ABSTRACT

SURIAATMAJA, DAHLIA. Mechanism of Meat Tenderization by Long-Time Low-Temperature Heating. (Under the direction of Dr. Tyre C. Lanier).

The tougher texture of the lesser desirable cuts of beef is primarily attributable to higher content of collagen and/or cross-linked collagen. Many chefs, and some recent scientific studies, have suggested that long-time, low-temperature (LTLT) isothermal (sous vide) heating can considerably tenderize such meat and still retain a steak-like (rather than pot roast) texture. We investigated the effects of extended LTLT heating at 50 – 59 °C as a pretenderizing treatment for a tougher beef cut (semitendinosus, eye of round), and compared this to a bromelain-injection meat tenderizing treatment (‘meat tenderizer’ addition with no preheating). Because our intended application envisioned finish cooking by grilling of these pretenderized steaks at restaurants, we subsequently grilled all steaks to 65 °C internal (medium done). To counteract water losses associated with extended LTLT heating, all steaks were pre-injected with 15% of a salt/phosphate solution. Tenderization, as monitored by a slice shear force (SSF) measurement, did not occur at 50 °C but did initiate above 51.5 °C (isothermal). At 56 °C, a LTLT temperature chosen for safe treatment of steaks, a tender yet steak-like texture was obtained after 24 hr heating. Sodium dodecyl polyacrylamide electrophoresis (SDS-PAGE) did not indicate proteolysis of myosin heavy chain, as would have been expected if cathepsin (protease) had been active during heating. Instead, a pronase-susceptability assay indicated a high association of tenderization with the conversion of collagen to a partially denatured amorphous (‘enzyme-labile’) form, suggesting that this partial denaturation of collagen is mainly responsible for the tenderizing effect. This occurred at much lower temperature than has previously been reported for the initiation of
collagen denaturation (>60 °C) by differential scanning calorimetry, which likely is accounted for by the longer time of LTLT heating. Bromelain injection also produced tenderization as indicated by SSF measurements, but these steaks evidenced mushy texture and uneven appearance. Thus LTLT at 56 °C can be an effective means of tenderizing less-tender cuts of beef in a central location, for distribution and finish grilling at restaurants, and simultaneously it pasteurizes meat which has been injected with salt/phosphate to enhance yield and succulence. This is accomplished without detriment to meat color such that these steaks can be finish grilled at restaurants to almost any desired degree of doneness with no compromise in food safety.
Mechanism of Meat Tenderization by Long-Time Low-Temperature Heating

by
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DEDICATION

I dedicate this thesis to my dear and loving husband, Daniel, for all of his supports and patience throughout the course of my graduate program. To my beloved daughters: Natalie, who assisted me with biochemistry, and Melissa, for her understanding that sometimes I had to miss her tennis tournaments.
BIOGRAPHY

Dahlia Suriaatmaja was born and grew up in Jakarta, Indonesia. She went to Singapore for her secondary school and completed the GCE “O” level. She attended Grade 13 High School in Toronto, Canada. Dahlia went to University of Southern California to study Chemical Engineering for her undergraduate degree and participated in undergraduate research with Dr. Theodore Tsostsis.

After completing a B.Sc in chemical engineering, Dahlia went back to Indonesia and worked for a coatings company. She began her work in quality control and then moved to the product development and purchasing divisions. After 10 years of employment, Dahlia took time off to concentrate on homeschooling her two daughters. During this time she helped start a dry cleaning business with her husband. Later, she developed a proprietary chemical cleaner for dry cleaning and marketed the chemical cleaner “Stain Free” for dry cleaning purposes.

Dahlia later moved to the United States with her husband and two children. Her love for food brought her to culinary studies at Alamance Community College in Burlington NC, which then led her to study food science at NCSU. Dahlia enrolled as graduate student and developed an interest in the LTLT (sous vide) cooking method introduced to her by Dr. Tyre Lanier who has been her mentor in this MS research project.
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Chapter 1. Literature Review

Introduction: meat texture and succulence

The major factors affecting consumer acceptability of cooked meat are tenderness, succulence, and flavor (Horsfield & Taylor, 1976). Sensory analysis has shown that these three factors are assessed independently of each other by consumers (Harries, Rhodes, & Chrystall, 1972) and among these, tenderness is the primary factor that influences consumer purchase decisions (Boleman et al., 1997). This review will focus primarily on factors affecting steak tenderness, with some attention also given to the attribute of succulence.

The term ‘succulence’ refers mainly to the moisture/juiciness/lubrosity aspects of cooked meat, and would relate to the ease of swallowing chewed meat (Horsfield & Taylor, 1976). Moisture loss during cooking will thus adversely affect succulence. The juiciness/succulence that comes from melting of the intramuscular fat content of meat is also an important factor (José, Maria, & Carlos, 2009). For example, the extreme succulence of Wagyu beef from Japan is associated with its very high degree of marbling/fat content. The most pleasant sensations seem to derive from oleic acid in the triglycerides, whereas linoleic acid is deemed to be less pleasant (Cyranoski, 2008). Succulence in very lean cuts of beef will however be mainly determined by its water holding properties, since the fat component will be low or missing.
Tenderness, or its counterpart, toughness, is a multi-dimensional quality comprised of the related sensory attributes of resilience, resistance and chewiness (Horsfield & Taylor, 1976). As will be subsequently discussed in more detail, tenderness/toughness is mostly determined by the concentration and composition of connective tissue proteins, because most meats offered commercially are properly handled and aged so as to insure desirable attributes of the myofibrillar proteins or sarcomeres (Kemp, Sensky, Bardsley, Buttery, & Parr, 2010). Interestingly, the water holding properties of meat affect not only meat succulence, but indirectly can have some effect on tenderness as well, since the density of both the myofibrillar and the connective tissue matrices increase as water is lost from meat, effectively increasing resistance to biting through the meat. An extreme example of this effect is the tougher texture of beef jerky. The major fraction of meat is water, 85% of which is held in the inter-filament spacing of meat myofibrils (Offer & Knight, 1988). The filament lattice separation is controlled by pH and ionic strength that determines the electrostatic repulsive forces between the filaments. The addition of salt (typically sodium chloride) and/or phosphates will thus increase the inter-filament spacing as shown in Figure 1-1, allowing more room for water to be held by capillary forces within the meat. With salt addition the thick filament in raw meat may also be partially depolymerized into myosin molecules which, upon heating, may aggregate to form a gel. Water is also held tightly within this gel fraction (Offer & Knight, 1988). Thus injection/tumbling of meat pieces with salt/phosphate solutions can enhance water binding, and increase cook yields, adding to meat succulence and possibly impart a slight tenderizing effect as well (Smith, Fletcher, & Papa, 1991).
Instrumental Measurement of Tenderness/Toughness in Meat

This approach to measuring tenderness/toughness is generally preferred in research studies because sensory analysis is time consuming and expensive (King, Wheeler, Shackelford, & Koohmaraie, 2008) and thus not suitable for testing large numbers of samples (Shackelford & Wheeler, 2009). Various instrumental methods and geometries have been used. Probe type penetrometers (Swatland, 2006; Tressler, Birdseye, & Murray, 1932) and the Allo-Kramer shear/compression test (Cavitt, Youm, Meullenet, Owens, & Xiong, 2004) have been used for this purpose, but the Warner Bratzler Shear Force test (WBSF) (Sullivan & Calkins, 2011; Wheeler, Shackelford, & Koohmaraie, 1997) has been more widely used. For WBSF testing, cooked meat samples are chilled overnight prior to coring. Cylindrical cores of 1.27 cm diameter are removed parallel to the orientation of the meat fibers with either a manual or automated coring device. Cores are sheared perpendicular to the muscle fiber direction with a V-notch blade attached to universal texture testing machine at room temperature (Warner, 1952; Wheeler, Koohmaraie, & Shackelford, 1995). A similar test, termed the Slice Shear Force (SSF) measurement, substitutes a single horizontal descending blade for the V-shaped blade of the WBSF cutting head to enable slicing of a greater surface of steak area (slices of steak rather than cored samples). SSF data can be compared to WBSF data via the following equation (Shackelford & Wheeler, 2009):

\[ \text{WBSF} = (0.1063 \times \text{SSF}) + 2.2718 \]

Somewhat similar to this is a slicing blade device termed the Meullenet Owens Razor Shear (MORS), which instead employs a sharp craft razor blade. It has primarily been used to
measure the tenderness of poultry meat (Cavitt, Youm, Meullenet, Owens, & Xiong, 2004). Since most poultry meat derives from extremely young animals, its tenderness is largely a factor of myofibrillar protein configuration, not connective tissue proteins.

For beef muscles cooked by a standardized protocol, a WBSF value between 3.9 and 4.6 kgf are categorized as “slightly tender” (Shackelford, Morgan, Cross & Savell, 1991). However, according to Smith et al. (1982) consumer satisfaction is obtained at WBSF < 4.1 kgf (Sullivan & Calkins, 2011). There is considerable variation in toughness among different muscles within an animal, and even within individual muscles depending on the sampling location (Rhee, Wheeler, Shackelford, & Koohmaraie, 2004).

**Structure and composition of muscle as affecting meat texture**

**Muscle Structure and Composition**

In general, mammalian muscle contains about 75% water, 19% protein, 2.5% lipid, 1.2% carbohydrate, and 2.3% non-protein substances (Lawrie, 2006). The proteinaceous structures are most important to imparting the textural properties of meat, and these also determine the water holding properties of meat.

A thin layer of connective tissue called the endomysium surrounds bundles of myofibrils, each of which is composed of bundles of myofilaments, to form a single muscle cell, termed the muscle fiber. Bundles of muscle fibers are in turn surrounded by a connective tissue layer termed the perimysium to form muscle fiber bundles which are also grouped together.
forming larger secondary bundles, surrounded by an outer connective tissue layer termed the epimysium (Figure 1-2). Directly beneath the endomysium layer is the basal lamina, which consists of two layers; the lamina densa and lamina lucida (Figure 1-3). The lamina densa forms a layer overlaying the endomysium, but the collagen fibrils of the endomysium cannot penetrate it. The lamina lucida borders on the sarcolemma or cell membrane. The sarcolemma and basal lamina are thus attached to one another and can only be separated by collagenase and/or acidic conditions.

**Meat Proteins**

The primary protein component of the connective tissues depicted in the two previous figures (Figure 1-2 and Figure 1-3) is collagen; whereas the myofilaments of the myofibrils are primarily myosin and the actin (the ‘myofibrillar proteins’). Meat proteins are commonly grouped into three categories on the basis of their solubility characteristics: (1) those soluble only in salt/high ionic strength solutions (the myofibrillar proteins; chiefly myosin and actin), (2) those easily soluble in water or low ionic strength (the ‘sarcoplasmic proteins’, which includes most enzymes and heme proteins) and (3) those largely insoluble in high or low ionic strength solutions (connective tissue proteins; chiefly collagen). Myofibrillar proteins comprise 50 to 55% of the total protein content, sarcoplasmic proteins constitute approximately 30 – 34% and the remaining 10 – 15% are connective tissue proteins. Myofibrillar and connective tissue proteins are fibrous in nature, being rod-shaped molecules of twisted polypeptide chains, whereas sarcoplasmic proteins are globular in conformation (Starsburg, Xiong, & Chiang, 2008).
Proteins under normal (physiological) conditions of pH and temperature remain in the native (folded) conformation. This conformation is related to the hydrophobicity, polarity and the steric hindrance of the amino acid side chains. Changes in the thermodynamic stability, such as may be caused by heating or a change in solvent conditions, can cause denaturation (unfolding) of proteins (Kauzmann, 1959). Heating, which leads to rupture of hydrogen bonds and strengthening of hydrophobic interactions, results in both denaturation and subsequent aggregation of proteins, since aggregation is also thermodynamically favorable. Whereas globular proteins tend to expand upon denaturation, fibrous proteins generally contract on cooking since dissolution of their folded helices leads to their compacting in a more globular form (Tornberg, 2005). Heat denaturation of proteins may thus effect changes in meat texture, and each type of protein in meat can affect texture differently as it denatures (Martens, Stabursvik, & Martens, 1982; Penfield, 1973).

**Sarcoplasmic Proteins**

The sarcolemma encompasses the cytoplasm of the muscle cell called the sarcoplasm. The major proteins dispersed in the cytoplasm are myoglobin and many enzymes in lysosomal compartments, dispersed throughout the sarcoplasm. Lysosomal enzymes include acidic proteases known as cathepsins with optimum activity within the pH range 2.5 to 6.0. Several different cathepsins have been identified, including cathepsins A, B, C, D, E, and L, but only cathepsin B, D, and L are known to degrade muscle proteins (Mikami, Whiting, Taylor, Maciewicz, & Etherington, 1987). Cystatin, an endogenous inhibitor of cathepsin, binds to cathepsins L and B and thus may inactivate their catheptic activity in meat (Wang & Xiong,
1999) while cathepsin D, an aspartate proteinase, apparently has no inhibitor in the lysosome (Etherington & Bardsley, 1995). Cathepsins B and D degrade actin and myosin in vitro (Okitani, Matsukura, Kato, & Fujimaki, 1980) while cathepsin L degrades actin, myosin, α-actinin, troponin and tropomyosin (Mikami, Whiting, Taylor, Maciewicz, & Etherington, 1987). Cathepsin D has also been hypothesized to attack collagen postmortem (Judge & Aberle, 1982) but these authors provided no evidence of this.

Another lysosomal protease that is optimally active at more neutral pH is calcium-dependent protease (CDP), better known as calpain. There are two types of calpain, which require different optimal calcium concentrations; m-calpain requires calcium at millimolar levels while μ-calpain requires calcium at micromolar concentrations (Camou, Marchello, Thompson, Mares, & Goll, 2007). Endogenous inhibitors, calpastatin, are also present in the sarcoplasm. μ-calpain is most likely to activate during ageing because its optimal calcium concentration is most likely to be attained post-mortem (Goll, Thompson, Li, Wei, & Cong, 2003). Meat tenderization induced by calpain is caused by disruption of the Z-lines of the myofibrils during early postmortem ageing; there is no known effect on connective tissues however. The amount of proteolytic activity occurring during the ageing period is regulated by pH, temperature and calcium concentration (Ceña, Jaime, Beltrán, & Roncalés, 1992).

**Myofibrillar Proteins**

Myofibrillar proteins are the main component of the myofibrillar contractile structure (Figure 1-4). The main such proteins are actin and myosin, the main myofilament proteins.
Regulatory myofibrillar proteins include tropomyosin, troponin complex, actinin, C-protein, \( \alpha \)- and \( \beta \)-actinin, M-protein and other minor proteins. Cytoskeletal myofibrillar proteins are titin, nebulin, desmin and other minor protein that support the myofibrillar structure (Huff-Lonergan, Mitsuhashi, Beekman, Parrish, Olson, & Robson, 1996). Myofibrils contain repeating units called sarcomeres, separated by boundary zones termed the Z-line. The striated appearance of the myofibrils is due to the contractile components of sarcomeres, the myofilaments. Myofilaments consist of thick filaments, which constitute the dark (A) band and thin filaments, which constitute the light (I) band. The thick filament is also referred as the myosin filament as it contains myosin as the main protein. The middle of the thick filament, or the H-zone, is composed of myosin rods with the globular heads external. The center of the H-zone is the M line. The thin filaments are also commonly referred to as actin filaments as actin is its main protein constituent. One end of the thin filament is attached to the Z-line while the other is oriented towards the A band. The overlapping space between thick and thin filaments is important as it affects hydration, tenderness, myosin extraction, juiciness and water holding capacity of the meat (Lawrie, 2006). In the relaxed state, the H band and the I band are at maximum length. For contracted muscle the actin filaments moves toward the center of the H band; minimizing the I band and the H band. The length of the A band is not affected by contraction, but the I band is shortened during contraction. In tightly contracted or cold shortened meat the overlap can be as much as 50%. This tightly contracted condition can contribute markedly to meat toughness, independent of the connective tissue content or degree of collagen cross-linking (Kinsman, Kotula, & Breidenstein, 1994).
The third myofilament present is known as the gap filament or intermediate filament, which is a fine line that runs parallel to the thick and thin filaments, connecting the Z-disk to the M-line of a sarcomere. This filament thus binds the thick filament to the Z-line. The main protein is titin, which has a molecular weight of about 1 million Daltons. Titin can be degraded during ageing by calpain as demonstrated by SDS-PAGE. However, this degradation alone seems not to markedly influence meat tenderness (Fritz, Mitchell, Marsh, & Greaser, 1993).

Myosin, which constitutes 50% of all myofibrillar protein, has a long rod-like asymmetrical structure that is 65 Å wide, 1600 Å long and 40 Å deep with a molecular weight of 500 kD. In 8 M urea, myosin disassociates into 6 polypeptides containing 2 heavy chains each of 200 kD and 4 light chains of 20 kD (x2), 15 kD and 25 kD. The two heavy chains form a double helix rod. One end of the rod is attached to 2 globular heads, S1 and S2. The S1 head protrudes to the thin filament to bind with actin. The long tail interacts with others to form the backbone of the thick filament.

Actin constitutes 22% of the total myofibrillar protein. In skeletal muscle, actin exists in the form of double helical filaments made of polymerized globular monomers, each being 43 kDa in size. Thin filaments each contain about 400 actin molecules and provide a binding site which allows myosin to temporarily bind during muscle contraction or permanently bind following rigor mortis.
**Connective tissue proteins**

The main extracellular connective tissue proteins are collagen, reticulin and elastin, which are insoluble proteins. Those which most affect meat texture are collagen and elastin. Elastin is present in only small amounts though its quantity varies among different muscles.

There are 12 types of collagen identified found in intramuscular, skin, tendon, bone, dentine, cartilage, disc, basement membrane, cartilage, vascular, and skin. The collagen types are categorized into three major groups; those related to meat tenderness are (Bailey, 1989):

- Fibre-forming collagen (types I, II, III and V)
- Non fibre-forming collagen (type IV)
- Filamentous collagen (type VI and VII)

The collagen molecule is composed of three polypeptide chains, or \( \alpha \) -chains. Individual chains are unstable but when three \( \alpha \) -chains entwine, they form a stable triple helix. The \( \alpha \) -chain is a left-handed helix with three amino residues per turn, but the trimer or tropoellogen, is a stable right-handed helix. Each \( \alpha \) -chain is comprised of more than 1000 amino acids and has molecular weight of 100 kD. The repeating unit of collagen is made up of Gly-X-Y, in which X and Y are most often proline and hydroxyproline but can also be other amino acids, with the exception of tryptophan. The collagen molecule contains approximately 33% glycine, 12% proline, and 11% hydroxyproline. Hydroxyproline is found in both elastin and collagen and in the enzyme acetylcholinesterase but since collagen is by
far a more abundant constituent of meat, hydroxyproline content is often used to quantify collagen content (Bailey, 1989).

Connective tissue proteins are often said to contribute a ‘background’ toughness in the sense that the toughness they contribute to meat is not related to handling or storage of the meat post-mortem. This toughness can be related to both the quantity of collagen in a particular muscle and/or to the prevalence of mature covalent cross-links in that collagen (Lepetit, 2007) (Table 1-1). Cross-links decrease the tendency of collagen to convert to soluble gelatin during cooking in the presence of water (Hill, 1966).

**Alterning meat structure to modify tenderness/toughness of cooked meat**

As was mentioned in the introduction, both connective tissue and myofibrillar proteins can affect tenderness/toughness in beef, though in those cuts of meat typically considered to be tough, or in meat of older animals (Tornberg, 2005), connective tissue seems primarily responsible for meat toughness (Shimokomaki, Elsden, & Bailey, 1972). Thus a naturally tender cut of beef is largely so because (a) its myofibrils are not tightly contracted (sarcomeres shortened) and/or the Z lines of sarcomeres have been weakened due to calpain activity during aging, and (b) the content and/or extent of cross-linking of collagen in its connective tissues is sufficiently low. Each of these factors will subsequently be explained in some detail, and effects of exogenous treatments, such as proteolytic enzyme applications,
heat-induced chemical/structural changes, and mechanical disruption of natural muscle structure, will be discussed as agents to tenderize otherwise tough cuts of beef.

**Rigor mortis, sarcomere contraction and ageing effects on toughness**

Post-mortem processing and storage conditions can play an important role in meat tenderness as affected by the myofibrillar structure. Kemp, Sensky, Bardsley, Buttery, & Parr (2010) and Dransfield (1977) stated that the sarcomere length varies according to carcass posture during rigor mortis. As previously discussed, shortened sarcomeres contribute to less tender meat. Another possible means of inducing shorter sarcomeres is cold shortening. After death, muscle undergoes anaerobic glycolysis, causing lactate build up which leads to the decline of meat pH from 7.4 to around 5.5. During this period the supply of ATP decreases which leads to the formation of actomyosin cross bridges. When the excised muscle is too rapidly chilled to below 10 °C while the meat pH is higher than 6.2, contraction of the sarcomeres can occur (Marsh & Leet, 1966). This occurs because at lower temperature the sarcoplasmic recticulum cannot supply enough calcium for muscle relaxation (Whiting, 1980) yet the muscle still has an ample supply of ATP. This results in muscle shortening by 35 - 40% (Locker, 1963) which causes cooked meat to be tougher in texture (King, Wheeler, Shackelford, & Koohmaraie, 2008; Marsh & Carse, 1974). When muscle has shortened by 35%, actin myosin filaments overlap causing the myosin filaments to either penetrate the Z-line or the myosin filaments to crumple as shown in (Figure 1-5). Thus cold-shortened meat would also have an increased cross section density of connective tissue, causing a higher
tension and shrinkage during heating (Dransfield & Rhodes, 1976). The phenomenon of cold shortening is independent of an animal’s age (Bouton, Harris, & Ratcliff, 1981).

Aging of meat post-rigor during chilled storage is necessary because meat toughens at rigor due to formation of the permanent bond between myosin and actin. Ageing usually is maximized at about 12 days of storage at 4 °C holding. The tenderization that occurs during ageing is due to proteolytic activity by calpains that disrupts the myofibrillar structure at the Z line of sarcomeres (Kinsman, Kotula, & Breidenstein, 1994). Calpain activation starts when the amount of calcium released is high enough to initiate proteolytic attack. At death, the muscle is at pH 7.5 to 8.0, gradually dropping to an ultimate level of 5.4 - 5.5 due to the formation of lactic acid during glycolysis in the absence of oxygen. While the optimum activity of calpain is said to be at pH 7.2 – 8.2, some workers have suggested that 15-25% of maximum activity remains even at pH 5.5- 5.9. Calpain degrades desmin, which weakens the binding of α - actinin to the Z disk, tropomyosin, titin, and M line proteins (Huff-Lonergan, Mitsuhashi, Beekman, Parrish, Olson, & Robson, 1996). The calpain system has however no effect on the integrity of actin and myosin myofilaments (Penny, 1980).

Cathepsins may possibly also take part in the ageing or conditioning process, especially when the pH has decreased and the temperature is elevated. Yates, Dutson, Caldwell, & Carpenter (1983) showed that degradation of myosin and troponin T increased at 37 °C. However, Koohmaraie, Whipple, Kretchmar, Crouse, & Mersmann (1991) showed that cathepsin, which though it may be active up to 70 °C during cooking, does not degrade myofibrillar
protein at 2 °C (chilled storage). Thus under normal conditions of ageing, cathepsin likely plays no role in tenderization of meat and the bulk of evidence suggests that calpain alone is responsible for tenderization during refrigerated ageing (Dayton, Goll, Zeece, Robson, & Reville, 1976; Dayton, Schollmeyer, Lepley, & Cortés, 1981; Nowak, 2011).

**Role of added (exogenous) proteases/activators in meat tenderization**

One approach to meat tenderization is the addition of proteolytic enzymes. Those which have been investigated to date include plant extracts, fungal proteases and bacterial proteases (Foegeding & Larick, 1986). Plant extract proteases are most commonly used commercially, primarily papain (papaya extract), bromelain (pineapple extract), and ficin (fig extract). Others studied for use include actinidin (kiwi extract) and zingiber (ginger extract) (Christensen et al., 2009; Ha, Bekhit, Carne, & Hopkins, 2012). Cucumis (cucumber extract) and ginger have been trialed to tenderize tough buffalo meat (Naveena & Mendiratta, 2004).

Papain has a broad substrate activity against both myofibrillar proteins and connective tissues (Ashie, Sorensen, & Nielsen, 2002; Kang & Rice, 1970). Using azocoll as a substrate, Foegeding & Larick (1986) showed that papain has optimal activity at 60 - 70 °C; thus this enzyme is most active during meat cooking. Etherington (1984) also found some activity of papain on meat at cold storage temperatures. A meat temperature of 80 °C is required for its inactivation; so often residual activity can occur post-cooking in steaks. Thus use of papain
can easily cause over-tenderization and result in a mushy meat texture (Ashie, Sorensen, & Nielsen, 2002).

Bromelain belongs to the cysteine plant proteinases that are known to attack myofibrillar proteins. It also is most active during cooking of meat, having an optimum temperature range of 60 - 70 °C (Foegeding & Larick, 1986). It shows homology to mammalian cysteine proteinases such as cathepsin B, L, H and calpains. The tenderization mechanism of bromelain is not clearly defined though there is evidence that bromelain has a greater activity towards collagen as compared to papain, and as compared with its activity towards myofibrillar proteins (Figure 1-6) (Etherington & Bardsley, 1995; Kang & Rice, 1970).

The application of exogenous enzymes to the meat surface or by injection into the meat, even with subsequent tumbling or massaging, does not really produce an even distribution of the added protease at the cellular level throughout the meat piece being treated. Enzymes are proteins, which on the cellular level are quite large and do not easily travel through meat membranes (Carvajal-Rondanelli, 2003). Therefore, the proteolytic effect is necessarily concentrated and localized at the point of injection or application, which is another possible contributor to the development of mushy texture in cooked meats, which have been treated by plant enzymes.

Calcium chloride injection of meat, even into the live animal’s blood stream or into meat prerigor, can be carried out to better activate m-calpains during ageing and thereby enhance
meat tenderization (Wheeler, Koohmaraie, & Shackelford, 1997). However, calcium chloride has a metallic taste that is often objectionable unless the meat is properly seasoned to mask the off-flavor.

**Blade tenderization**

Blade tenderization (BT) is commonly practiced by the meat industry to partially overcome myofibrillar- or connective tissue-associated toughness by severing the muscle in multiple locations, while attempting to minimize visual indication of this damage/tenderization. Sharp needles/blades are used to pierce the meat, which physically cut or tear the tissues at preset intervals. The USDA is presently promulgating rules to require retail labeling of meat treated by BT for retail and food service because such steaks could be contaminated internally with pathogenic bacteria as the needles/blades pierce through the meat tissue (U.S. Department of Agriculture, Food Safety and Inspection Service, 2013). USDA suggests that BT steaks should be cooked to at least 65 °C internal (coldest spot) for safe consumption, meaning that such steaks could not be presented to consumers in the ‘rare’ or ‘medium rare’ level of doneness (Alfaro, 2013; Tewari & Juneja, 2007).

The physical damage caused to the muscle structure by BT treatment also seems to facilitate greater cook loss during heating (Smith, Fletcher, & Papa, 1991). Consumers are able to differentiate the tenderness of mechanical tenderized steak from that of naturally tender steaks (Maddock, 2008).
**Effects of Cooking on Meat Texture**

The effects of cooking on meat texture are quite complex and do not always result in tenderization. Time, temperature, and heating rate are the three variables which ultimately determine how cooking affects meat texture.

Kinetic studies on the effects of temperature on changes in meat chemistry and tenderness/waterholding are most typically carried out by isothermal heating of meat over a range of temperatures. Under these conditions one would expect that changes might occur at much lower temperatures than may be observed for ramp cooking, especially when compared to heating at the relatively high rates (5 - 10 °C / min) that are typically used in differential scanning calorimetry (DSC) (Leikina, Mertts, Kuznetsova, & Leikin, 2002). Most commercial cooking of meats is conducted by relatively slow ramp heating (0.5 to 3 °C/min), necessitated by the relatively large thickness of most commercial meat products and physical limitations of heat transfer in commercial cookers. Many reported laboratory studies have however employed relatively fast heating rates, >3 °/min (Li, Zhou, & Xu, 2010), which are difficult to achieve commercially except when very thin meat pieces are being heated. The following discussion of cooking (heating) effects on proteins and meat structure, and the resulting effects on meat texture and water holding properties, will address heating under these three different conditions.

**Isothermal Heating Effects**

Shrinkage of muscle fibers in diameter is first observed at about 37 °C during isothermal heating. A greater degree of diameter shrinkage occurs at 45 °C, whereupon shrinkage in
length seems to only initiate (Figure 1-7) (Hostetler & Landmann, 1968). Both length and
diameter shrinkage increase with higher temperatures of isothermal heating. This study also
noted that cook losses increased in accordance with muscle fiber shrinkage, as would be expected.

Interestingly, extending the isothermal heating time beyond about 30 min does not seem to
induce much additional shrinkage in these fibers in either dimension (Hostetler & Landmann,
1968). This is counter-intuitive, since one would suppose that the activation energy for
changes in the meat proteins responsible for shrinkage would have been reached at the lowest
temperature where shrinkage is first detectable. Subsequently then, since proteins would
continue to denature, shrinkage would be expected to proceed at some rate proportional to
the temperature, the rate constantly increasing to a maximum at some higher temperature.
Maximum shrinkage of the fibers might be reached only when proteins were fully denatured
and the maximal amount of water had been expelled from the fibers.

Somewhat corresponding to this data, Davey (1974) measured toughening of meat (measured
by shear testing) during isothermal heating (Figure 1-8). Compared to raw meat, isothermal
heating at temperatures of 40 - 50 °C produced a significant toughening of fibers which they
attributed to myofibrillar protein denaturation. In the range of 50 – 65 °C the fibers became
more tender during isothermal heating, but when heated at 65 - 75°C fibers were again
observed to become more tough. The authors attributed the toughening at 65 - 75 °C to
collagen shrinkage. Presumably the logic of assigning the toughening that occurred at 40 - 50
°C to myofibrillar protein denaturation, and the toughening that occurred at 65-75 °C to collagen shrinkage, was that when measured by DSC (at high heating rates) myofibrillar proteins denature at temperatures somewhat lower than does collagen (Martens, Stabursvik, & Martens, 1982).

However, Christensen, Purslow, & Larsen (2000) reported hardening of isolated perimysium at 40 - 50 °C during isothermal heating (one hour) (Figure 1-9). According to Lewis & Purslow (1989) de-crimping of collagen (presumably due to its denaturation) most likely begins at 40 °C. Collagen is arranged in a ply formation and the de-crimping increases the collagen density which in turn increases the collagen strength (Lewis & Purslow, 1989). Similarly, Field, Pearson, & Schweigert (1970) demonstrated that the epimysium contracts during hardening and muscle with a more contracted epimysium exhibits a higher shear force value.

Since Li & Zhou (2010) measured denaturation onset temperatures of endomysium and perimysium to be 50 and 62 °C, respectively, by DSC (at a heating rate of 10 °C/min), it would be expected that under isothermal conditions, endomysium would denature at perhaps an even lower temperature than does perimysium, but certainly by the point at which perimysium apparently denatures and hardens (Christensen, Purslow, & Larsen, 2000). Based on this evidence it becomes difficult to assign changes in meat texture and morphology below 50 °C to any particular proteins or meat structures. Presumably either myofibrillar proteins, or collagen, or both could undergo denaturation and contribute to meat
shrinkage, toughening and water loss during isothermal holding at these temperatures. Clearly something different occurs in the 50 - 65 °C range that contributes to meat tenderization and this will be discussed further in the next section.

Field, Pearson, & Schweigert (1970) demonstrated that hardening of muscle due to contraction occurs even up to 90 °C and this likely is not caused by myofibril denaturation because those proteins would have completely denatured at much lower temperatures. Fiber length continuously decreases from 50 °C to above 80 °C when heated non-isothermally; this may be another indicator that collagen is predominantly contributing to texture changes at higher temperature. Toughening in the 65 -75 °C range (Beilken, Bouton, & Harris, 1986; Christensen, Purslow, & Larsen, 2000; Davey, 1974) has however only been measured during an isothermal heating time of 1-2 hr; longer times in this temperature range still lead to tenderization when sufficient time ensues (Machlik & Draudt, 1963).

Tenderization of Meat during Isothermal Heating, Long Time/Low Temperature (LTLT)

In the first 2 hr or so of heating at 50 - 59 °C there is no significant shear force decrease in tougher muscles; indeed meat toughens during such heating (Machlik & Draudt, 1963). This also has been attributed to initial shrinkage of the collagen induced by the heating treatment (Finch & Ledward, 1972). The degree of shrinkage and toughening during this initial phase of heating was found to depend on the concentration of mature cross-links in the muscle (Lepetit, 2007).
M. Christensen, L. Christensen, Ertbjerg, & Aaslyng (2011) reported there is no decrease of shear force with pork ST after heating 5 hours at 53 °C isothermal. The shear force decreased only after cooking was extended for 17 hours and decreased even further when heating was at 58 °C isothermal. This suggests that tenderization is ongoing with extended cooking time. Beilken, Bouton, & Harris (1986) reported that in meat of very old animals the shear force decreased at 50 °C and 55 °C after an extended heating time of 24 hr with further decreases noted upon holding for 48 hr at these temperatures. This decreased toughness is very significant when cooking temperature is elevated to 60 °C with holding for 24 - 48 hr.

Beilken, Bouton & Harris (1986) suggested that this tenderization noted between 50 - 60 °C at longer times of heating is due to weakening of connective tissue (Bouton & Harris, 1981; Bouton, Harris, & Ratcliff, 1981). Beilken, Bouton, & Harris (1986) found the meat of young animals begins to tenderize at 50 °C while that of very old animals tenderizes more readily at 60 °C.

Snowden & Weidemann (1977) found that isolated collagen fibrils heated at 60 °C exhibited a partially amorphous melted fraction under electron microscopy. This amorphous melted fraction was also shown to be susceptible to becoming soluble when subjected to pronase digestion. Extended cooking at 60 °C for one hour increased the fraction susceptible to pronase digestion. This suggests that isothermal cooking at 60 °C caused partial denaturation of cross-linked collagen, which did not result in its solubilization unless subjected to subsequent pronase digestion.
Powell, Hunt, & Dikeman (2000) used this approach to measure the pronase susceptibility of collagen after isothermal heating at 50, 60 and 70 °C. Tenderization increased as the pronase, or ‘enzyme’, labile fraction (ELF) increased. These treatments produced only a small amount of soluble collagen, suggesting that tenderization is caused mostly by weakening of partially denatured (cross-linked) connective tissue (Bouton & Harris, 1972; M. Christensen, L. Christensen, Ertbjerg, & Aaslyng, 2011; Lewis & Purslow, 1989).

M. Christensen, L. Christensen, Ertbjerg, & Aaslyng (2011) associated the decrease of meat toughness during LTLT with a measured increase in residual cathepsin activity. However, Wang & Xiong (1999) showed there is no breakdown of myosin heavy chain measurable by SDS-PAGE in bovine heart during cooking at 50 °C which argues against a role of cathepsin activity in tenderization over this range. The authors concluded that while the literature suggests cathepsin activity does exist in beef which could be active over this temperature range, natural inhibitors also exist which negate its effects.

Machlik & Draudt (1963) showed that collagen must shrink (denature) before tenderization can occur and noted a maximum rate of tenderization at 60 - 65 °C. Beilken, Bouton & Harris (1986) noted that cook loss at 60 °C after 24 hours was almost double that of the cook loss at 55 °C /24hr.
**Ramp Heating Effects**

Hostetler & Landmann (1968), who observed shrinkage of muscle fibers at 37 °C during isothermal heating (Figure 1-7) also found that during slow ramp heating (1.3 °C/min) of isolated muscle fibers the fiber diameter starts to shrink at 37 °C such that the diameter decreased to about 84% of its original width upon reaching 40 °C (Figure 1-7a). While they could detect initial shrinkage of muscle fiber length at 45 °C during isothermal heating (Figure 1-7b), this was not evident during ramp heating until a temperature of 50+ °C was attained (Figure 1-11). It is thus apparent that ramp heating can delay the onset of many events, which may onset at lower temperatures when measured under isothermal heating conditions.

When Laakkonen, Wellington, & Sherbon (1970a) measured water losses during very slow ramp heating (0.1 °C/min) they detected an increase also beginning at 37 °C, in agreement with the shrinkage of muscle fibers noted by Hostetler & Landmann (1968). As previously discussed, these associated changes in muscle morphology and water holding (and texture, noted previously) likely reflect the combined effects of heat-induced denaturation in myofibril and connective tissues.

The shrinkage in length of muscle fibers noted at 50+ °C during slow (1.3 °C/min) heating by Hostetler & Landmann (1968) (Figure 1-11) was accompanied by a loss in birefringence between 54 - 56 °C, which they attributed to denaturation of the myofibrils. It is possible however that changes in birefringence could also have indicated denaturation of collagen.
surrounding the myofibrils at this temperature since the melting of collagen also results in loss of birefringence (Engel, 1970).

In agreement with the isothermal studies of Beilken, Bouton, & Harris (1986) which showed tenderization of beef over the 50 - 60 °C range, Penfield (1973) also reported decreases in shear value of meat when slowly ramp heated (0.3 °C/min) from 50 - 60 °C, which correlated with a corresponding increase in hydroxyproline solubility (Figure 1-12). This also suggests that tenderization over this range primarily corresponds to changes in collagen of the connective tissues, though only a 5% solubilization in muscle having a high percentage of cross-linked collagen was reported by Powell, Hunt, & Dikeman (2000). As previously discussed, they noted that only partial denaturation of collagen, resulting in pronase susceptibility but not solubilization, is mainly associated with the tenderization occurring over this temperature range. And while residual collagenolytic activity was observed by some workers (Laakkonen, Wellington, & Sherbon, 1970a; 1970b) after heating meat in this range, it is not clear if this actually results in hydrolysis of collagen, as was previously discussed (Laakkonen, Wellington, & Sherbon, 1970a; 1970b).

Interestingly, when a very fast rate of heating is used, there is no tenderization effect noted in the temperature range 50 - 65 °C. Li, Zhou, & Xu (2010) found that meat is constantly increasing in shear force value when rapidly heated (> 3 °C/min) to endpoint temperatures ranging from 40 to 90 °C (Figure 1-10a). Muscle fiber diameter decreased dramatically over the same temperature range (Figure 1-10b). Thus clearly sufficient time within the
temperature range 50 - 65 °C is required for the tenderization reaction(s) to occur, and fast heating does not allow for tenderization to proceed.

The significant toughening that occurred above 65 °C (Figure 1-10a) might not be expected to derive from collagen denaturation since this is above the temperatures at which epimysium and perimysium were found to totally denature as measured by DSC (Brüggemann, Brewer, Risbo, & Bagatolli, 2010; Li, Zhou, & Xu, 2010; Martens, Stabursvik, & Martens, 1982). Actin denatures over the range of 66 - 73 °C as measured by DSC (Martens, Stabursvik, & Martens, 1982), but such a jump in meat toughness seems unlikely from this event alone. These authors (Li, Zhou, & Xu, 2010) concluded that this toughening at high temperatures is due to collapse of the epimysial/perimysial structures as manifested by the significant decrease of fiber diameter. Perhaps this decrease in fiber diameter is actually reflecting water losses, and drying out of the meat is inducing a stronger texture. Paul, McCrae, & Hofferber (1973) concluded that toughening at 70 °C is probably due to hardening of myofibrillar protein and the effect of tenderness cannot be completely explained by increases in heat labile collagen (Field, Pearson, & Schweigert, 1970).

Sous Vide (LTLT) Cooking

Sous vide as translated from French means simply “under vacuum”. It is one specialized method of LTLT cooking wherein the food (meat) is vacuum packed in a plastic pouch and heated in a controlled temperature water bath. The method allows for maximum heat transfer rate while also preventing contamination of the food by the water or during subsequent
chilled/frozen handling. Thus, if the food is pasteurized as well as tenderized by the LTLT treatment, that state of pasteurization is maintained until the seal of the package is broken. This method of LTLT cooking is favored by many chefs, some of whom have made specific claims that meat is tenderized by the treatment when it is conducted within certain parameters. For example, Myhrvold, Young, & Bilet (2011) in their seminal book *Moderist Cuisine* noted that “weakening the [collagen] mesh is the job of cooking. To do that, collagen fibers must be heated with sufficient moisture; the minimum temperature varies from one kind of meat to another, but is about 52 °C for most red meats.”

**Food Safety Considerations in LTLT Processing of Meat**

The greatest concerns with LTLT processing of meats derive from the possible growth and/or toxin production of *Clostridium botulinum*, *Clostridium perfringens*, and *Bacillus cereus* because they are able to survive low heat treatments. They are gram positive, spore-forming bacteria, and all can cause acute food poisoning. They produce toxins, which are harmful to the human intestinal track and generally cause even more severe illness in elderly or health-compromised persons. *C. botulinum* and *C. perfringens* are anaerobic bacteria, whereas *B. cereus* is a facultative bacteria. Beside these spore-forming bacteria, *Listeria monocytogenes*, *Salmonella* spp, *Staphylococcus aureus* and *Escherichia. coli* are non-spore forming and facultative bacteria that also can impose illness (Jay, 2005; Tewari & Juneja, 2007) . *C. botulinum* grows most rapidly at 12 to 50 °C (Jay, 2005). The spores of *C. botulinum* are heat resistant requiring a temperature in excess of 100 °C for destruction. *C. perfringens* grows most rapidly from 20 to 52 °C with an optimum temperature from 37 to 45 °C and optimum
pH ranging from 5 to 9 (Jay, 2005; Willardsen, Busta, Allen, & Smith, 1978). B. cereus has an optimum growth region from 5 - 50 °C and optimum pH range of 4.9 – 9.3 (Jay, 2005). To control the growth of these spore forming bacteria an LTLT heated product should be rapidly cooled afterwards to < 4 °C to prevent spore germination and inhibit growth of vegetative cells (Ghazala, Ramaswamy, Smith, & Simpson, 1995). The growth of vegetative cells can be stopped by heating and the minimum treatment as set by the U.S. Department of Agriculture, Food Safety and Inspection Service (1999) for pasteurization (6.5 D reduction, Salmonella) is 54.4 °C for 112 min.

This U.S.D.A., F.S.I.S. (1999) recommended process is not adhered to by chefs of many leading restaurants today. For example, the very popular chef-authored manual on sous vide cooking Modernist Cuisine (Myhrvold, Young, & Bilet, 2011) suggested that a 6.5 D reduction of Salmonella can be accomplished at a lower pasteurization temperature, longer time: 52 °C for 314 min. However, according to Willardsen, Busta, Allen, & Smith (1978), C. perfringens is able to grow at up to 52 °C. The authors of Modernist Cuisine did however inject a disclaimer in their book, saying “This book cannot and does not substitute for legal advice about food regulations in the United States as a whole or in any U.S. legal jurisdiction. Nor can we guarantee that following the information presented here will prevent foodborne illness. Unfortunately, the many variables associated with food contamination make eliminating all risk and preventing all infections virtually impossible. We cannot accept responsibility for either health or legal problems that may result from following the advice
presented here. If you operate a commercial establishment and serve food to the public, consult the rules and health regulations in your area”.

Summary and implications for future development

It seems clear from this review of literature that any tenderization that may occur at temperatures below 50 °C (such as during refrigerated ageing) is not likely the result of any changes to connective tissue/collagen. Indeed, isothermal heating at temperatures below 50 °C actually seems to toughen meat and connective tissue and no tenderization occurs afterwards despite very long holding times (Bouton & Harris, 1981; Christensen, Christensen, Ertbjerg, & Aaslyng, 2011). From the evidence it would appear that collagen may denature (with extended time of isothermal heating) at temperatures as low as 40 °C (Christensen, Purslow, & Larsen, 2000), resulting initially in its contraction and shrinkage. This likely also contributes to water loss from the meat, which may be accentuated by denaturation of the myofibrillar proteins at 40 - 50 °C. If calpains or cathepsins are active at these temperatures there should be some degree of tenderization detectable as they degrade myofibrillar proteins, but this has not been reported to date.

At >50 °C, however, it would appear that the activation energy for some new change in meat structure is initiated. From the evidence presented thus far in the literature this seems most likely to involve a particular type of unfolding/denaturation specific to cross-linked collagen, which can be measured as ELF (pronase susceptible) and which dramatically softens tough connective tissue. This reaction appears to occur at all temperatures between just above 50
°C up to the mid 60s °C and possibly higher. But this tenderization reaction is never apparent when meat is heated at a rapid heating rate to an internal endpoint of 65 °C or higher, such as occurs in normal grilling of beef steaks.

The beef industry does fully cook and simultaneously tenderize many tough cuts of meat (high in cross-linked collagen content) but typically at temperatures above 65 °C and for sufficient time to convert most collagen to gelatin. Such meat is uniformly brown in color to its core because of the higher temperatures and times used. In home food preparation the crock pot is often used to achieve similar tenderization of tough beef cuts; this is carried out near 80 °C (Sundberg & Carlin, 1976) and typically results in a ‘pot roast’ texture (meat falls apart) rather than a ‘steak-like’ texture typical of grilled meat.

LTLT heating of meat in the 50 – 60 °C range might result in a less cooked internal appearance, offering a means of tenderizing meat without fully cooking it, allowing for subsequent grilling to almost any desired level of internal ‘doneness’ at restaurants. Clearly however there would be a concern that using LTLT to merely tenderize meat would result in excessive water loss, with accompanying loss of meat succulence. However, if LTLT heating were carried out under the correct parameters, the parcooked meat would be pasteurized. This would allow for the enhancement of the meat by injected solutions to improve its yield and succulence. LTLT heating by the sous vide method (vacuum packaged meat in a water bath) would facilitate both the heating and the subsequent safe
storage/handling of the product until the package was opened and steaks were grilled at restaurants.
### Tables

Table 1-1. Relationship between number of Hp cross-links and tenderness (*Lepetit, 2007*).

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Q</th>
<th>$n_0$(Hp)</th>
<th>$Q\ n_0$ (Hp)</th>
<th>Tenderness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biceps Femoris</td>
<td>3</td>
<td>0.5</td>
<td>1.50</td>
<td>1</td>
</tr>
<tr>
<td>Semimembranosus</td>
<td>3</td>
<td>0.5</td>
<td>1.5</td>
<td>$\geq 1$</td>
</tr>
<tr>
<td>Gluteus Medius</td>
<td>2.77</td>
<td>0.35</td>
<td>0.97</td>
<td>2</td>
</tr>
<tr>
<td>Longissimus dorsi</td>
<td>1.86</td>
<td>0.36</td>
<td>0.67</td>
<td>3</td>
</tr>
<tr>
<td>Psoas Major</td>
<td>1.41</td>
<td>0.45</td>
<td>0.63</td>
<td>4</td>
</tr>
</tbody>
</table>

$Q$, collagen content of muscles (%), $n_0$ (Hp), Hydroxylxylpyridinoline (mole/mole collagen)
Figure 1-1. Schematic diagram illustrating the effect of swelling with aid of a salt/phosphate solution, due to charge repulsion of myofibrils. *Adapted from Offer & Knight* (1988).
Figure 1-2. Organization of striated muscle. *Adapted from Tortora, Funke, & Case (2001).*
Figure 1-3. Sarcolemma and basal lamina. Adapted from Huijing (1999).
Figure 1-4. Schematic diagram of sarcomere skeletal protein. *Adapted from Au (2004).*
Figure 1-5. Schematic diagram of cold shortened meat (1) penetration of Z line and (2) rejection by Z line. *Adapted from Marsh & Carse (1974).*
Figure 1-6. Relative activity of enzymes on three fractions of meat. *Adapted from Kang & Rice (1970).*
Figure 1-7. Relative percentage change of (a) fiber diameter and (b) fiber length with time at various temperatures. *Adopted from Hostetler & Landmann (1968).*
Figure 1-8. The effect of isothermal heating at different temperatures for one hour: toughening occurs in two separate phases. Adapted from Davey (1974).
Figure 1-9. Tensile strength of perimysial connective tissue isolated from semitendinosus muscle after 1 hour heating at different temperatures. Adapted from Christensen, Purslow, & Larsen (2000).
Figure 1-10. (a) Changes in shear force value during ramp heating and, (b) percentage change in diameter, with a fast heating rate (>3 °C/min). Data is reconstructed from Table 2 (Li, Zhou, & Xu, 2010).
Figure 1-11. Percentage change of fiber diameter or length with slow heating rate ($<1.3 ^\circ$C/min). Adapted from Hostetler & Landmann (1968).
Figure 1-12. The effect of non-isothermal heating on shear values (A) and percentage of soluble hydroxyproline with heating 0.3 °C/min. Adapted from Penfield (1973).
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Chapter 2. Beef Steak Tenderization by Long Time, Low Temperature Heating

Introduction

Tenderness is a primary determinant of beef consumer acceptance (Boleman et al., 1997). Except when the meat has been cold-shortened or improperly aged, a tough beef steak is likely to have a relatively high collagen content and/or a high percentage of cross-linked collagen in its connective tissues (Lepetit, 2007).

Typically tougher meat cuts are somewhat tenderized to improve palatability by either blade tenderization (Maddock, 2008; Pietrasik & Shand, 2011) or the use of exogenous proteolytic enzymes (‘meat tenderizers’) (Etherington & Bardsley, 1995; Sullivan & Calkins, 2010). Neither approach is capable of producing steaks from tougher beef cuts that compare in palatability to naturally tender steaks however (Maddock, 2008). Enzyme tenderization is particularly prone to producing an objectionably mushy texture in steaks arising from difficulty in evenly distributing the enzymes within muscle tissue (Carvajal-Rondanelli, 2003) and/or residual activity following cooking (Ashie, Sorensen, & Nielsen, 2002).

Beilken, Bouton, & Harris (1986) showed that tenderness of a typically tough beef muscle, semimembranosus, increases during holding at 55 °C or above, which they attributed to weakening of the connective tissues (Beilken, Bouton, & Harris, 1986). Christensen, Purslow, & Larsen (2000) showed that while isolated perimysium from semitendinosus (ST) muscle increased in fracture stress after one-hour isothermal treatment at 40 or 50 °C, a
decrease in fracture stress occurred upon incubation at higher temperatures. Snowden & Weidemann (1978) found that, upon sufficient heating, a relatively large fraction of cross-linked collagen, while remaining insoluble, nonetheless becomes readily susceptible to digestion by pronase. Powell, Hunt, & Dikeman (2000) found that this pronase susceptibility correlated with increasing tenderness of ST when heated from 50 to 70 °C.

Moisture losses from beef are initiated at relatively low temperatures, caused by shrinkage of collagen and the sarcomere in combination with lowered water holding capacity of denatured myofibrils (Hostetler & Landmann, 1968; Laakkonen, Wellington, & Sherbon, 1970a). Both heating time and temperature affect water loss from meat. For example, Bouton & Harris (1986) also noted that cook loss after holding at 60 °C for 24 hours was almost double that at 55 °C for the same holding time.

The objectives of the present study were to first pinpoint the minimum temperature at which tough beef steaks can be tenderized during isothermal heating, and then to explore the mechanism whereby this tenderization proceeds, and how water loss is affected as well. The desired heating process would be sufficient to insure safe processing yet gentle enough to minimize color change (relatively long time, low temperature LTLT). Thus the research could lead to development of a method for tenderizing beef steaks that does not rely on blade tenderization or the use of exogenous proteolytic enzymes (‘meat tenderizer’ enzymes) and therefore allow for finish cooking of steaks to any desired degree of doneness by grilling in restaurants.
Material and Methods

Materials

USDA choice grade beef psoas major (PM) and semitendinosus (ST) muscles, aged by chilling 14 - 21 days post-mortem, were obtained from a local supplier. The pH ranged 5.4 – 5.6 in the muscles selected. PM was used as a ‘naturally tender’ control for comparison while ST was chosen for tenderization experiments because it is a representative ‘tough’ beef cut and because it is largely free of heavy bands of fat or connective tissue and therefore more homogeneous in composition, thus reducing variability in texture testing (Huffman, Miller, Hoover, Wu, Brittin, & Ramsey, 1996; Shackelford, Morgan, Cross, & Savell, 1991; Sullivan & Calkins, 2011). Bromelain (Enzeco® bromelain 240) was supplied by Enzyme Development Corp. (New York, NY) and stored at 4 °C until used. Sodium tripolyphosphate (STPP, Brifisol® STPNEW) was obtained from B.K. Giuliani Corp. (Simi Valley, CA) and non-iodized salt (NaCl) was purchased from the supermarket.

Methods

Experiment 1. Initiation Temperature for Tenderization

This experiment explored the changes in meat texture during LTLT cooking as an effect of heating temperature (isothermal) at one or two long heating times. Both PM and ST muscles were trimmed of visible surface fat and cut to steaks of approx. 1.25 cm thickness. ST steaks for this experiment were obtained from a single intact muscle. Prior to finish grilling, ST steaks (3 per treatment) were initially (experiment 1a) isothermally heated at 50, 53, 56, or 59
°C (+/- 0.5 °C) for 24 or 48 hr (times are determined subsequent to the internal temperature attaining within 1.5 °C of the water bath temperature, denoted as “time zero”). Additionally 3 PM and 3 ST steaks remained refrigerated prior to grilling to serve as ‘tender’ and ‘tough’ controls. For LTLT heating, steaks were weighed and vacuum packaged in Cryovac bags (product 97390 type B 620) using a JVR Busch RB 0016 DIZ0 vacuum sealer (Maulburg Germany) prior to heating in a water bath. To monitor meat temperature, a thermocouple (K-type, Omega Engineering, Stamford CN) was inserted into the center of one additional steak, which was placed in an open bag, weighted with a bottom clip to hold it immersed in the water bath while the bag top was kept above the water bath, to effectively evacuate the package. All steaks were completely submerged in water baths, which were isothermally controlled at each of the predetermined temperatures. Steaks were placed within a divided rack to ensure bags did not touch during cooking. Following the LTLT treatments the steaks were immediately chilled in ice water and held overnight before grilling. All steaks were grilled on a unit with two hot plates (top and bottom, Breville) preheated to 177 °C to an endpoint internal temperature of 65 °C, as measured within one steak (the grill held 4 steaks per grilling pass). A temperature of 65 °C was chosen as the end point of grilling to represent a ‘medium’ done steak since, following removal from the grill at this point, the internal temperature actually reached 70 – 71 °C before the center of the steak cooled (American Meat Science Association, 1995).

Experiment 1a was regarded as only preliminary to experiment 1b and thus was not replicated. Experiment 1b was carried out identically to experiment 1a except a more narrow
range of water bath temperatures was used (50, 51.5 and 53 °C) (+/- 0.5 °C) and heating time was 24 hr only. A randomized block design was used wherein 3 separate ST muscles were used, each being portioned into 18 steaks. The steaks within each of the three muscles were randomly assigned to one of the three temperature treatments.

All steaks in experiment 1 (a and b) were subsequently evaluated for cook loss and slice shear force (texture) as explained later.

The hypothesis for this experiment is that meat tenderizes at the temperature range 50 – 59 °C.

**Experiment 2. Effect of salt/phosphate solution enhancement on cook loss during LTLT heating**

A single intact ST muscle was cut into two portions after trimming as described previously. One portion (approx. 3/5 of the muscle) was injected with 16% (w/w, above the initial weight) of a 6% NaCl+ 2% STPP (sodium tripolyphosphate) solution so as to achieve a target meat concentration of 0.83 % NaCl and 0.28 % STPP. The remaining portion was not injected (control). Both injected and non-injected portions were tumbled (packaged separately in sealed bags but tumbled separately) for 30 min at 3 °C in a Polymaid tumbler (Key Laboratories, Inc., Largo, Fl) of 20 cm dia., 20 cm depth at 24 rpm. Following brief (2-3 hr) exposure to -20 °C to firm the portions, the injected portion was then cut into 9 steaks of 1.25 cm thickness. These were packaged as in experiment 1 and randomly (3 per
temperature) subjected to 0 (as previously defined), 24 and 48 hr heating at 53 °C (+/- 0.5 °C), then immediately cooled in an ice water bath. The non-injected portion was sliced into only 6 steaks of the same thickness and cooked identically. Cook loss was measured as described later. This experiment was not replicated as it was only done in confirmation of accepted industry practice for improvement of cooked yield.

Our hypothesis is that salt phosphate can be used to increase cook yield during treatment.

**Experiment 3. Comparison of tenderization by LTLT heating vs. by injection of bromelain**

This experiment was conducted similarly to that of experiment 1 except that all ST steaks were tenderized by LTLT treatment only at 56 °C for 24 hr. Also, all muscles used in this experiment were enhanced by injection (prior to heat treatment) with a 15% salt/phosphate solution as in experiment 2 so as to minimize cook loss during the LTLT treatment. Additionally, some steaks were derived from muscle portions to which bromelain, a widely used meat tenderizing enzyme, had been added to the salt/phosphate solution prior to injection and tumbling. In order to better understand the mechanism of tenderization occurring, steaks in this experiment also received more extensive evaluation subsequent to LTLT treatment and grilling, including not only cook loss and SSF (texture) measurements, but also analysis for evidence of myosin heavy chain proteolysis and changes to the collagen component of the meat which may have occurred during the treatments. Informal sensory evaluations of the grilled steaks from this experiment were also conducted.
Thus three main treatments, prior to grilling of all steaks, were compared: (a) LTLT treatment at 56 °C for 24 hr, (b) bromelain injection to a final concentration 14.5 ppm, and (c) an untreated control. For each replicate of the experiment (3 total replications) one whole ST muscle was used. The muscle was cut into approximately three equal portions, which pertained to each of the three treatments mentioned above. After injection (treatments a and b only), all portions (a, b and c) were separately tumbled (packaged within separate bags) for 30 minutes at 3 °C as in experiment 2. Following brief (2-3 hr) exposure to -20 °C to firm the portions, five steaks were then cut from each of the three portions for subsequent evaluations, 3 of these being grilled and evaluated for cook loss and SSF while the remaining 2 were vacuum packaged and finish cooked in a 100 °C water bath to the same internal temperature as by grilling (65 °C) so as to avoid losses of liquid that would interfere with the chemical analyses conducted on these (heating time for both grilling and water bath cooking was essentially identical).

The hypothesis for this study is that at >50 C something initiates as the activation energy is met, most likely some particular type of unfolding specific to crosslinked collagen, which can be measured as ELF and which dramatically softens tough connective tissue.

**Sample preparation for chemical analysis.**

Following finish cooking to 65 °C in boiling water to simulate grilling, sample pouches were immediately plunged in ice water for 30 min. Pouches were then stored in at -20 °C for 5 days prior to freeze drying (Model 75035, Labconco Corp., Kansas City, MO) to a moisture
content to below 7%. Freeze dried samples were powdered with a food processor (MiniPro, Black & Decker) for 5 min followed by a mortar and pestle. Moisture content of wet samples prior to drying and of freeze dried samples were measured according to AOAC (1995).

**pH determination of raw meat prior to treatments**

A digital pH meter (Fisher model 420 digital with Accunet probe) was used to measure pH of raw meat at room temperature. Only muscles in the pH range 5.4 to 5.6 are used. 10 mg of meat samples are chopped coarsely mixed with 90 mg of deionized water until homogenized. The homogenized solution was measured for pH.

**Cook loss determination**

Cook loss from the untreated, raw (‘green’ weight) was determined as the difference in weight before and after treatment for grilled samples. Following grilling each sample was patted dry with a paper towel and weighed.

\[
\text{cook loss \%}= \frac{\text{weight after treatment} - \text{weight before injection}}{\text{weight before injection}} \times 100\%
\]

Measurements were made for at least 3 steaks per treatment.

**Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) measurement**

The protein concentration of each treatment for SDS-PAGE was determined by measuring the difference in absorbance 280 nm and 260 nm, diluting the soluble sample with DI water in a 1:20 ratio. Details of the sample preparation are described in Ruilova-Duval (2009) and
Segel (1976). SDS-PAGE was used to assess myofibrillar proteolysis by measuring disappearance of myosin heavy chain (MHC). The procedure for SDS-PAGE was conducted according to manufacture’s instruction with Bis-Tris Mini Gel (Invitrogen Inc.). Gels were scanned using densitometry (Alpha Innotech Corp., San Leandro, CA) to determine protein band degradation. The density of the MHC band was expressed as a percentage of the density of all bands in each lane.

**TCA precipitation absorbance value**

The TCA precipitation and salt soluble extraction procedure is determined according to Joo, Kauffman, Kim and Park (1999) and Zipprerer’s (1972) procedure. Briefly, 20% w/v of FD sample is extracted with 0.3 M potassium chloride phosphate buffer (pH 7.0). Optical density readings were recorded at 280 nm using spectrophotometer (Spectronic 1001 Bausch & Lomb Rochester, NY).

**Modified slice shear force (SSF) measurement of meat tenderness**

A slight modification of the SSF procedure of Shackelford, Wheeler, & Koohmaraie (1999) was used to measure the relative tenderness of samples. Figure 2-1 illustrates the methodology used for SSF. The only modification made was the use of samples less than one inch in thickness at the time of grilling. This modification was made in order to minimize the come up time to reach the designated isothermal heating temperature (time to reach time zero of heating) and also to maximize the number of steaks that could be obtained from any one intact muscle.
Separation of collagen fractions based on solubility and pronase susceptibility

Total collagen was measured by a modification of the method of Hill (1966) by first dissolving 0.125 g of freeze-dried sample in 10 ml 6N HCl. This was then digested with 6 N HCl for 14 hours at 130 °C. Two ml of this was then neutralized with KOH solution and the volume adjusted to 50 ml volume with addition of water. The procedure of Blumenkrantz & Asboe-Hansen (1975) was then used to assay hydroxyproline using a single toluene extraction. A conversion factor of 7.25 was used to convert readings to collagen content. The acid hydrolysis was conducted in duplicate and the hydroxyproline assay was read in triplicate.

The soluble collagen or Ringer soluble fraction (RSF) was determined using a modification of published methods (Hill, 1966; Powell, Hunt, & Dikeman, 2000). Briefly, 0.125 g of freeze dried sample was mixed with 2.5 ml of quarter-strength Ringer’s solution which had been warmed to 50 °C. The solution was held for 2 hr at room temperature to allow the freeze-dried sample to fully rehydrate. The sample was then vortexed and centrifuged at 6,000 x g for 10 min. The supernatant was collected and the residue was re-extracted as before. The supernatants were then combined and digested with 10 ml of 6 N HCl for 14 hours at 130 °C for hydroxyproline assay as previously described.

Powell, Hunt, & Dikeman’s (2000) procedure was used to quantitate the relative percentages of total unalterable fraction (TUF) and enzyme labile fraction (ELF) except that pronase digestion was carried out by shaking for 16 hours with shaking, stopped by
centrifugation, then centrifuged for 2 hr at 20,000 x g. The supernatant was then decanted and the residue transferred carefully to a glass test tube with the aid of added distilled water for acid digestion and hydroxyproline determination. This determined the TUF; ELF was calculated as total collagen minus TUF+RSF.

Figure 2-2 is a schematic of the procedure used to assess the relative percentages of TUF, ELF and RSF fractions.

Statistical analysis
Experiments 1b and 3 were conducted using a randomized complete block design with three replicates. The least square means procedure of the Proc general linear models method (SAS, Cary NC, version 9.2) was used to separate the means for each treatment.

Results and Discussion

Experiment 1. Initiation temperature for tenderization.
SSF results (Figure 2-3) showed that ST muscle held isothermally at 50 °C for up to 48 hr toughened with no subsequent tenderization. At 53 °C some tenderization was noted, and when the heating temperature was increased to 56 °C a greater tenderization was noted. Increasing the temperature to 59 °C did not seem to induce additional tenderization however. Except for 53 °C, much greater change was noted in SSF during the first 24 hr of heating than in the second 24 hr.
It is clear that LTLT heating at 53 °C or above could produce tenderization of ST comparable that of a naturally tender muscle (PM) without a tenderization treatment. 48 hr heating at 53 °C produced a SSF almost equal to the PM control. A similar low SSF was achieved after only 24 hr isothermal heating at 56 or 59 °C.

Cook loss in these same samples appeared to increase with increasing temperature (Figure 2-4) over the initial 24 hr of isothermal heating, with little further increase in the subsequent 24 hr of heating. Cook losses were quite large, ranging from about 25 to 36% after 24 hr of isothermal heating.

To more precisely determine the temperature of onset of tenderization (isothermal heating, 24 hr minimum), the experiment was repeated using heating temperatures over a more narrow range (Figure 2-5). The SSF data clearly revealed that tenderization initiated within the narrow range of 50 - 51.5 °C.

Only small changes in SSF were measured at the zero time of heating (point at which the core of steaks was within 1.5 °C of the bath temperature) as compared to raw steaks (control) (Figure 2-3 and Figure 2-5). The heating time required to reach this ‘zero time’ temperature was about 15 minutes. At this point presumably myofibrillar proteins would be highly denatured since Penny (1967) saw denaturation of myosin within 10 min at temperatures as low as 35 °C. Several workers have attributed toughening of meat during heating at temperatures below 60 °C primarily to myofibrillar denaturation (Davey & Niederer, 1977;
Martens, Stabursvik, & Martens, 1982), so it seems odd that little or no toughening was measurable at time zero in these steaks.

In the first 2 hr or so of heating over the entire range 50 - 59 °C meat is known to toughen in texture (Machlik & Draudt, 1963). This has been attributed to initial shrinkage of collagen induced by the heating treatment (Finch & Ledward, 1972). The degree of shrinkage and toughening during this initial phase of isothermal heating was found to depend on the concentration of mature cross-links in the muscle (Lepetit, 2007). According to Lewis & Purslow (1989) de-crimping of collagen (presumably due to its denaturation) most likely occurs well below 50 °C. Collagen is arranged in a ply formation and the de-crimping increases the collagen density, which in turn increases the collagen strength (Lewis & Purslow, 1989; McCormick, 1999). Similarly, Field, Pearson, & Schweigert (1970) demonstrated that the epimysium contracts during hardening and muscle with a more contracted epimysium exhibits a higher shear force value.

Christensen, Purslow & Larsen (2000) later confirmed that perimysium toughens markedly when heated isothermally for one hour at 40 °C, becoming even stronger when incubated at 50 °C. Since Li and Zhou (2010) measured denaturation onset temperatures of endomysium and perimysium to be 50 and 62 °C, respectively, using DSC which employs a fast heating rate (10 °C/min), it would be expected that under isothermal conditions endomysium would denature at an even lower temperature than does perimysium, but certainly by the point at
which perimysium apparently denatures and hardens at 40 °C (Christensen, Purslow, & Larsen, 2000).

Thus it seems likely that the toughening of ST meat that occurred during extended heating at 50 °C (Figure 2-3 and Figure 2-5) is primarily attributable to collagen shrinkage. This shrinkage also likely gives rise to the expelling of water from muscle during LTTLT heating, measurable as cook loss (Figure 2-4 and Figure 2-6). Water is held within muscle primarily by capillarity, within the myofibrils (Offer & Knight, 1988). The myofibrils are attached to the basal lamina, which contains type IV collagen (Bailey, 1989). When myosin denatures it aggregates, freeing water to migrate from the myofibril (Offer & Knight, 1988). As explained previously, the perimysium and endomysium apparently begin to denature at temperatures as low as 40 °C and this eventually leads to their contraction and the expelling of water, now freed from the capillarity of an intact myofibrillar structure, from the meat.

The initiation of tenderization upon holding at temperatures above 50 °C (Figure 2-3 and Figure 2-5) likely indicates some dramatic change over extended heating time in the collagen of a tougher meat cut such as ST. Many workers have considered this to be a type of ‘accelerated aging’ (Dutson, 1983); that is, a result of proteolytic attack (in this case primarily from endogenous cathepsins, since calpains are largely inactivated at temperatures above 37 °C (Goll, Thompson, Li, Wei, & Cong 2003). However the dramatic onset of tenderization within such a narrow temperature range (50 – 51.5 °C), and above 50 °C, does not seem to correlate with known temperature activity ranges of cathepsins present in beef.
muscle. For example, beef cathepsins are reported to have an activity range extending from 35 °C up to only 55 – 60 °C (Draper & Zeece, 1989; Okitani, Matsukura, Kato, & Fujimaki, 1980). Therefore, a tenderization reaction which only initiates at 50 °C would not seem to implicate cathepsins. Wang & Xiong (1999) presented evidence that argued against a role of cathepsin activity in tenderization over this temperature range. The authors concluded that while the literature suggests cathepsin activity does exist in beef which could be active over this temperature range, natural inhibitors also exist which negate its effects.

Snowden & Weidemann (1978) showed that upon sufficient heating a relatively large fraction of cross-linked collagen, while remaining insoluble, nonetheless becomes readily susceptible to digestion by pronase. Powell, Hunt, & Dikeman (2000) found that this pronase susceptibility correlated with increasing tenderness of ST when heated from 50 to 70 °C. This would indicate that there might be partial denaturation of cross-linked collagen that leads to a molten globule structure, which is less tough than the native cross-linked state, but nonetheless still insoluble.

**Experiment 2: Effect of salt/phosphate solution enhancement on cook loss during LTLT heating.**

The large cook losses observed in ST meat after LTLT heating at 50 - 59 °C (Figure 2-4) would likely be unacceptable when using the LTLT for meat tenderization in commercial practice. Enhancement of meat by injection/tumbling with solution of NaCl and phosphates is a well accepted practice for reducing cook losses in meat (Smith & Young, 2007) and
derives from the ability of added salt and phosphate to increase the charge on neighboring myofibrils, promoting their separation and thus allowing more room for water uptake (Offer & Trinick, 1983; Offer & Knight, 1988). When ST muscle chunks were injected to 15% above green weight and tumbled prior to LTLT heating at 53 °C isothermal for 24 hr, this enhancement treatment resulted in reduction of cook losses in the steaks sliced from about 32% to only 6% (Figure 2-7). For subsequent work on this LTLT approach to tenderization (experiment 3) all meat was injected with this same salt/phosphate solution prior to any further tenderizing treatment.

**Experiment 3. Comparison of tenderization by LTLT heating (56 °C only) vs. by injection of bromelain.**

LTLT treatment was conducted at 56 °C since, per the results of experiment 1, this effected significant tenderization of meat and represents a safe temperature of treatment that could be considered for commercial application. The U.S. Department of Agriculture, Food Safety and Inspection Service (1999) requires beef to be cooked at minimum temperature of 54.4 °C for at least 112 minutes to achieve a minimum lethality of 6.5 log reduction of Salmonella. This temperature also insures virtual elimination of vegetative cells of C. perfringens (Juneja, Marks, Huang, & Thippareddi, 2011; Willardsen, Busta, Allen, & Smith, 1978).
Changes in SSF

As expected from the results of Experiment 1, significant decreases (P < 0.05) were noted in SSF of grilled steaks, which had received the LTLT treatment as compared to the control (Figure 2-8). Likewise SSF of the bromelain-treated steaks decreased upon grilling, to a level not statistically different from the LTLT treatment in SSF (P < 0.05). Informal tasting of these steaks revealed a quite uneven and more mushy texture in steaks treated by bromelain injection as compared to by LTLT (Figure 2-10), the latter having a more consistent steak-like texture.

This result was obtained despite that the bromelain treatment used (14.5 ppm) being that suggested by Sullivan (personal communication, 2013) as the maximum which would effect significant tenderization without any negative textural characteristics (typically, mushiness). However, proper distribution of an injected plant-derived protease like bromelain within muscle is quite difficult to achieve, even when the solution is injected via multiple locations and tumbling is employed, as in the present experiment. This is because proteases are proteins, which are too large in molecular weight to easily move through muscle membrane (Carvajal-Rondanelli, 2003). The result then is a localized tenderizing effect at the points of injection where the enzyme solution pools, resulting in a localized mushy texture.
Changes in Collagen

The soluble collagen fraction in steaks cooked to 65 °C internal (about 6-10% of total collagen) did not differ significantly due to treatment (Figure 2-11, P < 0.05). The TUF fraction decreased significantly (p < 0.005) accompanied by a significant increase in the ELF fraction (P < 0.01) in LTLT steaks as compared to both the bromelain treatment and the control (the latter two treatments did not differ significantly in ELF or TUF (P < 0.05)). This suggests that cross-linked collagen only partially denatures during LTLT, to a form that becomes pronase-susceptible, and that it is this conversion of collagen from the native to an intermediate molten globule form (not to fully denatured gelatin), which may be largely responsible for tenderization of ST meat (Powell, Hunt, & Dikeman 2000).

Evidence of proteolysis

No evidence of marked proteolytic degradation of myosin heavy chain (MHC) was noted for any of the steak treatments by SDS-PAGE (Figure 2-12). Some decrease in the MHC band could be noted for the LTLT and bromelain treatments but the change was small (lsd = 0.01) as compared to the control. A slight increase of TCA absorbance (possible evidence of peptide production by proteolysis) was observed during LTLT (Figure 2-13, lsd = 0.1291) but not in the bromelain-treated steaks, as compared to the control.

Laakkonen, Wellington, & Sherbon (1970b) postulated that tenderization during LTLT heating could be due to catheptic activity. M. Christensen, L. Christensen, Ertbjerg, & Aaslyng, (2011) associated the decrease of meat toughness during LTLT with a measured
increase in residual cathepsin activity after heating; however, they did not directly measure proteolysis of meat proteins in this case. Wang & Xiong (1999) however suggested that cystatin, a cathepsin inhibitor inhibitor, binds to cathepsin L and B in beef muscle, preventing catheptic activity in intact meat during heating.

We were particularly surprised that the bromelain treated steaks, which clearly decreased in SSF during grilling and which exhibited a mushy texture, did not show evidence of MHC breakdown by SDS-PAGE. However, Foegeding & Larick (1986) and Kang & Rice (1970) both presented evidence that bromelain preferentially degrades collagen as opposed to myofibrillar protein. Although collagen degradation is not easily measured by SDS-PAGE it seems likely that tenderization of ST by bromelain in this experiment was largely the result of collagen degradation by the enzyme since marked tenderization occurred and yet no MHC degradation was noted.

Clearly a role of autolytic activity by cathepsins in the tenderization by LTLT heating cannot be absolutely ruled out by the present evidence. However, cathepsins are known to be quite active against myosin and other myofibrillar proteins (Draper & Zeece, 1989; Okitani, Matsukura, Kato, & Fujimaki, 1980) such that, if this was the chief mechanism of this tenderization by LTLT heating, more marked degradation of MHC would have been expected in the LTLT treated steaks. Lack of evidence for MHC degradation in bromelain
treated steaks is more easily explained by the greater affinity of bromelain for collagen than for myofibrillar proteins (Foegeding & Larick 1986; Kang & Rice 1970).

**Cook Yields**

The pH of ST meat after salt/phosphate injection was about 5.8 (data not shown); this increase in meat pH is partially reflective of the increase in water holding capacity effected by salt/phosphate injection. As expected, the cook yields for all treatments were reasonably high and no significant differences were noted between treatments (Figure 2-9, p < 0.05).

**Effects on Meat Color**

The visually assessed color of LTLT steaks after grilling was quite pink, indicating that the LTLT treatment, though rigorous enough to produce a pasteurized product with marked increase in tenderness, was yet sufficiently mild to still allow grilling to a ‘medium’ degree of doneness, which is desired by many restaurant consumers.

**Conclusions**

Tenderization of ST meat initiates at temperatures just above 50 °C for isothermal heating. The present evidence would suggest that tenderization results primarily from the partial unfolding of cross-linked collagen as revealed by a significant increase in ELF, while
conversion to gelatin (RSF) was not related to grilled steak tenderness. Proteolysis of MHC in these samples was slight at most, and thus it would seem unlikely that catheptic-induced autolysis could explain the marked increase in meat tenderness induced by LTLT at 56 °C/24 hr. It remains unclear why this heat-induced tenderization reaction is so dramatically switched on at just above 50 °C (isothermal) and further study is needed to better understand the changes that occur in cross-linked collagen in the 50 – 59 °C range of isothermal (LTLT) heating.

The practical implications of this work are that LTLT heating can possibly provide a good way to more uniformly and consistently tenderize tough, lower valued cuts of meat to be suitable for grilling than by blade tenderization or the application of plant-derived meat tenderizing enzymes. Enhancement with salt and phosphate helps retain the yield and succulence of meat during the LTLT treatment, which besides tenderizing the meat provides a pasteurization treatment to the injected meat. Because the meat color appears not to be strongly affected by LTLT at 56 °C for up to 24 hr, tenderization by this method assures the safety of a pasteurized product regardless of the final degree of doneness to which the steak is grilled.

**Acknowledgement**

The authors gratefully acknowledge the contribution of Enzyme Development Corporation for providing Bromelain® 240 for this present work.
1. ½ inch thick steak is cut to 5 cm width
2. Slice 5 cm wide section
3. Run test with SSF blade fixed to Instron

Photo shows the 1x5 cm strips on their sides (1/2 inch thick; cooked surfaces at either side)

Shear line

Figure 2-1. SSF apparatus
Figure 2-2. Schematic flow chart of pronase susceptibility assay (Powell, Hunt & Dikeman, 2000)
Figure 2-3. Effect of LTLT on SSF when semitendinosus muscle was held isothermally at 24 hr and 48 hr.
Figure 2-4. Effect of LTLT on cook loss when semitendinosus muscle was held isothermally at 24 hr and 48 hr.
Figure 2-5. Effect of LTLT heating at 50 °C, 51.5 °C, and 53 °C on SSF of semitendinosus muscle.
Figure 2-6. Effect of LTLT heating at 50 °C, 51.5 °C, and 53 °C on cook loss on semitendinosus muscle
Figure 2-7. Effect of 15% salt/phosphate (S/P) injection after LTLT heating at 53 °C / 24 hr on semitendinosus muscle
Figure 2-8. Effect of SSF of semitendinosus muscle on LTLT at 56 °C / 24 hr, bromelain and control after grilling to 65 °C
Figure 2-9. Effect of cook yield of semitendinosus muscle on LTLT at 56 °C/24 hr, bromelain and control grilled to 65 °C internal temp.
Figure 2-10. Photograph of ST muscle sliced steaks obtained from LTLT at 56 °C and bromelain treatment grilled to 65 °C internal temp.
Figure 2-11. Relative content of three measured collagen fractions of LTLT 56 °C/24 hr, bromelain, and control grilled to 65 °C internal temp.
Figure 2-12. Effect of LTLT at 56 °C/24 hr, bromelain and control on MHC proteolysis measured by SDS PAGE
Figure 2-13. Effect of LTLT at 56 °C/24 hr, bromelain, and control measured by TCA absorbance at 280 nm
References


