The effects of postmortem aging time, animal age and sex on selected characteristics of bovine *longissimus* muscle

by

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GENERAL INTRODUCTION

Researchers have studied the palatability attributes of beef tenderness for many years. Although much data has been accumulated, a fully satisfactory explanation of what causes a wide variation in beef steak tenderness continues to elude researchers. Meat is an extremely complex tissue and any answer to the question of why some beef is more tender than other beef must also be complicated, and will, by necessity have to take into consideration numerous variables.

Quantifying the differences in palatability attributes by using sensory and mechanical methods has required much time and effort. Because meat is a multiple component system, any method used to quantify the tenderness and other palatability attributes should also be differentiated into several components. Cover and coworkers (1962) proposed that the tenderness descriptors used in sensory panel work should be partitioned into several categories. Likewise, it has been suggested that the traditional mechanical methods used to assess tenderness, such as the Warner-Bratzler shear device, be re-evaluated. Bouton and Harris (1972) compared several instrumental methods of measuring meat tenderness with measures that directly determine some texture characteristics of meat. They suggested that compression as measured by the Instron and adhesion measurements made on muscle samples are highly related as are the peak (maximum) force measurements made with the Warner-Bratzler shear device and the

fiber tensile strength measurements. Møller (1981) proposed that the deformation curves obtained by using the Warner-Bratzler attachment on the Instron be divided into multiple components when analyzed. That is, the initial yield observed on the curve represented the myofibrillar component of tenderness, while the final yield point on the curve represented the connective tissue component of tenderness.

Certain factors and procedures have been identified as aiding in predicting types of beef that may be more or less tender than average. Some of these factors include the age and sex of the animal. Numerous research studies have shown that beef from older animals is significantly less tender than beef from younger animals (Tuma et al. 1963; Dikeman and Tuma, 1971; Smith et al., 1982). When comparing beef from intact males (bulls) with castrated males (steers) of the same age, conflicting results have been reported. Albaugh et al. (1975) showed that beef from bulls was less tender than that from steers, while Hedrick et al. (1969) showed that there was no difference in tenderness in beef from bulls and steers of less than 16 months of age. The practice of postmortem aging has been shown to greatly improve the tenderness of beef. Field et al. (1971) showed that shear force values declined from 2 to 21 days of postmortem storage. Busch et al. (1967) showed that a greater increase in tenderness was observed when beef was aged at 16°C rather than at 2°C.

In search for the answer to the question of what causes these differences in tenderness, changes in the structural components of the myofibril have been examined. Parrish et al. (1973) observed

fragmentation of myofibrils due to the disintegration of Z-lines in bovine longissimus samples that had been aged at 16°C for 1 day or at 2°C for 3 days. Their sensory panel studies also showed a marked increase in tenderness at these time/temperature periods. Parrish et al. (1973) suggested, based on these observations, that the reduced size of the myofibril brought about by fragmentation increased sensory panel tenderness because of a reduction in the amount of force required to cut through the fragmented sample. MacBride and Parrish (1977) and others (Olson et al., 1977; Olson and Parrish, 1977) have shown that a degradation product of the myofibrillar protein troponin T, termed the 30,000-dalton component was observed to occur more often in tender than in less tender beef. Other components of the myofibril have also been examined. Locker (1982) suggested that filaments he termed 'gap filaments' played a major role in determining the tenderness of meat. These gap filaments are now believed to be titin, an elastic protein found in the myofibril (Locker, 1982; LaSalle et al., 1983). Titin is one of two extremely large molecular weight proteins first extensively examined by Wang et al. (1979). Titin has an approximate molecular weight of 28,000 kDa (Kurzban and Wang, 1988) and migrates on SDS-PAGE gels as a doublet. The two bands are commonly referred to as T_1 and T_2, with T_2 migrating just ahead of T_1. The second protein, nebulin, is another large molecular weight protein first identified as band 3 (Wang et al., 1979). Nebulin has now been shown to have a molecular weight of between 600 and 900 kDa (Wang and Wright, 1988; Jin and Wang, 1991). These two proteins, titin and

nebulin, have been shown to degrade under postmortem conditions (Lusby et al., 1983) as well as to undergo postmortem degradation more rapidly and more extensively in tender than in less tender beef (Anderson and Parrish, 1989).

One of the major enzymes that is believed to be responsible for some of the observed postmortem degradation that occurs in meat is that protease which is known as calcium activated factor (CAF). CAF is a protease with optimum activity at pH levels near 6.5. CAF consists of two subunits, one approximately 80 kDa and the other approximately 28 kDa (Goll et al, 1985). CAF, as the name implies, requires the presence of calcium for its activation. CAF has been shown to exist in two forms based upon its calcium requirement. These two forms differ in that one form requires μmolar quantities of calcium for activation, while the other requires millimolar quantities. Research has shown that CAF will degrade several proteins of the myofibril including titin, desmin, troponin T and troponin I (Goll et al., 1983; Zeece et al., 1986).

This project was developed to determine the effects of sex, chronological age and postmortem aging time on tenderness differences observed in meat samples as well as to determine if specific myofibrillar/cytoskeletal proteins such as titin and nebulin from specific sex and age categories were degraded differently during postmortem aging.

Explanation of Thesis Format

This thesis is written in an alternate style format consisting of a general introduction, a general review of literature, a publishable paper and a concluding summary. The paper represents the work done by the first author to fulfill requirements for the degree of Master of Science. The paper consists of a title page, abstract, introduction, materials and methods, results and discussion, summary, implications and references. The format of the paper is in accordance with the Journal of Food Science style guide for research papers.

GENERAL REVIEW OF LITERATURE

Skeletal Muscle Structure and Postmortem Changes

Muscle structure

Muscle is one of the most complex tissues found in nature. The details of muscle structure and function play a vital role in determining the quality and palatability attributes of meat and meat products. A knowledge of the structure of muscle is necessary for one to understand how and why some of these differences in quality occur. A short discussion of the structure of muscle follows.

Muscle tissue is classified into three categories: striated, cardiac and smooth. Both striated and cardiac muscle exhibit a banding pattern that is seen as being transverse to the long axis of the muscle fiber when viewed microscopically. Smooth muscle does not exhibit this banding pattern. Both smooth muscle and cardiac muscle are classified as involuntary as their function is not normally consciously controlled by the organism. Striated muscle is generally referred to as that muscle making up the bulk of the skeletal muscle. Skeletal muscle is classified as voluntary muscle as it is usually controlled by the will of the organism (Judge et al., 1989; Pearson and Young, 1989). Because skeletal muscle is the primary tissue that comprises meat, the remainder of this discussion will focus on it.

Organization of muscle

A muscle is covered by a thin sheet of connective tissue known as the epimysium. A muscle itself is comprised of a number of structures known as muscle bundles. These bundles are covered by a sheath of connective tissue known as the perimysium. Muscle bundles are made up of structures known as muscle fibers. These fibers are long, cylindrical, multinucleated cells that can be several centimeters in length. The diameter of these cells can range from 10 μm up to greater than 100 μm (Judge et al., 1989). The outer cell membrane of the muscle fiber is known as the sarcolemma which in turn is surrounded by a connective tissue sheath known as the endomysium.

The main functional unit of the muscle fiber is known as the myofibril. The myofibrils are long, cylindrical organelles that average 1-2 μm in diameter and extend the entire length of the muscle fiber. Within the myofibril is an array of interdigitating thick and thin filaments. These two sets of filaments are aligned parallel to each other. These thick and thin filaments overlap each other in specific regions giving the muscle fiber its typical banding pattern or "striated" appearance. This striation appears as regions of light and dark bands. The light bands are described as being isotropic when viewed using polarized light and so they are termed "I-bands". The dark bands appear anisotropic under the same conditions, and so are termed the "A-bands". Bisecting the I bands is a dark band known as the Z line. The region between two Z lines is termed the sarcomere. Within the

sarcomere there is one A band located between two half I bands. This structure is repeated through the myofibril. In resting muscle, the typical length for a sarcomere is 2.5 μm. The A band is bisected by a narrow band known as the M line. In addition, in the center of the A band is a slightly lighter region known as the H zone.

Muscle filaments

In normal muscle cells, the thick filaments are approximately 14-16 nm in diameter and are 1.5 microns long. These filaments are primarily made up of hundreds of myosin molecules. Myosin molecules are rod shaped (tail region) with a globular two headed region on one end. In the thick filament, the myosin molecules are arranged in bundles with the tail regions making up the main shaft of the thick filament. The head regions of the myosin molecules project outward from the main body of the thick filament. The myosin filaments are arranged so that the heads are oriented toward the two ends of the thick filaments leaving a bare zone in the middle of the filament.

The thin filaments of muscle average around 6-8 nm in diameter and are approximately 1.0 μm in length. The primary protein that is found in the thin filaments is the protein actin. G-actin (globular form) has a molecular weight of approximately 42,000. The actin molecules themselves are spherical in shape and are arranged in twin strands that are twisted around each other to form the main portion of the thin filament. These actin particles seem to have a "front" and a "back"

giving the entire filament directional polarity. The thin filaments are anchored to the Z line and are attached to the thin filaments on the opposite side of the Z line in an array of cross connections that give the Z line its characteristic zigzag pattern (Pearson and Young, 1989).

The thin filaments of muscle also contain the proteins tropomyosin and troponin. Tropomyosin is the second most abundant protein in the thin filament, accounting for approximately 7% of the total protein in the myofibril. Tropomyosin is made up of two chains that have an approximate weight of 34,000 each. The native tropomyosin molecule has a molecular weight of approximately 68,000 and has a length of 40 nm. In the myofibril, tropomyosin is found as long, thin filaments on the actin strand. The tropomyosin molecule is located near the groove between the paired strands of actin molecules (Murray and Weber, 1974).

Troponin is a complex made up of three subunits, troponin C (MW 18,000), troponin I (MW 23,000) and tropinin T (MW 37,000). The three subunits have separate biochemical properties. Troponin C has the function of binding calcium. Troponin I can inhibit the interaction between myosin and actin, while troponin T binds strongly to tropomyosin (Pearson and Young, 1989). The overall shape of the troponin molecule is thought to contain both a globular region as well as a rodlike portion. The total length of the molecule is about 26.5 nm while the rodlike portion comprises around 16.5 nm of the length (Flicker et al., 1982). The entire troponin molecule therefore interacts with approximately two-thirds of the tropomyosin molecule.

Muscle contraction

The basic principle in contraction involves the shortening of the sarcomere and thus ultimately the muscle by the sliding of the thin filaments past the thick filaments. The force that causes this sliding is generated by the formation and dissolution of cross-bridges between the thick and thin filaments. The myosin heads attach to specific sites on the actin chain and swivel. This movement draws the thin filaments past the thick filaments before the heads detach and attach again. This cycle of attaching and detaching causes a relative movement of the thin and thick filaments by about 100 angstroms (Murray and Weber, 1974).

Regulation of contraction is accomplished by the actin-troponin-tropomyosin system, as well as by the concentration of ATP and calcium in the myofibril. Phillips et al. (1986) discussed the interaction of this system. In the relaxed state (off-state) the troponin complex binds the tropomyosin and holds it on the outer part of the actin helix thus prohibiting the interaction of myosin and actin. When the concentration of Ca^{2+} reaches the proper levels the troponin complex binds to the calcium and releases the tropomyosin from its position on the actin strand. The filaments are then in the "on" or active position and the myosin heads are able to bind to actin.

The energy for contraction is provided by the hydrolysis of adenosine triphosphate (ATP) into two lower energy compounds, adenosine diphosphate (ADP) and inorganic phosphate. This hydrolysis

reaction takes place on the head of the myosin molecule. The reaction occurs as follows as described by Murray and Weber (1974). ATP has a very strong affinity for myosin and binds to the head very readily. The myosin-ATP complex is then raised to a charged intermediate form that can then bind to actin. Hydrolysis can then occur releasing ADP and inorganic phosphate and energy to power contraction. The myosin-actin complex (rigor complex) is maintained until ATP is again bound to myosin and the sequence can be repeated again.

Myofibrillar/cytoskeletal proteins

The Z line bisects the I band and is the region in which the actin filaments of opposing sarcomeres are tied together. Some of the proteins that have been identified as being located in or near the Z line include desmin, filamen, vimentin, synemin, α-actinin (Greaser et al., 1981), zeugmatin, and vinculin (Maher et al., 1985).

Desmin

Desmin has an molecular weight of 55,000 and is highly susceptible to proteolysis. Desmin is arranged around the periphery of the Z line and serves to bind the myofibrils together in this region (Robson et al., 1981). Desmin may play a role in influencing the water-holding capacity of the muscle.

α-actinin

α-actinin has a molecular weight of roughly 200,000 and is made up of two subunits of approximately 100,000 each (Robson et al., 1981). The dimensions of the molecule are approximately 4 x 50 Å and it has been suggested that it is the Z-filament of the Z-disc (Robson et al., 1981).

It has been suggested by Robson et al. (1981) that α-actinin may play a three-fold role in skeletal muscle. First, it may serve to anchor the thin filaments into the Z line, second, it may modify the structure of actin in the thin filaments and finally, it may help to determine the directionality and regulate the growth of the thin filaments.

Filamin

Filamin has been shown to be a protein made of two subunits of a molecular weight of 240,000 (Wang, 1977). The exact role of filamin in the myofibril is not known, however, it has been shown to bind to F-actin and may form an important part of the structure of the Z discs (Bechtel, 1979).

Synemin

Synemin has been shown to have a molecular weight of 23,000 (Granger and Lazerides, 1980), and has been identified as part of the intermediate filaments (10 nm). Synemin, desmin and vimentin appear to have closely related roles in that they seem to surround the myofibril at the Z line (Granger and Lazarides, 1979).

Vimentin

Vimentin is a protein with a molecular weight of approximately 57,000 and has been shown to exist with desmin at the Z line (Franke et al., 1978). Like synemin and desmin, it appears that vimenten plays a role in maintaining the lateral register of the myofibrils.

Vinculin

Vinculin has a molecular weight of 130,000. Its role in the myofibril is not known at the present time. Some studies suggest that it may play a role in linking actin to the cell membrane (Geiger, 1979).

Zeugmatin

Zeugmatin has a molecular weight of approximately 500,000. When it is observed on SDS-polyacrylamide gels it migrates only slightly below nebulin (Maher et al., 1985). Zeugmatin appears to be especially susceptible to proteolysis, therefore, only fresh muscle can be used for its isolation and purification (Pearson and Young, 1989).

Titin

In 1979, Wang and his associates reported finding an extremely large protein that showed up on 4% polyacrylamide gels as a doublet with an approximate molecular weight of 1×10^6. Wang named this protein titin from the Greek word meaning anything of great size. In his studies he reported that a titin antibody was found to react with

the myofibril in certain locations. The antibody reacted strongly at the junctions of the A and the I bands and in the central region of the A band. He also noted that weak staining was seen throughout the entire A band. In addition, the M and Z lines exhibited labeling. They also reported that their results showed titin to be present in striated muscles, (both skeletal and cardiac) and in vertebrate and invertebrate species.

Maruyama and coworkers (1976, 1977) earlier had also reported the isolation of an elastic and highly insoluble protein which they termed connectin. King and Kurth (1980) showed that this preparation consisted of some protein polypeptides that were larger than myosin. Maruyama later showed (1981) that his connectin preparation also contained the same extremely high molecular weight polypeptide as was found in Wang's titin preparation.

Maruyama et al. (1980) reported that their connectin preparation showed strong immunoflourscence starting at the A band and the Z line of the myofibril, while the I bands showed weak staining. These results lead them to the conclusion that connectin was located throughout the entire myofibril.

Electron microscopy of rotary-shadowed samples of titin extracted under nondenaturing conditions showed that titin is an asymmetrical molecule that appears as a string-like structure with lengths of up to 8000 Å and a diameter of 40 Å. Negative staining of titin molecules has shown it to be similar in appearance to a string of beads (Trinick et al., 1984; Hainfield et al., 1989).

Titin appears to be the third most abundant protein found in the myofibril (Trinick et al., 1984) making up 8% of the total protein of the myofibril. Titin is third in abundance behind myosin (43%) and actin (22%). While not found to any great extent in smooth muscle, titin has been found to exist in all types of striated muscle, including cardiac (Maruyama et al., 1977). Titin appears to be found throughout the length of the sarcomere. Titin is a very elastic protein and the entire length of the strands of the protein seem to be very flexible.

Maruyama et al. (1985) reported studies in which they examined frog skeletal muscle using immunoelectron microscopy. From their studies, they concluded that connectin structures were directly linked to the Z lines from the myosin filaments. They found five antibody labeling stripes in each half of the sarcomere and two in the A-I junction. Antibody deposits were also found in the I bands and in the A bands. They proposed that the connectin filaments run alongside the thick filaments beginning approximately 0.15 μm from the central portion of the A-band.

Fürst et al. (1988) utilized immunolocalization microscopy to observe the binding sites of ten distinct titin antibodies that each gave a pair of decoration lines per sarcomere. The decoration lines were centrally symmetric to the M line. They found one location at the Z line, five along the I band, one in the A-I junction and three within the A band. When they looked at immunoblots the two antibodies decorating at or just before the Z line recognized the T_1 component, but not T_2. The rest of the antibodies recognized both T_1 and T_2. Their

results suggest that the length of the titin molecule is greater than 1 μm, which is long enough to span the region between the Z line and the M line. Barnoili et al. (1989) also supported the theory that the titin molecule connects the M line and the Z line of the sarcomere. In their study, they also observed that areas in the sarcomere located between the A-I junction and the N_1 band recognized by the anti-titin antibodies moved away from the Z disc when the sarcomere was lengthened. This action was mirrored on opposing sides of the M line. The areas decorated by the anti-titin antibodies remained the same distance from the M line when the sarcomere was stretched to approximately 2.8 μm. The anti-titin epitopes moved away from the M line and the elastic character of the molecule was seen when the sarcomere was stretched beyond 2.8 μm, indicating that the elastic portion of the molecule is in the region between the N_1 line and the A/I junction.

Fürst et al. (1989a) showed an anti-titin antibody labeling site that was located 55 nm from the center of the M band showing that titin extends into the structure of the M band. Their preparation of titin for electron microscopy studies showed that the T_2 form of titin possesses a globular head that is possibly the suggested M band anchoring domain. When they used immunoblotting techniques they found that two epitopes located at the Z-line and at a point 0.04 μm before the Z line were found only on the T_1 band. They suggested that the T_1 band is the portion of the molecule that is located at the Z line. They proposed that the proteolytic cleavage site that converts the

nonextractable form (T_1) into the extractable form (T_2) may be in the region between the N_2 line and the M band.

The exact function of titin is not well understood at this time. Several different theories have been proposed. One function that has been proposed is that of assisting in the assembly of the myofibril. Fürst and coworkers (1989b) examined muscle cell development in mouse embryos. They found that titin was expressed before myosin heavy chain in *in vivo* conditions. In addition, the Z line section of titin was found to be brought into register in the myofibril before the A band region of the molecule was brought into register. These results lead them to hypothesize that these early titin filaments act as integrators during skeletal muscle development.

Whiting et al. (1989) proposed that titin may regulate the length of the thick filaments. This suggestion was based on several facts. Antigenic sites of titin located in the A band do not change their relative positions to the M line over varying sarcomere lengths. This suggested that titin is bound to the outside of the thick filament shaft. Also, many epitopes were found to be located in the A band. Since the length of the titin molecule is 1 μm or more, they proposed that titin may regulate the length of the thick filaments by forming a template that spans the length of the filament.

King et al. (1981) looked at the effects of heating meat samples to 50-70°C on connectin (titin). They found that when heated to 55°C, the titin from homogenized muscle (pH 5.5) was extensively degraded.

When they examined the relationship between the ultimate pH of the muscle and the degradation of titin, they found an inverse relationship.

Lusby and coworkers (1983) showed that titin is degraded in meat samples over time, and that this rate of degradation is influenced by storage temperature. They found that over time the top band of the titin doublet disappeared. The lower band disappeared after 3 days storage at the higher temperatures (37°C), but remained up to and including 7 days postmortem storage at 22 °C.

Paxhia and Parrish (1988) showed that in light poultry muscle, the T_1 (top band of the titin doublet) band disappeared after 1 day of storage at both 4°C and 22°C, while in dark poultry muscle the T_1 band was still present after 1 day of storage at 4°C. The opposite was found when light and dark pork muscles were examined. The T_1 band in light pork muscle was still seen at 3 days postmortem when stored at 4°C. In dark pork muscle, the T_1 band disappeared after 1 day at 22°C and 7 days at 4°C.

Paterson et al. (1988) showed that suspending myofibrils in a solution containing 10 mM pyrophosphate resulted in swelling of the myofibrils, at the same time the extraction of titin and the water holding capacity were both increased, suggesting that titin may provide some of the structural restraints that hold the sarcomere together. This observation suggests the extraction of titin allows greater water holding capacity. Paterson and Parrish (1986) found that both the T_1 and T_2 bands of titin were present in the less tender *rhomboideus* muscle while only the T_2 band was present in the more tender

infraspinatus muscle. Further evidence as to the role that titin plays in meat/muscle systems was provided by Anderson and Parrish (1989) when they examined the degradation of titin in beef steaks from samples that exhibited differences in tenderness. They found that titin was more rapidly degraded in tender than in less tender steaks suggesting that titin may indeed play a role in influencing meat tenderness.

Fritz and Greaser (1991) examined the postmortem degradation of titin and nebulin in bovine *psoas major* muscle. They found that nebulin (pg 20) had alomost completely degraded by 48 hours postmortem, however, they still noted the presence of titin after 2 weeks of postmortem storage. They also used immunofluorescence microscopy to determine the number of titin antibody reactive (anti-titin) bands that could be detected over time postmortem. They found that at 45 minutes postmortem, less than 1% of the myofibrils exhibited four anti-titin bands per sarcomere (most exhibited 2), while at 48 hours postmortem, 65% of the myofibrils had four anti-titin bands per sarcomere. They also noted that this two to four band conversion occurred over the same time frame as did the degradation of nebulin. These scientists noted that the *psoas* muscle is one that exhibits most of its change in tenderness in the first few days postmortem. Coupling this knowledge with the results they obtained, they proposed that whatever processes are occuring to cause the change from two to four anti-titin bands per sarcomere may also be

coupled to the tenderness changes observed in postmortem muscle tissue.

Nebulin

Nebulin is another extremely large protein found to exist in the sarcomere. Nebulin was first described by Wang and his associates (1979) as band 3 due to its position on SDS-PAGE gels in relationship to the two bands of titin. Nebulin was initially reported to have a molecular weight of 500 to 600 kDa; however, this figure has been modified and it is now thought to have a molecular weight in the range of 600-900 kDa (Wang and Wright, 1988; Jin and Wang, 1991). Nebulin has been estimated to make up 3-4% of the total myofibrillar protein in mammalian skeletal muscles (Wang, 1982). Nebulin has not as yet been shown to exist in smooth muscle (Robson and Huiatt, 1983) or cardiac muscle (Locker and Wild, 1986; Fürst et al., 1988).

Nebulin received its name as it was originally observed by some researchers to be associated with the N-lines of the sarcomere which are rather "nebulous" in their appearance (Wang, 1981). When seen in electron micrographs, the N_2-line appears as a dark line parallel to the Z-line and across the I-band (Locker and Leet, 1976). Some researchers have described the N_2-lines as being four lines that appear to be on either side of the Z-line (Locker and Wild, 1984). The exact function of the N-lines is not known, however, they have been observed to change position as the sarcomere changes in length while maintaining the same proportional distance between the M-line and the Z-line (Locker and Leet, 1976).

In light of research within the past few years, nebulin is now thought to be an inextensible filament that is close to 1 μm in length. These nebulin filaments are now proposed to be associated with actin (rather than the N_2 lines) and to run parallel with the actin filaments (Robson et al., 1991) It has been shown that nebulin binds to α-actinin (Nave et al., 1990) and to F-actin (Jin and Wang, 1991). This suggests the possibility that nebulin may play a role in attaching or anchoring the actin filament to the Z-line in the sarcomere (Robson et al., 1991).

Several researchers have shown that nebulin is degraded rapidly in meat (Lusby et al.; 1983, Paterson and Parrish, 1987; Paxhia and Parrish, 1988; Anderson and Parrish, 1989; and Fritz and Greaser, 1991) An involvement of nebulin in the tenderization process has been suggested by Anderson and Parrish (1989). They showed that nebulin bands were less intense or non-existent in myofibrils from steaks that were categorized as 'tender" by sensory panel evaluations when compared to myofibrils from "less-tender" steaks. As nebulin seems to be intimately involved with proteins of the Z-line and the actin filaments, it has been suggested that nebulin may be important in regulating the structure of the myofibril in early postmortem muscle (Robson et al., 1991), and thus somewhat affect the tenderness of meat.

Rigor mortis

Following the death of an animal, a complex series of physical changes occur which lead to the muscle being in a stiffened or rigid state termed "rigor mortis". This state is a stage of the conversion of muscle to meat and its resolution plays an important role in determining the perceived tenderness of meat. During the past half-century much time and effort has been devoted to studying the factors affecting the rate of the onset and the resolution as well as the degree of stiffening obtained throughout the course of the development and decline of rigor mortis.

When an animal is first slaughtered, ATP and creatine phosphate are present in the muscle and the pH of the muscle is near neutrality, pH 6.7-7.2. During normal metabolic processes in the muscle the supply of ATP is continually replenished by oxidative phosphorylation; however, when the supply of oxygen from the blood is terminated the muscle goes into an anaerobic state and the level of ATP can no longer be maintained. For a short time during this anaerobic period the level of ATP needed can be maintained by the conversion of ADP to ATP by drawing upon the reserves of creatine phosphate in postmortem muscle. Once these reserves are exhausted the ATP level in the muscle falls. This drop in the level of ATP in the system causes the anaerobic production of lactate from glycogen resulting in a drop of the pH of the muscle. Within a short period of time, the pH can drop from 7.2 to 5.5 (Penny, 1980).

The stiffness that is observed during rigor mortis is caused by the formation of permanent cross-bridges between the actin and myosin filaments. This is essentially the same interaction that occurs in living muscle during contraction, except that when the reserves of ATP are depleted after death there is no longer a constant supply of energy (ATP) available to assist in breaking the actomyosin bond (Judge et al., 1989).

Many physical changes occur during the process of converting muscle to meat. One of the more easily quantified changes is the loss of extensibility when the muscle is subjected to a load. Immediately following death, the muscle is rather extensible, it passively stretches under load up to 140% of its resting length (Penny, 1980) and will readily return to its resting length as allowed by the natural elasticity of muscle. During this stage very few actomyosin cross-bridges have been formed which would prevent this extension. This phase is termed the delay phase of rigor mortis (Bate-Smith and Bendall, 1949). Once the stores of glycogen and creatine phosphate are exhausted, the rephosphorylation of adenosinediphosphate (ADP) to adenosinetriphosphate (ATP) becomes inadequate to continue to break the actomyosin bonds being broken. This end result is reduced extensibility of the muscle. The stage during which a loss of extensibility is observed is known as the onset phase. This phase begins when the muscle starts to lose its elasticity and lasts until the completion of rigor mortis. The completion of rigor mortis occurs when all of the creatine phosphate has been used. Characteristically the

muscle is no longer extensible at this point. The time that is required for muscle to pass through these stages of rigor varies from animal to animal and even from muscle to muscle. If muscle that has undergone the effects of rigor mortis is "aged" or held for a certain length of time the meat will again attain some measure of increased tenderness and pliability (Whitaker, 1959).

Busch et al. (1967) and Goll et al. (1971) suggested that the stages of rigor mortis be defined in terms of the isometric tension that develops in meat (muscle) samples over time postmortem. Isometric tension is that amount of tension that is measured when a muscle strip is held at one end, while the other end is attached to a sensing device and the amount of tension or shortining that develops or declines is measured using a physiograph. Busch et al. (1967) and Goll et al. (1968) proposed that the period of increasing isometric tension be identified with the onset of rigor mortis, while the decrease in isometric tension be identified with the resolution of rigor. Busch and coworkers (1972) showed through a series of experiments that the measurement of the development of isometric tension is a sensitive method for evaluating the onset of rigor mortis. They proposed that the development of postmortem isometric tension patterns is strongly related to the development of changes in muscle length that occur in postmortem muscle during the development of rigor mortis.

Postmortem aging

The practice of holding beef for extended periods of time postmortem has long been recognized by those working with meat as a way of improving tenderness. Many studies have been conducted to determine what causes these changes and at what point optimum tenderization occurs. Paul et al. (1944) examined several factors in beef roasts aged at 1.7°C for 0, 1, 2, 4, 9, and 18 days postmortem. They found that the greatest increase in palatability scores occurred at 9 days of storage for small cuts of meat. More recently, Jennings et al. (1978) showed that aging for periods up to twenty days significantly decreased shear force measurements. Other studies have shown that by increasing the storage temperature of beef, the rate of tenderizing due to the aging process can be accelerated. This acceleration in the tenderizing process has been observed at temperatures up to 60°C (Davey et al., 1976).

The histological structure of beef muscle has been examined to determine what structural changes occur that may affect the perceived tenderness of beef. Paul et al. (1944) saw the development of dense "nodes of contracture" in fibers which were associated with extreme stretch on either side of these "nodes" resulting in the appearance of "waves" and "kinks" in the muscle fibers. As the meat was allowed to age or ripen, they noted the appearance of breaks and ruptures in those fibers that showed the development of the "nodes". Essentially two types of histological changes were found by these researchers.

They noted that the fiber striations became more fragile and that the fiber striations were lost over a more extensive area. These changes were shown to coincide with the differences in tenderness measured either mechanically or by the use of a sensory panel.

Schmidt and Parrish (1971) examined at death and seven day postmortem bovine muscle samples using both phase contrast microscopy and electron microscopy. Phase contrast examination revealed that in those samples which were aged seven days, the myofibrils appeared to be slightly out of register and were not as sharply defined as were those from the unaged samples. In addition, they noted a certian degree of shrinkage that had occurred in the muscle fibers. Electron microscopy studies showed that the aged samples had lost some of the structural integrity that was observed in at-death samples. They also noted a shortening of the sarcomere, the disappearance of the H zone and an apparent degradation of the Z-line. They noted essentially no changes in the M lines and the thick and thin filaments.

The change in the structure of the Z line of the myofibril has been examined by many researchers. Davey and Gilbert (1967) reported that in meat aged at 15°C for 3 days the most recognizable changes were the lengthening of the A bands with the concurrent shortening of the I zones and the complete disappearance of the Z line. Fukazawa and Yasui (1967) looked at the structure of the Z line in postmortem muscle stored at 0°C for 24 hours. In muscle that had been left attached to the skeleton for the aging period, the Z line appeared to

have been removed. They suggested that the fragmentation of the myofibril that had been noted in previous studies was due to the disintegration of the Z line. Davey and Gilbert (1969a) examined myofibrils from beef samples that had been aged up to 20 days. They again found that the most notable change that occurred was the apparent disappearance of the Z lines and a weakening of the lateral attachments that held the myofibrils in place within the muscle. In those samples in which the Z lines had disappeared, the myofibrils still retained their inherent integrity, suggesting that substances still remained in this region that helped to hold the sarcomere together. Davey and Dickson (1970) used the electron microscope to examine the changes that occurred in beef muscle during aging. They found that a reduction in the tensile strength of the myofibrils of meat aged for 90 hours postmortem coincided with a weakening of the interaction between I filaments and Z discs. When meat aged for 55 days at 2°C was examined, they saw that the Z discs had essentially disappeared.

Parrish and coworkers (1973) reported a study in which they aged bovine muscles in the carcass at higher temperatures (16°C versus 2°C). They found that postmortem tenderization and myofibrillar fragmentation occurred more rapidly under the high temperature aging conditons. On the basis of their observations, they proposed that this increase in tenderness may be due, in part, to their observations of greater fragmentation of the myofibrils at the Z-line in these samples.

In order to determine the weakest points in the structure of the filament, Davey and Graafhuis (1976) examined raw and cooked muscle

that had been stretched to twice its resting length. In this condition the thin filaments were pulled away from the thick filaments. They found that the fibers in this highly stretched state broke specifically at the A-I junction. Their results pointed to the disruption of filaments that may hold the structure of the sarcomere in register.

Because of the breakage that occurs in the myofibril, there has often been reported the observation of a higher number of myofibrillar fragments in aged meat than in unaged meat. This increase in fragmentation has been used by a number of workers to characterize the aging that has occurred (Fukazawa et al., 1967; Olson et al., 1976; Culler et al., 1978). The method utilized by Fukazawa and coworkers involved counting the number of myofibrils that had one to four sarcomeres. They found that as postmortem aging time increased, so did the number of myofibril fragments. Another more widely used method for determining the amount of fragmentation that has occurred in the myofibril involves measuring the change in the turbidity at 540 nm. As the length of the postmortem aging time increases so does the turbidity of the sample (Olson et al., 1976; Culler et al., 1978). Several researchers have shown that as the degree of fragmentation in the native myofibrils increases so does the tenderness of the corresponding cooked meat, so much so that a significant correlation has been observed (Culler et al., 1978). These observations on the changes in myofibrillar fragmentation values over increasing time postmortem lend further credence to the idea that the degradation of Z-disc structures is one of the major changes occurring during postmortem

aging that weakens the myofibrillar structure and leads to increased tenderness.

A few researchers have examined microscopically the changes that occur in meat samples after cooking. Schmidt and Parrish (1971) noted in electron microscopy studies that as meat was heated to progressively higher temperatures greater shrinkage and degradation of the myofibril occurred. At 60°C significant changes in the structure of the myofibril occurred. Some of the more notable changes included the loss of the M-line structure and the beginning of the disruption of the thin filaments and the start of thick filament coagulation. At 70°C they noted severe disruption of the thin filaments and coagulation of the thick filaments. When they observed samples with light and phase contrast microscopy, they saw that as temperatures increased from 50 to 90°C, the connective tissue fibers underwent shrinkage and fragmentation. They also noted as temperature increased, the myofibrils tended to shrink leading to the expression of fluid from the tissue. They concluded that while heating to higher temperatures should break down the connective tissue and cause an increase in tenderness, cooking to high temperatures also coagulates and hardens the myofibrillar proteins leading to a decrease in tenderness.

In order to more completely understand the processes that take place during postmortem aging, one should first have an understanding of exactly which structural elements or proteins are altered to allow the gross changes in the characteristics of meat to occur. Many

researchers have been engaged for several years trying to identify these changes.

30,000-dalton component

In the search for clues to what structural changes affect the postmortem tenderization of meat, several methods have been employed. One of these methods is the application of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) to the study of the degradation of muscle proteins. Employing this method, Hay et al. (1973) examined aged myofibrils from chicken. They noted the appearance of a band in the molecular weight range of 30,000 daltons that appeared at 48 hours postmortem and increased in intensity as the aging period progressed. Samejima et al. (1976) also reported observing a 30,000-dalton band in the myofibrils of chicken breast muscles that increased in intensity with time. Penny (1974) reported finding a 30,000-dalton band in preparations of bovine myofibrils that had been held for a minimum of 24 hours. Later, Olson et al. (1977) observed that troponin T disappeared from SDS-PAGE gels of myofibrils from bovine *longissimus* and *semitendinosis* muscles after postmortem storage at 25°C. Along with the disappearance of troponin T, they noted the appearance of a 30,000-dalton component. The appearance of this component appeared to coincide with the disappearance of troponin T in the same samples. They also noted that total activity of the protease calcium activated factor (CAF) was high in *longissimus* and

semitendinosus muscles. In order to determine a relationship between these observations, they incubated purified CAF with purified preparations of troponin T. They discovered that this incubation resulted in the degradation of troponin T to a 30,000-dalton component, suggesting that the 30,000-dalton component arises from the action of CAF on troponin T.

Olson and Parrish (1977) examined myofibril samples from bovine *longissimus* muscle using several methods to determine relative differences in tenderness. One method they used was the myofibril fragmentation index (MFI). MFI is a procedure whereby the amount of breakage or fragmentation of myofibrils in suspension is quantitated by measuring the light absorbance of a suspension of myofibrils at 540 nm. Samples that are more turbid would contain more small pieces or fragments than would less turbid samples with the same protein concentration. Therefore, those samples with a greater absorbance would be expected to be more fragmented (Olson et al., 1976). MFI has been shown to coincide with Warner-Bratzler shear values and is related to the tenderness of the sample (Culler et al., 1978). Olson and Parrish (1977) showed that the intensity of the 30,000-dalton component and the degradation of troponin T paralleled the Warner-Bratzler shear force values and the sensory tenderness scores for samples from both A and C maturity carcasses. Those samples in their study that showed the presence of the 30,000-dalton component and the corresponding absence of troponin T also had high MFI values and sensory tenderness scores as well as low Warner-Bratzler shear values.

Conversly, those samples in their study that were less tender (based on MFI values, Warner-Bratzler shear force values and sensory scores) exhibited less degradation of troponin T and had less intense 30,000-dalton bands. They concluded that those muscles that were less tender had less degradation of the myofibrillar proteins than did those that were tender. This was evidenced by differences in the MFI values and the relative differences in the intensities of the troponin T and the 30,000-dalton band. MacBride and Parrish (1977) showed that bovine *longissimus* samples that were significantly more tender after one day storage at 2°C exhibited the presence of the 30,000-dalton band. MacBride and Parrish (1977) showed that bovine *longissimus* samples that were significantly more tender after one day storage at 2°C exhibited the presence of the 30,000-dalton component, while those samples designated as tough did not have the 30,000-dalton component present at one day postmortem. This indicated to them that even at one day postmortem, significant differences in the degradation of myofibrillar proteins can be detected between tough and tender meat samples.

In summary, much has been learned about the relationship between the 30,000-dalton component, the degradation of myofibrillar proteins and the tenderness/toughness of bovine longissimus samples. First, the 30,000-dalton component appears as time postmortem increases and its appearance seems to be related to increased tenderness. Secondly, CAF appears to play a role in the appearance of the 30,000-dalton component by degrading troponin T (Olson et al.,

1976; Olson and Parrish, 1977; Olson et al. 1977; MacBride and Parrish, 1977). Based upon this evidence, MacBride and Parrish (1977) suggested that the term "myofibril fragmentation tenderness" be used to describe the increase in tenderness that occurs as the 30,000-dalton component increases in intensity and as the myofibril is concurrently fragmented at the Z-disc.

Proteolytic enzymes

The main building blocks of the majority of the animal kingdom are proteins. In order for an organism to achieve growth and maintenance, these building blocks must be continually broken down and resynthesized. This process is accomplished by a specific class of proteins collectively known as proteolytic enzymes. These enzymes are responsible for the hydrolysis or breakdown of proteins into their constituent amino acids (Whitaker, 1972).

In a review of the subject of proteolytic enzymes Asghar and Bhatti (1987) described five basic types of reactions that can be catalyzed by proteinases. These included: a) cleaving of a peptide bond, b) cleavage of the amide bond, c) synthesis of a peptide bond and transpeptidation, d) cleaving of the ester bond of amino acids and e) the exchange of oxygen between water and carbonyl group of amino acids.

The majority of the proteolytic enzymes in mammals are found in the organs (e.g., liver, spleen, kidney etc.). However, certain proteolytic

enzymes are also found to exist in muscle, although when compared to organs such as the liver, their activity is much lower. These enzymes appear to be involved in protein turnover and to the tenderizing process that takes place during the postmortem aging of beef (Asghar and Bhatti, 1987)

In muscle tissue, the proteases are generally classified into three categories which are determined for the most part by the optimum pH of the enzyme. These categories of enzymes include the neutral proteases, the alkaline proteases and the cathepsins or acidic proteases. The first two classes, the neutral and alkaline proteases are thought to be found throughout the sarcoplasm, while the catheptic enzymes are generally located in the muscle lysosomes (Bandman, 1987).

The alkaline proteases are those proteases whose optimum activity is in the pH range of approximately 8.0 or higher. Since this range is considerably higher than the ultimate pH of most normal meat products (pH 5.5) their ability to affect the characteristics of meat postmortem is expected to be rather limited. One of the proteases that falls into this category is alkaline protease which has a pH optimum of 8.5 to 9.0 and was first purified from rat skeletal muscle (Koszalka and Miller, 1960). Muscle alkaline protease is another protease that has been described. Its pH optimum is in the range of 9.5 to 10.5 and it is stable at temperatures up to 47°C (Noguchi and Kandatsu, 1976).

The acidic proteases are a class of proteases that have received a significant amount of attention over the years. These proteases, often known as cathepsins are the most active in the lower pH ranges. The

cathepsins that are the most studied are the cathepsins B, C, D and L. Although the cathepsins are all generally thought of as lysosomal enzymes, they seem to have widely different substrate specificities (Canonico and Bird, 1970). Briefly the major characteristics of the cathepsins are described below. Cathepsins B and C have been shown to split myosin and actin (Bandman, 1987). Cathepsin L degrades actin, myosin, α-actinin, troponin and tropomyosin (Okitani et al., 1980).

Cathepsin D is another lysosomal protease that has been found to be present in various organs such as the spleen and liver (Okitani et al., 1981). Samarel et al. (1984) examined the multiple forms of cathepsin D in rabbit cardiac muscle. They reported that cathepsin D is initially produced as a 53,000 dalton precursor. Following a limited proteolysis in the lysosome, they showed that an active form of 48,000 daltons was produced. In a study by Schwartz and Bird (1977), the pH optimum for cathepsin D purified from rat liver was 4.0. They showed that cathepsin D could degrade F-actin at pH 5.0 and was inhibited by pepstatin. The rate of hydrolysis of F-actin however was only found to be 10% of that reported for myosin. All three of the isomers of cathepsin D they examined revealed the same product when they were incubated with actin and myosin.

Robbins et al. (1979) reported a study in which they examined the effect of a partially purified cathepsin D extract from two different sources (bovine muscle and bovine spleen) on bovine myofibrils using SDS-PAGE techniques and scanning electron microscopy. They found that at temperatures of 25°C or 37°C (pH 5.2-5.3), the structure of the

Z-disc was disrupted to the point of almost total degradation by forty minutes to one hour. When they followed the breakdown of myosin, they found that the heavy chain fraction, beginning at a weight of 200,000 daltons was degraded to smaller fragments of approximately 170,000, 150,000 and 80,000 daltons. They found that cathepsin B had relatively little effect on either α-actinin or actin. In 1981, Okitani et al. reported a more detailed study of the properties of cathepsin D and of its action on myofibrils. They found purified cathepsin D from rabbit muscle to be a single polypeptide with a molecular weight of 42,000 daltons. Their cathepsin D preparation optimally hydrolyzed myofibrils at a pH value of 3.0. When they looked specifically at proteins affected by cathepsin D, they found myosin heavy chain to be degraded and a 30,000 dalton component to be produced at pH 3.8 after a 22 hour incubation at 37°C. These researchers suggested that the proteinase cathepsin D, with its peak proteolytic activity at pH 3.0 and 4.5 may not play as important a role in postmortem proteolysis as other enzymes such as cathepsin L and calpain.

Matsumoto et al. (1983) showed that at pH 3, the proteins that were optimally degraded by muscle cathepsin D included troponin I, troponin T, myosin heavy chain, and the slower degradation of tropomyosin and α-actinin. They did not note any degradation of actin or troponin C. When they more closely examined the degradation of myosin heavy chain, they found four major fractions were produced, 155,000, 130,000, 110,000 and 90,000 daltons. Troponin I was broken down into fragments of 13,000 and 11,000 daltons, while troponin T

was hydrolyzed into fragments of 33,000, 20,000 and 11,000 dalton components. The degradation of tropomyosin produced a fragment of 30,000 daltons. They noted that their results indicated that cathepsin D from muscle was markedly different in its action toward actin than was the cathepsin D preparation obtained by Schwartz and Bird (1977).

Zeece et al. (1986) reported that purified preparations of cathepsin D revealed two bands with molecular weights of 47,000 daltons and 29,500 daltons when subjected to SDS-PAGE. When they incubated bovine myofibrils with purified cathepsin D at pH 5.5 and 37°C, they found that myosin heavy chain was degraded as was titin. They also noted a slight increase in the degradation of the proteins actin, tropomyosin, troponin T, troponin I and myosin light chain. When the degree of fragmentation of the myofibrils was examined, they found no significant increase in the amount of fragmentation in the cathepsin D treated myofibrils when compared with untreated myofibrils. When they raised the pH and/or lowered the temperature they found that cathepsin D was not as active. From their data they concluded since cathepsin D was active only in such a limited pH and temperature that it probably does not play a major role in the postmortem tenderization process.

Cathepsin B has been characterized as a thiol protease with a molecular weight of 27,000 daltons. In order to observe the maximum activity of this cathepsin, EDTA must be added as the enzyme is inhibited by the presence of heavy metals. In addition, it is also inhibited by leupeptin and iodoacetate (Penny, 1980). Schwartz and

Bird (1977) showed that cathepsin B had a pH optimum of 5.2 on native myosin. When cathepsin B is incubated with myosin at a pH of 5.2 and a temperature of 37° C, the heavy chain portion is broken down to a product with a molecular weight of 150,000 daltons and to components with molecular weights ranging from 100,000 to 50,000 daltons (Penny, 1980). The action of cathepsin B on F-actin was found to be very similar to that of cathepsin D, producing a major fragment at 35,000 daltons (Schwartz and Bird, 1977). While the pH optimum of both cathepsins B and D appears to be outside the normal pH range of postmortem muscle, it is important to note that the activity of cathepsin B (optimum pH 5.2) is still 50% at pH 5.6 and 20% at pH 6.0. Cathepsin D (optimum pH 4.0) retains 30% of its activity at pH 5.5 (Penny, 1980).

Okitani et al. (1980) reported that the enzyme known as cathepsin L has a molecular weight of 24,000 by estimation with gel filtration. Its activity to degrade myosin was determined to be optimum at a pH of 4.1, however, the enzyme was stable in the pH range of 4.5 to 6.5. They found the most potent inhibitors to be iodoacetate, leupeptin and antipain.

Matsukura et al. (1981) reported a study in which they examined the degradation of myofibrillar proteins with cathepsin L. They found that cathepsin L degraded myosin heavy chain, α-actinin, actin, troponin T and troponin I. They found the most intense degradation to occur around the pH of 4.8. When they looked specifically at the action of cathepsin L on myosin they found the heavy chain to be degraded,

and the light chains to decrease with fragments of 160,000, 92,000, 83,000 and 60,000 being produced. The most optimum pH for the enzyme to degrade myosin heavy chain appeared to be at a pH value of 4.2. Actin was degraded by this enzyme most intensely at a pH of 4.7, with resulting fragments occurring at 40,000, 37,000 and 30,000 daltons. They reported no degradation of tropomyosin and troponin C, but at pH 3.7-6.7, troponin T and troponin I were degraded producing fragments of 30,000 and 13,000 daltons. They also observed the degradation of α-actinin at pH 3.0-3.5 into fragments, the major one occurring at 80,000. From their data, they suggested that cathepsin L may play a role in actin catabolism in living muscle. They also suggested that it may contribute to the tenderizing process that occurs during postmortem aging of meat by possibly causing the breakdown of troponin T with the concurrent production of a 30,000-dalton component in a similar manner as has been shown with the protease Calcium Activated Factor (CAF) (pg 41). However, in their study this activity was shown in the presence of EDTA and not Ca^{++}.

Mikami et al. (1987) designed a study in which they specifically analyzed the degradation of myofibrils by cathepsin L and with lysosomal lysates. In this study, they found unlike Matsukura et al. (1981), that a α-tropomyosin was degraded after a four hour incubation at 30°C, while ß-tropomyosin was not degraded. They also found actin not to be initially degraded, however, they did see slight degradation at four hours. They found myosin heavy chain to be degraded at pH 5.0 after a four hour incubation period. Troponin T,

troponin I and C-protein were found to be quickly degraded, while titin, nebulin, myosin heavy chain, α-actinin and myosin light chains LC_1 and LC_2 were more slowly degraded. The action of cathepsin L was faster at a pH value of 5.5 than at a pH of 6.0, however, the action at pH 5.5 was slower than at pH 5.0. They noted that after incubation with cathepsin L, several new protein bands appeared on SDS-PAGE gels at 130,000, 120,000, 90,000, 85,000, 80,000, 31,000, and 30,000 daltons. When they incubated with only the lysosomal lysate, they found essentially the same degradation pattern that they found with cathepsin L except that there appeared to be a reduced degradation of actin and of the 30,000 dalton component. When they examined electron micrographs of myofibrils incubated with cathepsin L, they found preferential degradation of the Z-lines, with the disappearance of the Z line after four hours of incubation in the presence of the enzyme. From their results, they concluded that at pH 5.5, cathepsin L may be the most important lysosomal enzyme in postmortem tenderization.

When examining the rate at which cathepsins are active, one must keep in mind several factors. These include the release of the cathepsins from the lysosomes, the removal of the inhibitors and the temperature and pH of the system under observation. An increase in the temperature and/or lowering of the pH will accelerate the reactions by most of the catheptic enzymes (Penny, 1980).

Calcium Activated Factor (CAF)

In 1969, Weiner and Pearson reported a study in which they examined the effects of three different chelators (EDTA, EGTA and CDTA) on the progress of rigor mortis and the ensuing quality factors associated with its development. They found that by injecting any of the three chelators they could significantly inhibit the shortening of the *semitendinosus* muscle. They theorized that the chelating agents formed a complex with free calcium and thereby inhibited shortening. In a second portion of the experiment, $CaCl_2$ was injected into the muscle in micro amounts. This injection resulted in significant shortening when compared with control muscles. These results were in contrast with the results obtained with micro-injections of $MgCl_2$ which had no significant effect on postmortem muscle shortening. Weiner and Pearson's study seemed to show that the calcium concentration in postmortem muscle had a direct effect on some of the ultimate physical properties of the muscle.

As mentioned earlier one of the most striking microscopical changes that occurs in postmortem muscle during aging is the weakening and subsequent disappearance of the Z-lines. Since this discovery and the realization that calcium concentration has an effect on some postmortem aspects of muscle, scientists searched for an agent that could cause these changes. In 1972, Busch and coworkers described studies in which they observed the complete removal of Z-lines from rabbit skeletal muscle that had been incubated in a saline solution containing 1 mM Ca^{2+} and 5 mM Mg^{2+} for 9 hours at a

temperature of 37°C and a pH of 7.1. They noted no other major changes at the microstructural level of the myofibril. In support of previous studies (Weiner and Pearson, 1969), they also found that the addition of EGTA and EDTA instead of Ca^{2+} produced little if any changes in the structure of the Z-lines. They were able to isolate the protein fraction that they believed was responsible for the observed action on the Z-line in myofibrils. This protein was shown to produce the described changes in the Z-line at calcium concentrations greater than 0.1 mM and a pH of 7.0. They hypothesized that the low level of endogenous calcium in muscle cells could regulate the activity of the protein. This discovery made them the first researchers to report the presence of a protein native to muscle that can initiate degradation of the myofibril.

This proteinase has been called a variety of names since its discovery. Some of these include, KAF (Kinase Activating Factor), CASF (Calcium Activated Sarcoplasmic Factor), CANP (Calcium Activated Neutral Protease), CDP (Calcium-Dependent Protease), CDSP (Calcium Dependent Sulfhydryl Protease) and Calpain (Cal- because of its dependence on calcium and - pain because it is a thiol protease) (Goll et al., 1985). In addition, the two forms of the enzyme have been called low Ca^{2+} and high Ca^{2+} CAF, L-CAP and H-CAP, CANP I and CANP II, and Calpain I and Calpain II (Goll et al., 1985).

CAF is a molecule made up of two polypeptide chains of 80 kDa and 28 kDa (Goll et al., 1985) which autolyze rapidly in the presence of calcium. The 80 kDa unit degrades to a 74-76 kDa peptide while the 28

kDa unit degrades to an 18 kDa unit (Goll et al., 1985) One result of this autolysis is that the calcium requirement is reduced (Goll et al., 1985, Murachi, 1989). CAF has been shown to exist in two forms based upon its calcium requirement. These two forms differ in that one form requires μmolar quantities of calcium for activation (μmolar CDP, calpain I) while the other requires millimolar quantities (millimolar CDP, calpain II) (Goll et al., 1985). Originally CAF was shown to be maximally active between the pH values of 6.5 and 8.0 and required 1-2 mM Ca^{2+} for activation. A second form of CAF has been shown to be fully active at 70 μM Ca^{2+} and shows activity at 10 μM Ca^{2+} which is more consistent with the intracellular levels of free calcium (Goll et al., 1983).

An inhibitor to CAF has been described by Shannon and Goll (1985) and Murachi (1989) and is found to be widely distributed within muscle cells. One molecule of inhibitor has been shown to inhibit six moles of CAF. When larger quantities of the enzyme are encountered, the inhibitor is hydrolyzed.

Calcium activated factor has since been shown to be found to exist in many types of cells and tissues and the connective tissue matrix surrounding them (Goll et al., 1985). Dayton and Schollmeyer (1981) and Ishiura and coworkers (1980) showed that CAF was located on the Z discs of individual myofibrils near the plasma membrane. Goll and coworkers (1985) examined sections of bovine muscle and found that CAF was located throughout the muscle cell without any preferential localization at the Z-line. They concluded that this absence

of localization at the Z-line in muscle sections that had been fixed indicated one of two things, either the mildly hydrophobic calpain molecule is drawn to this region during the isolation of myofibrils or that only a small portion of the total cellular CAF is bound to the Z-line and is visable only when the large quantities of CAF in the I band are washed away during the myofibril isolation procedure.

Since the discovery of the calcium activated factor, much work has been done to further purify the protein and to determine its action on specific muscle proteins in order to more accurately determine the role it plays in postmortem changes that occur in muscle. Penny (1974) demonstrated that the proteinase used tropomyosin, α-actinin and troponin as substrates. Their results also showed that actin and actomyosin were not digested, but actomyosin was shown to have reduced Mg^{2+}-activated ATPase activity after treatment with the enzyme, while the Ca^{2+}-activated ATPase activity appeared not to be affected. Penny put forth the hypothesis that the degradation of the Z-lines results from the digestion of α-actinin, a protein thought to be one of the main structural elements of the Z-line. However, since their report, several studies have shown that CAF does not appear to actually degrade α-actinin, (Goll et al., 1983; Goll et al., 1985). It appears that relatively undegraded α-actitnin is released from the myofibrils that have been treated with a CAF preparation (Reddy et al., 1975).

CAF appears to have a fairly limited specificity. CAF has not been shown in most studies to degrade actin, α-actinin, or troponin C and a majority of the studies have failed to show activity against myosin (Goll

et al., 1983). CAF does show activity to several other contractile proteins, however, the main effect of the enzyme is to make only a few specific cleavages and leave relatively large polypeptide fragments remaining (Goll et al., 1983). As mentioned earlier, one of the major structural changes that is made is the removal of the Z-line. Speculation still exists as to which proteins are degraded to allow the removal of the Z-line. Two proteins that are removed at about the same rate as the Z-line are troponin T and desmin. It would seem unlikely that troponin T, as it is not located near the Z-line, would play a major structural role in maintaining the integrity of the Z-line. Desmin, on the other hand, is located around the periphery of the Z-disc and its degradation may play a role in the removal of the Z-line (Goll et al., 1983).

Olson et al. (1977) described a study in which they analyzed myofibrils isolated from bovine *longissimus*, *semitendinosis* and *psoas major* muscles at at-death, 1, 2, 3, 6 and 10 days postmortem storage times. At-death samples that were incubated with a crude preparation of calcium activated factor showed that the Z-discs were degraded. In additon, troponin T disappeared and a 30,000-dalton component appeared. When purified troponin T was incubated with CAF, they again noted the production of the 30,000-dalton component. They concluded that since these changes mimicked some of those seen in meat following postmortem storage, certain specific structural changes could be attributed to the action of CAF. Olson et al. (1977) also noted that the CAF activity was high in *longissimus* and *semitendinosus*

muscles. The activity noted in the *psoas major* was less than one-half that found in the *longissimus* and *semitendinosus*.

Koohmaraie et al. (1988a) also found that CAF activity was high in the *longissimus dorsi* and *biceps femorus* and low in the *psoas major* muscle. They also noted that those muscles with a high CAF activity also showed a greater increase in tenderness with postmortem aging time (Koohmaraie et al., 1988a). Koohmaraie et al. (1988b) observed that when they incubated muscle strips with EDTA and EGTA that many of the changes normally found to occur over increasing time postmortem failed to occur. They suggested that the normally seen changes are associated with the presence of divalent cations. To further support this they found that when they infused lamb carcasses with calcium chloride, accelerated postmortem tenderization occurred and that an agent dependent on the presence of divalent cations was active (Koohmaraie et al., 1988c).

Factors Influencing Tenderness

Animal age

Age of the animal has been shown to affect the palatability characteristics of beef. Breidenstein et al. (1968) examined the carcasses from sixty cow and heifer carcasses of A, B and E maturities. They found E maturity had significantly coarser texture than did A and B maturities. Meat from E maturity cattle was also darker than meat from A maturity. Taste panel studies revealed significantly lower

tenderness scores for meat from E maturity cattle than for either A or B. Warner-Bratzler shear force values were also significantly higher for those steaks from E maturity animal than for those from A and B maturities, indicating the steaks from those carcasses with more advanced maturities were less tender. Covington et al. (1970) when examining histological charcteristics of muscle fibers, found that as maturity increased so did fiber diameter. Berry et al. (1974) described a study in which they evaluated the palatability characteristics of various maturity levels of beef. Panel scores revealed that those samples from animals in the most advanced age categories were significantly less mealy, had more adhesion between fibers, and were more difficult to fragment than those from younger animals.

When the literature concerning sensory panel studies was examined, reports showing differences in tenderness between young (A, B maturities) and older animals (C maturity and older) exist.

Smith et al. (1982) reported that there were large differences in the sensory panel ratings for more mature beef (C maturity and greater) versus young beef (A and B maturity). Similarly, Dikeman and Tuma (1971) reported a significant decrease in sensory panel tenderness scores between samples from A and C maturity animals, but little or no difference between samples from C and E maturity animals, was observed.

Many ways of improving the tenderness and palatability of beef from older animals have been examined. Hawrysh and Wolfe (1983) studied the effect of electrical stimulation on the quality and

palatability attributes of meat from animals 6 - 9 years of age. They found essentially that electrical stimulation had little effect on color, firmness or texture, nor did panel scores seem to be affected.

Production of bulls verses steers and heifers

The castration of male cattle for the purpose of beef production has long been an accepted practice in the beef industry. Castration produces a relatively docile animal that fits into the normal procedures for raising beef. Certain advantages exist however, that favor the production of bulls for beef. The intact male of most domestic species tends to be the fastest gaining, most efficient producer of red meat. One of the major reasons for this phenomenon is the difference in the hormones being produced by the intact male versus the castrate. Once the intact male animal reaches puberty the production of the sex hormones, androgens, increases relative to the castrate. The functions of the androgens include an increase in general of the earlier growth of the more early maturing tissues such as muscle and bone, as well as stimulation of the growth of muscles in the forequarter. Therefore, the intact male has, in general, a higher proportion of muscle and bone when compared to the castrate (Kay and Houseman, 1974), leading to an increasing interest in the use of the intact male for lean red meat production. The remainder of this section of the literature review will focus on a discussion of the available research on the production of bulls verses steers for red meat production.

Growth and carcass characteristics

Numerous studies have been conducted to document that bulls are more efficient gainers in the feedlot when compared with steers and heifers (Cahill, 1964; Glimp et al., 1971; Nichols et al., 1966). Nichols et al. (1966) reported that Holstein bulls, when compared with steers of the same breed, reached their targeted slaughter weights earlier and required less feed to accomplish their desired weights. Other studies with more traditional beef breeds have also shown that bulls gain faster and are more efficient in converting feed to live weight gain than their counterpart steers and heifers (Hedrick et al., 1969; Glimp et al., 1971; Kay and Houseman, 1974; Bailey et al., 1966; Field, 1971; Arthaud et al., 1977).

When the carcass characteristics of bulls and steers are compared, bulls tend to possess an advantage in yield grade (Jacobs et al., 1977a) producing heavier carcasses with less fat and a higher proportion of lean (Arthaud et al., 1977; Albaugh et al., 1975; Arthaud, et al., 1969; Hedrick et al., 1969; Landon et al, 1978; and Glimp et al., 1971). Jacobs et al. reported a study in 1977 in which he documented that bulls produced more edible meat, had more crude protein and less crude fat than steers. When boxed beef yields and cutting losses were examined, bulls yielded more boxed beef and had lower cutting losses. In a review by Kay and Houseman (1974), they concluded that bull carcasses contained an average of 8% more muscle and 38% less fat

than steer carcasses. Landon and coworkers (1978) also reported that bulls produced more total retail cuts than did steers.

Palatability and Consumer Acceptance

Many studies throughout the years have been done to determine the differences in the palatability of beef from bulls and steers. These studies have often resulted in with conflicting results. Hedrick and coworkers (1969) reported that Warner-Bratzler and sensory panel studies revealed beef from bulls at sixteen months of age was comparable (with respect to shear force values) to beef from steers and heifers of the same chronological age. Prost and coworkers (1975) also found that the sex of the animal (bulls versus heifers and steers) did not significantly affect the tenderness values obtained from sensory panel and shear force tests, however, the age of the animal did have an effect with meat from older animals being less tender than younger animals. Jacobs et al. (1977b) reported a study in which they utilized steers and bulls of approximately 555 days (18.5 months) of age. In their study, a trained taste panel was unable to detect a significant difference in the tenderness of steaks from bulls and steers. The Warner-Bratzler shear force values showed that bulls were slightly less tender than steers. In the same report, they analyzed a retail study in which consumers were asked to evaluate the tenderness of beef from steers versus beef from bulls. The consumer responses tended to show that they preferred the tenderness of steer beef to that of bull beef. As many as 85% of the consumers in this study rated the beef from the

bulls as being "as good" or "better" than the beef they normally purchase, indicating adequate consumer acceptability of bull beef.

Other studies have shown differences to exist in the perceived tenderness of beef. Albaugh and coworkers (1975) reported sensory panel studies that indicated beef from steers was significantly more tender than bull beef at sixteen to seventeen months of age. Field and coworkers (1966) showed that sensory panel tenderness ratings and shear force values for bulls 500 to 699 days (sixteen to twenty-three months) of age indicated that bulls were less tender than steers and heifers of the same age. When the marbling of the steers and heifers was held constant, they did not see any difference in palatability due to age. When the age of the animal was held constant, higher sensory panel ratings were associated more closely with higher marbling scores in bulls than for either steers or heifers. Forrest (1975) reported that for the sensory panel evaluations of tenderness, juiciness, flavor of lean, flavor of fat and overall desirability, beef from bulls received significantly lower scores than did beef from steers. Reagan et al. (1971) also reported that sensory panel scores for tenderness, flavor and overall desirability were significantly higher for rib steaks from steer carcasses than for those from bull carcasses. In a study by Johnson et al. (1988), beef from young steers was shown to be significantly more tender than beef from young bulls (fourteen to fifteen months of age). In addition, higher shear force values were recorded for loin steaks from bulls, at six days postmortem aging, but after thirteen days of postmortem aging, the shear force values were

roughly equivalent. Crouse et al. (1983) reported a study in which they slaughtered Hereford bulls and steers. The right sides of the carcasses were electrically stimulated and the carcasses were chilled at either 16°C or 2°C for twelve hours. Their results showed that the tenderness scores for beef from bulls was significantly lower than for beef from steers. When they examined the effects of electrical stimulation and cooler temperature, they found electrical stimulation had no effect on the beef from bulls. High temperature aging also did not appear to increase palatability scores for meat from bull carcasses.

Several researchers have attempted to pinpoint why the reported differences in tenderness and palatability occur. One possible explanation is that carcasses from bulls may have a higher collagen content than carcasses from steers. Boccard et al. (1979) examined differences in the amount and the solubility of the collagen from the carcasses of bulls and steers. They reported that bulls had a higher muscle collagen content at birth than they did at all other ages, and that the solubility of the collagen decreased over the time period from birth to sixteen months of age. Correspondingly, shear force values for meat from bulls increased between the ages of eight and sixteen months. They also noted that the muscle collagen from bulls was higher than for steers. The solubility of the collagen decreased sharply between the ages of twelve and sixteen months in the bulls, but the same relationship was not noted in steers. They suggested that the increase in collagen for bulls eight to twelve months of age may be related to sexual development and hormonal functions in the animal.

In their study, Boccard et al. (1979) showed that the changes were not the same for Afrikaner and Friesian bulls suggesting that the interaction between collagen and age of the animal may be related to the onset of puberty. Wilson et al. (1954) and Richey and Cover (1962) did not find a relationship between the sex and the connective tissue content of the muscle.

Calkins et al. (1987) examined the relationship between growth rate, endogenous muscle enzymes and palatability of thirteen month old Angus bulls. They found that enzyme (cathepsins B and H and ß-glucoronidase) and palatability measures were not affected by the rate of growth, nor were collagen amount and solubility. However, they were able to account for one-half of the variation in serum hydroxyproline, muscle collagen sensory panel ratings and shear force values by the collective differences in enzyme activities. They suggested that a relationship may exist between meat tenderness and proteolytic activity.

There are several reasons why the United States beef industry is reluctant to produce bulls for beef on a large scale. One reason that has been reported is the fact processing costs for bulls can be significantly higher than for steers and heifers due to the fact that the hides of bulls are generally thicker and more difficult to remove. Another factor that must be taken into consideration is that the USDA places bulls in a separate classification. Carcasses that are identified as coming from bulls are designated as "bullocks" in the current quality grading

system, and as such receive a lower premium than do carcasses from steers and heifers (Seideman et al., 1982).

Another disadvantage that has been noted when raising bulls for slaughter is the increased incidence of dark cutting beef in the carcasses from bulls. This incidence has been reported to be as much as twice as high in beef from bulls when compared to their counterpart steers (Brown et al., 1990). Dark cutting beef is characterized as being darker than normal in color and having a high ultimate pH (6.0 or greater). This meat has two advantages in that it has a higher water holding capacity and greater tenderness, however, it is generally not acceptable to the meat industry (Kauffman and Marsh, 1987). Dark cutting beef has been reported to have an unusual texture and flavor. In addition, because its dark color resembles beef from older animals, the quality grade of the carcass is often reduced significantly (Judge et al., 1989). This type of meat is also more prone to microbial spoilage due to its high pH (Kauffman and Marsh, 1987). Stress appears to be the basic cause of the dark cutting problem (Judge et al., 1989) especially in bulls which appear to be more susceptible to stress than do steers (Field, 1971). These effects can be reduced by not mixing strange animals together, or by eliminating the mounting and aggressive behaviors by some external means (Bartos et al., 1988).

Methods To Assess Tenderness

Sensory assessment

Two of the most important attributes of meat that contribute most highly to the overall palatability of meat are flavor and tenderness. Of these two attributes, tenderness has perhaps received the most attention from researchers. Traditionally, two methods have been used to assess the tenderness of meat. These include subjective methods (sensory panels) and objective methods (e.g. shear force values). The use of each of these has its advantages and disadvantages. Objective methods have the advantage of not being subject to some external factors such as personal biases and in most cases they can be calibrated more easily. They have certain disadvantages also. One of these is that there is always an inherent uncertainty as to whether or not the measurement accurately reflects what is perceived by humans. Sensory assessment on the other hand does provide a means by which one can measure how the attribute is perceived by humans. In order to make the most effective use of sensory panels, one important consideration is the type of panel to use. The use of an inappropriate test or the incorrect use of tests can generate not only useless data, but can lead to inaccurate conclusions (Sidel et al., 1981).

Lindsay (1986) identified three different categories that describe the need for sensory data. These include: the need to analytically

characterize certain properties of foods, to determine quality-related factors of foods, and to predict consumer reactions to foods. When running a panel that determines consumer reactions to foods, one generally uses an untrained panel that is made up of a large number of consumers. Responses are generally limited to a measure of like or dislike.

When a more precise measurement of the amount, the intensity, or other, more specific measurements is desired, one must employ the use of a trained sensory panel, usually consisting of smaller numbers of people than consumer panels. When using a trained sensory panel extreme care must be used in selecting and training the panelists. Zook and Wessman (1977) described the selection and training of panelists. They recommended screening a number of prospective judges to obtain 10-12 who exhibited the greatest discrimination for the attribute being tested. Once the judges were selected, they recommended a training period before the actual panel was to be run. During this training period the panelists should be actively involved in developing or modifying the terminology to be used in scoring the samples. In this way, the panelists became comfortable with the panel sheet, and collectively have a more uniform understanding of the descriptors being used.

Stone et al. (1974) described a method by which one could quantitate perceived differences in the sensory attributes of foods. In their method they used a six-inch long, unstructured line that was anchored $^1/_2$-inch from either end by pairs of terms. Each judge

recorded their perception of the intensity of each attribute by placing a vertical mark across the line. For the purpose of statistical analysis the mark was converted into a score ranging from 0 to 60. The use of an unstructured scale provides the judges with more freedom in reporting their scores and can eliminate some biases that can exist with structured or numbered scales.

In addition to careful selection of the panelists and type of score sheet, one must also pay particular attention to the physical environment in which the testing occurs. The sensory panel room must be made as comfortable for the panelists as possible, including adjusting the temperature and the humidity of the room if possible. The panel room should be in an area that is easily accessible to the panel members. The preparation area should be kept separate from the testing area and panelists should not be allowed access to the preparation area prior to the panel evaluation. Odors from preparation and any foreign or unusual odors should also be kept from the room. Since most types of testing require the panelist to make independent judgements, individual booths should be used to eliminate distractions and to prevent communication between the panelists during testing. The lighting that is present in the testing room should be uniform. If a researcher is interested in minimizing the differences in product color, colored lights may be used. The samples that are served to the panelists should be representative of the product. In addition, all the samples from different treatments should be identical in all ways except for those characteristics that are being tested in order to

eliminate extraneous differences that may cause unwanted bias. Serving temperature of the product must also be maintained so that all samples are served at the same temperature. This can be accomplished by the use of a warming oven, a hot water bath or warming pans to name a few methods. Care must be taken when using these methods not to cook the product past the desired endpoint temperature. The utensils that are used to present the samples to the panelists should be chosen carefully to ensure they do not provide any taste or odor to the product. Additionally, all samples should be served in identical containers to eliminate any bias from the container. Sample size should also be controlled throughout the course of the panel. Enough sample should be served at one time to ensure the panelists can sample enough product to base their decision. The recommended amounts in most cases are $^1/_2$-ounce of a liquid or 1 ounce of a solid. The order in which the samples are presented can affect the panelists perception of the product. For this reason, the order of presentation of the sample should be either randomized or balanced. In an experiment with a small number of samples, the order can be arranged so that every sample is presented in every possible order, a balanced arrangement. If the number of samples is large, the samples can be presented in a random order. Samples presented to the panelists should be identified or coded in such a way that the identity of the sample is not revealed to the judges. The recommended method of coding the samples is by using a three digit number generated by a table of random numbers (Larmond, 1973).

Panelists should be provided with a substance to rinse their mouths between samples. Room temperature water is most often preferred by many researchers. In addition, crackers, apples, celery and bread have all been used to remove any flavors of the previous sample from the mouth. The same pattern of rinsing and/or eating between samples should be followed consistently by the panelists. By controlling as many of the external and environmental factors as possible, the researcher can eliminate many of the psychological errors that can occur (Larmond, 1973).

Meat is an extremely complex system made up of multiple components each of which contribute in some way to the perceived tenderness of meat. Since meat is such an intricate tissue, any method used to quantify the tenderness and palatability attributes must also be broken down into many components. Cover and coworkers (1962a, 1962b, 1962c, 1962d) described in a series of studies, a way that tenderness descriptors could be broken down into six components. These components included those attributes related to the connective tissue component, the muscle fiber component and to softness attributes. The connective tissue component was evaluated simply as one score that represented at the panelists determination of the amount and the hardness of the connective tissue present. The softness attribute was evaluated with two descriptors, softness to tongue and cheek and softness to tooth pressure. Softness to tongue and cheek was simply evaluated by the feel of the sample in the mouth, while softness to tooth pressure was evaluated as the amount of

muscular force required to bite the sample. The muscle fiber component was evaluated as three descriptors, ease of fragmentation across the grain, mealiness and apparent adhesion between the fibers. Ease of fragmentation described the breaking of the fibers across the grain. Mealiness was a specific type of fragmentation. It was described as a feeling of tiny hard and dry particles that clung to the cheek, gums and tongue. Apparent adhesion between fibers was scored as low when the fibers "felted" together. Juiciness was also evaluated (Cover, 1962a).

Cover and coworkers (1962b) indicated the measurement they termed softness to tooth pressure is an attribute that is related to the elasticity of meat, or the amount of strain (measure of the deformation of the sample) and the stress (measure of the force used to push into the sample). A high softness score would then relate to a high strain (large amount of deformation) and a low stress (low force required to push into the sample). Low softness scores would relate to high stress and low strain.

Mealiness, according to Cover et al. (1962c) was not present in samples cooked to 61°C, as the samples were not dry enough to exhibit this characteristic. The trait was more often scored in samples cooked to between 80°C and 100°C. Cover et al. (1962c) stated that increased mealiness occurred with an increase in the tenderness response rather than a toughening response to meat. The fibers of more tender meat they concluded tended to more readily separate into tiny particles.

Cover and coworkers (1962d) also related the measures of shear force and fiber extensibility to their measurements of tenderness and juiciness. They found the shear force values of *longissimus dorsi* steaks were significantly higher at 80°C than when they were cooked at 61°C. When they examined the correlations between the panelists scores for connective tissue tenderness and shear force values, they found the correlations were low. They found that the highest correlation coefficients existed between shear force values and those panel scores for ease of fragmentation, softness to tooth pressure and adhesion.

Shear force measurements

Much work has been done in past years centering on instrumental methods to measure textural properties of foods. Interest in these instruments is high due to the desire to develop a highly reproducible method to quantify specific differences in the textural properties of foods. There have been a relatively large number of instruments developed for this purpose, each designed to measure a particular property in a certain way. Much of the research in the past thirty years has centered on trying to define exactly what parameter each instrument is quantifying. Szczesniak (1973) pointed out that since many times poor correlations have been reported between instrumental and sensory evaluations, one must keep in mind the fact that instruments measure only a small portion of the entire realm of the parameters being evaluated by human beings.

Most instrumental methods consist of certain basic elements. These include a device that actually contacts the food sample, a mechanism that provides the force for contacting the sample, a device that measures or senses the resistance provided by the substance being contacted and a device to register and/or record the force encountered (Szczesniak, 1973).

The Warner-Bratzler shear device is one of the most commonly used mechanical methods for determining the amount of force required to reduce the size of a meat particle. The Warner-Bratzler device was developed in the late 1920's by Warner and modified by Bratzler in the 1930's. The mechanism consists of a blade 1 mm thick with a triangular hole that is pulled through a slot approximately 1.2 mm wide (Voisey, 1976). In most versions of the instrument, a cylindrical sample of cooked meat is sheared by the force of the V-shaped blade passing through the sample at right angles to the meat fibers. The result is that the sample is subjected to a combination of tensile, compression and shear stress forces (Harris, 1976).

Currently many researchers utilize the attachment of the Warner-Bratzler shear device on the Instron Universal Testing Machine. The Instron uses a load cell to measure the forces generated by a particular test. The unit is driven by a crosshead that is moved in a vertical motion up and down by a screw drive mechanism. Use of the Instron has numerous advantages, some of which include the ability to run the test at a constant speed, the ability to calibrate the instrument so one knows the values for the force and distance axis on the recorder

chart, and the fact that the results are given in calibrated units of force, distance and/or time. Additional advantages include the ability to set the crosshead for a maximum distance or load, upon the attainment of which the crosshead stops, the ability to program the instrument for an automatic return, its cycling capability and its use for relaxation tests to name a few (Szezesniak, 1973).

Many researchers have reported data that indicate that the toughness/tenderness associated with the myofibrillar structure of the meat is most closely associated with the initial yield force values. When force deformation curves are obtained, those treatments that affect primarily the muscle fibers (aging, cooking etc.) change mainly the initial yield force values, while differences between the initial yield and peak force values seem to mirror changes in the connective tissue component (Harris, 1976).

Warner-Bratzler shear force studies have indicated that the myofibrillar component of toughness is more closely related to the peak shear force than the connective tissue component (Bouton and Harris, 1972). Møller (1981) reported a study in which he examined the interrelationships between the initial and final yield forces and the myofibrillar and connective tissue components of tenderness. By stopping the crosshead movement after the first yield force value was recorded, he observed that the muscle fibers had been nearly sheared through, while the connective tissue elements continued to hold the sample together. When the movement of the crosshead was resumed, the yield obtained corresponded to the final yield value on typical

deformation curves. In addition, when the samples were heated to higher endpoint temperatures (60°C vs 80°C) the initial yield value (myofibrillar value) increased while the final yield value (connective tissue component) decreased, lending further support to the hypothesis that the yield values and the muscle tissue components were related. Møller also found that by using the initial and final yield force values instead of peak force values he was able to more accurately predict the sensory evaluations of tenderness and chemical measurements of collagen solubility (Møller, 1981).

Another factor that must be controlled is the core of meat itself. Research has shown that variations in the diameter of the sample core diameter can significantly influence the shear force value readings obtained by the Warner-Bratzler device (Davey and Gilbert, 1969b; Poole and Klose, 1969). In addition to sample diameter, core location may provide a source of variation. Crouse et al. (1989) found that in steak samples that were classified as being more tough overall, the shear force values increased significantly as the cores were obtained from the dorsal to the lateral areas within the muscle.

SECTION I. THE EFFECTS OF POSTMORTEM AGING TIME, ANIMAL AGE AND SEX ON SELECTED CHARACTERISTICS OF BOVINE *LONGISSSIMUS* MUSCLE

TITLE: The effects of postmortem aging time, animal age and sex on selected characteristics of bovine longissimus muscle

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ABSTRACT

Strip loins were removed from 10 bulls and 10 steers of approximately 14 months of age and 10 cows of 44 months of age and older. Samples were removed at 3, 7, 14, and 28 days postmortem. Shear force values were obtained by using the Warner-Bratzler accessory of the Instron. Sensory evaluation of the samples was done by a 15 member trained panel. Purified myofibrils were isolated from the longissimus muscle representing each sex and age category at the four postmortem aging periods and were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) to determine changes in myofibrillar/cytoskeletal proteins. Sensory panel results revealed that steaks from the steer carcasses were significantly ($p< 0.05$) softer and were scored as being easier to fragment across the fiber than those from cow carcasses. Steaks from cow carcasses were scored significantly ($p< 0.05$) higher for connective tissue residue than steaks from bull or steer carcasses. Steaks from bull carcasses were significantly ($p< 0.05$) less mealy than those from steer carcasses. No other differences were found in steaks from bull and steer carcasses. A significant ($p< 0.05$) increase in softness and ease of fiber fragmentation was observed with an increase in postmortem aging time, with an exception between days 7 and 14. The steaks from 14 and 28 day aging periods were significantly ($p< 0.05$) more mealy than those at 3 and 7 day periods. The steaks at the 28 day period had significantly ($p< 0.05$) less detectable connective tissue residue than

the other 3 periods, while the 3 day aging period had significantly ($p< 0.05$) more detectable connective tissue residue than the other periods. The Warner-Bratzler shear force test showed steaks from cow carcasses had significantly ($p< 0.05$) higher compression and residual force values than steaks from bull and steer carcasses. The steaks from the 3 day aging period had the highest compression and residual force values while the steaks from the 28 day period had the lowest. No interactions were observed between sex/age and postmortem aging time.

SDS-PAGE analysis revealed that the 30,000-dalton component intensified with increasing time postmortem. This corresponded to the increase in sensory panel measures for tenderness and the decrease in shear force values over all postmortem aging periods. In addition, increasing postmortem aging time was accompanied by a loss of the T_1 band of titin. However, in older animals the T_1 band of titin persisted and was more frequently observed through 14 days postmortem. SDS-PAGE analysis also revealed that a nebulin band was more frequently present at 3 days postmortem in muscle samples from bull carcasses and also from those steer and cow carcasses with greater shear force values and lower sensory tenderness scores.

The results of this study suggest that structural proteins such as titin and nebulin persist longer in skeletal muscle from certain animals and could play a role in some of the tenderness differences observed.

The increase in intensity of the 30,000-dalton component with increasing time postmortem also seems to be related to the relative change in tenderness observed over all sex and age categories.

INTRODUCTION

Researchers have studied the palatability attributes of beef tenderness for many years. Although much data has been accumulated, a fully satisfactory explanation of what causes a wide variation in beef steak tenderness continues to elude researchers. Meat is an extremely complex tissue and any answer to the question of why some beef is more tender than other beef must also be complicated, and will, by necessity have to take into consideration numerous variables.

Quantifying the differences in palatability attributes by using sensory and mechanical methods has required much time and effort. Because meat is a multiple component system, any method used to quantify the tenderness and other palatability attributes should also be differentiated into several components. Cover and coworkers (1962) proposed that the tenderness descriptors used in sensory panel work should be partitioned into several categories. Likewise, it has been suggested that the traditional mechanical methods used to assess tenderness, such as the Warner-Bratzler shear device, be re-evaluated. Bouton and Harris (1972) compared several instrumental methods of measuring meat tenderness with measures that directly determine some texture characteristics of meat. They suggested that compression as measured by the Instron and adhesion measurements made on muscle samples are highly related as are the peak (maximum) force measurements made with the Warner-Bratzler shear device and the

fiber tensile measurements. Møller (1981) proposed that the deformation curves obtained by using the Warner-Bratzler attachment on the Instron be divided into multiple components when analyzed. That is, the initial yield observed on the curve represented the myofibrillar component of tenderness, while the final yield point on the curve represented the connective tissue component of tenderness.

Certain factors and procedures have been identified as aiding in predicting types of beef that may be more or less tender than average. Some of these factors include the age and sex of the animal. Numerous research studies have shown that beef from older animals is significantly less tender than beef from younger animals (Tuma et al., 1963; Dikeman and Tuma, 1971; Smith et al., 1982). When comparing beef from intact males (bulls) with castrated males (steers) of the same age, conflicting results have been reported. Albaugh et al. (1975) showed that beef from bulls was less tender than that from steers, while Hedrick et al. (1969) showed that there was no difference in tenderness in beef from bulls and steers of less than 16 months of age. The procedure of postmortem aging has been shown to greatly improve the tenderness of beef. Field et al. (1971) showed that shear force values declined from 2 to 21 days of postmortem storage. Busch et al. (1967) showed that a greater increase in tenderness was observed when beef was aged at 16°C rather than at 2°C.

In search for the answer to the question of what causes these differences in tenderness, changes in the structural components of the myofibril have been examined. Parrish et al. (1973) observed

fragmentation of myofibrils due to the disintegration of Z-lines in bovine *longissimus* samples that had been aged at 16°C for 1 day or at 2°C for 3 days. Their sensory panel studies also showed a marked increase in tenderness at these time/temperature periods. Parrish et al. (1973) suggested, based on these observations, that the reduced size of the myofibril brought about by fragmentation increased sensory panel tenderness because of a reduction in the amount of force required to cut through the fragmented sample. MacBride and Parrish (1977) and others (Olson et al., 1977; Olson and Parrish, 1977) have shown that a degradation product of the myofibrillar protein troponin T, termed the 30,000-dalton component was observed to occur more often in tender than in less tender beef. Other components of the myofibril have also been examined. Locker (1982) suggested that structural elements he termed gap filaments, played a major role in determining the tenderness of meat. These gap filaments are now believed to be composed of an elastic protein, titin, found in the myofibril (Locker, 1982; LaSalle et al. 1983). Titin is one of two extremely large molecular weight proteins first extensively examined by Wang et al. (1979). Titin has an approximate molecular weight of 28,000 kDa (Kurzban and Wang, 1988) and migrates on SDS-PAGE gels as a doublet. The two bands are commonly referred to as T_1 and T_2, with T_2 migrating just ahead of T_1. The second protein, nebulin, is another large molecular weight protein first identified as band 3 (Wang et al., 1979). Nebulin has now been shown to have a molecular weight of between 600 and 900 kDa (Wang and Wright, 1988; Jin and Wang,

1991). These two proteins, titin and nebulin, have been shown to degrade under postmortem conditions (Lusby et al., 1983) as well as to undergo postmortem degradation more rapidly and more extensively in tender than in less tender beef (Anderson and Parrish, 1989).

One of the major enzymes that is believed to be responsible for some of the observed postmortem degradation that occurs in meat is that protease which is known as calcium activated factor (CAF). CAF is a protease with optimum activity at pH levels near 6.5. CAF consists of two subunits, one approximately 80 kDa and the other approximately 28 kDa (Goll et al., 1985). CAF, as the name implies, requires the presence of calcium for its activation. CAF has been shown to exist in two forms based upon its calcium requirement. These two forms differ in that one form requires μmolar quantities of calcium for activation, while the other requires millimolar quantities. Research has shown that CAF will degrade several proteins of the myofibril including titin, desmin, troponin T and troponin I (Olson et al., 1977; Goll et al., 1983; Zeece et al., 1986).

This study was designed to examine the relationship between the tenderness differences and postmortem time, sex and age categories as well as changes and differences in specific myofibrillar/cytoskeletal proteins.

MATERIALS AND METHODS

Sources and storage of *longissimus* muscle samples

Strip loins were obtained from the carcasses of ten bulls and ten steers from a single management group. The bulls and steers in this study were all approximately 14 months of age (A maturity). Strip loins were also obtained from the carcasses of ten cows 44 months of age to 108 months of age (C^{50} to E maturity). Marbling scores for the carcasses in all groups ranged from $Slight^{60}$ to $Small^{70}$. None of the carcasses in this study were electrically stimulated. The strip loins were removed from the carcasses at approximately twenty-four hours postmortem. Each of the thirty strip loins were individually vacuum packaged and stored at 2°C. Two 2.86 cm steaks and two 1.27 cm steaks were removed from each strip loin at three, seven, fourteen and twenty-eight days postmortem. Steaks were vacuum packaged, frozen and stored at -20°C until analysis.

Sensory analysis

Steaks were removed from the freezer and thawed at 2°C for 36 hours. After thawing, the steaks were removed from the vacuum bags and were trimmed to no more than 0.51 cm of external fat. Steaks were cooked in an electric (General Electric CB60) broiler oven that had been previously preheated a minimum of 30 minutes to 204°C. Samples were placed on aluminum broiler pans at a distance of no

more than 12.7 cm and no less than 11.0 cm away from the heat source. During cooking, each steak had a copper constantan thermocouple placed in the geometrical center. Temperatures were monitored with an Omega Digital Trendicator (Omega Engineering, Inc. Stamford, CN). The steaks were turned at half doneness (32.5°C) and were cooked to a final endpoint temperature of 65°C. Steaks were wrapped in aluminum foil and placed in a preheated oven (65°C) to maintain the temperature until they were served to the panelists. Prior to serving, steaks were removed from the oven and carved into 1.27 x 1.27 x 2.86 cm cubes using an 11.4 x 11.4 cm Plexiglass mold. Two cubes per steak per panelist were removed at random, placed into preheated 6.0 cm diameter aluminum pans and served to each of fifteen panelists. Panelists were seated in individual booths during the evaluation of the samples. Red fluorescent lights were used to eliminate any personal biases due to the color of the sample. Panelists used a fifteen cm line scale (anchored at 1 cm from each end) to evaluate the samples by placing a vertical mark on the horizontal line to indicate their score for each of the following attributes: softness to tooth pressure, fiber fragmentation, mealiness, residue and juiciness. Each attribute was described as follows. Softness to tooth pressure was a measure of how easily the sample compressed between the molars on the first one or two chews. Fiber fragmentation was defined as how easily the sample broke down into smaller pieces upon chewing. Mealiness was described as the breakdown of the meat sample while chewing into small, hard and dry particles that clung to the cheek and

gums. Residue was an estimation of the amount of insoluble connective tissue and/or other insoluble material that remained in the mouth after the sample was thoroughly chewed. Juiciness was the panelists' estimation of the amount of free fluids released from the meat while chewing. Scores were recorded in millimeters and had a possible range of from 0 [very hard (for softness to tooth pressure), very difficult (for fiber fragmentation), very mealy (for mealiness), large amount (for residue) or very dry (for juiciness)] to 150 [very soft (for softness to tooth pressure), very easy (for fiber fragmentation), none (for mealiness), none (for residue) and very juicy (for juiciness)]. Panelists were asked to refresh their palate between samples by eating an apple slice or an unsalted soda cracker and drinking room temperature water between each sample. Two panel sessions were held each day, one at 10:00 am and the other at 2:00 pm. A total of twenty panel sessions were held over a period of four weeks.

Warner-Bratzler shear force determinations

Cores were taken from each of three sections (central, medial and lateral locations) of the cooked steaks after they had been allowed to equilibrate to room temperature (25°C). The three 1.27 cm cores were removed parallel with the axis of the muscle fiber and were used for Warner-Bratzler (W-B) shear force determinations of tenderness. Shear force measurements were made using the Warner-Bratzler shear force attachment of the Instron Universal Testing Machine (Instron Corporation, Canton, MA). A 50 kg load cell and a cross-head speed of

200 mm/minute were used. Shear force deformation curves were recorded with the use of a chart recorder set at a chart speed of 200 mm/minute. Each core was sheared twice along the long axis, and the values were averaged. The Warner-Bratzler curves obtained for each sample were divided into two parts. The first yield point of the curve was identified as the compression force and corresponds to the myofibrillar component of tenderness. The second yield point of the curve was identified as the residual force and corresponds to the connective tissue component of tenderness (Møller 1981). These values were initially recorded in pounds of force per 1.27 cm diameter core. This value was later converted to kg of force per square cm by using a factor of 0.36.

Preparation of myofibrils

Purified myofibrils used for analysis by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) were isolated from raw samples taken from 1.27 cm thick steaks adjacent to the 2.86 cm thick steaks of each sex and age category at the four postmortem aging periods by using a modification of the method by Lusby et al. (1983). Four grams of minced muscle tissue were homogenized for ten seconds in a Waring blender in 10 volumes (w/v) of a standard salt solution (SSS),(100 mM potassium chloride, 20 mM potassium phosphate, 2 mM magnesium chloride, 1 mM EGTA, 1 mM sodium azide). This homogenate was centrifuged 10 minutes at 1000 x g. The supernatant was decanted and the pellet was suspended in 6 volumes of SSS,

homogenized again for 10 seconds and centrifuged 10 minutes at 1000 x g. The supernatent was decanted and the pellet was twice resuspended in eight volumes of SSS, homogenized 10 seconds, strained through a nylon strainer and again twice centrifuged at 1000 x g. This was followed by centrifugation of the resuspended pellet at 1500 x g for 10 minutes in 2 changes of 6 volumes of SSS and 1% Triton, then centrifuged again at 1500 x g for 10 minutes in 2 changes of 8 volumes of 100 mM KCl. Samples were then centrifuged at 3020 x g for 10 minutes in 2 changes of 10 volumes of 5 mM Tris-HCl, pH 8.0. Following centrifugation, the pellet was suspended in 4 volumes of 5mM Tris-HCl, pH 8.0. The modified biuret procedure of Robson et al. (1968) was used to determine the protein content of the final myofibril suspension. Following protein determination, one ml of protein solution was added to 0.5 ml of tracking dye solution [30 mM Tris-HCl, pH 8.0, 3 mM EDTA, 3% (wt/vol) SDS, 30% (v/v) glycerol, and 0.3% (w/v) pyronin Y] and 0.1 ml of 2-mercaptoethanol (MCE) and heated at 50°C for 20 minutes (Wang, 1982) Eighty μg of protein per lane were loaded on the slab gel.

SDS-Polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was done according to the procedure of Laemmli (1970) with modifications to accommodate separation of proteins with widely different molecular weights. A 5% polyacrylamide (acrylamide/bisacrylamide = 100:1, w/w) slab gel was used to monitor changes in extremely high molecular weight proteins (e.g., titin and

nebulin) and a 12% polyacrylamide (acrylamide/bisacrylamide = 37:1, w/w) slab gel, with a 5% polyacrylamide stacking gel, was used to monitor changes in lower molecular weight myofibrillar/cytoskeletal proteins. A 10x running buffer [25 mM Tris, 192 mM glycine, 2 mM EDTA and 0.1% SDS] was used in both the upper and lower buffer chambers of the SE 400 Sturdier Vertical Slab Gel Unit (Hoefer Scientific Instruments, San Francisco, CA). Gels were run at room temperature and a current setting of 20 mA. Staining was done for six hours in an excess of 0.1% (w/v) of Coomassie brilliant blue R-250, 50% methanol and 30% glacial acetic acid, and were then destained in an excess of the same solution excluding the Coomassie brilliant blue.

Statistical Analysis

The Statistical Analysis System (SAS, 1985) was used to determine the means, standard deviations and analysis of variance. Least Significant Differences (LSD) were calculated and used to separate the means. An alpha level of 0.05 was used to determine significance.

RESULTS AND DISCUSSION

Sensory panel studies

The results of the sensory panel study showed that the steaks from the carcasses of young steers were scored as being significantly ($P < 0.05$) easier to fragment across the fiber than were steaks from older animals (Table 1). Samples from bull and steer carcasses were not found to be significantly different ($P > 0.05$) for the attribute of fiber fragmentation. A similar trend was noted for the attribute of softness to tooth pressure. The steaks from the young steers were scored as being significantly ($P < 0.05$) softer than steaks from the older animals (cows) (Table 1). Sensory panelists found no differences ($P > 0.05$) in samples from bull and steer carcasses for the measurement of softness to tooth pressure. These sensory panel results seem to show that for the attributes which reflect some component of muscle tenderness (softness to tooth pressure and fiber fragmentation), those samples from more mature animals tended to receive significantly lower scores than did those samples from A maturity animals. This indicates that maturity plays an important role in predicting the tenderness of beef. Smith et al. (1982) also reported that there were large differences in the sensory panel tenderness ratings for more mature beef (C maturity) versus young beef (A and B maturity). Similarly, Dikeman and Tuma (1971) reported a significant decrease in sensory panel tenderness scores between samples from A and C maturity animals, but

little difference between samples from C and E maturity animals was observed.

When the characteristic of mealiness was examined, steaks from young steers were scored as significantly ($P < 0.05$) more mealy than those from old animals (cows) (Table 1). Steaks from bulls were significantly ($P < 0.05$) less mealy than steaks from steers.

The sensory panel scored the steaks from older animals as having significantly ($P < 0.05$) more detectable connective tissue residue than steaks from either young bulls or steers. Cross et al. (1973) also discovered that as animal age increased, so did sensory panel scores for the amount of connective tissue remaining after chewing. They found that the total amount of chemically determined connective tissue however, was not closely related to the panel ratings for the amount of connective tissue. They did observe that their determinations of percent soluble collagen were significantly related to the panel rating for connective tissue. Because detectable connective tissue was defined to the panelists in the present study as the amount of insoluble connective tissue that remains in the mouth after thorough chewing, it is possible that this parameter measures the amount of relatively indestructible collagen/connective tissue present in the samples evaluated. According to Bailey and Light (1989) there is little solid evidence to support the theory that the entire amount of collagen in muscle increases as the animal ages, however the size of collagen fibers and fiber bundles becomes larger (Bailey and Light, 1989), and the amount of cross-linking appears to increase leading to an increase in

the strength of the collagen (Goll et al. 1964; Bailey and Light, 1989). There was no significant difference ($P > 0.05$) between steaks from bulls and steers for the amount of connective tissue residue. Hunsley et al. (1971) found also that sex (bulls verses steers) did not significantly affect collagen levels in steaks as determined by hydroxyproline analysis.

When the results for juiciness were examined, the samples from the carcasses of older animals were scored as being significantly ($P < 0.05$) more juicy than the samples from the carcasses of young bulls and steers (Table 1). There was no significant difference ($P > 0.05$) observed between steaks from bulls and steers for the attribute of juiciness. Reagan and coworkers (1976) found that while percentage moisture was not significantly correlated with their sensory panel evaluations of juiciness, collagen content of the samples was related to panel scores of juiciness. When the collagen content of their samples rose, so did their panel juiciness scores. In the present study, the steaks from the most mature animals (cows) were scored as having a higher amount of detectable connective tissue while also being scored the most juicy.

When the results from the four aging periods were examined, (Table 2) sensory panelists scored the steaks from three day postmortem aging period over all ages and sexes as having the least ($P < 0.05$) softness to tooth pressure and having the most ($P < 0.05$) resistance to fiber fragmentation of the four periods. Conversely, the steaks from the twenty-eight day aging period were scored as being

significantly ($P < 0.05$) softer and easier to fragment than the other three periods. Panelists found no differences ($P > 0.05$) between steaks from the seven and fourteen day postmortem aging periods (over all ages and sexes) for the attributes of softness to tooth pressure and fiber fragmentation. These results may lend further support to the concept of myofibrillar fragmentation tenderness (MacBride and Parrish, 1977). Researchers have shown that as postmortem aging time increases, myofibrils become fragmented as the Z-discs become more degraded (Parrish et al., 1973; Olson et al., 1976). Parrish et al (1973) suggested that the reduced size of the myofibril brought about by fragmentation was sufficient to cause enough reduction in the force required to cut through the sample that this force reduction could be detected by sensory panelists.

The steaks from the three and seven day aging periods were evaluated as being significantly ($P < 0.05$) less mealy than the steaks from the fourteen and twenty-eight day periods over all sexes and ages.

When the panelists scored the steaks for the amount of connective tissue residue, steaks from the three day postmortem aging period were scored as having the most ($P < 0.05$) detectable connective tissue residue and steaks from the twenty-eight day postmortem aging period having the least ($P < 0.05$). Stanton and Light (1987) showed that they could extract significantly greater amounts of collagen or collagen fragments from muscles aged 14 days than from unaged muscles indicating some limited proteolysis of muscle collagen may

occur upon postmortem aging. Whether or not panelists in this study were able to detect an actual decrease in the tensile strength of collagen or whether they were responding to a general increase in tenderness of the samples over time cannot be determined on the basis of these sensory panel results.

Juiciness was scored the highest ($P < 0.05$) for the seven and twenty-eight day postmortem steaks, while the fourteen day postmortem steaks were scored the lowest ($P < 0.05$) for this palatability attribute.

Warner-Bratzler shear force tests

The Warner-Bratzler shear force results revealed that samples from the carcasses of more mature animals (cows) had significantly higher ($P < 0.05$) compression (initial yield) and residual shear (final yield) force values than did the samples from young bull and steer carcasses. On the other hand, the compression and residual shear force values from the steaks from the young bull and steer carcasses were not significantly different ($P > 0.05$) (Table 3) indicating that essentially no differences occurred in tenderness between the two sexes. In this study, the effects of maturity seemed to be more important than any differences caused by sex condition. The results obtained by Prost et al. (1975) substantiates this observation. They found that the sex of the animal (bulls verses heifers and steers) did not significantly affect the tenderness values obtained from sensory panel and shear force

tests, however, the age of the animal was shown to have an effect, with the steaks from older animals being less tender than younger animals.

Analysis of the shear force values for the postmortem aging periods (Table 4) showed that the days of postmortem aging (over all ages and sexes) were significant ($P < 0.05$) in the model for the compression (initial yield), while the days of postmortem aging (over all ages and sexes) were not found to be significant ($P > 0.05$) in the model for the residual (final yield) values. A comparison of the means for the compression values indicated all four postmortem aging periods were significantly ($P < 0.05$) different from each other, with the three day postmortem aging period requiring the greatest amount of force. The values for the remaining three periods (seven, fourteen, and twenty-eight days postmortem) continued to show a significant ($P < 0.05$) downward decline in the amount of force required to produce the initial yield value. This is consistent with the results of Jennings et al. (1978) who showed that tenderness of beef samples continued to increase over time.

In this study, the compression (initial yield) values were shown to decrease steadily as time postmortem increased, a response that could be expected if the myofibrillar component of the sample were to undergo structural disruption with increasing time postmortem as several researchers have reported (Schmidt and Parrish, 1971; Davey and Gilbert, 1967; Olson et al. 1977). In addition, the fact that days of postmortem aging were not significant with respect to residual shear force values may lend some support to the idea that the residual shear

force values are related to the connective tissue component on tenderness as suggested by Møller (1981). Since the response of connective tissue to the aging process is generally thought to be less than that of the myofibrillar component (Bailey and Light, 1989), this decreased response may be reflected in the lack of difference in the residual value of the samples over the four postmortem aging times observed. This further indicates that connective tissue has less to do with postmortem tenderization than does the myofibrillar tenderization that occurs as myofibrils become more fragmented with increasing time postmortem (Olson et al., 1976; Olson and Parrish, 1977,).

No significant interactions were observed between animal age and postmortem aging time, nor were there any significant interactions found between the sex (intact males versus castrates) and postmortem aging time for either the sensory panel or the Warner-Bratzler shear force results. This portion of the study seems to confirm that animal age and postmortem aging time have more influence on the tenderness attributes than does the sex of the animal. The results of this section of the study also indicate that increased postmortem aging time improved the tenderness attributes under consideration (especially myofibrillar tenderness attributes) regardless of the sex or age of the animal. This evidence of improved tenderness over postmortem aging time seems to further substantiate the concept of myofibrillar fragmentation tenderness of beef steaks.

SDS-PAGE analysis

Figure 1 is a representative 12% SDS-PAGE electrophoretogram showing the banding patterns observed from myofibrils isolated from samples aged for 3, 7, 14 and 28 days postmortem. This figure represents samples that were the extremes for the loin steaks from steers in toughness/tenderness as evaluated by both Warner-Bratzler compression (initial yield) forces and sensory scores for fiber fragmentation at 3 days postmortem. While this figure represents samples from steers, similar patterns (not shown) were also seen in samples from bull and cow carcasses. The SDS-PAGE analysis revealed that the 30,000-dalton component intensified with increasing time postmortem in all samples (Figure 1), regardless of the sex or age of the animal. Those samples that had low Warner-Bratzler shear force values and high sensory scores for fiber fragmentation (more tender samples) tended to show the appearance of the 30,000-dalton component at an earlier time postmortem than did those samples with higher Warner-Bratzler shear force values and lower sensory fiber fragmentation scores (tougher samples). Figure 1 shows that in the more tender sample at day 3 postmortem, the 30,000-dalton component band is faintly present, while at days 3 and 7 postmortem in the more tough sample, the 30,000-dalton component band is not yet observed. The greatest increase in intensity of the 30,000-dalton component seemed to coincide with the largest reduction in shear force values and the largest increase in sensory panel determinations of fiber fragmentation. The 30,000-dalton component was observed however,

to have appeared in all samples in this study by 28 days postmortem. The disappearance of troponin T appeared to coincide with the appearance of the 30,000-dalton component. Days 3 and 7 postmortem in the samples from the tougher animals showed the presence of a band in the region corresponding to troponin T, while the 30,000-dalton component was not evident at those days. At days 14 and 28 the troponin T band disappeared and the 30,000-dalton component increased in intensity. In the more tender samples in which the 30,000-dalton component appeared at 3 days postmortem, the troponin T band was already beginning to appear very faint (Figure 1). This indicates that the 30,000-dalton component is a degradation product of troponin T. Its appearance seems to be an indicator of some of the degradative processes that occur in postmortem muscle leading to changes in the tenderness of the samples. Olson et al. (1977) showed that increasing time postmortem was accompanied by a decrease in troponin T and an increase in the intensity of the 30,000-dalton component band. When they incubated troponin T with preparations of the protease calcium activated factor (CAF), they saw the appearance of the 30,000-dalton component on SDS-PAGE electrophoretograms. This led them to conclude that the appearance of the 30,000-dalton component over time postmortem was due to the action of CAF on troponin T. The appearance of the 30,000-dalton component and the disappearance of tropinin T in the samples from this study may also be indicative of CAF activity. MacBride and Parrish (1977) also showed that the appearance of the 30,000-dalton component is related to the

tenderness of beef *longissimus* and myofibril fragmentation tenderness.

Figure 2 is a 5% SDS-PAGE electrophoretogram of the same samples in Figure 1, showing the changes that occurred in the high molecular weight proteins titin and nebulin over the four postmortem storage periods. Again, while these samples were from steers, similar patterns were also seen over increasing time postmortem in myofibrils isolated from the loin muscles of cows and bulls. Titin is normally shown to migrate under SDS-PAGE conditions as a doublet with the upper band commonly referred to as T_1 and the lower band, regarded to be a degradation product of T_1, referred to as T_2 (Wang et al. 1979) The samples in the current studies showed a third band appearing between T_1 and T_2, presumably also a degradation product of T_1. Figure 2 shows that increasing the time postmortem was accompanied by a loss of the T_1 band of titin (upper band) and by a loss of the nebulin band in those samples in which it was still present at 3 days postmortem. This is in accordance with the results of Lusby et al. (1983) who showed that titin slowly disappeared as postmortem aging time increased. While the specific rates of degradation differed among animals, in all samples in this study the T_1 band of titin disappeared by 28 days postmortem and the T_2 band became correspondingly more intense. This corresponding increase in intensity of the T_2 band appears to indicate the breakdown of titin into slightly lower molecular weight components that migrate further down the gel under SDS-PAGE conditions. Nebulin was not seen at 7 days postmortem or later in any

of the samples in this study. Among those samples that initially had relatively high shear force values and lower sensory scores (tough, Figure 2), the nebulin band was present and the T_1 band of titin was more intense at 3 days postmortem. The T_1 band of titin also tended to remain visible beyond the 3 day aging period in tougher samples. In those samples that had initially (3 days postmortem) lower shear force values and higher sensory fiber fragmentation scores (tender, Figure 2) the nebulin band was not seen at 3 days postmortem and the T_1 band of titin was less intense than at 3 days postmortem in the tougher samples. In addition, the T_1 band tended to disappear earlier in the more tender samples (Figure 2). This indicates that titin and nebulin were degraded faster in those samples that were classified as more tender. This coincides with the work of Anderson and Parrish (1989) who showed that tender *longissimus* samples exhibited more rapid degradation of titin and nebulin than did less tender samples.

Figure 3 shows a 5% SDS-PAGE electrophoretogram of purified myofibrils isolated from *longissimus* samples from bulls, steers and cows at 3 days postmortem. This figure shows that a nebulin band was consistently present in samples from bulls (lanes 4, 5, 6) and older animals (cows, lanes 7, 8, 9), while it was not always present at day 3 postmortem in samples from steers (lanes 1, 2, 3). The myofibrils isolated from the *longissimus* samples of the most tender steer (lane 1) showed the absence of the nebulin band. In the samples from the bulls and cows, while nebulin was detectable in all samples at 3 days postmortem, its intensity was greatest in those samples that

represented the upper extremes for toughness as measured by both shear force values and sensory scores for fiber fragmentation (lanes 5 and 9). The T_1 band of titin, while present in all samples at 3 days postmortem tended to be more intense in those samples which also showed the more intense nebulin band. This indicates the possibility that the intensity of the bands of these two proteins are reflecting differences among animals in the activity of a protease that acts on both titin and nebulin. This further suggests that titin and nebulin degradation patterns may help in developing a practical process for indicating those samples that are more or less tender.

Figure 4 is a 5% SDS-PAGE electrophoretogram of purified myofibrils isolated from the *longissimus* samples of steers, bulls and cows at 14 days postmortem. Those samples that had the lowest sensory scores for fiber fragmentation (lanes 5 and 9) exhibited the most prominent T_1 band at the 14 day aging period. In addition, this figure shows that, while often faint, the T_1 band of titin was more frequently seen at 14 days postmortem in samples from older animals (cows, lanes 7, 8, 9) than in samples from steers and bulls (lanes 1 through 6). The samples from older animals (cows, lanes 7, 8, 9) also had the heaviest T_2 bands (lower bands). This may be indicative of less proteolytic degradation due either to lower CAF activity or higher amounts of an inhibitor such as calpastatin. No evidence of the T_1 band of titin was observed in any of the samples from steer carcasses (lanes 1, 2, 3) at 14 days postmortem possibly indicating that more proteolytic degradation was occuring in those samples. The T_1 band of

titin was also not consistently seen in 14 day postmortm samples isolated from *longissimus* samples from bulls (lanes 4, 5, 6).

The reduced degradation of titin over increasing time postmortem in the samples from cows may help to explain why, as a group, cows were rated significantly lower than bulls and steers for the sensory tenderness attributes of softness to tooth pressure and fiber fragmentation. It may also help explain why cows had significantly higher compression shear force values (indicative of lower myofibrillar tenderness) than did bulls and steers. The explanation of why tenderness may be related to titin is possibly two-fold. First, the prolonged presence of titin in older animals (cows) that were identified as being significantly less tender by both sensory panel and Warner-Bratzler measures, may lend further support to the idea that titin plays a role in determining the tenderness of meat. Secondly, the presence or absence of titin over time postmortem may be an indicator of the amount of proteolysis in general that is occurring in the samples. As titin is an extremely large protein that makes up a relatively high percentage of the total myofibrillar protein (Trinick et al., 1984), the time required to degrade a large enough proportion of it to be detected on SDS-PAGE would seem to be longer than that required for smaller molecular weight proteins. Therefore, by the time changes in titin are noted, enough other proteins may also be degraded or disrupted that measurements of tenderness differences may be due to gross changes in the structure of the sarcomere. The evidence presented seems to suggest the possibility that a decreased activity of a protease such as

CAF or possibly an inhibitor such as calpastatin may be responsible for the decreased tenderness seen in some samples, especially in the older animals. Likewise the more tender samples may be reflective of an increased activity of a protease such as CAF, or a decreased amount of an inhibitor such as calpastatin. Whipple et al. (1990) reported that *Bos indicus* cattle, which are traditionally less tender than *Bos tarus* cattle, exhibited a higher activity of calcium dependent protease inhibitor activity (calpastatin). This led them to conclude that this difference in activity may influence the tenderness of the *longissimus*. A similar relationship may exist in those samples from older animals. It may be possible that as an animal ages, the activity of a protease such as CAF may decrease or the action of its inhibitor may increase, thereby influencing the rate and extent of postmortem tenderization.

SUMMARY

The results of the sensory panel study showed that the steaks from the young steers were scored significantly softer than steaks from the older animals. Sensory panelists found no differences for the attributes of softness to tooth pressure between bulls and steers. The same trend was observed for fiber fragmentation. Steaks from young steers were scored significantly easier to fragment across the fiber than were steaks from older animals. Again, steers and bulls were not significantly different for the attribute of fiber fragmentation. When the attribute of mealiness was examined, steaks from young steers were scored significantly more mealy than those from older animals. Steaks from bulls were significantly less mealy than steaks from steers. Sensory panelists scored the steaks from older animals as having significantly more detectable connective tissue residue than either young bulls or steers. When juiciness was examined, older animals were scored significantly more juicy than young bulls and steers. No significant difference in juiciness was observed between bulls and steers.

The Warner-Bratzler values showed that steaks from older animals required significantly more force for the compression or initial yield values than did young steers. Again there was no significant difference in the compression of steaks between bulls and steers.

When the four aging periods were examined, sensory panelists scored the three day aging period as having the least softness to tooth

pressure and having the most resistance to fragmentation of the four periods. Conversely, the twenty-eight day aging period was scored as being significantly softer and easier to fragment than the other three periods. Panelists found no difference between the seven and fourteen day periods of softness and fragmentation. The three and seven day aging periods were evaluated as being significantly less mealy than the fourteen and twenty-eight day periods. When the panelists scored steaks for the amount of connective tissue residue, day three was scored as having the most, while day twenty-eight was scored as having the least detectable connective tissue residue. Juiciness was scored the highest for the seven and twenty-eight day periods and lowest for the fourteen day aging period.

The Warner-Bratzler results showed that as postmortem aging time increased, the amount of force required for the initial yield or compression value decreased, indicating an increase in the myofibrillar tenderness over time. For the final yield or residual value, day three again required the most force and day twenty-eight the least, however, day fourteen was not significantly different from days three and seven postmortem indicating more time postmortem was required to see a difference in the final yield value.

SDS-PAGE analysis of purified myofibrils revealed that the 30,000 dalton component intensified with increasing time postmortem in all samples observed. In addition, the greatest increase in intensity appeared to correspond with the largest reduction in shear force values and the greatest increase in sensory panel scores for ease of fiber

fragmentation (tenderness) for a particular *longissimus* sample over time. This was especially evident in the samples that were initially less tender. Increasing postmortem aging time was also accompanied by a loss of the T_1 band of titin and the nebulin band in all sex and age classifications. The rate of the disappearance of these bands differed among animals. Among the steaks from steer carcasses, those that were more tender also exhibited a faster rate of titin and nebulin degradation than those that were less tender. When comparing the rate of titin degradation across sex and age categories, the T_1 band of titin persisted longer and was seen more frequently up to and including fourteen days postmortem in steaks from cows and also in tougher bulls. SDS-PAGE analysis also showed that a nebulin band was consistently present at 3 days postmortem in samples form bull carcasses and from steer and cow carcasses that had steaks which were characterized as being tougher.

The results of this study show that animal age and postmortem aging time had more influence on the tenderness attributes examined than did the sex of the animal. In addition, increased postmortem aging time improved the tenderness attributes under consideration regardless of the sex or age of the animal. This study also shows that differences exist in the degradation patterns of some of the myofibrillar/cytoskeletal proteins from samples of steaks from different sex and age categories and from steaks that differ in tenderness within the same sex and age categories. The tenderness differences observed may be dependent in part upon the rate of

degradation of the large proteins titin and nebulin. The postmortem degradation of these proteins and their filamentous forms, which may serve to hold some of the basic elements of the sarcomere together, ultimately leads to a weakening of the basic structure of the muscle fiber. These results lend credence to the concept of myofibril fragmentation tenderness, namely the tenderization that occurs within the myofibril at or near the Z-line due to the degradation of certain myofibrillar cytoskeletal proteins.

IMPLICATIONS

The results of this study show that certain differences exist in the degradation patterns of some of the myofibrillar/cytoskeletal proteins from samples of steaks from different sex and age categories and from steaks that differ in tenderness within the same sex and age categories. The tenderness differences observed may be dependent in part, upon variations in the rate of degradation of the large proteins titin and nebulin. The appearance of the 30,000-dalton component may prove to be an effective tool in tracking the rate of protein degradation and postmortem tenderization for different meat samples. The postmortem degradation of these structural proteins (titin and nebulin) and their filamentous forms, which may serve to hold some of the basic elements of the sarcomere together, ultimately leads to a weakening of the basic structure of the muscle fiber. The possibility exists that differences in CAF and/or its inhibitor (while not specifically examined in this study) may contribute to the changes noted. Further research into the interactions between the myofibrillar/cytoskeletal proteins and the protease systems in cattle during various phases and stages of their life and development may prove useful in understanding why palatabilty differences occur.

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Table 1. Means of sensory scores for loin steaks from different sex and age categoriesover all 4 postmortem aging times

CLASSIFICATION	SOFTNESS TO TOOTH PRESSURE[1]	FIBER FRAGMENTATION[2]	MEALINESS[3]	RESIDUE[4]	JUICINESS[5]
COW	102.7[a]	98.4[a]	124.0[a]	108.1[a]	107.4[a]
BULL	107.8 [ab]	107.9[ab]	119.8[a]	120.7[b]	98.4[b]
STEER	118.3[b]	119.7[b]	112.7[b]	128.4[b]	95.9[b]
SEM[d]	0.79	0.86	0.73	0.81	0.81

[a-b] Means with different superscripts within the same column are significantly different ($P < 0.05$).
[d] SEM is the standard error of the mean.
[1] Possible range of scores: 0 = very hard to 150 = very soft.
[2] Possible range of scores: 0 = very difficult to 150 = very easy.
[3] Possible range of scores: 0 = very mealy to 150 = none.
[4] Possible range of scores: 0 = large amount to 150 = none.
[5] Possible range of scores: 0 = very dry to 150 = very juicy.

Table 2. Means of sensory scores for loin steaks for four postmortem aging periods and three sexes

DAYS POSTMORTEM	SOFTNESS TO TOOTH PRESSURE[1]	FIBER FRAGMENTATION[2]	MEALINESS[3]	RESIDUE[4]	JUICINESS[5]
3	99.2[a]	97.7[a]	120.9[a]	110.5[a]	99.2[a]
7	109.7[b]	107.9[b]	123.5[a]	120.3[b]	105.0[b]
14	110.1[b]	109.7[b]	114.1[b]	119.5[b]	94.4[c]
28	121.1[c]	121.7[c]	115.9[b]	127.9[c]	103.1[b]
SEM[d]	0.79	0.86	0.73	0.81	0.81

[a-c] Means with different superscripts within the same column are significantly different ($P < 0.05$).
[d] SEM is the standard error of the mean.
[1] Possible range of scores: 0 = very hard to 150 = very soft.
[2] Possible range of scores: 0 = very difficult to 150 = very easy.
[3] Possible range of scores: 0 = very mealy to 150 = none.
[4] Possible range of scores: 0 = large amount to 150 = none.
[5] Possible range of scores: 0 = very dry to 150 = very juicy.

Table 3. Means for Warner-Bratzler shear force values for loin steaks from different sex and age classifications over all postmortem aging times

CLASSIFICATION	COMPRESSION (kg/cm^2)	RESIDUAL (kg/cm^2)
COW	3.3[a]	3.2[a]
BULL	2.7[b]	2.6[b]
STEER	2.5[b]	2.3[b]
SEM[e]	0.03	0.04

[a-b] Means with different superscripts within the same column are significantly different ($P < 0.05$).

[e] SEM is the standard error of the mean.

Table 4. Means for Warner-Bratzler shear force values for loin steaks from four postmortem aging periods over all sexes and ages

DAYS POSTMORTEM	COMPRESSION (kg/cm^2)	RESIDUAL (kg/cm^2)
3	3.1[a]	2.8
7	2.9[b]	2.7
14	2.8[c]	2.8
28	2.3[d]	2.2
SEM[e]	0.03	0.04

[a-d] Means with different superscripts within the same column are significantly different ($P < 0.05$).

[e] SEM is the standard error of the mean.

Figure 1. 12% SDS-PAGE gel of purified myofibrils from "tough" and "tender" *longissimus* samples from two steer carcasses over all postmortem aging times

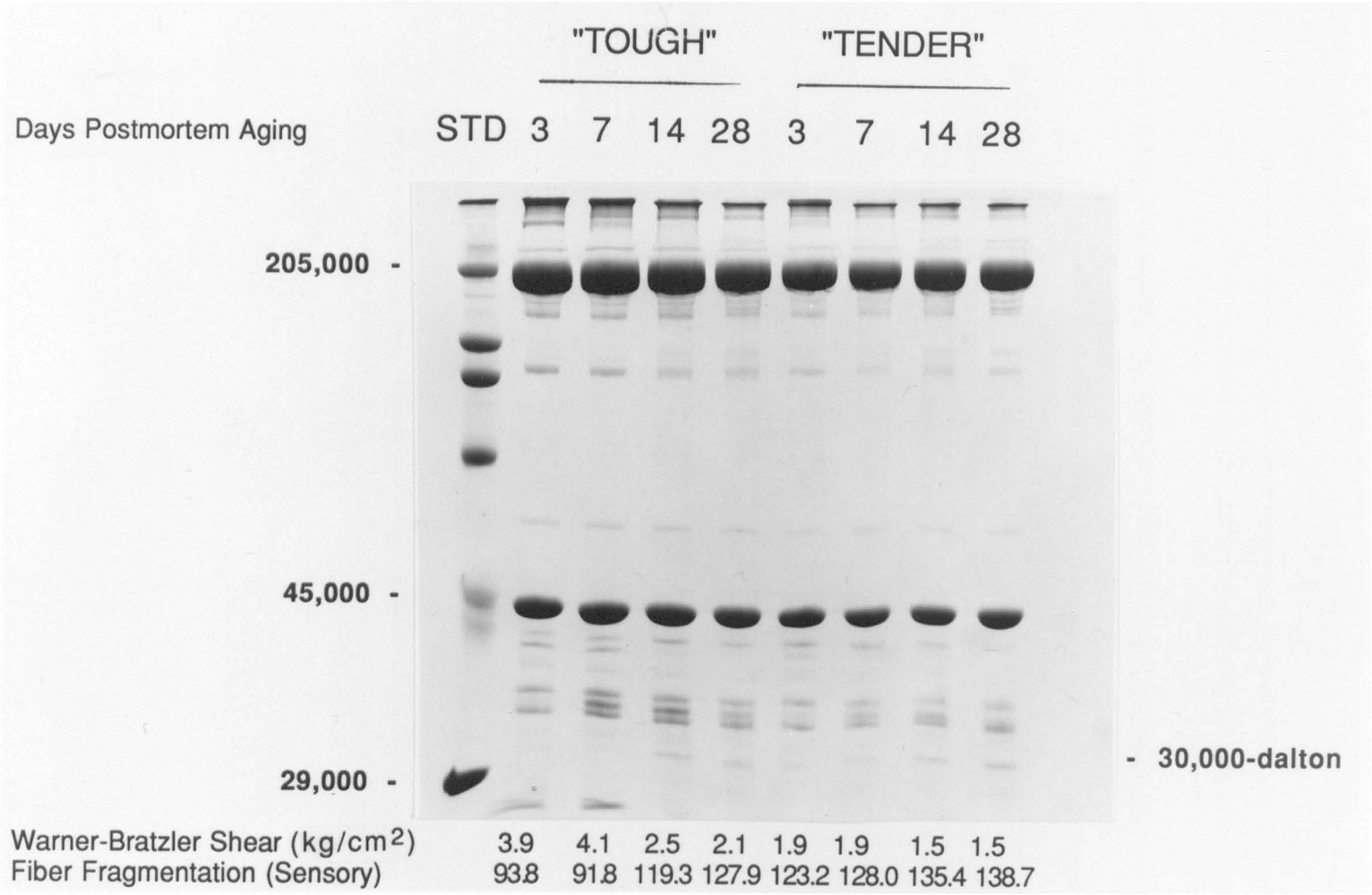

"TOUGH"
"TENDER"
Days Postmortem Aging
STD 3 7 14 28 3 7 14 28
205,000 -
45,000 -
29,000 -
- 30,000-dalton
Warner-Bratzler Shear (kg/cm2) 3.9 4.1 2.5 2.1 1.9 1.9 1.5 1.5
Fiber Fragmentation (Sensory) 93.8 91.8 119.3 127.9 123.2 128.0 135.4 138.7

Figure 2. 5% SDS-PAGE gel of purified myofibrils from "tough" and "tender" longissimus samples from two steer carcasses over all postmortem aging times

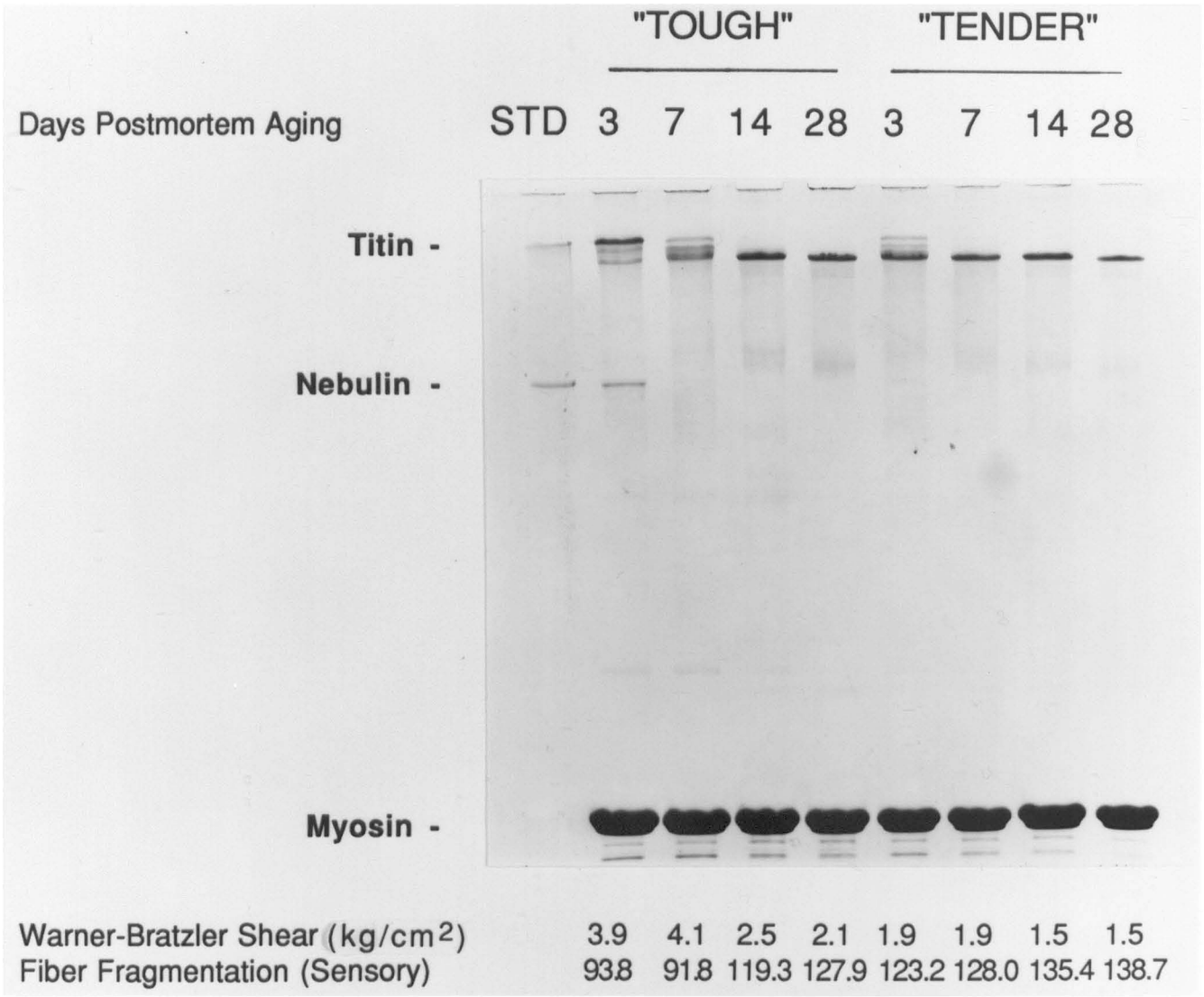

"TOUGH"
"TENDER"
Days Postmortem Aging
STD 3 7 14 28 3 7 14 28
Titin -
Nebulin -
Myosin -
Warner-Bratzler Shear (kg/cm2) 3.9 4.1 2.5 2.1 1.9 1.9 1.5 1.5
Fiber Fragmentation (Sensory) 93.8 91.8 119.3 127.9 123.2 128.0 135.4 138.7

Figure 3 5% SDS-PAGE gel of purified myofibrils from *longissimus* samples from carcasses of bulls, steers, and older animals (cows) at 3 days postmortem

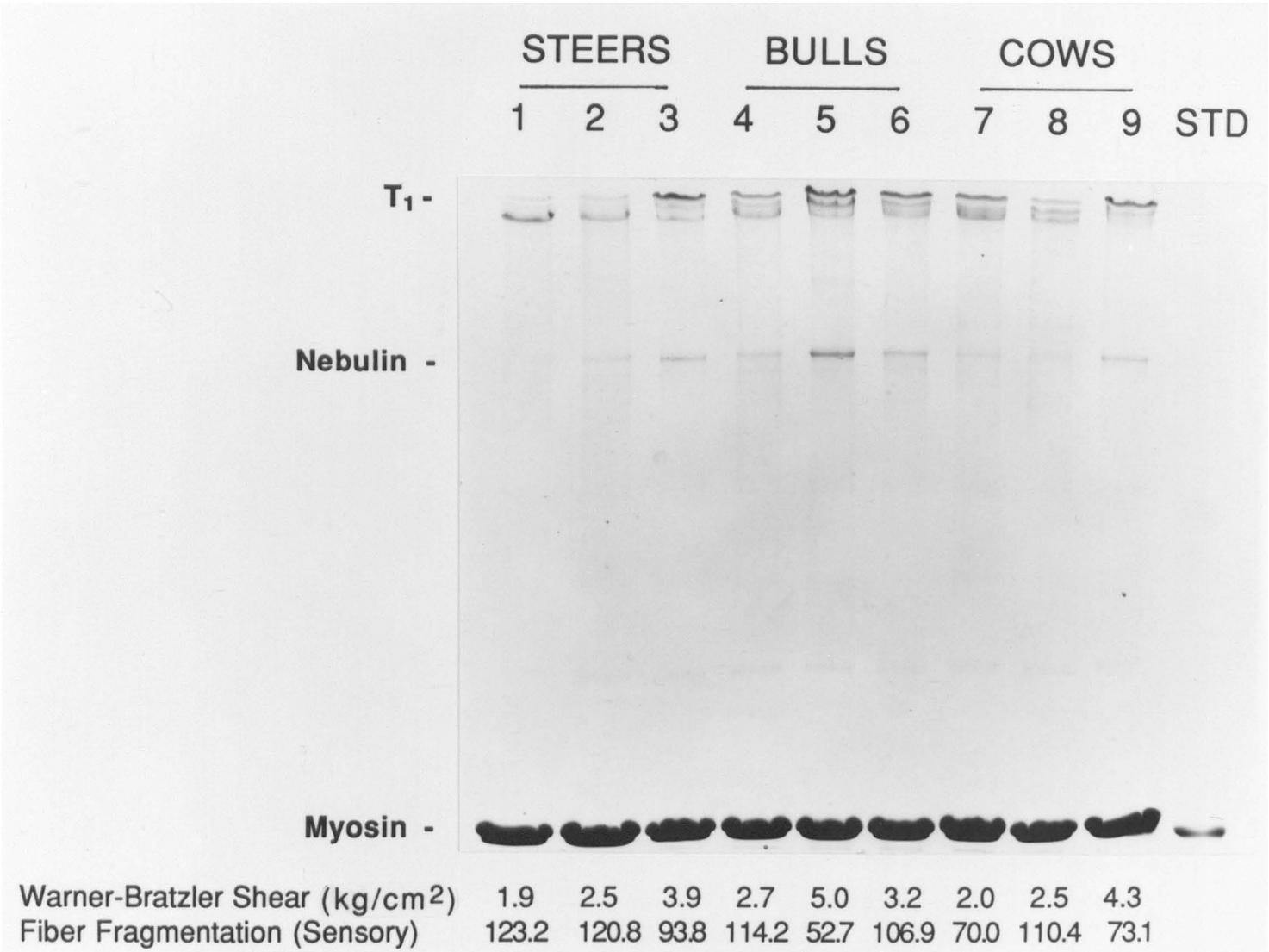
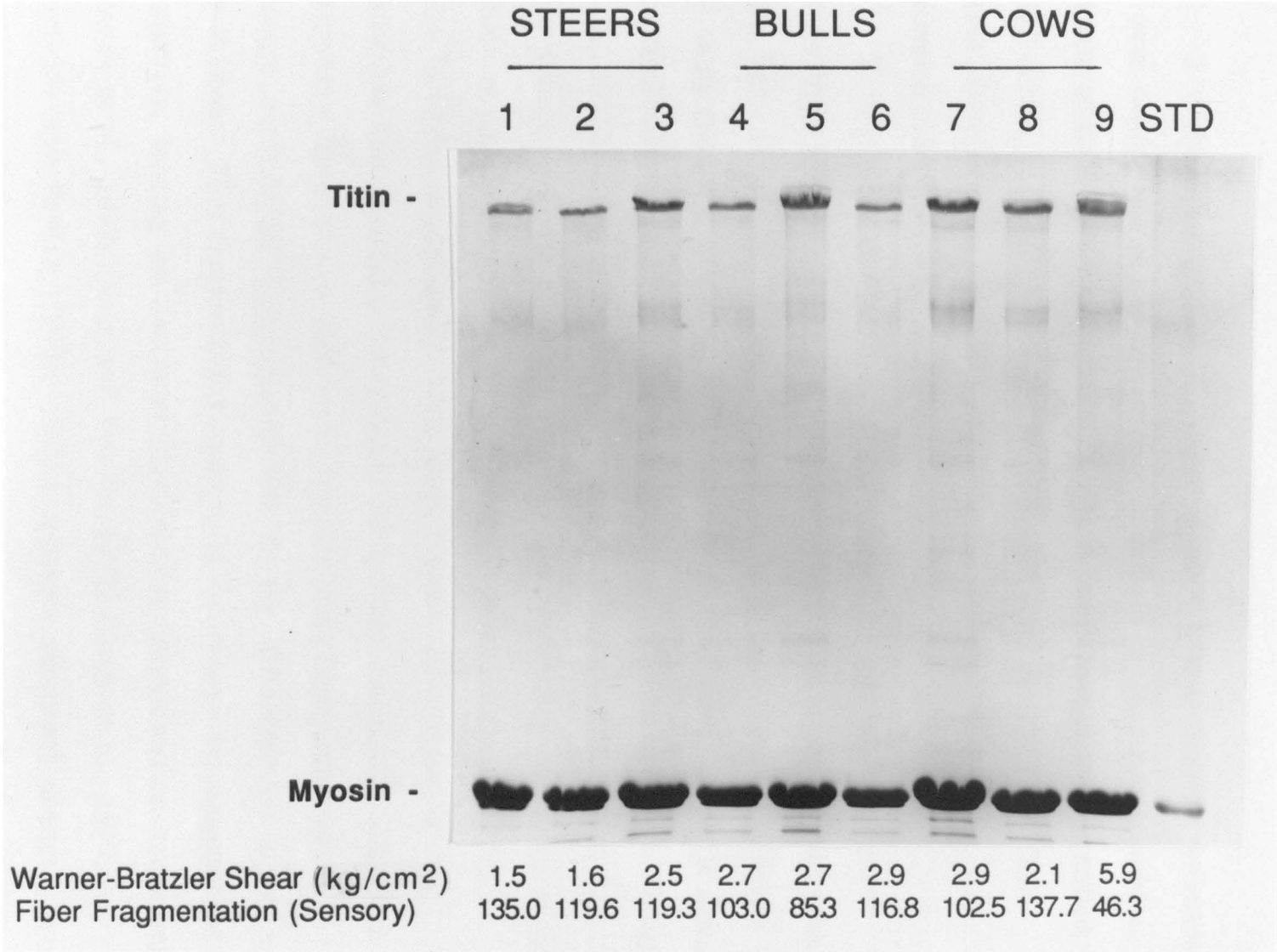

STEERS
BULLS
COWS
1 2 3 4 5 6 7 8 9 STD
T1 -
Nebulin -
Myosin -
Warner-Bratzler Shear (kg/cm2) 1.9 2.5 3.9 2.7 5.0 3.2 2.0 2.5 4.3
Fiber Fragmentation (Sensory) 123.2 120.8 93.8 114.2 52.7 106.9 70.0 110.4 73.1

Figure 4. 5% SDS-PAGE gel of purified myofibrils from *longissimus* samples from carcasses of bull, steers, and older animals (cows) at 14 days postmortem


STEERS
BULLS
COWS
1 2 3 4 5 6 7 8 9 STD
Titin -
Myosin -
Warner-Bratzler Shear (kg/cm2) 1.5 1.6 2.5 2.7 2.7 2.9 2.9 2.1 5.9
Fiber Fragmentation (Sensory) 135.0 119.6 119.3 103.0 85.3 116.8 102.5 137.7 46.3

GENERAL SUMMARY

A fully satisfactory explanation of what causes a wide variation in beef steak tenderness has long been sought by many researchers. Certain characteristics and handling procedures have been identified as being useful in predicting types of beef that may be more or less tender than average, yet what specifically causes tenderness differences is still under investigation.

Factors such as the sex and the age of the animal have been shown to predict beef steak tenderness. Beef from older animals has often been shown to be less tender than beef from younger animals (Tuma et al. 1963; Smith et al., 1982). Beef from intact males is often less tender than that from castrates (steers) of the same age (Albaugh et al., 1975). Postmortem aging is one handling procedure that has been shown, in most cases, to improve the tenderness attributes of beef (Busch et al., 1967; Parrish et al., 1973; Olson and Parrish, 1977).

In order to determine what causes these differences in tenderness the structural components have been examined. Several researchers have shown that meat samples that have been aged undergo myofibrillar fragmentation due to the disintegration at or near the Z-lines. This fragmentation appears to be related to measures of tenderness, leading to the term 'myofibrillar fragmentation tenderness' (Parrish et al., 1973; Olson and Parrish, 1977; Olson et al., 1977; MacBride and Parrish, 1977). Other apparent structural changes have been investigated. A degradation product of troponin T, the 30,000-

dalton component, has been observed to occur more often in tender than in less tender beef (MacBride and Parrish, 1977; Olson et al., 1977; and Olson and Parrish, 1977). Two very large molecular weight myofibrillar/cytoskeletal proteins, titin (MW = 28,000 kDa) and nebulin (MW = 600-900 kDa) have been implicated as playing a role in the tenderness of meat. These proteins have been shown to undergo postmortem tenderization more rapidly in tender beef than in less tender beef (Anderson and Parrish, 1989).

The goal of this project was to determine the effects of sex, chronological age and postmortem aging time on tenderness differences observed in meat samples as well as to determine if specific myofibrillar/cytoskeletal proteins such as titin and nebulin from samples from specific sex and age categories were degraded differently during postmortem aging.

Sensory panel tenderness evaluations and Warner-Bratzler shear force values revealed that animal age and postmortem aging time has more influence on the attributes tested in this study than did sex of the animal. Furthermore, the length of the postmortem aging period improved the tenderness attributes observed by both sensory panel scores and Warner-Bratzler shear force values regardless of sex or chronological age. SDS-PAGE analysis revealed that the 30,000-dalton component intensified with increasing time postmortem. This corresponded to the increase in sensory panel measures for tenderness and the decrease in shear force values over all postmortem aging periods. In addition, increasing postmortem aging time was

accompanied by a loss of the T_1 band of titin. However, in older animals the T_1 band of titin persisted and was more frequently observed through 14 days postmortem. SDS-PAGE analysis also revealed that a nebulin band was more frequently present at 3 days postmortem in muscle samples from bull carcasses and also from those steer and cow carcasses with greater shear force values and lower sensory tenderness scores.

The results of this study suggest that structural proteins such as titin and nebulin persist longer in skeletal muscle from certain animals and could play a role in some of the tenderness differences observed. The increase in intensity of the 30,000-dalton component with increasing time postmortem also seems to be related to the relative change in tenderness observed over all sex and age categories. The appearance of the 30,000-dalton component may prove to be an effective tool in tracking the rate of protein degradation and postmortem tenderization for different meat samples. The tenderness differences observed may be dependent in part, upon variations in the rate of degradation of the large proteins titin and nebulin. The postmortem degradation of these structural proteins (titin and nebulin) and their filamentous forms, which may serve to hold some of the basic elements of the sarcomere together, ultimately leads to a weakening of the basic structure of the muscle fiber. The possibility exists that differences in CAF and/or its inhibitor (while not specifically examined in this study) may contribute to the changes noted. Further research into the interactions between the myofibrillar/cytoskeletal proteins

and the protease systems in cattle during various phases and stages of their life and development may prove useful in understanding why palatability differences occur.

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APPENDIX A: SENSORY PANEL EVALUATION FORM

Name_____

SENSORY ANALYSIS OF BEEF

INSTRUCTIONS: Place a vertical mark across the horizontal line according to the intensity of the attribute you are testing. Please put the number of the sample above the vertical line when you make it.

Softness to tooth pressure: Measure of how easily the sample compresses between the molars

very hard — very soft

Fiber fragmentation: Measure of how easily the sample breaks down into smaller pieces

very difficult — very easy

Mealiness: Breakdown of the meat into small, hard and dry particles that cling to the cheek and gums

very mealy — none

Residue: The amount of insoluble connective tissue that remains in the mouth after thorough chewing

large amount — none

Juiciness: The sensation of free fluids released from the meat upon chewing.

Very dry — Very juicy