Z-line network is connected to one end of the thin filament array in its adjacent half sarcomere. The other end of actin forms cross-bridges with thick filaments in the overlap region. This assembly of the filament lattices reinforces the Z-line network mechanically. It is noteworthy that I-band and M-line are two regions with similar flexibility and are slightly more flexible than the overlap region in myofibrils (Yoshikawa

et al., 1999). This is according to the flexible nature of actin and connectin (or titin) which are the primary components of these sites. Yamada et al. (2003) indicated that the rupture distance and the rupture force of various predominant components are interlocked in sarcomere, changing in the ranges of 50–100 nm and 30–150 pN, respectively (Yamada et al., 2003). This rupture force is comparable in magnitude to the force required to break the rigor complex of actomyosin, which is ca. 15 pN (Nishizaka et al., 2000), to extend titin filaments, 150–300 pN, to break actin filament by stretching, ca. 108 pN (Kishino and Yanagida, 1988), and to break actin filament from α -actinin, ca. 18 pN (Miyata et al., 1996).

3. TOUGHENING PHASE

When contraction occurs, the thick and thin filaments interact via the head region of myosin, forming a complex which is usually referred to as actomyosin. In electron micrograph images of contracted muscle, the actomyosin bonds look like cross-bridges between thick and thin filaments. The globular head of myosin also has enzymatic activity; it can hydrolyze ATP and release energy. Before death, the ATPase activity of myosins provides the necessary energy for their bond to actins and then pulls actins toward the center of the sarcomere. Following this interaction, the myofibril is shortened and eventually the muscle goes to contraction. Dissociation of myosin and actin may occur when an action potential is exerted to the cell and a new molecule of ATP is attached to the myosin head (Huff Lonergan et al., 2010). After death, muscle filaments are in a continuous state of contraction and relaxation, and as anaerobic glycolysis proceeds, the sources of ATP synthesis are soon exhausted, ATP level is diminished, and the ability of calcium pump ion protein to maintain calcium reduces (Hwang et al., 2003). These events increase the number of myosin cross-bridges with actin that permanently bond to actin filaments to form the actomyosin complex (Hopkins and Geesink, 2009) causing filaments to enter rigor and a constant contracted state as stiffness develops in the postmortem muscle (Huff Lonergan et al., 2010). In fact, the toughening phase is created by muscle shortening during rigor development (Wheeler and Koohmaraie, 1994; Koohmaraie et al., 1996).

Wheeler and Koohmaraie (1994) measured variation in longissimus muscle stiffness of lamb during 14 days and reported an intermediate shear force value of 5.07 kg immediately after slaughter that increased during the first 24 h to 8.66 kg (maximum toughness was achieved at 9–24 h). They concluded that the main reasons for toughening in the muscle are the shortening of sarcomere from 2.24 μm at death to 1.69 μm at 24 h postmortem and the development of rigor (Wheeler and Koohmaraie, 1994). During rigor development, both lateral and longitudinal contractions occur. It has been shown that the cross-sectional area of the myofibrils decreases by about 9% during rigor (Offer et al., 1989). As actin-myosin cross-bridge is the main cause of lateral contraction during rigor, the extent of this type of contraction increases with shorter sarcomeres, giv-

ing rise to a larger viscose component (Tornberg, 1996). Some studies have related rigor toughening to decreased sarcomere length (Herring et al., 1965; Dutson et al., 1976; Wohlfart et al., 1977; Koohmaraie et al., 1996), whereas others have not found any relationship between these two factors (Culler et al., 1978; Seideman et al., 1987). The reasonable augment for the role of sarcomere length in meat toughness might be related to the increase observed in myofibrillar structure density in the contracted sample. Goll et al. (1995) argued that a change in the actin/myosin interaction from a weak-binding to a strong binding state results in toughening during the first 24 h of post-mortem, and that this increase in toughness is not necessarily accompanied by shortening (Goll et al., 1995).

Better understanding of the events during rigor development may be gained by studying force changes in the myofibrillar scale. In this regard, nanometer observations have been made under an optical microscope and piconewton forces generated by single molecular motors have been measured (Table 1) (Ishijima et al., 1998; Mehta et al., 1999). Single-fluorophore imaging has revealed that individual ATPs degrade by myosin and the swirl movement between actin and myosin (Sase et al., 1997), suggesting a hopping character for myosin. However, the exact mechanisms of motor function are still unclear. During one cycle of ATP hydrolysis, myosins form different orientations with different affinities toward their substrate filaments with alternate binding and unbinding (Fig. 2) (Nishizaka et al., 2000). Nishizaka et al. (1995) measured the force required to unbind a rigor bond formed between an actin filament and a single heavy meromyosin (HMM) molecule in the absence of ATP using optical tweezers. The average unbinding force was 2–5 times larger than the sliding force (Finer et al., 1994; Ishijima et al., 1994) which was measured to be approximately 9 pN (Nishizaka et al., 1995). Unbinding under a constant force was a haphazard process, and the lifetime of the rigor bond decreased by a factor of 10^2 – 10^3 along with 10 pN increase in the load (Nishizaka et al., 2000). Nishizaka et al. (2000) also measured the force between heavy meromyosin and actin in the rigor bond and indicated that when the myosin filament is bent at an acute angle, a force less than 60 pN is needed for an actin filament to break. Torsional strength was estimated by analyzing the rotational Brownian motion of a short actin filament tethered to a single HMM molecule, and a force of $(2.3 \pm 1.9) \times 10^{-22}$ N.m/rad was measured. This small stiffness must have originated from the flexible part at the joint between S1 and S2 regions, and/or within the S2 region of the HMM molecule (Nishizaka et al., 2000). Yoshikawa et al. (1999) measured stiffness of different parts of myofibril in rigor and relaxed states by using AFM and indicated that the upward slope in the force-distance curves was less steep in the order Z-line > overlap region > M-region \geq I-band. According to their findings, the transverse stiffness at Z-line, I-band, overlap region, and M-region of myofibrils increased from 1.2 ± 0.2 , 0.8 ± 0.2 , 1.0 ± 0.2 , and 0.7 ± 0.2 pN/nm in the relaxed state to 7.7 ± 1.8 , 3.3 ± 1.4 , 4.6 ± 0.7 , and 3.0 ± 1.2 pN/nm in the rigor state, respectively (Yoshikawa et al., 1999). Akiyama et al. (2006) also used AFM to survey force

Table 1 Young's modulus and Transverse stiffness of skeletal muscle and myofibrils

Myofibril types	Commentary	Transeverse stiffness (pN/nm)	Young modulus modulus (kPa)	Ref.
Myofibrillar bundles	During relaxed state	4.4	39.8	(Nyland and Maughan, 2000)
	During active state	5.9	54.7	
	During rigor state	10.3	93.7	
Skeletal myofibrils	During relaxed state	2.10		(Ogneva et al., 2010)
	During active state	4.1		
	During rigor state	8.3		
Skeletal myofibrils	During relaxed state	3	5	(Akiyama et al., 2006)
	During rigor state		61	
Skeletal myofibrils	During relaxed state		11.5	(Yoshikawa et al., 1999)
	During active state		37.5	
	During rigor state		84.0	
Skeletal muscle cells			10–17	—
Fibroblast	Healthy cell		5.1	(Pelling et al., 2009)
	During apoptosis		1.6	
Fibroblast			3–5	(Rotsch and Manfred, 2000)
Fibroblast			2.68	(Guo et al., 2006)
Fibroblast			4–5	(Bushell et al., 1999)
Fibroblast	Spatial heterogeneity		3–12	(Rotsch et al., 1999)
Fibroblast	Changes at adhesion		0.6–1.6	(Mahaffy et al., 2004)
Fibroblast			4–5	(Wu et al., 1998)
Murine C2C12 myoblasts	Change at differentiation		11–45	(Collinsworth et al., 2002)
Murine C2C12 myoblasts	Changes at differentiation, Treating with L-arginine		8–14	(Zhang et al., 2004)
Murine C2C12 myoblasts			12	(Engler et al., 2004)
Murine C2C12 myoblasts			21–28	(Mathur et al., 2001)
Murine C2C12 myofibrils			61	(Defranchi et al., 2005)

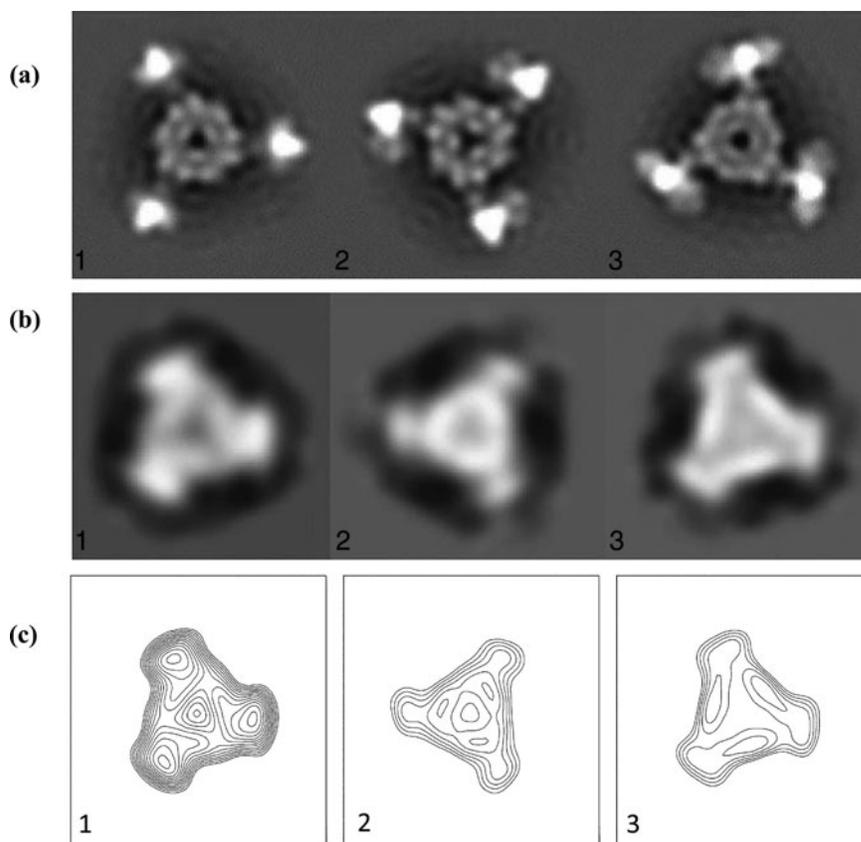


Figure 2 Different orientation of three individual myosin head. (a) Cross-sectional view of myosin heads for the X-ray model, (b) the corresponding heads in the EM map, and (c) contour plot corresponding to cross-sectional view (AL-Khayat et al., 2006).

variation during rigor development and found that relaxed myofibrils were less rigid compared to rigor myofibrils (Akiyama et al., 2006). In another study, myofibrillar bundles in a rigor (without ATP) and relaxing solutions (pCa 8.0) were studied using AFM in the contact mode where indentation depth was 10 nm. The values thus obtained for transverse stiffness were 10.3 ± 5.0 and 4.4 ± 2.0 pN/nm in the rigor and relaxation states, respectively (Nyland and Maughan, 2000). Such difference in stiffness was associated with the transverse “flexibility” of the myosin molecule, meaning that myosin heads can be at various angles to the actin filament, therefore affecting transverse stiffness. The authors estimated the transverse stiffness of the cross-bridge to be 13–15% of the longitudinal stiffness. The relatively low stiffness may reflect a greater flexibility of the myosin molecule in the radial direction due to proline residues at the so-called hinge points (Nyland and Maughan, 2000). A similar conclusion is reached if rigor stiffness in the transverse and longitudinal directions are compared (93.7 kN m^{-2} vs. 940 kN m^{-2}) (Dickinson et al., 1997).

In rigor myofibrils, the Z-lines are anchored by the actomyosin filament lattice and strongly support them since rigor complexes are formed between thin and thick filaments, but in relaxed myofibrils, it would not support Z-bands vigorously because no rigor complexes are formed (Akiyama et al., 2006). It can also be noted that I-band and M-line are similarly flexible, particularly the overlap region in rigor myofibrils. Considering the force required for dissociating myosin head from thin filaments, it is evident that a greater transverse stiffness of myofibrils is recorded due to the greater affinities of myosin head to actin. Hence, the actomyosin filament lattice in sarcomere is stabilized in the transverse direction dominantly by the attachment of cross-bridges with thin filaments (Yoshikawa et al., 1999).

Expression of the myofibril elasticity in terms of the Young's modulus of elasticity will be helpful to understand the structural stability of myofibrils at the molecular level (Table 1). The “apparent” transverse Young's modulus of elasticity in myofibrils has been estimated to be 84.0 ± 18.1 kPa in rigor solution (Yoshikawa et al., 1999). Nyland and Maughan (2000) found that Young's modulus of elasticity in myofibrils may increase from 40 ± 17 kPa in the relax state to 94 ± 41 kPa in the rigor state (Nyland and Maughan, 2000). On the other hand, the longitudinal Young's moduli of elasticity for a single myofibril were 10 and 0.2 Mpa in the rigor and relax states, respectively. Sarcomere structure of myofibril is actually more rigid in a longitudinal structure than in a transverse one. These results indicate that the formation of cross-bridges between actin and myosin after slaughter increase myofibril rigidity in both transverse and longitudinal directions and that a greater shear force is, therefore, required to cut the meat during rigor development.

4. BACKGROUND TOUGHNESS

Background toughness of meat is defined as “the resistance to shearing of the unshortened muscle” (Koochmaria and Geesink,

2006). Background toughness is stable at the time of slaughter and during the storage period. This type of toughness is due to the connective tissue component of the muscle. Individual muscle cells are coated with a collagenous connective tissue that can be subdivided into a basement membrane that is in close contact with the cell and a more distal portion called the endomysium. A bundle of muscle cells is surrounded by a thicker collagenous connective tissue network called the perimysium (Swartz et al., 2009). Since a general relationship has been found in some studies between the characteristics of perimysium and those of muscle tenderness for both beef and chicken, it is concluded that the organization of perimysium presumably affects background toughness (Hopkins and Geesink, 2009). An anatomical muscle consists of a number of bundles surrounded by epimysium (Swartz et al., 2009).

Collagen is present in muscles in amounts ranging from 1.5% to about 10% of dry weight. Raw collagen fibers are very stiff, their elastic modulus being about 0.5–1 GPa. The elastic modulus of elastin (another protein of the connective tissue), which is much less than that of the collagen, is about 0.1–0.4 MPa. In beef, it is present in small amounts (usually lower than 0.4% dry weight) in most muscles except in *Semitendinosus* and *Latis-simus dorsi* muscles which comprise approximately 2% of dry weight (Lepetit, 2008). During maturation of the animal's tissues, collagen becomes much more resistant to breakdown. This is not due to an increase in the number of intermolecular cross-links – indeed they may decrease – but to the formation of nonreducible links involving three or more chains whereby a three-dimensional network characterized by a high tensile strength is formed. Although the nature of the links is not yet clear, there is evidence indicating that hydroxyaldohistidine and pyridinoline are involved in the structures. Glycosylation of lysine residues may also be significant over the maturation of collagen. Ageing of animals is primarily controlled by changes in the protein structure of elastin and collagen, in the form of increasing cross-links between the molecules which develop due to nonenzymatic processes. Indeed, cross-linking leads to stiffening of tissue (Lawrie and Ledward, 2006). Collagen cross-linking occurs in two forms. Intramolecular cross-linking occurs between tropocollagen molecules and intermolecular cross-linking occurs between collagen fibrils. Because intermolecular cross-links are responsible for stabilization of the collagen fiber, they have been the focus of investigation detailing the relationship between collagen and meat tenderness (Brooks, 2000). Buehler (2008) surveyed the influence of cross-linking on the deformation mechanics of collagen fibrils and argued that for larger cross-link densities, the collagen becomes stronger and as the cross-link density increases, the collagen fibril becomes more “brittlelike” (Buehler, 2008). The increase of fiber diameter during maturation has also been considered as an additional contributory factor (Lawrie and Ledward, 2006).

Intramuscular collagen has been shown to have adverse affects on meat tenderization (Hill, 1966; Cross et al., 1972). Measurement of total collagen, heat soluble/insoluble collagen, and the proportion of stable cross-links between collagen fibers has indicated that total collagen content is sufficient for

increasing toughness in beef with increasing chronological age (Hill, 1966). The shear force required to cut different raw meats is highly correlated with collagen content (in beef: $r^2 = 0.95$, (Dransfield et al., 2003); in chicken: $r^2 = 0.94$, (Liu et al., 1996)) and this has still maintained the conviction that collagen content is important in determining meat tenderness. Some significant correlations between muscle collagen content and cooked meat toughness have been observed (in beef: $0.66 < r^2 < 0.82$, (Riley et al., 2005)); however, these correlations between collagen content and cooked meat toughness have been shown to be lower (in beef: $0.09 < r^2 < 0.30$ (Serra et al., 2008); $r^2 = 0.18$, (Dransfield et al., 2003); in lamb: $r \leq 0.48$ (Tschirhart-Hoelscher et al., 2006)). Cross et al. (1972) showed that the percentage of soluble collagen decreased with age, possibly explaining why tenderness decreases with increasing age (Cross et al., 1972). Bosselmann et al. (1995) reported that in estimating beef tenderness, total collagen, and percentage of soluble collagen might not be as important as the degree of soluble collagen cross-linking (Bosselmann et al., 1995). Although high correlation has been reported between tenderness and collagen solubility (in beef: $0.77 < r^2 < 0.81$, (Herring et al., 1967)), lower correlation has been also evidenced (in beef $r^2 = 0.01$, (Campo et al., 2000)). Lepetit (2007) concluded that measurement of cross-links, particularly pyridinoline, is a reasonable predictor of tenderness (Lepetit, 2007).

Studies focused on the physical properties of perimysium and endomysium for their effects on tenderness have indicated that background toughness changes during long time ageing. Takahashi (1996) divided tenderness period to two slow and rapid phases. In the slow phase, intramuscular connective tissue (IMCT) weakens just after a rapid phase of myofibril degradation by which the breaking strength of the perimysial connective tissue in raw beef decreases during postmortem ageing (Lewis et al., 1991). Nishimura et al. (1998) observed that mechanical strength of IMCT during postmortem ageing of beef remained unchanged up to 10 days, then decreased linearly until day 35 of postmortem (Nishimura et al., 1998). Observation with light microscopy indicated that structures of chicken endomysium and perimysium disintegrate into several thin sheets within 12 h postmortem so that many gaps appear in the endomysium and perimysium (Liu et al., 1994). Nishimura et al. (2008) indicated that perimysial sheets in pork aged for 8 days had separated into collagen fibers and fibrils, leaving a crimp structure, and endomysium had disintegrated into individual collagen fibrils which were arranged loosely, though neither broken nor torn (Fig. 3) (Nishimura et al., 2008). These results clearly show that structures of the IMCT are weakened and, as Takahashi (1996a) stated, contribute to tenderization of beef and chicken at the latter phase of postmortem ageing. Hannesson et al. (2003) demonstrated a breakdown of a large proteoglycan and hyaluronic acid in beef during the first 24 h postmortem (Hannesson et al., 2003). Since proteoglycans play an important role in the stabilization of collagen fibrils (Scott and Thomlinson, 1998), their degradation may lead to the weakening of IMCT. This is because of the weakening of linkages between collagen fibrils and fibers in the

endomysium and perimysium, resulting in meat tenderization during postmortem ageing.

5. TENDERIZATION PHASE

While under similar processing conditions, toughening occurs in the carcass of different animals, a large variation is observed in both the rate and extent of tenderization. Generally speaking, meat tenderness improves during refrigerated storage and it was well indicated almost a century ago that this happens following enzymatic activity. It is also well-established that postmortem proteolysis of myofibrillar and associated proteins is responsible for this process. The major myofibrillar protein that maintain the organization of muscle structure and their degradation affect on mechanical properties of meat, presented in Table 2. However, it is a matter of debate which proteases are responsible for tenderization (Table 3) and which muscle proteins are degraded (Koochmaraie and Geesink, 2006; Ouali et al., 2006).

5.1. Muscle Proteinase

Efforts have been made over decades to show the tenderizing functions of two best known enzymatic systems, namely cathepsins and calpains (Herrera-Mendez et al., 2006). However, two other protease systems have also been considered in this regard which include the multicatalytic proteinase complex and, to a lesser extent, caspases (Table 2).

Cathepsins are a group of enzymes comprised of both exo- and endo-peptidases, categorized into cysteine (cathepsins B, H, L, and X), aspartic (cathepsins D and E), and serine (cathepsin G) peptidase families (Sentandreu et al., 2002), each with its own inhibitors designed as cystatins (Herrera-Mendez et al., 2006). Many of the studies on cathepsins and their role in meat tenderization have been focused on B, L, and D, which are endopeptidases, although cathepsins B and L have been shown to exhibit exopeptidase activity. Cathepsins are located in and released from lysosomes and, thus, play a role in myofibril degradation. Incubation of myofibrils or glycerinated fibers with a lysosomal extract results in some of the ultrastructural changes observed during meat ageing including the degradation of myofibrils near the N₂ lines and at the A-I junction. Moreover, a few fragments with molecular masses close to 155 and 90 kDa, probably originating from myosin heavy chain degradation, have been identified in stored meat (Sentandreu et al., 2002). Like myosin, actin seems to degrade partially but only 7–10 days postmortem (Taylor et al., 1995a). Cathepsins degrade many other structural and contractile proteins, so that they may show more affinity for these other proteins than for actin and myosin filaments in the muscle.

Many efforts have been directed over the last decades toward finding relationships, if any, between meat tenderization and cathepsin proteolytic activity (Maribo et al., 1999; Jung et al.,

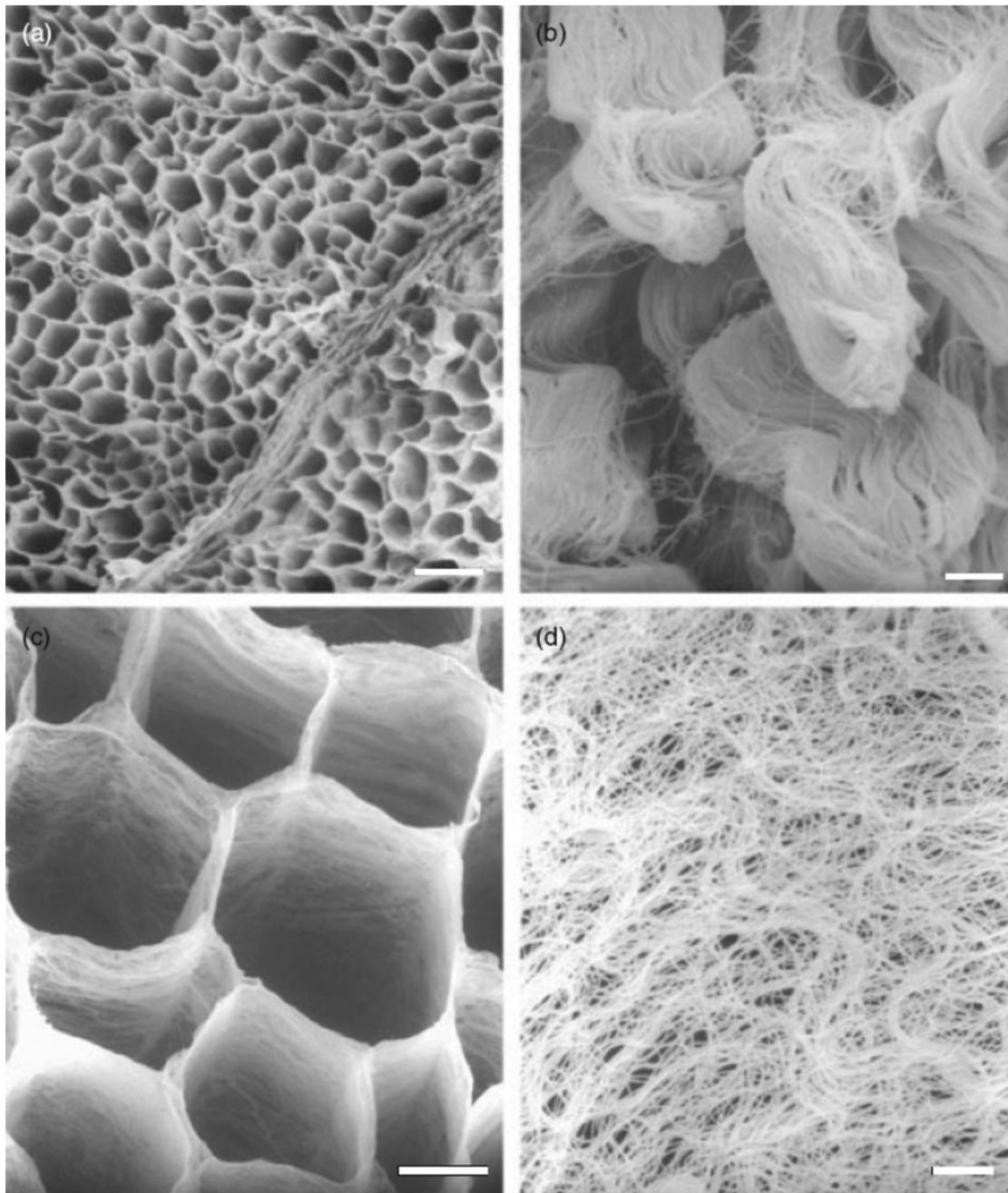


Figure 3 Scanning electron micrographs of intramuscular connective tissue (IMCT) of pork aged for 5 days at 4°C. (a) Low magnification view of IMCT. (b) A part of the premyosium. (c) Endomysium sheaths. (d) A closer view of a part of c. Scale bars indicate 100 (a), 5 (b), 25 (c), and 1 μ m (d) (Nishimura et al., 2008).

2000; Chéret et al., 2007; Buckow et al., 2010; Fontanesi et al., 2010); the role of endogenous cathepsins in tenderization of meat postmortem has, however, remained a controversial issue. That cathepsins are a major contributor to postmortem meat tenderization may be challenged by the following observations: (1) these enzymes require acidic conditions for optimum activity (Hopkins and Geesink, 2009); (2) they require to leak from lysosomes into the cytosol (Koochmaraie, 1996); (3) proteins such as myosin and actin do not degrade under normal chilling conditions although they may rapidly degrade when incubated with

cathepsins (Koochmaraie et al., 1991); (4) despite the fact that certain cathepsin inhibitors are not able to suppress postmortem proteolysis, some general cysteine peptidase inhibitors such as E-64, which inhibits both calpains and cathepsins, effectively prevents postmortem proteolysis (Sentandreu et al., 2002); and (5) no substantial study has been reported to demonstrate a link between tenderization and these enzymes (Whipple et al., 1990).

The members of the calpain superfamily have also been classified in the following three groups: ubiquitous calpains (μ , m and μ /m calpains), tissue specific calpains (calpain 3 or p94,

Table 2 Major Myofibrillar protein and their characteristics

%MF protein (w/w)	MW (kDa)	Location	Subunits	Protein
43	520	Thick filament	Heterohexamer:	Myosin
	220		Heavy chain	
	23		Essential (proximal) light chain	
	20		Regulatory (distal) light chain	
22	43	Thin filament	Homopolymer	Actin
10	3200–3700	Z-line to M-line	Homohexamer	Titin
5		Z-line to M-line	Monomer	Nebulin
5	73	Thin filament	Heterotrimer:	Troponin
			Troponin T	
			Troponin I	
			Troponin C	
5	66	Thin filament	Homo/heterodimer:	Tropomyosin
			Alpha-tropomyosin	
			Beta-tropomyosin	
2	130	Cross-bridge region	Monomer	MyBP ^a -C
<1	55	Cross-bridge region	Monomer	MyBP-H
2	165–185	M-line	Homodimer	Myomesin (1–3)
2		Z-line	Homodimer	α -actenin
<1	40	Thin filament	Monomer	Tropomodulin
<1	68	Z-line	Heterodimer:	CapZ
			Alpha-subunit	
			Beta-subunit	
			32	
<1	43	M-line	Homodimer	Creatine kinase
		—	Monomer	Adenylate kinase
<1	54	Near Z-line	Homopolymer	Desmin
<1	185	Z-line	Heteropolymer (w/desmin)	Synemin
<1	240	Z-line	Homodimer	Filamin
<1	19	Z-line	Monomer	Telethonin/T-cap
<1	145	Z-line	Monomer	Myopalladin
<<1	427	Z-line	Monomer	Dystrophin

^aMyosin binding protein.

calpains nCl-2, nCl-4, Lp85, and Rt88, . . .), and atypical calpains (calpains 5, 6, 10, . . .). The most investigated calpains, i.e., μ -calpain or calpain 1 (active at μ M calcium concentrations), m-calpain or calpain 2 (active at mM calcium concentrations), and μ /m-calpains (active at intermediate calcium concentrations), are heterodimers composed of two subunits. The catalytic subunit with a molecular weight of 80 kDa is unique for an enzyme and exhibits peptidase activity while the regulatory subunit with a molecular weight of 30 kDa is common to all of them (Sentandreu et al., 2002). The inhibitor of calpains is calapstatin (Herrera-Mendez et al., 2006).

Early studies have shown that the action of the calpains leads to the release of α -actinin from the Z-disk and the degradation of troponin-T and -I and C-protein (Hopkins and Geesink, 2009). In myofibrillar proteins, as in striated muscle, the calpains readily cleave titin and nebulin just near the Z-disk, thereby severing their attachment to the proteins in the Z-disk. In addition, the calpains remove the intermediate filament protein desmin that attaches the Z-disk to the sarcolemma; hence, proteins constituting the Z-disk, including α -actinin, are released and the Z-disk disappears leaving a space in the myofibril (Dayton et al., 1976; Akiyama et al., 2006; Kayamori et al., 2006). Consequently, the net effects of calpains on striated muscle myofibrils involve progressive disruption of the Z-disk leading eventually to its complete loss; subsequent release of myosin and actin filaments from

the surface of the myofibril; and production of single fragments of titin (some as large as 500 kDa), nebulin, desmin, and other calpain susceptible proteins. It is noteworthy that due to the limited specificity of calpains, further degradation of titin, nebulin, and other fragments along with actin and myosin to amino acids requires the participation of other proteases. It seems that it is the proteasome which has a major role in the degradation of the released actin and myosin molecules and the other myofibrillar protein fragments (Goll et al., 2003). This is while a recent study indicates that degradation of actin and myosin is not generally observed even after cytoskeleton proteins have been degraded by calpains (Hopkins and Geesink, 2009). On the other hand, evidence has been provided to demonstrate the involvement of calpain in post-mortem muscle tenderness: (1) myofibrils and those treated with calpain undergo similar ultrastructural degradation; (2) myofibrillar proteins, whether with calpain or not, exhibit similar electrophoretical degradation patterns; (3) the Z-disk, where the calpain is localized, is susceptible to calpain-catalyzed hydrolysis; (4) the higher the level of calpains in the muscle, the faster the rate of postmortem tenderization (Dayton et al., 1976; Dayton et al., 1981); (5) calcium has been observed to accelerate proteolysis and tenderization whereas infusion or injection of calpain inhibitors inhibits tenderization (Koochmaraie, 1990; Wheeler et al., 1992); (6) the greatly reduced rate and lower proteolysis and tenderization in callipyge

Table 3 Overview of the studies conducted on protease activity and role of various enzymes in tenderness of different meats

Enzyme	Animal	Objects	Results	Ref.
Cathepsin L Cathepsin S	Italian Large White pigs	Identify and analyse DNA markers in two cathepsin genes, cathepsin L and cathepsin S and study their association with meat quality parameters (including cathepsin activity of post mortem muscle) and several production traits	Association between cathepsin S polymorphism and feed: gain ratio and average daily gain No association between the analysed markers and meat quality parameters (pH ₁ , pH _u , lactate, glycogen, glycolytic potential, and cathepsin activity)	(Fontanesi et al., 2010)
Cathepsins B, B+L, H Cysteine proteinase inhibitors	Sow	Study the influence of several common types of terminal sires on the activity of muscle enzymes, implicated in pork meat quality	Terminal sire type and sex have significant influence on enzyme activity	(Armero et al., 1999)
Cathepsin B and B+L CDP ^a and CDP inhibitor activities	Herford and Brahman Steer	Determination the role of postmortem muscle ultrastructure, sensitivity to ionic strength, and (or) endogenous protease activity in the differences in tenderness of meat from <i>Bos indicus</i> vs. <i>Bos taurus</i> cattle.	Cathepsin B or cathepsin B+L activities and the myofibrillar response to elevated ionic strength were not different between breeds, but both could be related to the postmortem increase in tenderness of both breeds. Calcium-dependent protease activity, as modulated by calcium-dependent protease inhibitor, seems to play a major role in the inherent tenderness differences between breeds	(Wheeler et al., 1990)
Cathepsin B + L Calpains	Steer	Examination the relationship of Ca ²⁺ -dependent protease and lysosomal enzymes with breed type and beef tenderness	Differences in meat tenderness among breed types may be explained partially by differences in proteolytic enzyme activity	(Johnson et al., 1990)
Cathepsin B Calpains	Pig	Investigation the ability of m-calpain, μ -calpain and cathepsin B to degrade desmin, and to identification specific cleavage sites on the desmin molecule. Discussion about the substrate specificity regulating the proteolytic activity of these cysteine proteases	m-calpain or μ -calpain primarily cleaved desmin in the head and tail region leaving the rod domain relatively intact Cathepsin B produced a sequential C-terminal degradation. The substrate primary structure was not found to be essential for regulation of the proteolytic activity of the cysteine peptidases studied. Calpains are involved in degradation of desmin early postmortem	(Baron et al., 2004)
Cathepsins B, D, H, and L Calpains Calpastatin	Dairy cows Sea bass fish	Characterizing and comparing fish flesh and meat initial amount of proteolytic activity in order to evaluate the possible differential role of proteases in both types of muscles.	The cathepsin B and L activities were in more important amounts in sea bass than in bovine muscle. Cathepsin D activity was higher in meat than in fish muscle	(Chéret et al., 2007)
Cathepsin (B+L) Cathepsin D Calpains Calpastatin	Pig	Investigation the activities of several muscle enzymes in two pig lines, one stress sensitive line and one stress resistant line	Cathepsin H was negligible in both muscles. Calpain activities were similar in both types of muscle. Calpastatin level was higher in sea bass white muscle Lower activities of m-calpain, μ -calpain in stress sensitive pig compared with stress resistance. Activities of calpastatin, cathepsin (B+L) and cathepsin D were not different. Significant correlation between activities of several enzymes and pH values 1 h postmortem. Lower enzyme activities are a consequence of a more pronounced denaturation due to a faster post mortem pH decline	(Claeys et al., 2001)

CDP ^a -I Cathepsins B and H	Bull and Steer	Comparison the relative importance of the CDP and catheptic enzyme systems to meat tenderness	CDP-I helps to establish initial (d 1) meat tenderness but that cathepsins B and H are responsible for the tenderization that occurs during aging	(Calkins and Seidemam, 1988)
Cathepsins B, H, D, and L m-, μ -calpain Calpastatin	White-red bulls	Assessing the proposed role of calpain activity in tenderization. Investigate the involvement of endogenous cathepsin B, H, D, and L and serine proteinases	Cathepsin B, D, H, and L inhibitors did not affect texture or proteolysis. Calpains are the main proteases involved in beef tenderization	(Uytterhaegen et al., 1994)
Cathepsins m-calpain	Ovine	Determination the extent of myofibrillar hydrolysis by partially purified m-calpain and lysosomal cysteine proteinases at their respective physiological concentrations. Determination the effectiveness of zinc in inhibiting proteolysis by these enzymes	The degradation by the lysosomal extract is far more extensive than the degradation that occurs with normal postmortem storage and that possibly a non-cysteine protease is present that is capable of hydrolyzing some myofibrillar proteins under this in vitro condition, because Zn ²⁺ did not block all proteolysis. Similar changes were induced by m-calpain incubation and postmortem storage	(Koochmarate and Whipple, 1991)
μ -calpain	Steer	Evaluation that the exposure of postmortem bovine muscle tissue to oxidation (via irradiation at 24 h postmortem) would result in inactivation of μ - and m-calpain and decreased proteolysis of myofibrillar proteins and higher shear force values in aged beef steaks	Irradiation decreased μ -calpain activity and autolysis, whereas m-calpain activity was not affected by diet or irradiation. Inactivation of μ -calpain by oxidation during early times postmortem decreased the amount of myofibrillar proteolysis, thereby decreasing the extent of tenderization of beef steaks	(Rowe et al., 2004)
μ -calpain, m-calpain, Calpastatin	Steers or Heifers	Use of antibodies to monitor changes in the calpains and calpastatin during postmortem storage and clarification the role of the calpains in postmortem tenderization	Slightly change of extractable m-calpain during the first 7 d after death. Rapidly decrease of extractable calpastatin and μ -calpain activity during this period. Over 50% of total muscle μ -calpain was tightly bound to myofibrils 7 days after death and this calpain was nearly inactive proteolytically	(Boehm et al., 1998)
μ -calpain, m-calpain, Calpastatin	Pig	Determination the extent to which pH and ionic strength influence μ - and m-calpain activity and the inhibition of calpains by calpastatin	Higher ionic strength decreased μ -calpain activity at all pH conditions. Inhibition percent of μ -calpain by calpastatin was not affected by pH; however, it was influenced by ionic strength.	(Maddock et al., 2005)
μ -calpain Calpastatin	Cow	Examination the effect of postmortem conditions on μ -calpain and myofibrillar proteins in a system that closely mimics postmortem muscle	m-calpain activity is greater in alkaline and neutral pH Increasing the amount of calpastatin in the incubations limited the rate and extent of proteolysis of myofibrillar proteins and autolysis of μ -calpain. Excess calpastatin did not inhibit proteolysis completely. Proteolysis of myofibrillar proteins virtually ceased after 7 days of incubation. Inhibition of μ -calpain activity by calpastatin and instability of autolyzed μ -calpain probably limit the rate and extent of postmortem proteolysis and, as a result, meat tenderization	(Geesink and Koochmarate, 1999a)
Calpains Calpastatin	Turkey	Examination the effects of bird age and muscle tissue type on calpain and calpastatin activities in turkey skeletal muscle	Breast muscle from younger birds had higher m-calpain, μ -calpain, and calpastatin activities than older birds. Thigh calpastatin activity was increased with age	(Northcutt et al., 1998)

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Table 3 Overview of the studies conducted on protease activity and role of various enzymes in tenderness of different meats (*Continued*)

Enzyme	Animal	Objects	Results	Ref.
μ -calpain m-calpain	Cow	Determination the effects of time, postmortem storage, pH, and temperature on activities of μ - and m-calpain activity by zymography in bovine skeletal muscle	μ -calpain activity decreased nearly to zero within 48 h postmortem in <i>longissimus dorsi</i> , <i>psaos major</i> , <i>semimembranosus</i> unlike <i>triceps brachii</i> . Activity of m-calpain decreased at a much slower rate. Neither μ - nor m-calpain was proteolytically active at pH 5.8 after 24 h	(Camou et al., 2007b)
μ -calpain m-calpain	bovine	Evaluation the effects of pH, temperature, and inhibitors on the proteolytic activity of skeletal muscle μ -calpain	Postmortem conditions have a significant effect on the rate of autolysis, proteolytic activity, and inactivation of bovine μ -calpain. By decreasing pH and temperature, autolysis of μ -calpain is significantly increased and decreased, significantly. Autolysis and exogenous proteolytic activity of calpains are two independent processes	(Koochmarai, 1992a)
μ -calpain m-calpain	Lamb	Measurement of m- and μ -calpain activity using casein zymography for better understanding of calpain role in postmortem proteolysis	Calcium concentration in postmortem muscle is only high enough to activate μ -calpain. μ -calpain-mediated proteolysis of key myofibrillar and cytoskeletal proteins is responsible for postmortem tenderization	(Veiseth et al., 2001)
m-calpain Calpastatin	Bovine	Characterization the effects of pH and ionic strength on bovine m-calpain activity and calpastatin inhibition of m-calpain	Although m-calpain and calpastatin activities decrease with increasing ionic strength, their activities in the presence of myofibrils were not affected by ionic strengths typically found in postmortem muscle	(Kendall et al., 1993)
μ -calpain	Normal and callipyge sheep	Study the μ -calpain binding to myofibrillar protein postmortem. Differences in myofibril bound calpain in callipyge and normal sheep	Activities and properties of the myofibril-bound calpain were identical from callipyge and normal sheep. It seems unlikely that the myofibril-bound calpain has a significant role in postmortem tenderization of ovine longissimus	(Delgado et al., 2001b)
μ -calpain, m-calpain, Calpastatin	Callipyge and normal sheep	Evaluation the role of calpastatin in postmortem tenderness	Postmortem tenderization is related to the rate of calpastatin degradation in postmortem muscle and that calpastatin inhibition of the calpains in postmortem muscle is modulated in some as yet unknown manner	(Delgado et al., 2001a)
Calpastatin	Lamb	Determination the protease(s) responsible for degradation of muscle calpastatin during postmortem storage	Among m-calpain, μ -calpain, cathepsin B, proteasome, trypsin, or chymotrypsin, it seems that m- and (or) μ -calpain to be responsible for calpastatin degradation during postmortem storage of meat	(Dounit and Koochmarai, 1999)
Calpain 10	yearling sheep	Measurement of the relative level of calpain 10 proteins in sheep and evaluation of the temporal changes in calpain 10 proteins during postmortem tenderization	Tenderness improved gradually during post-mortem storage and was accompanied by a decline in intact desmin level. Calpain 10 is present in sheep Longissimus as two proteins (MW 73.6 and 70.7 kDa). Increases in the myofibrillar calpain 10 proteins were strongly correlated with the rate of tenderization. Sub-cellular distribution of calpain 10 proteins is dynamic during postmortem storage	(Ilian et al., 2004)

Calpain 3	Calpain 3 knockout Mice	Test the effect of postmortem storage on proteolysis and structural changes in muscle from normal and calpain 3 knockout mice	Postmortem proteolysis of muscle occurred similarly in control and in calpain 3 knockout mice. Desmin degradation did not differ and there were no indications that degradation of nebulin, dystrophin, vinculin, and troponin-I were affected by the absence of calpain 3 in postmortem muscle. Structural changes were affected by time postmortem but not by the absence of calpain 3 from the muscles. Calpain 3 plays a minor role, if any, in postmortem proteolysis in muscle	(Geesink et al., 2005)
Calpain 3	Porcine	Investigation the possibility of a relationship between p94 characteristics and variable tenderness of porcine longissimus muscle	The variability in tenderness of porcine longissimus muscle can not be attributed to an underlying variability in calpain 3	(Parr et al., 1999)
Caspase 3	Steer	Evaluation the relationship between caspase 3 activity and beef tenderness and muscle growth. Activation of caspase 3 in postmortem beef muscle	No significant activation of caspase 3 activity, even reduction of activity after 72 h postmortem. Caspase 3 activity was not associated with Warner-Bratzler shear force at slaughter. Caspase 3 is not anticipated to be involved in postmortem tenderization of beef	(Underwood et al., 2008) (Caine et al., 2003)
Caspase 3/7 Caspase 9	Large White gilts	Investigation the protease family caspases in skeletal muscle and their potential contribution to postmortem proteolysis and meat tenderization	Changes in caspase activity and caspase-mediated cleavage in muscle during the conditioning period. Association between the caspase activity and development of tender meat	(Kemp et al., 2006a)
Caspases 3, 8 and 12	Large White pig	Characterization of the caspase system in various porcine skeletal muscles and investigation the relationship between muscle type and caspase expression	Significant difference in caspases activity and their inhibitor ARC levels between muscles but no difference in mRNA abundance. Expression of multiple components of the caspase system in porcine skeletal muscle and variable of their levels but no distinct association of expression with a particular muscle	(Kemp et al., 2006b)
Proteasome Calpains	Young bull calves	Address the question of whether and how proteasomes and calpains are involved in postmortem proteolysis from another angle, by investigating inhibition of the enzyme already present in the meat	Inhibition of the proteasome activity results in a lack in degradation of several proteins including metabolic and structural proteins, which are also degraded in meat post-mortem. Inhibition of the ubiquitous calpain only resulted in minor changes in the degradation pattern. Involvement of the proteasome in postmortem proteolysis in beef	(Houbak et al., 2008)
Proteasome	Bovine	Evaluation the proteasome activity during postmortem aging	Considerable postmortem stability of proteasome activities, despite the marked decrease in pH, allows them to play a role in meat tenderization in synergy with other proteolytic systems. Proteasomes may be involved in other modifications, such as the change in Z-line density in type I fibres	(Lamare et al., 2002b)
Proteasomes	Bovine	To seek the evidence for attack by the proteasome on specific muscle structures	The 20S proteasome completely and rapidly hydrolyses purified myofibrillar proteins and nebulin, myosin, actin, and tropomyosin, are degraded during the incubation with proteasome. 20S proteasome probably plays a role in the postmortem hydrolysis of cellular proteins	(Robert et al., 1999)

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Table 3 Overview of the studies conducted on protease activity and role of various enzymes in tenderness of different meats (*Continued*)

Enzyme	Animal	Objects	Results	Ref.
MCP ^b	Ovine	Determination the existence of MCP ^c in ovine skeletal muscle and to characterize its biochemical properties and effects on myofibrils	The MCP was maximally active at pH 7.5 to 8.0 and 45°C. Troponin C and myosin light chains-2 and -3 are degraded by MCP. MCP is not involved in the initial steps of myofibril disassembly	(Koochmarate, 1992b)
Serine proteinase	Crucian carp	Elucidation the existence of MBSP ^c in silver carp muscle and identifies its involvement in the degradation of myofibrillar proteins	Myofibril-bound serine proteinase in crucian carp myofibril efficiently cleaves MHC ^d and tropomyosin	(Cao et al., 2006)
MBSP ^d	Mice	Determination the action of MBSP and its inhibitor on whole myofibrils	Myosin, actin, troponin T, α -actinin, and tropomyosin are degraded by MBSP. This enzyme would be implicated in skeletal muscle myofibrillar ATP-independent proteolysis	(Sangorrin et al., 2002)

^aCalcium dependent proteases.

^bMulticatalytic proteinase complex.

^cMyofibril-bound serine proteinase.

^dMyosin heavy chain.

lamb which has been attributed to elevated levels of calpastatin in these animals (Geesink and Koohmaraie, 1999b); and (7) calpastatin is overexpressed in transgenic mice, which has led to a considerable reduction in postmortem proteolysis of muscle proteins (Geesink et al., 2006). In spite of the overwhelming evidence supporting the calpain proteolytic effect, the following uncertainties still prevail awaiting answers. (1) μ -calpain is so rapidly inactivated that it cannot account for tenderization beyond 24–48 h postmortem (Camou et al., 2007a); (2) muscles have twice as much calpastatin activity as μ -calpain activity; (3) μ - and m-calpains are easily autolyzed (Jiang, 1998); and (4) calpain sensitive proteins (nebulin, metavinculin, vinculin, dystrophine, desmin, and troponin T) in mice whose μ -calpain gene is disrupted are degraded (Camou et al., 2007a).

On the other hand, a considerable debate is still going on as to which of the μ - or m-calpains is responsible for postmortem changes. Adequate evidence has been provided indicating that it is μ -calpain, rather than m-calpain, that plays an important role in postmortem degradation of muscle during refrigerated storage (Geesink and Koohmaraie, 2000; Hopkins and Thompson, 2002). In bovine and ovine postmortem muscles, the extractable activity of μ -calpain declines, whereas the activity of m-calpain remains remarkably stable (Geesink and Koohmaraie, 1999b). This observation has led to the conclusion that μ -calpain, but not m-calpain, is responsible for postmortem tenderization (Koohmaraie and Geesink, 2006). Moreover, m-calpain, requires high Ca^{2+} concentrations for proteolytic activity, and it is unclear whether enough Ca^{2+} concentrations are accessible to initiate m-calpain activity (Camou et al., 2007a).

The proteasome is a multicatalytic protease complex involved in the regulation of a number of basic cellular pathways, which is due to their effect on degradation of proteins in the cytosol and nucleus. Each proteasome (26S) consists of a 19S regulatory subunit and a 20S multicatalytic structure with proteolytic enzyme activities (Kemp et al., 2010). Although the 26S proteasome comprises a protein unfolding system, i.e., the 19S subunit, the 20S proteasome is sometimes considered to be unable to hydrolyze native proteins, especially muscle proteins. Indeed, the 20S proteasome has been shown to degrade myofibrils and to impose significant damages on the M- and Z-lines as do calpains (Sentandreu et al., 2002). It has been found that bovine proteasomes are capable of causing proteolysis in such myofibril proteins as nebulin, myosin, actin, and tropomyosin in bovine myofibrils (Taylor et al., 1995b). Proteasome maintains its activity during development of rigor, and even post rigor, and a substantial increase in its activity has been detected after 7 days postmortem and at pH levels below 6 (Lamare et al., 2002a). Accordingly, it has been suggested that under high pH conditions, these enzymes may also play a role in myofibrillar degradation (Taylor et al., 1995b). Based on some specific ultrastructural changes observed in high pH meat and in postmortem slow-twitch oxidative or type I muscles, recent investigations suggest that the 20S proteasome might be the main proteolytic system of interest in postmortem tenderization of these meat types. In slow twitch bovine muscles, e.g., diaphragm pedialis

muscle, the main ultrastructural change was indeed an enlargement of the Z-line with more or less density loss (Sentandreu et al., 2002).

Caspases constitute a new family of cysteine peptidases characterized by their ability to cleave proteins where aspartic acid residues are present. Their primary function is to contribute to the programmed cell death, or apoptosis (Sentandreu et al., 2002; Fuentes-Prior and Salvesen, 2004). Up to now, three classes of caspases have been distinguished: caspase involved in initiating apoptosis (caspases 8, 9, and 10); effector caspases (caspases 3, 6, and 7); and caspases involved in inflammatory processes (caspases 1, 4, and 5) (Hopkins and Geesink, 2009). These have been found in many different subcellular locations (including the endoplasmic reticulum, mitochondria, and the cytosol) and have been shown to degrade a number of key structural components of the cytoskeleton such as spectrin, actin, and gelsolin (Nakagawa et al., 2000). The enzymes are tightly regulated by inhibitors such as apoptosis repressor with caspase recruitment domain (ARC) (Earnshaw et al., 1999). Studies have also shown that caspases are involved in skeletal muscle development and remodeling, with expression being essential for normal muscle differentiation during myogenesis (Fernando et al., 2002). Caspases are upregulated in conditions like sarcopenia (Dupont-Versteegden, 2005) and muscular dystrophies (Sandri et al., 2001), and are activated early in pathological events associated with hypoxia/ischemia (Gustafsson and Gottlieb, 2003), which is similar to the hypoxic conditions that occur postmortem. If we keep this unique relationship between skeletal muscle and caspases in mind, it will then be easy to understand why caspases are also involved in postmortem proteolysis and meat tenderization (Kemp et al., 2006a). The presence of active caspase 3 in beef muscle has been evaluated but its activity has not been detected (Underwood et al., 2008). This finding is different from that of a previous study with pigs which found that caspase 3 was activated in postmortem porcine skeletal muscle (Kemp et al., 2006b). Also, the highest activity of caspase 3/7 and 9 was measured 2 h after slaughter; however, the activity of these enzymes decreased after 192 h. Moreover, a negative relationship has been reported between shear force and the 0:32 h caspase 3/7 and 9 activity ratios (Kemp et al., 2006a).

5.2. Mechanical and Nanomechanical Changes

There have been many attempts to evaluate changes in mechanical properties and tenderness using objective physical methods. Instrumental texture assessment on meat is made by means of a texturometer, a device that allows tissue resistance both to shearing and to compression to be measured. The most widespread method normally used as an indicator of meat hardness (tenderness) is the Warner–Bratzler shear test, almost the sole methodology used in raw meat (de Huidobro et al., 2005). Other apparatuses have also been developed for evaluation of meat texture like “Armor Tenderometer” and “Torque

Tenderometer” (de Huidobro et al., 2005). Warner–Bratzler shear force, referred to in most papers, decreases during post-mortem storage; however, it depends on many factors including extent of shortening (Marsh and Leet, 1966), pH (Watanabe et al., 1996), temperature decline, as well as amount and solubility of collagen (Seideman et al., 1987). Modeling studies based on μ -calpain activity as a primary cause of postmortem tenderization predict toughness at death time to be about 7.2–11.0 kg, toughness at 3 days postmortem as ca. 5.5–7.2 kg, toughness at 5 days as ca. 5–7 kg, and as approximately 3.5–4.0 kg at 25 days which is usually considered to be the ultimate toughness. Hence, this model suggests that shear force decreases by almost 30%, 34%, and 59% during 3, 5, and 25 days postmortem, respectively (Dransfield, 1992). Warner–Bratzler shear measurements of bovine semitendinosus muscle stored at 2–4°C have been reported to change from 7.3 kg at death to 4.1 kg after 3 days postmortem and to 3.4 kg after 13 days of postmortem storage. Consequently, the shear force in this study decreased by 44% and 53% after 3 and 13 days postmortem, respectively (Goll et al., 1964). Based on the maximum toughness at 24 h, Wheeler and Koochmarai (1994) showed that shear force decreased by approximately 47% and 64% after 3 and 14 days postslaughter (Wheeler and Koochmarai, 1994). Generally speaking, these studies suggest that shear force reduced by 30–47% after 3 days and by 53–64% after 13 days of storage.

In order to evaluate changes in mechanical force on a smaller scale, Christensen et al. (2003) investigated the effect of added μ -calpain and postmortem storage on breaking stress and strain of a single muscle fiber and found that treatment with μ -calpain (pH 7.5) decreased the breaking stress by 66% (from 137.5 kPa for the control to 45.8 kPa after treatment with μ -calpain) and the breaking strain by 68% (from 22.3% for the control to 7.0% for the μ -calpain treated sample) (Christensen et al., 2004). In another study, the same authors used a μ -calpain concentration lower by four times and reported that after incubation with the enzyme, the breaking stress of the single muscle cell reduced by ca. 50% but no significant difference was observed in its breaking strain. Incubation of myofibrils with cathepsin B (pH 5.6) showed the same trend. In this case, a higher concentration of cathepsin B was used over longer incubation times compared to those for μ -calpain and the same cathepsin B-induced changes were observed in the fracture properties of muscle fibers. This could be due to the premier role of μ -calpain substrate, i.e., costameric and structural protein, compared to that of cathepsin B substrate in the mechanical strength of muscle cells. Post-mortem storage for 10 days led to a decrease of 60–70% in the force needed to fracture the fibers, i.e., 141.6 kPa at 24 h vs. 42.9 kPa after 10 days postmortem (Christensen et al., 2004). In general, force reduction in the single muscle fiber is greater than that in intact meat, probably due to the lack of connective tissues.

Wakayama et al. (2000) measured transverse stiffness of different loci along myofibrils before and after treating with calcium activated neural protease (CANP) using AFM and the force-distance curves. All the loci in CANP-treated myofibrils

exhibited lower values of transverse stiffness than the various loci in intact preparation. A drastic decline was reported in the stiffness of Z-band in the absence of Z-band in CANP-treated myofibrils. Stiffness values of 1.8–4.1 pN/nm were estimated in all the loci of CANP-treated myofibrils, which were four- to five times less rigid than the Z-band of intact myofibrils (ca. 10 pN/nm) (Wakayama et al., 2000). A decrease has also been reported in the transverse stiffness at Z-disk while the calpain treatment advanced. Actin and myosin remained unchanged after digestion with calpain, and the transverse stiffness of the overlap region did not change extensively (Akiyama et al., 2006).

5.3. Ultrastructural Changes and Protein Degradation

The changes after death of the muscle cell and its subsequent events have been extensively studied. Sarcolemma in at-death muscle has periodic areas of invaginations at the level of the Z-disk and the M-line. The sarcolemmal invaginations, however, disappear during the first 24 h of postmortem storage, and the sarcolemma is pulled away from the underlying myofibril in almost 50% of the cells. Since costameres are evidently responsible for attachment of the sarcolemma to the myofibril, these structural changes indicate that it is the costameres that are extensively degraded within the first 24 h postmortem leading to the loss of sarcolemmal integrity. After 72 h postmortem, the sarcolemma is completely moved away from the myofibrils. Another considerable ultrastructural change during postmortem aging of muscle is the separation of adjacent myofibrils within the muscle fiber. These changes are most frequently observed in postmortem muscle as “tears” or gaps in the I-band area. These tears or breaks often occur near intact Z-disks in the area previously occupied by the N2 line. “Tears” or breaks in the I-band begin to appear after 3 days of postmortem and increase prevalently over the following days. Despite evidence that most Z-disks in postmortem muscle stored at 4°C remain almost unchanged ultrastructurally even after 16 days of postmortem storage (Taylor et al., 1995a), many researchers have shown that one of the most important events during postmortem storage is loss of Z-line (Olson et al., 1977; Ho et al., 1996; Nagaraj et al., 2005). Using phase contrast microscopy for comparison between intact myofibril and CANP-treated myofibrils, it was noted that both of the myofibrils had essentially the same structure except that Z-bands were absent in the CANP-treated preparation (Fig. 4) (Wakayama et al., 2000). Akiyama et al. (2006) reported that decline in Z-band integrity and rigidity is accompanied by digestion of titin and α -actinin. They concluded that digestion of α -actinin, a major Z-band component, would directly lead to the reduction of the transverse stiffness of Z-bands. It is well-known that titin filaments link Z-bands to the thick filaments in skeletal myofibrils, strengthening the sarcomere structure in the longitudinal direction and that, therefore, their digestion by calpain occurs almost concurrently with the transverse stiffness changes (Akiyama et al., 2006).

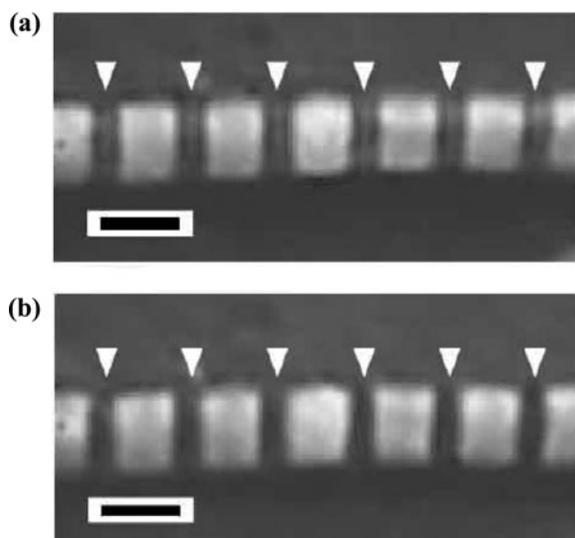


Figure 4 Phase contrasted images of single myofibrils. (a) An intact myofibril and (b) a calpain-treated myofibril. Arrows indicate locations of Z-band. Scale bars, 2 μm (Wakayama et al., 2000).

Both heavy weight titin and nebulin that are crucial to the ordered structure of the sarcomere have been clearly shown to degrade into fragments during postmortem period (Huff-Lonergan et al., 1996a). Degradation of both proteins is suggested as reason for the increased fragility of myofibrils in the I-band region (Taylor et al., 1995a). Proteins associated with muscle contraction such as myosin, actin and α -actinin have been reported to be resistant to degradation under normal postmortem conditions (Hopkins and Geesink, 2009). Troponin T is a regulatory protein that is degraded during aging, giving rise to a 30 kDa protein fragment (Ho et al., 1994; Nagaraj et al., 2005). Among the costameric proteins, vinculin (Taylor et al., 1995a; Taylor and Koochmaraie, 1998), desmin (Huff-Lonergan et al., 1996a; Wheeler and Koochmaraie, 1999) and filamin (Huff-Lonergan et al., 1996a; Taylor and Koochmaraie, 1998) have been investigated for their postmortem degradation.

The structure of titin is extremely complex, containing numerous repeating large domains. Most of the mass consists of 90–100 amino acid modules arranged in seven stranded β -sheets. These modules are of two types—those similar to the backbone-folding pattern of Ig and those similar to the third

domain of fibronectin (FN3). The portion of titin that lies in the A-band has both FN3 and Ig domains but the I-band region contains only the Ig-type domains. Because titin is linked at both Z- and M-lines, the structure must be elastic in nature to accommodate changes in sarcomere length during contraction and relaxation (Swartz et al., 2009). Moreover, it is now well established that the passive elasticity of striated muscle is mainly regulated by titin (Garcia et al., 2009). When a muscle is extended from its rest length, the supercoiled titin molecules are straightened. With greater extension forces, an exclusive amino acid sequence called the PEVK region (proline (P), glutamate (E), valine (V), and lysine (K) residues) will be lengthened (Fig. 5). This region is very sensitive to protease action (Swartz et al., 2009). Single-molecule experiments with optical tweezers and atomic force microscope have demonstrated that titin behaves as an unusual spring, where reversible domain unfolding plays an important role in its elasticity (Li et al., 2002). The molecule unfolds in a high-force transition beginning at 20–30 and refolds in a low-force transition at ~ 2.5 both at pN levels (Kellermayer et al., 1997). Reversible Ig-like domain unfolding is thought to serve as a safety mechanism that protects titin and the whole sarcomere from mechanical damage in case of extreme stretch during stress (Garcia et al., 2009). It is important to note that it is the degradation of titin, rather than the absolute amount of titin in postmortem muscle, that contributes to postmortem tenderization (Fritz et al., 1993). In SDS-PAGE of muscle samples taken at death, titin is found to migrate primarily as a single band referred to as T1; however, it initially migrates as a doublet. The upper band is intact T1 and the lower migrates only slightly faster and is indeed a degraded part of T1, refer to as T2. This product migrates at approximately 2400 kDa. Another high-molecular weight degradation product of mammalian titin migrates at approximately 1200 kDa and appears with additional time postmortem (Huff-Lonergan et al., 1995). The latter has been shown to contain the portion of titin that extends from Z-line to near N2 line in the I-band (Kimura et al., 1992; Huff-Lonergan et al., 1996a) although the exact position of 1200 kDa polypeptide in the sarcomere is still not certain. The 1200-kDa polypeptide has been documented to appear earlier in the postmortem period in myofibrils from aged beef that had a lower shear force (and more desirable tenderness scores) than in samples from products with less favorable tenderness scores

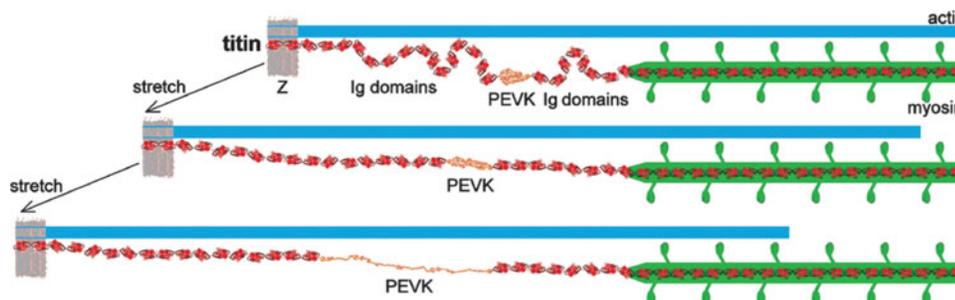


Figure 5 Model for the extensibility of structurally distinct titin region in the half-sarcomere. Sequential extension of Ig-domain regions and the PEVK-domain in titin of skeletal muscle (Neagoe et al., 2003). (Color figure available online.)

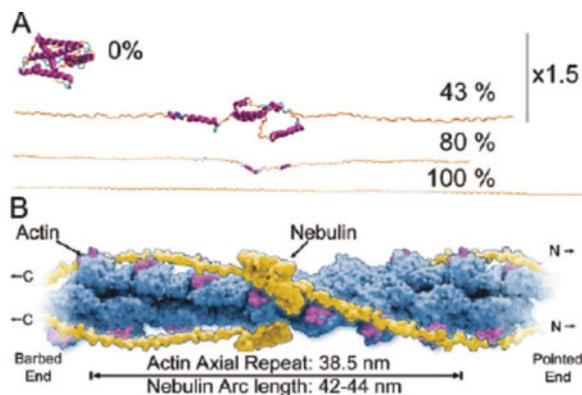


Figure 6 (a) structural simulation of stretching one nebulin superrepeat to 0, 43, 80, and 100%. (b) Full-length nebulin associates along and around actin filaments in the sarcomere (Yadavalli et al., 2009). (Color figure available online.)

(Huff-Lonergan et al., 1995; Huff-Lonergan et al., 1996b). The T2 polypeptide can also be subsequently degraded or altered during normal postmortem aging.

Nebulin is also a large protein with a weight in the range of 750–850 kDa. Nebulin is also composed of a large number of repeating domains and it is alternatively spliced. It contains an SH3 domain at the C-terminus and this sequence is proposed to bind to proline-rich sequence near the N-terminal of titin in the Z-line. The protein α -actinin, desmin and CapZ also bind to the nebulin carboxyl end. Nebulin has been suggested to function as a molecular ruler to determine the thin filament length (Fig. 6) (Swartz et al., 2009) and may aid in anchoring thin filaments to the Z-line and, thus, like titin, may play a significant role in maintaining structural order and integrity in the myofibril (Huff-Lonergan et al., 1996b). Nebulin is an attractive candidate for study by force spectroscopy because it plays a direct role in the force generating machinery of the muscle and is likely to play a mechanical role in the stability and function of the muscle sarcomere. The average stiffness of a single nebulin at ~ 1 pN/nm indicates that single nebulin is compliant with only ~ 3 – 5% of the stiffness of the F-actin filament at 20–34 pN/nm (Yadavalli et al., 2009) that makes it vulnerable to lateral forces. Degradation of nebulin is completed 3 days after death (Huff-Lonergan et al., 1996b) and could weaken the thin filament linkages at the Z-line, and/or of the thin filaments in the nearby I-band regions (Taylor et al., 1995a), thereby weakening the structure of the muscle cell. Portions of nebulin that span the A–I junction have the ability to be bound to actin, myosin, and calmodulin. More interestingly, this portion of nebulin has been shown to inhibit actomyosin ATPase activity, and has also been suggested to inhibit the sliding velocities of actin filaments over myosin. If the latter role is confirmed, then it is also possible that nebulin's postmortem degradation may alter actin–myosin interactions in such a way that the alignment and interactions of thick and thin filaments in postmortem muscle are disrupted. This phenomenon could also lead to an increase in postmortem tenderization. Some researchers believe that nebulin degradation does not seem to be correlated to postmortem tenderization

(Huff Lonergan et al., 2010). Taylor et al. (1995a), however, observed that the intact nebulin band at 3 days postmortem in less-tender meats was still easily detectable (Taylor et al., 1995a) while in the more-tender samples, only a trace of the intact nebulin could be observed (Huff-Lonergan et al., 1995). Overall, nebulin degrades more easily than titin. Destruction of titin and nebulin might be the cause of sarcomere lengthening and the emergence of the gap (Nagaraj et al., 2005).

Degradation of troponin T and appearance of 30 kDa peptide have been used as an indicator of meat tenderness for many years (Huff-Lonergan et al., 1995; Huff-Lonergan et al., 1996a). The potential importance of the degradation of troponin-T on myofibril integrity was discussed by Huff-Lonergan et al. (1996a) (Huff-Lonergan et al., 1996a). Troponin-T is a regulatory protein that is bound to thin filaments and its degradation changes myofibril integrity. Often, more than one fragment of troponin-T can be identified in postmortem muscle (each is likely a closely spaced doublet of polypeptides) at approximately 30 and 28 kDa, which is related to troponin-T isoforms (Huff Lonergan et al., 2010).

α -actinin is an antiparallel homodimer found in the Z-line. It has a length of about 35 nm. The monomer has three major domains; a globular actin binding domain near the N-terminus composed of a pair of calponin homology domains. This is followed by four triple α -helical, spectrin-like domain repeats. Near the C-terminal, there is a calmodulin-like domain, which is the muscle isoform that does not bind calcium. As the name implies, the actin binding domain binds to actin, and the residue on actin involved in this situation has some overlap with the residue. α -actinin also binds to titin, nebulin and CapZ. These numerous interactions of the protein with other sarcomeric proteins lead to very high affinity for binding of α -actinin to the Z-line of sarcomere (Swartz et al., 2009). The result of SDS-PAGE and Western blots showed that postmortem storage did not noticeably affect α -actinin.

Desmin is another insoluble structural protein that appears to form a network of collars within the plane of the Z-line, and may be responsible for maintaining the alignment of adjacent sarcomeres. This protein and connectin ("T-filament" or "gap-filament" protein) thus constitute a filamentous cytoskeleton in the muscle which is additional to the well-established system of actin and myosin filaments (Lawrie and Ledward, 2006). It also links the Z-disks to costameres and desmosomes at the periphery of the muscle fiber, contributing to the mechanical integrity of muscle tissue. The main features of the tensile properties of a desmin are a yield stress of 10 MPa, a strain-hardening behavior that becomes prominent above 50% extension, an extensibility of 240%, and a tensile strength of at least 240 MPa (Kreplak et al., 2008). Desmin is also subjected to degradation during postmortem storage (Huff-Lonergan et al., 1996b) and is a very light band by 28 days on SDS-PAGE (Ho et al., 1996). It has been documented that desmin is degraded more rapidly in myofibrils that are more tender ones (Huff-Lonergan et al., 1996b). A major degradation product that is often seen in beef is a polypeptide of approximately 38 kDa. This degradation product also has been

shown to be present in μ -calpain digested myofibrils (Carlin et al., 2006).

Three types of α , β , and γ filamins with molecular weights of 300 kDa have been identified in muscle cells, all with similar molecular structures. The γ -filamin locates around Z-disk and creates a strong linkage between sarcolemma and sarcomeric cytoskeleton. Filamin exists at an early development stage of Z-disk and may involve in Z-disk formation (Strasberg et al., 2007). Thus, postmortem degradation of filamin could conceivably disrupt key linkages that serve to hold myofibrils in the lateral register. Degradation of filamin may also alter linkages connecting the peripheral layer of myofibrils in muscle cells to the sarcolemma by weakening interactions between peripheral myofibrillar Z-disks and the sarcolemma via intermediate filament associations or costameres (Robson et al., 1995). A study using myofibrils from beef showed that some degraded filamins form an approximately 240 kDa product that migrates as a doublet in both myofibrils from naturally aged muscle and in μ -calpain-digested myofibrils (Huff-Lonergan et al., 1996a).

6. CONCLUSION

After slaughter and over the time, meat shows extremely different mechanical properties that can be classified in three phases. In the preliminary stage and just after slaughtering the meat has its original characteristics, however, after a while it enters into the second stage with increase in both common and nano scales shear stress and transverse stiffness that is attend to increase due to the movement of myosin heads toward actin along with sarcomere shortening in longitudinal and vertical directions. In the third stage, meat proceeds toward tenderness phase and reduction in shear force is evidenced, in which various proteins and enzymes are involved. There is still uncertainty in relation to which enzyme is the main responsible of this event however what can be said practically is that more than one enzyme is participated in last phase. Studies indicated that after enzyme treatment of muscle fiber not only whole mechanical properties altered but also different loci along with the sarcomere show a number of changes in stiffness. It is clear that the cytoskeleton proteins play a structural role along with participation in mechanotransduction and thereby modulates the functional possibilities of muscle fibers. Such alterations in nanomechanical properties of muscle fiber may be related to degradation of these proteins as it has been confirmed with ultrastructural observations and SDS-PAGE analysis. This is an interesting paradox since structural proteins have been considered for many years as “mechanical integrators of cellular space” but there is not any research about change in their mechanical properties during tenderness. Moreover, despite the much attention that has been focused on mechanical properties of meat during tenderness phase, the origin of these properties that is related to changes in stiffness of muscle cells particularly in pN scale has not be considered. Except the three stages, mechanical properties of meat are also influenced by connective tissue that has not any

distinct alternation during short time aging, however, long storage of meat accompanied with moderate improvement in the texture which is related to disintegration of endomysium and perimysium, though there is no report on mechanical properties of connective tissue fibers.

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