

**SELECTED SKELETAL ALTERATION  
TO IMPROVE BEEF TENDERNESS**

by

Cathy Jean Cotroneo

Thesis submitted to the Faculty of the  
Virginia Polytechnic and State University  
in partial fulfillment of the requirements for the degree of

**Master of Science**

in

Food Science and Technology

APPROVED:

  
James R. Claus, Chairman

  
Norman G. Marriott

  
Janet M. Johnson

July 16, 1992  
Blacksburg, Virginia

C.2

LD  
5605  
V555  
1992  
C687  
C.2

SELECTED SKELETAL ALTERATION TO  
IMPROVE BEEF TENDERNESS

by

Cathy Jean Cotroneo

Committee Chairman: James R. Claus  
Food Science and Technology

(ABSTRACT)

A prerigor cut was made through the 12th thoracic vertebrae of one side of a beef carcass, while the other side, processed conventionally, served as the control. The effect of this cut upon yield grade, sensory attributes and tenderness of *Longissimus* steaks was determined. The treatment caused no differences ( $P>0.05$ ) in yield grade or in CIE  $L^*$   $a^*$   $b^*$  values and oxymyoglobin, reduced myoglobin and metmyoglobin values measured on samples at the treatment site. Sensory panel ratings of visual attributes of color and overall appearance were less desirable ( $P<0.05$ ) for the treated sides, while for visible texture ratings, there was no significant difference. Purge, cooking loss, percentage moisture, fat and protein, and total collagen were not affected by the treatment ( $P>0.05$ ). Sarcomere length, and sensory panel ratings of myofibrillar tenderness, connective tissue, and overall tenderness were significantly more desirable for the treated sides. There was no significant difference in fragmentation index or peak force values between treatments, although these values tended to be lower for the treated sides than for the control sides.

## ACKNOWLEDGEMENTS

The author wishes to express sincere thanks to Dr. James R. Claus for his patience, guidance, and friendship throughout the course of this study and thesis preparation. She would also like to recognize her committee members, Dr. Norman G. Marriott, and Dr. Janet M. Johnson for their counsel. Special acknowledgement is extended to the Virginia Cattle Industry Board for the financial support provided.

The assistance of Dr. Hengjian Wang with methodology, and the sensory panel was greatly appreciated. The author would like to recognize Joe Boling for his invaluable assistance with statistical analysis, and Dennis Shaw for the unlimited use of his computer. Thanks are due to the panelists for their participation in sensory evaluation and for their outstanding performance.

The author is grateful to Stephen and Karina Sylvia for their help during research and writing, and for their friendship.

Thanks are extended to James and Florence Cotroneo, the author's parents, for their continued support during the author's educational career, without whom, it would not have been possible. The author is especially grateful for the love, friendship, and support of her husband, Dale Richard Ludwig.

## Table of Contents

	Page
1.0	Introduction..... 1
2.0	Review of Literature..... 7
2.1	Physiological Maturity..... 7
2.2	Muscle Characteristics..... 7
2.2.1	Tenderness Differences Among Muscles..... 8
2.2.2	Fiber Size..... 8
2.2.3	Sarcomere Length..... 9
2.2.4	State of Crossbridges..... 10
2.2.5	Fiber Orientation..... 11
2.2.6	Myofibrillar Fragmentation..... 11
2.2.7	Connective Tissue..... 12
2.3	Chemical Characteristics..... 14
2.3.1	Moisture.....14
2.3.2	Intramuscular Fat..... 15
2.3.3	Collagen Solubility..... 16
2.3.4	Endogenous Enzymes..... 17
2.3.4.1	Calcium Dependent Proteases..... 17
2.3.4.2	Cathepsins.....18
2.3.5	pH.....20
2.3.6	Water Holding Capacity..... 21
2.3.7	ATP Degradation..... 22
2.4	Factors Influencing the Contractile State of Muscles.....23
2.4.1	Postmortem Temperature..... 23
2.4.2	Chilling Rate..... 24
2.4.3	Aging Time..... 25
2.5	Postmortem Technologies..... 26
2.5.1	Alternative Carcass Suspension..... 26
2.5.2	Skeletal Restraint and Stretching..... 27
2.5.3	Electrical Stimulation..... 29
2.5.4	Hot-boning Prerigor..... 30
2.5.5	High Temperature Aging..... 32
2.5.6	Prerigor Cookery..... 33
2.5.6.1	Cooking Conditions..... 33
2.5.6.2	Cooking Methods..... 36
2.5.7	Vacuum Aging..... 37
2.5.8	Mechanical Tenderization..... 38
2.6	Added Ingredients..... 39
2.6.1	Exogenous Enzymes..... 39
2.6.2	Calcium Chloride and Sodium Chloride..... 40
2.6.3	Calcium Chelators..... 42
2.6.4	Organic Acids..... 42

<b>3.0</b>	<b>Materials and Methods.....</b>	<b>45</b>
3.1	Sampling and Storage.....	45
3.2	Sensory and Instrumental Color Determinations.....	47
3.3	Thaw and Cooking Loss Evaluation.....	48
3.4	Warner-Bratzler Shear Determinations.....	49
3.5	Sensory Evaluation.....	49
3.6	Laser Sarcomere Measurements.....	50
3.7	Fragmentation Index.....	51
3.8	Chemical Determinations.....	51
3.9	Statistical Analysis.....	52
<b>4.0</b>	<b>Results and Discussion.....</b>	<b>57</b>
4.1	Treatment Effects on Physical Carcass Characteristics.....	57
4.2	Chemical Analyses.....	59
4.3	Thaw and Cooking Loss Evaluation.....	59
4.4	Tenderness Evaluation.....	61
4.4.1	Warner-Bratzler Shear Force.....	61
4.4.2	Fragmentation Index.....	69
4.4.3	Sarcomere Length.....	71
4.4.4	Sensory Panel.....	75
<b>5.0</b>	<b>Summary and Conclusions.....</b>	<b>82</b>
<b>6.0</b>	<b>References.....</b>	<b>86</b>
<b>7.0</b>	<b>Appendix A.....</b>	<b>97</b>
<b>8.0</b>	<b>Appendix B.....</b>	<b>102</b>
<b>Vita.....</b>		<b>121</b>

## List of Tables

Table		Page
1.	Effects of prerigor treatment on various physical and sensory traits of beef longissimus muscle.....	58
2.	Effects of zone on various physical and sensory traits of beef longissimus muscle.....	60
3.	Effects of aging on various physical and sensory traits of beef longissimus muscle.....	62
4.	Effects of aging on various physical and sensory traits of beef longissimus muscle by treatment.....	63
5.	Effects of core location on various physical and sensory traits of beef longissimus muscle.....	65
6.	Pearson correlation coefficients of sensory palatability with instrumental measures of tenderness.....	70

## List of Figures

Figure		Page
1.	Location and allotment of steaks for specific analysis.....	54
2.	Core locations within a longissimus muscle steak.....	55
3.	Experimental Design.....	56
4.	Effect of zone upon Warner-Bratzler peak force and total energy values and sarcomere length by treatment.....	66
5.	Peak force values by zone and core location for beef steaks.....	68
6.	Effect of zone on sarcomere lengths of beef steaks by treatment.....	72
7.	Effect of zone upon sensory panel ratings of beef steaks.....	74
8.	Effect of treatment upon sensory panel ratings of beef steaks.....	76
9.	Effect of aging time upon sensory panel ratings of overall and myofibrillar tenderness of beef steaks by treatment.....	78
10.	Overall tenderness ratings by zone and core location.....	81
11.	Total energy values by zone and core location....	98
12.	Sarcomere lengths by zone and core location....	99
13.	Myofibrillar tenderness ratings by zone and core location.....	100
14.	Connective tissue ratings by zone and core location.....	101

## CHAPTER I

### INTRODUCTION

Meat tenderness has been researched more than any other palatability factor (Bailey, 1972). This phenomenon is attributable to the importance of tenderness to the consumer's judgment of quality (Judge et al., 1989). Indeed, research results on beef tenderness improvement are abundant. Physical, chemical, and postmortem technologies for improving beef tenderness have been explored, although most tend to have some disadvantages.

Because of diet and health concerns, contemporary consumers are demanding leaner beef (Sweeten et al., 1990). The beef industry has responded to this need by physically removing adipose tissue and by genetically producing beef with more muscle and less fat (Sweeten et al., 1990). However, quality has suffered. Smith et al. (1976) found that lamb carcasses with more fat chilled more slowly, sustained less sarcomere shortening, and were more tender than those with less fat, suggesting that meat from the latter would not be as palatable as the former. Indeed, steaks from beef containing minimal subcutaneous fat (Tatum et al., 1982) and marbling (Berry and Leddy, 1990) were less tender than those with more subcutaneous fat and marbling. The amount of subcutaneous fat

can affect tenderness by having an insulative effect during chilling, thereby decreasing the rate of temperature decline, which can lessen the extent of cold shortening (Smith et al., 1976).

Tenderness variability among animals and muscles, and even within muscles, is a major problem in the beef industry today. Differences in tenderness between animals are attributable to variation in chronological age (Herring et al., 1967), collagen solubility (Culler et al., 1978), and marbling (Romans et al., 1985). Tenderness variation among muscles is obvious. Differences in sarcomere length, collagen solubility, protease activity, and overall tenderness can be found among *Psoas major*, *Biceps femoris*, and longissimus muscles (Koochmaraie et al., 1988b). Many researchers have discovered differences due to location within a muscle. Crouse et al. (1989) and Howard and Judge (1968) reported that medially located cores were less tender than laterally located cores, while other researchers (Tuma et al., 1962; Williams et al., 1983) observed the exact opposite.

Physical alteration of carcasses to improve beef tenderness has been considered. Hostetler et al. (1972) studied the effect of carcass suspension on bovine muscles. They investigated five different carcass suspension methods: conventional; horizontal; neck-tied, suspended by cervical vertebrae; hip-free, suspended by obturator foramen with limbs free; and hip-tied, the same as previous, but with limbs tied;

and concluded that the hip-free method resulted in the longest sarcomeres, and therefore, the greatest tenderness. This treatment, however, has not been adopted because it requires a different hanging method than what is currently used in the industry.

Stouffer et al. (1971) patented a prerigor method of tenderizing muscles by applying tension with weights or a mechanical device designed for this purpose. The device targets the middle meats, front leg, rear leg, and rump portions of bovine, ovine, and porcine species, by serving as a weight to cause stretching. Improved tenderness can result from stretching of the muscle by using another device also proposed by Stouffer et al. (1971). This instrument consists of a number of pins attached to each end of an adjustable bar. The pins can be inserted directly into the muscle of interest and anchored at another point, allowing extension of the prerigor muscle. While all three methods of tensioning reduced shear force values, they require extra time and equipment for tensioning and are not currently used.

Blade tenderization is a method of mechanical manipulation to produce a more tender product. It reduces shear force values significantly (Savell et al., 1982). However, the process can decrease moisture in meat (Savell et al., 1982).

Electrical stimulation is used to hasten the onset of rigor in order to reduce processing time (Judge et al., 1989)

and has been found to improve tenderness (Cross and Tennent, 1980). This improvement can be attributed to three factors: prevention of cold shortening by acceleration of glycolysis and rigor onset before chilling; proteolytic enzyme activation due to low pH and high temperatures; and physical disruption of fiber structure due to extensive muscle contraction. Electrical stimulation can however have adverse effects on water-holding capacity and color (Judge et al., 1989).

High temperature aging, the holding of postmortem muscle at temperatures of 30-40°C, often used in combination with hot-boning, does not lower shear values compared to conventionally processed muscle (Cecchi et al., 1988). When high temperature aging is combined with hot-boning and electrical stimulation, improved tenderness results. High temperature aging can have adverse effects on color and should be used in conjunction with electrical stimulation to minimize this problem (Babiker and Lawrie, 1983).

Chemical methods of tenderness improvement have also been investigated. Miyada and Tappel (1956) found that proteolytic enzymes, including papain, bromelin, ficin and trypsin were able to hydrolyze collagen, therefore making beef more tender. These enzymes have also been found to degrade myofibrillar proteins, which again, results in improved tenderness. However, use of these enzymes requires extra cost and time and may cause over-tenderization (Romans et al., 1985).

Stanton and Light (1990) investigated the effects of

prerigor lactic acid injection to accelerate conditioning in bovine muscle. They found that lactic acid injection may be used to improve acceptability of poorer quality muscles. However, they did not perform sensory analyses to verify that the method had no adverse effects on flavor or texture.

Weiner and Pearson (1969) studied the effect of calcium chelators (e.g. EDTA) on the shortening of rabbit and porcine muscle. They found that chelators inhibited shortening of the muscle, but observed no significant difference in shear values.

Koohmaraie et al. (1988a) found that calcium chloride ( $\text{CaCl}_2$ ) injections resulted in the acceleration of postmortem tenderness, due to the action of calcium-dependent proteases. Shear force values have been reduced by  $\text{CaCl}_2$  injections (St. Angelo et al., 1991). Injecting  $\text{CaCl}_2$  reduces the time necessary for postmortem storage (Morgan et al., 1991) in order to produce tender meat.  $\text{CaCl}_2$  injections, however, have been found to reduce sarcomere lengths (Morgan et al., 1991) and to cause extensive muscle shortening (Weiner and Pearson, 1969). Metallic (Morgan et al., 1991) and warmed over flavor (St. Angelo et al., 1991) can also result from  $\text{CaCl}_2$  injection.

At present, no single technique has been accepted industry-wide for improving the tenderness of fresh meats due to inconvenience, inefficiency, high cost, or unacceptable

changes in sensory attributes. Despite existing technologies, tenderness problems, including unacceptable and high variability of tenderness still remain. The objective of this research was to explore the effects of selected prerigor skeletal, connective tissue and muscle cuts on USDA yield grade and various physical and sensory properties of *Longissimus* beef steaks.

## CHAPTER II

### REVIEW OF LITERATURE

Most consumers consider tenderness to be the most important attribute of meat (Judge et al., 1989). Thus, meat tenderness has been researched extensively during this century. Tenderness is influenced by many factors and conditions. The review that follows will discuss how these factors relate to tenderness.

#### 2.1 Physiological maturity

As a young animal ages, sarcomere length decreases (Herring et al., 1967), muscle fiber diameter increases (Tuma et al., 1962), shear force values increase and tenderness decreases (Herring et al., 1967). Although the amount of collagen is not age-dependent, the chemical nature of the collagen is affected by animal age (Hill, 1966). Muscles from older animals have less soluble collagen and are consequently less tender (Culler et al., 1978; Herring et al., 1967).

#### 2.2 Muscle Characteristics

Physical characteristics of muscle have a great effect

upon tenderness. Tenderness differs among muscles, and can be influenced by fiber size and orientation, sarcomere length, crossbridge formation, myofibrillar fragmentation, and amount of connective tissue.

### **2.2.1 Tenderness differences among muscles**

It is well documented that bovine muscles differ in tenderness. The *Psoas major* is considered to be very tender, with an average Warner-Bratzler shear value of about 3.2 kg, the *Semimembranosus* is less tender at 5.4 kg and the *Biceps femoris* is intermediate in tenderness at 4.1 kg. The longissimus muscle is rated as slightly tender at 3.8 kg (Judge et al., 1989). Differences in sarcomere length, collagen content and solubility, and protease activity have been found among various muscles (Koochmaraie et al., 1988b). At 1 day postmortem, Koochmaraie et al. (1988b) found that the *Psoas major* was more tender than the *Longissimus dorsi* and *Biceps femoris*, which agrees with the previously stated shear values of Judge et al. (1989).

### **2.2.2 Fiber size**

Swanson et al. (1965) reported that the larger the fiber diameter in beef longissimus muscle, the higher the shear force. Herring et al. (1965) found that as fiber diameter

increased, tenderness decreased. More tension imposed on the muscle prior to rigor completion decreases the postrigor fiber diameters, resulting in more tenderness (Herring et al., 1965; Gillis and Henrickson, 1969). Because the fiber diameter increases as the fiber shortens (Herring et al., 1965), contracted muscle should be less tender. Herring et al. (1967) found that contracted muscle had a larger percentage area of fibers and a smaller percentage area of endomysial and perimysial material than stretched or resting muscle.

### **2.2.3 Sarcomere length**

A sarcomere is the basic contractile unit of the myofibril between two adjacent Z-disks. Locker (1960) discovered that muscles with short sarcomeres were related to less tender meat. Sarcomere length was found to account for 12% of the variation in tenderness among muscles (Hostetler et al., 1972). Herring et al. (1965) confirmed that, in general, the most tender muscles of a beef carcass had the longest sarcomeres. They found sarcomere length to be related to tenderness ( $P < 0.025$ ) as measured by the Warner-Bratzler shear method (Herring et al., 1965). As sarcomere length increased, there was a decrease in shear force and an increase in tenderness (Herring et al., 1965). The opposite was true of decreasing sarcomere lengths. Marsh and Leet (1966) found that muscle shortening of 20-40% resulted in decreased

tenderness. However, they also determined that further shortening of up to 60% of the muscle resulted in increased tenderness. Hostetler et al. (1972) found that tenderness increased up to a length of 2.5  $\mu\text{m}$ , but further increase in sarcomere length did not cause an improvement in tenderness in all muscles studied. This concurs with earlier research in that equal changes in sarcomere length did not produce the same changes in tenderness (Hostetler et al., 1970). Buege and Stouffer (1974a) found that sarcomere lengths were not greatly affected by postmortem storage for up to 10 days. Only minor increases or decreases in sarcomere length were observed during aging.

#### **2.2.4 State of crossbridges**

In muscle contraction, crossbridges between the myofibrillar proteins, actin and myosin, are formed. During rigor, these crossbridges become permanent (Judge et al., 1989). Decreased formation of complexes between actin and myosin appears to be associated with improved tenderness (Weiner and Pearson, 1969). The association of actin with myosin during rigor onset has a negative effect on the solubility of myofibrillar proteins, causing a decrease in tenderness (Honikel et al., 1981a). Marsh and Leet (1966) proposed that the toughness associated with shortened sarcomeres may be the result of increased actomyosin

formation during rigor. Maximum actomyosin formation occurs when muscle is fully contracted (Hostetler et al. 1970).

#### **2.2.5 Fiber orientation**

Eisenhut et al. (1965) have found evidence that fiber angles affect textural properties of the muscle. In a comparison between horizontal and vertical hanging methods, larger fiber angles were found in the muscles from carcasses hung vertically, suggesting that the tension on the muscle was released, resulting in a less tender product (Eisenhut et al., 1965). It was also found that there were differences in fiber arrangement, with the vertical suspension, allowing fibers to shorten (Eisenhut et al., 1965). Fiber angle was also found to affect muscle shape, which is an important consideration of consumers (Eisenhut et al., 1965).

#### **2.2.6 Myofibrillar fragmentation**

Myofibrillar fragmentation is defined as the breaking of myofibrils into shorter segments at or near the Z-disk during postmortem storage of the muscle (Olson et al., 1976). Myofibrillar fragmentation index has a significant correlation with shear force of 0.61 (Moller et al., 1973). Olson et al. (1976) found that a large decline in shear force corresponded to an increase in myofibrillar fragmentation. This

observation suggested that myofibrillar fragmentation was related to tenderness (Moller et al., 1973; Olson et al., 1976). Olson and Parrish (1977) discovered that less tender muscles exhibited reduced myofibrillar degradation, therefore indicating the direct relationship between tenderness and myofibrillar degradation. Culler et al. (1978) stated that myofibrillar fragmentation was a more important effector of tenderness than collagen solubility or sarcomere length. In fact, they concluded that myofibrillar fragmentation accounted for more variation in tenderness than any other factor (Culler et al., 1978).

Olson et al. (1976) found that fragmentation varied with storage temperature and muscle. Myofibrillar fragmentation index was higher in muscle stored at 25°C, than when stored at 2°C (Olson et al., 1976). In general, higher temperatures and longer storage time resulted in more fragmentation, which related to increased tenderness (Olson et al., 1976). Olson and Parrish (1977) also noted that myofibrillar fragmentation increased with postmortem storage time from 1 to 7 days.

### **2.2.7 Connective tissue**

Collagen is abundant in the animal body and significantly influences meat tenderness. Though collagen is the main protein in connective tissue, elastin and reticulin fibers are present and contribute to toughness (Judge et al., 1989). The

distribution of connective tissue is not uniform among muscles, and the amount varies with the physical activity of the muscle (Judge et al., 1989). For example, a less used muscle, as in the spine region, would be more tender than a limb muscle (Judge et al., 1989). Collagen is a component of tendons, ligaments, bones and cartilage. Type I collagen is found in tendons, Type II collagen is the major component of cartilage, and Type III collagen is found in skin and vascular tissues (Bandman, 1987). Collagen is relatively insoluble and strong due to intermolecular crosslinkages, which are fewer and weaker in young animals (Judge et al., 1989). Crosslinking in fibers of often used muscles is extensive, giving a background toughness to meat. The crosslinks in mature collagen are heat stable and retain strength after cooking (Judge et al., 1989). Collagen is resistant to physical force, but can be converted to gelatin by boiling water. Papain, ficin, and bromelin have been found to hydrolyze collagen (Wang et al., 1957; Miyada and Tappel, 1956). Reticulin is thin, branched, and associated with the endomysium (Cassens, 1987). Elastin is yellow connective tissue that is heat stable to 150°C (Bandman, 1987) and can stretch up to 150% of its original length (Cassens, 1987). Elastin is much less abundant than collagen, and does not have as great an effect on tenderness (Judge et al., 1989). Trypsin, chymotrypsin, pepsin and cathepsins have no effect on elastin, though it can be degraded by some enzymes (Bandman,

1987).

## **2.3 Chemical characteristics**

The chemical characteristics as well as the physical characteristics of a muscle are important to meat tenderness. How these characteristics change postmortem is associated with variations in tenderness.

### **2.3.1 Moisture**

Fresh meat is approximately 75% moisture (Judge et al., 1989). Increases in fat cause decreases in water content (Wismer-Pedersen, 1987). During postmortem processes, the changes of water structure and movements are important to the quality of meat, especially tenderness and juiciness (Honikel, 1989). Water, along with intramuscular lipids are the main sources of juiciness in meat (Judge et al., 1989). Moisture loss occurs from exposed muscle surfaces, affecting weight, color, texture and firmness of raw meat, and juiciness and tenderness of cooked meat (Judge et al., 1989). This loss can occur during chilling, freezing, thawing, processing and heating, all of which may have adverse effects on tenderness (Honikel, 1989). Judge et al. (1989) stated that aging muscle improves water holding capacity, thereby improving tenderness.

Cooking at lower temperatures causes improved water retention (Judge et al., 1989).

### **2.3.2 Intramuscular Fat**

A certain level of marbling is necessary to ensure juiciness and flavor of meat (Romans et al., 1985). Marbling present in the exposed beef longissimus muscle at the 12-13th rib interface, along with maturity, form the basis of the USDA quality grades of U.S. Prime, Choice, and Good [now Select] (Savell et al., 1986). Meat is separated by the USDA into 8 categories according to marbling level, from moderately abundant to practically devoid (Savell et al., 1986). In a study where the steaks were separated into 3 groups according to marbling level 1) small or less 2) modest and moderate and 3) slightly abundant or greater, the total cooking time was the longest for the steaks with the lowest marbling (Cross, 1977). The moisture content was highest in the low marbling groups, and evaporative or drip cooking losses did not differ among groups (Cross, 1977). Even or uneven distribution of marbling did not significantly affect cooking losses, degree of doneness, or percentages of fat or moisture (Cross, 1977). Cross (1977) concluded that cooking times were affected by amount, distribution, and texture of marbling. Savell et al. (1986) found that the highest marbling level was associated with the highest percentage of ether extractable fat and the

lowest percentage moisture. In a consumer panel, marbling level and degree of doneness significantly affected overall desirability ratings (Savell et al., 1987). A decrease in marbling level corresponded to a decrease in rating of overall desirability with slightly abundant marbling steaks receiving the highest rating and traces of marbling steaks receiving the lowest rating (Savell et al., 1987). U.S.D.A. requirements for marbling, in general, increase with the age of the animal. Therefore, meat from older animals must have a higher degree of marbling to obtain the same grade as a younger animal with less marbling (Romans et al., 1985). In general, the highest grade of meat has the most marbling (Romans et al., 1985).

### **2.3.3 Collagen solubility**

Cross et al. (1973) found that percentage soluble collagen was important in sensory and mechanical measures of tenderness. Increased solubility results in improved tenderness (Judge et al., 1989). In a study comparing different muscles, it was expected that the muscle with the highest percentage of solubilized collagen would be the most tender (Cross et al., 1973). However, it was found that the amount of solubilized rather than total collagen was a significant factor in determining muscle tenderness (Cross et al., 1973).

### **2.3.4 Endogenous enzymes**

#### **2.3.4.1 Calcium dependent proteases**

Calcium dependent proteases, known by a variety of names including CAF (calcium activated factor) and currently calpains, represent a class of endogenous neutral proteases active in postmortem bovine muscle that are responsible for myofibrillar protein degradation during postmortem aging (Olson and Parrish, 1977). Calcium dependent protease (CDP) has been found to degrade titin, also known as connectin (Lusby et al., 1983). The presence of CDP causes myofibrillar fragmentation, thereby increasing tenderness (Zeece et al., 1986). Myofibrils treated with CDP exhibited a release of soluble protein dependent on pH and temperature (Zeece et al., 1986). CDP has an optimum pH of around 7.5 (Zeece et al., 1986) and an optimum temperature of 37°C (Bechtel and Parrish, 1983; Lusby et al., 1983). At this temperature it was found that there was more protein degradation than at temperatures of 2-4°C and 23-25°C (Bechtel and Parrish, 1983; Lusby et al., 1983). Decreasing the temperature resulted in a reduced action of the enzyme, which in turn caused a decreased production of solubilized protein (Zeece et al., 1986). Zeece et al. (1986), like the others, found that at 5°C, only a small amount of protein was solubilized. At the optimum pH, CDP removes Z-disks from intact myofibrils, degrades troponin,

tropomyosin, and C-protein components of muscle (Robbins et al., 1979). Robbins et al. (1979) claimed that since CDP has an optimum pH of 7.5, it is not expected to be an important factor in the postmortem breakdown of myofibrils due to the postmortem pH conditions. However, Koohmaraie et al. (1988a) have found that calpains do play a major role in postmortem improvements of tenderness. Increases in CDP inhibitor activity are associated with higher Warner-Bratzler shear values (Johnson et al., 1990). Infusion with calcium chloride results in loss of CDP-I and inhibitor activities, and a decrease in CDP-II activity within 24 hr, which should result in increased tenderness (Koohmaraie et al., 1988a).

#### **2.3.4.2 Cathepsins**

Cathepsins are proteolytic enzymes found in muscle cells. They are held in an inactive state in lysosomes and are released as the pH of the muscle drops postmortem and begin to degrade protein structures of the muscle (Judge et al., 1989).

Cathepsin B degrades myosin and troponin T (Ouali et al., 1987). Cathepsin L degrades myosin, alpha-actinin, actin, and troponin, tropomyosin, or both (Okitani et al., 1980). As postmortem aging time continues, the correlation between Warner-Bratzler shear and total cathepsin B & L activity increased, suggesting that cathepsins B & L activity could significantly influence shear values during postmortem aging

(Johnson et al., 1990). Increasing levels of cathepsins B & L activity were associated with tenderness improvement in response to aging (Johnson et al., 1990). Since the activity of cathepsins B & L is optimum at pH 4.0-6.0, maximum activity should occur following rigor and during aging (Johnson et al., 1990). Yates et al. (1983) reported that a combination of 37°C for 5 hr postmortem produced conditions that favored the activity of acidic cathepsins, including B and L. A lower temperature (4°C) and a higher pH (7.0) were not as conducive to cathepsin activity (Yates et al., 1983).

Cathepsin D degrades Z-disks of the myofibrils (Robbins et al., 1979). Cathepsin D has been found, by Robbins et al. (1979) to have a significant role in postmortem proteolysis associated with tenderization. However, Zeece and Katoh (1989) found that cathepsin D is probably only a minor contributor to tenderization during proteolysis. Zeece and Katoh (1989) showed that cathepsin D alters the Z-line structure and causes narrowing of the A-band. They also found that at high temperatures (37°C or higher) cathepsin D may play a role in myofibrillar degradation, but acknowledged that myosin degradation could also be due to the action of cathepsins B & L (Zeece and Katoh, 1989). Robbins et al. (1979) found that cathepsin D degrades myofibrils at pH between 5.1 and 5.3, causing alteration in Z-disk structure. Robbins et al. (1979) found that cathepsin D breaks the myosin heavy and light chains into fragments and causes some loss of

proteins in the troponin-tropomyosin complex while actin is unaffected. Incubation of bovine myofibrils (pH 5.5, 37°C) resulted in the degradation of the myosin heavy chain into two major groups of fragments after 30 min (Zeece and Katoh, 1989). Titin was also susceptible to hydrolysis by cathepsin D (Zeece and Katoh, 1989).

Although the optimum pH of Cathepsin D is acidic (3.0-4.5), it has activity at pH 3.0-6.0 (Zeece and Katoh, 1989). The optimal temperature for cathepsin D is 45°C (Zeece and Katoh, 1989). Cathepsin D is stable up to 60°C, and it may be active during cooking processes (Zeece and Katoh, 1989). After 120 min of incubation at 37°C, myosin and actin had been degraded and traces of titin, tropomyosin and troponin remained (Zeece et al., 1986b)

#### **2.3.5 pH**

The pH of muscle drops postmortem because of the production of lactic acid. The pH of the muscle seems to affect many tenderness factors. In a comparison between bulls and steers, the effect of pH was found to be highly significant in Warner-Bratzler shear force, sarcomere length, expressed juice, and cooking loss (Purchas, 1990). Purchas (1990) found that an ultimate pH of 6.1 of the *Longissimus dorsi* muscle, after cooking by immersion in a 70°C water bath for 90 min, gave the highest shear values with tenderness

improving on either side of this pH. Above pH 6.1, tenderness may have improved due to changes in proteolytic activity caused by calcium-activated proteases which have a pH optimum close to 7.0. The increase in shear values from pH 5.5 to 6.2 may be due to the effect of pH on sarcomere length (Purchas, 1990). Although Honikel et al. (1981a) found that prerigor pH had no influence on sarcomere length, Purchas (1990) found that as the pH was elevated, sarcomere length decreased until a pH of 6.3 was attained, after which it increased. Marsh and Leet (1966) showed that a rapid decline of pH in rapidly chilled muscles could result in cold shortening. Conditions of high early postmortem temperature and low pH may stimulate enzyme activity and therefore cause increased tenderness (Marsh, 1981).

A postmortem fall of pH causes a decrease in water holding capacity as measured by cooking loss (Honikel et al., 1981a; Honikel et al., 1981b; Purchas, 1990). A decrease in pH was also found to cause a decrease in myofibrillar protein solubility between pH 6.6 and 6.1. Below 6.1, a large decrease in protein solubility was noted (Honikel et al., 1981a). An increase in pH caused a decrease in expressed juice (Purchas, 1990).

### **2.3.6 Water holding capacity**

Many of the physical properties of meat, including

tenderness and juiciness of cooked meat, are affected by water holding capacity (Judge et al., 1989). Judge et al. (1989) defined water holding capacity as the ability of meat to retain its water during application of external forces such as cutting, heating or grinding. The postmortem pH decline causes a decrease in the number of reactive groups on proteins available for water binding (Judge et al., 1989). This occurs because as the pH approaches the isoelectric point of myofibrillar proteins, the positively and negatively charged groups of proteins are attracted to each other, and only those not paired are able to bind water (Judge et al., 1989). Honikel et al. (1981a) found that shortening of sarcomeres may have some relation to water retention. The postmortem fall of pH causes decreases in water holding capacity of both salted and unsalted muscle (Honikel et al., 1981a). If the pH of rigor muscle were raised to that of prerigor muscle, the result would be higher water holding capacity (Honikel et al., 1981a). Honikel et al. (1981a) determined that 66% of the decrease of water holding capacity postmortem was due to the development of rigor and 33% was due to the fall of pH.

### **2.3.7 ATP degradation**

Shortly after death, ATP is depleted from the muscle. Energy is no longer available to break the actomyosin bonds and the result is the formation of permanent crossbridges,

which, in the absence of ATP, are irreversible (Judge et al., 1989). Rigor mortis occurs at an ATP level of about 1  $\mu\text{mol/g}$  (Honikel et al., 1981a). Only after ATP is depleted (at pH of about 5.9), does the calcium pump become inactive, causing the release of calcium. Under these conditions, rigor shortening can occur (Honikel et al., 1981b). A rapid rate of ATP degradation was found to have no adverse effect on shear resistance (Busch et al., 1967).

#### **2.4. Factors influencing the contractile state of muscles**

The state of contraction of muscle is a major influencing factor upon tenderness. Postmortem temperature and chilling temperatures, as well as rate of chilling and aging time can affect the state of contraction of the muscle.

##### **2.4.1 Postmortem temperature**

Temperature is an important factor when considering meat tenderness. Low temperatures (0-10°C) have been found to cause shortening in beef and lamb muscles excised prerigor, a phenomenon known as cold shortening (Locker and Hagyard, 1963). Muscles removed immediately postmortem can shorten by 50-60% when placed at 2°C (Bechtel, 1986). Locker and Hagyard (1963) found that maximum shortening of the muscle occurred at 0°C, intermediate shortening at 37°C, and minimum shortening

at 14-19°C. This observation suggests that the optimum postmortem temperature of meat is between 14 and 19°C. This conclusion is supported by the discovery that storing meat at 16°C until rigor mortis occurs prevents cold shortening and toughening (Bechtel, 1986). Of the prerigor temperatures of 15°C, 24°C, 28°C, 34°C, and 37°C, it was found that at 15°C the least amount of shortening of the muscle occurred (Locker and Daines, 1975). Busch et al. (1967) found that high postmortem muscle temperature accelerated the rate of pH decline in muscle. Marsh and Leet (1966) found that as postmortem muscle pH decreased, the amount of cold shortening was lessened. To avoid cold shortening, prerigor muscle should be maintained at 15-16°C (Pearson, 1987) until the pH is less than 6.0 (Marsh and Leet, 1966).

Heat rigor is the shortening that occurs under high temperatures (up to 50°C) during rigor onset. It is the result of rapid depletion of ATP stores (Judge et al., 1989). Heat shortening may have adverse effects on tenderness through shortened sarcomeres and decreased protein solubilities (Koh et al., 1987). Heat rigor can be prevented by maintaining postmortem muscle at 15-16°C (Pearson, 1987).

#### **2.4.2 Chilling rate**

There are two considerations when chilling: the rate of chilling and the length of time between slaughter and

chilling. Stanton and Light (1990) found that the best quality meat, as measured by tenderness, was obtained when the carcass was chilled slowly and then allowed to hang. Carcasses are chilled slowly to avoid cold shortening (Dransfield et al., 1981). Marsh and Thompson (1958) found that early chilling followed by freezing before completion of rigor onset resulted in tough meat. In lamb, it was shown that the tenderness of the longissimus muscle increased as the interval between slaughter and chilling was lengthened resulting in better eating quality (McCrae et al., 1971; Marsh et al., 1968).

#### **2.4.3 Aging time**

Storage time of meat is a concern when considering tenderness. There is a decrease in toughness with storage time at 1°C (Dransfield et al., 1981). It was found that in beef, 50% of tenderizing occurred within 4.2 days and 90% occurred within 9.5 days (Dransfield et al., 1981). Shear force decreased with time after death from 0 to 20 days, leveling off after 20 days (Dransfield et al., 1981). These results were similar to those reported by Buege and Stouffer (1974a) who found that shear force decreased with time after death from 2 to 10 days postmortem, in tensioned and non-tensioned muscle. Olson et al. (1976) found a decrease in shear force with postmortem storage time for *Longissimus*

*dorsi*, *Semitendinosus*, and *Psoas* muscles.

## **2.5 Postmortem technologies**

There are certain postmortem technologies that can be applied to improve beef tenderness. Some methods that have been researched include alternative carcass suspension (Herring et al., 1965), skeletal restraint and stretching (Buege and Stouffer, 1974a), and prerigor cookery techniques. Methods currently in use include electrical stimulation, high temperature and vacuum aging, and mechanical tenderization.

### **2.5.1 Alternative carcass suspension**

Some question the conventional method of hanging the carcass by the Achilles tendon. Herring et al. (1965) found that the conventional method of hanging released tension in some muscles, particularly the longissimus muscle. But, according to Buege and Stouffer (1974b), data suggested that in the conventionally hung carcass, the longissimus muscle was naturally under tension due to its attachment to the tubera coxae crest of the ilium. Hostetler et al. (1970) found that the conventional method caused shortening of the longissimus muscle, while hanging by the obturator foramen lessened this shortening.

Although Herring et al. (1965) found that horizontal

suspension (i.e. lying on a table) increased tension on some muscles, particularly the longissimus, Hostetler et al. (1970) pointed out that this method has little practical value. When Hostetler et al. (1972) later tested the five different methods of suspension that have been mentioned, they found that all the methods except the conventional method lengthened the *Longissimus dorsi* muscle because the pelvic limb was brought forward relative to the vertebral column. These workers discovered that all treatments except the neck-tied were found to increase sarcomere length. The hip-free method was found to be the best in improving tenderness and in lengthening sarcomeres, as well as being easy to hang (Hostetler et al., 1972). Quarrier et al. (1972) reported that the conventional method, as opposed to the tenderstretch (hung by obturator foramen) had higher shear force values and shorter sarcomeres.

### **2.5.2 Skeletal restraint and stretching**

Since Locker (1960) found that the final state of a muscle appeared to depend on the strain imposed by the hung carcass, several methods have been sought to improve muscle tenderness through stretching and restraint. The effects of stretching upon sarcomere length, myofibrils, shear force values, drip loss, and tenderness has been well studied.

Stouffer (1975) reported that stretching the back muscles

by hanging weights from the neck of a beef carcass increased the back length by 5-15% and reduced shear force values by 8-19%. The application of prerigor tension as accomplished through weighted or mechanical techniques, was found to reduce shear values, though not uniformly, in the longissimus muscle of both ovine and bovine carcasses (Buege and Stouffer, 1974a). Tenderness differed between the rib and loin regions of both lamb and beef, with shear values reduced more in the rib than in the loin for ovines and vice versa for bovines (Buege and Stouffer, 1974a).

Stretching has also been shown to have an effect on sarcomere length. In the beef *Semitendinosus* muscle, maximum stretch resulted in the longest sarcomeres, followed by slightly stretched and unrestrained, respectively (Cross et al., 1981). Buege and Stouffer (1974a) found that sarcomeres of the longissimus muscle were lengthened when exposed to the lowest tension level treatment, but were not significantly lengthened with a higher amount of tension.

Bruce and Ball (1990) found that there were no differences in drip or cooking loss between the stretched muscle and the muscle at rest. They also discovered that stretching decreased the myofibrillar fragmentation index.

Extension of muscles, especially the *Longissimus dorsi*, has been found to alter tenderness (Martin et al., 1971). Buege and Stouffer (1974a) concluded that a small amount of tension applied to prerigor intact muscle causes an

improvement in tenderness. Buege and Stouffer (1974b) also found that prerigor tension improves tenderness in some muscles, except where optimum tension already exists (i.e. lateral position in loin). In relation to tenderness, however, Herring et al. (1967) found it was more important to prevent postmortem shortening than to ensure maximum stretch.

### **2.5.3 Electrical stimulation**

The effects of electrical stimulation (ES) on specific carcass traits have been variable, due to chilling rate, outside fat cover, pH, and many other factors (Cross and Tennent, 1980). Cross and Tennent (1980) found electrically stimulated carcasses to be more tender than non-stimulated carcasses at all postmortem excision times. ES may tenderize through fiber disruption (Bruce and Ball, 1990). The sarcomeres of electrically stimulated muscle were longer than those from non-stimulated muscle (Koolmees et al., 1986). Shear force was decreased by a small amount by ES (Cross and Tennent, 1980). ES was found to have no significant effect on sensory panel ratings of connective tissue (Cross and Tennent, 1980). ES produces a rapid pH decline early postmortem (Bruce and Ball, 1990).

ES did not affect juiciness, flavor intensity of cooked muscle, and water holding capacity (Cross and Tennent, 1980), although Bruce and Ball (1990) found that ES adversely

affected drip loss. Cross and Tennent (1980) found that the percentage of intramuscular fat, cooking time and degree of doneness were not affected by ES. ES has been found to have no effect upon pelvic fat, heart fat, or fat over the rib eye, or on lean firmness, lean texture, heat ring or marbling (Cross and Tennent, 1980). They also found that ES resulted in positive effects on subjective color evaluations (Cross and Tennent, 1980).

#### **2.5.4 Hot-boning prerigor**

Hot-boning is the removal of muscle or muscle groups from the carcass prior to chilling (West, 1983). The process of hot-boning saves energy by reducing requirements for refrigeration facilities, transportation costs and labor (Ray et al., 1980). The practice of hot-boning is said to give the superior functional characteristics of prerigor meat (West, 1983). However, prerigor excision can result in muscle shortening and toughening (Locker, 1960).

Locker (1960) discovered that beef boned 2 hr after slaughter and chilled was tougher than that chilled on the carcass. This result was later supported by Herring et al. (1965). They found that if the muscles remain attached to the skeleton during the early postmortem phase, tenderness increased. McCrae et al. (1971) showed that prerigor excised muscles shortened more and had higher shear values than those

skeletally attached.

Although Cecchi et al. (1988) determined that cold-boned muscles had lower shear force values than those which were hot-boned [1 hr postmortem], other research refutes these findings. Schmidt and Gilbert (1970) found no difference in shear values for cold-boned and hot-boned muscles [2 hr postmortem] chilled for 24 hr. In addition, muscles hot-boned and chilled for 48 hr were more tender than the controls. Schmidt and Keman (1974) found no significant differences in shear force between hot [1 hr postmortem] and cold-boned muscles. Kastner and Russell (1975) reported that muscles hot-boned at 6 hr postmortem had higher shear values than cold-boned counterparts, whereas muscles hot-boned at 8 and 10 hr postmortem did not have different shear force values than cold-boned muscles. Schmidt and Gilbert (1970) found that hot-boned muscles [2 hr postmortem] chilled for 24 and 48 hr exhibited the same tenderness or better than the cold-boned controls.

Kastner and Russell (1975) found that hot-boning did not affect pH decline and the ultimate pH when compared to those which were cold-boned. In contrast, Cecchi et al. (1988) found that the ultimate pH values were significantly higher in certain hot-boned muscles as compared with those which were cold-boned. Cold-boned muscles were found to have longer sarcomeres than hot-boned counterparts (Cecchi et al., 1988).

Problems with hot-boning include shortening of

sarcomeres, decrease in brightness of color (Cecchi et al., 1988; Cross and Tennent, 1980) and unacceptable tenderness and dryness when roasted (Dransfield et al., 1976). Cross and Tennent (1980) recommended combining electrical stimulation with hot-boning to reduce possible tenderness problems.

#### **2.5.5 High temperature aging**

The process of high temperature aging, or the holding of postmortem muscle under temperatures of 30-40°C (Babiker and Lawrie, 1983) may save time and therefore expenses in meat processing. High temperature aging has been investigated for any effects it may have on the physical traits or palatability of meat. High temperature aging, in combination with hot-boning, resulted in lower shear values than hot-boning alone, but not lower than those that were cold-boned (Cecchi et al., 1988). Babiker and Lawrie (1983) found that hot-boned, electrically stimulated, high temperature aged (30 or 40°C for 5 hr) muscles were significantly more tender than cold-boned, non-stimulated controls held at 5°C. Also, electrically stimulated, hot-boned, high temperature aged muscles were more tender than those which were non-stimulated, hot-boned and held at the same temperature, suggesting that perhaps ES was the key factor (Babiker and Lawrie, 1983). However, hot-boned, electrically stimulated muscles stored at 2°C were significantly more tender than hot-boned electrically

stimulated or non-stimulated muscles stored at 40°C (Babiker and Lawrie, 1983). Indeed, Babiker and Lawrie (1983) concluded that aging at 40°C was less effective than at 30°C. High temperature aging did not affect sarcomere length or collagen content (Cecchi et al., 1988).

Problems with high temperature aging include: increase in microbial load (Babiker and Lawrie, 1983), decrease in water holding capacity at 40°C, and adverse effects on color at temperatures as high as 40°C, though these effects were not as pronounced at 30°C (Babiker and Lawrie, 1983). The adverse effects of high temperature aging on color and water holding capacity were worse in the non-stimulated muscle than in the stimulated muscle, suggesting that when implementing high temperature aging, electrical stimulation should also be used (Babiker and Lawrie, 1983).

## **2.5.6 Prerigor cookery**

### **2.5.6.1 Cooking conditions**

During cooking, two general changes occur: muscle fibers become tougher and connective tissue becomes more tender (Cross, 1987). Therefore, it is appropriate to cook meats containing a large amount of connective tissue for a long period of time and those containing a small amount of connective tissue for a short period of time, to reduce

toughening of muscle fibers (Cross, 1987). Heating results in coagulation and disappearance of I-band filaments, coagulation of A-band filaments, Z-line breakdown, and reduction of sarcomere length (Leander et al., 1980). As temperatures become increasingly higher, disintegration of Z-disks occurs (Judge et al., 1989). Research by Leander et al. (1980) indicated that although higher temperatures altered muscle fiber ultrastructure, these changes did not result in increased tenderness, instead, the muscles became less tender at higher temperatures (Leander et al., 1980). Temperatures as low as 56°C can cause collagen shrinkage, which is accompanied by increased collagen solubility (Judge et al., 1989). At low cookery temperatures (56-58°C), however, tenderization is slow. By raising temperatures to 62-64°C, collagen shrinkage occurs more quickly, temperatures of 72-74°C promote rapid shrinkage of collagen and protein hardening and toughening (Judge et al., 1989).

Increased toughness associated with higher cooking temperature is due to contracted filamentous material in A-bands of sarcomeres (Leander et al., 1980). Bouton et al. (1974) reported that shear force values increased from 60 to 90°C. Differences in tenderness at various temperatures depends on myofibrillar contraction state as well as on animal age (Bouton et al., 1974). A decrease in shear values as cooking temperature was raised from 50 to 60°C was dependent on cooking time (Bouton and Harris, 1981). For stretched

muscle heated for 1 hr at temperatures between 40 and 95°C, shear values decreased between 50 and 60°C and increased until 80°C, after which they decreased again (Beilken et al., 1986).

Heating to 68°C shortened sarcomere length from 2.6  $\mu\text{m}$  (of raw meat) to 2.0  $\mu\text{m}$ , while heating to 73°C reduced sarcomere length to 1.85  $\mu\text{m}$  (Leander et al., 1980). Cooking losses have been shown to increase with higher cooking temperatures (Sanderson and Vail, 1963; Bouton and Harris, 1981). High cooking temperatures reduce water holding capacity (Judge et al., 1989). Bouton et al. (1974) found that adhesion values, a measure of connective tissue strength, for 50°C were 5 times higher than those at 60°C, regardless of myofibrillar contracted state (Bouton et al., 1974). Extending cooking temperatures to approximately 60°C caused tenderization of meat by accelerating aging and reducing connective tissue strength (Beilken et al., 1986).

Time of cooking, as well as cooking temperature affects tenderness. Duration of cooking at 50°C did not affect shear values, but increasing cooking time at 55 or 60°C caused significant decreases in shear values (Beilken et al., 1986). Longer cooking time caused an increase in shear force values for 50°C, but decreased shear force values for steaks cooked at 55, 60, and 65°C (Bouton and Harris, 1981). Cooking at 50°C for 30 min caused no change in shear force values, but increased after 2 hr of cooking at the same temperature (Bouton and Harris, 1972). Increased cooking time showed

higher cooking losses (Bouton and Harris, 1981). Adhesion values decreased by 5 to 1 when cooking time at 90°C was increased from 1 to 3 hr (Bouton et al., 1974). Extended cooking times at temperatures between 55 and 60°C caused a decrease in connective tissue toughness and a significant reduction in myofibrillar toughness (Beilken et al., 1986).

#### **2.5.6.2 Cooking methods**

Several cooking methods have been investigated as to their effects upon the sensory attributes of meat. Noble et al. (1990) found that roasts cooked in conduction ovens were juicier than those prepared by other methods. Meat cooked in a steam oven showed less shortening than meat cooked in a hot water vat or convectional oven, as measured by change in length, width and depth of meat cuts from fresh to cooked meat (Ray et al., 1980). In the same study, cooking in the hot water vat required less time than steam or convectional ovens. The convectional oven method caused the highest cooking losses. This study found that the steaks cooked in the steam oven were the most tender, followed by the convectional oven, then the hot water vat (Ray et al., 1980).

In a study comparing microwaving and boiling water methods of cooking prerigor and postrigor steaks, it was shown that the prerigor samples shortened much more than those that were cooked after rigor onset (Cia and Marsh, 1976).

Microwaving caused more shortening than boiling water, but the difference was not significant (Cia and Marsh, 1976). Ray et al. (1980) found that prerigor cooking has the disadvantage of reduced tenderness and distortion of shape, while Cia and Marsh (1976) found that meat cooked early postmortem was found to be very tender relative to that prepared after rigor onset. Microwaving meat resulted in less cooking loss than boiling (Cia and Marsh, 1976). Overall, microwaving resulted in more tender meat than boiling, possibly due to faster heating (Cia and Marsh, 1976).

Coleman et al. (1988) observed the effects of removing external fat before cooking. They found that removing the external fat before cooking did not affect the cooking yield of strip loin steaks, eye of round and top round roasts, or the sensory properties of these cuts (Coleman et al., 1988). In addition, removal of external fat did not significantly affect shear force values, moisture content, or fat content of the cooked meat. Furthermore, removing the external fat also had the advantage of reduced cooking time.

#### **2.5.7 Vacuum aging**

Vacuum packaging has the advantages of reducing moisture loss and facilitating boxing and handling of meat (Hodges et al., 1974), and lowering the risk of later bacterial contamination (Schmidt and Keman, 1974). Time and temperature

are important factors when considering vacuum aging. Hodges et al. (1974) found that 15 days storage before vacuum packaging increased tenderness, but also increased off-flavors and microbial load. In ground beef patties, microbial numbers increased with storage and became microbiologically unacceptable at the end of 21 days (Bentley et al., 1989). Lee et al. (1990) found an increase in drip loss with increased storage time, although cooked roasts aged 24 and 38 days were rated softer to tooth pressure than those aged at 3 and 10 days. Various optimal aging times for conventionally processed, vacuum packaged beef have been recommended including: 10 days, 2°C (Swatland, 1982), 11 days at 1°C (Smith et al., 1978) and 14 days at 2°C (Hodges et al., 1974).

#### **2.5.8 Mechanical tenderization**

Blade tenderization is a method of mechanical manipulation of meat to produce a more tender product. Some of the less tender cuts may even benefit from blade tenderization twice (Rolan et al., 1988). Savell et al. (1982) found that blade tenderization reduced shear force values significantly and decreased toughness of steaks as measured by muscle fiber tenderness and overall tenderness. Blade tenderization did not affect drip or cooking losses and had little or no effect on juiciness and flavor (Flores et al., 1986; Rolan et al., 1988), although Savell et al. (1982)

found that the process increased dryness of steaks. Rolan et al. (1988) found that blade tenderization resulted in higher overall satisfaction ratings from the sensory panel. Savell et al. (1982) found that blade tenderization improved overall palatability.

## **2.6 Added ingredients**

The practice of injecting substances into meat to improve tenderness has been investigated. Exogenous enzymes, including papain, ficin, and bromelin, and salts such as NaCl and CaCl<sub>2</sub>, have been tested, with some success. Calcium chelators and organic acids also have been researched. The effects of these added ingredients is discussed below.

### **2.6.1 Exogenous enzymes**

Enzymes have been found to have a desirable effect upon tenderness. Enzyme-treated steaks were found to have lower shear values and higher tenderness ratings than control steaks (Rolan et al., 1988). Enzymes degrade muscle fibers and reduce muscle fiber extensibility (Wang et al., 1957). Papain, bromelin, trypsin, ficin and Rhozyme P-11 readily hydrolyze meat proteins, especially actomyosin, with papain having the greatest activity (Tsen and Tappel, 1959). Rolan et al. (1988) reported that while papain and ficin decreased

the amount of detectable connective tissue, at high concentrations, off-flavors were detected. Ficin and papain had no effect on thaw and cooking losses, and while ficin increased frozen storage life, papain decreased it (Rolan et al., 1988). Tsen and Tappel (1959) found that papain hydrolyzed actin and collagen to a very limited extent. Papain, ficin, and bromelain also have been found to degrade collagen and elastin (Wang et al., 1957; Miyada and Tappel, 1956).

#### **2.6.2 Calcium chloride and sodium chloride**

Extensive research on calcium infusions and calcium chelators has been conducted. Koohmaraie et al. (1988a) found that infusion of lamb carcasses with 0.3M calcium chloride (10% of live weight) resulted in acceleration of postmortem tenderization, due to activation of calcium-dependent proteases. St. Angelo et al. (1991) reported that shear force values of electrically stimulated lamb carcasses infused with 0.3M calcium chloride (10% of live weight) were reduced when compared to electrically stimulated non-infused or normally processed carcasses. Koohmaraie et al. (1988a) found that infusion of calcium chloride lowered longissimus muscle shear values during the first 24 hr, but did not change after that. There is evidence that infusion with calcium chloride results in the attainment of the same shear force values within 24 hr

that non-infused treatments showed after 3-7 days of aging (Koochmaraie et al., 1988a). Infusion of prerigor carcasses with calcium chloride eliminated the requirement for extended postmortem storage (Koochmaraie et al., 1988a; St. Angelo et al., 1991).

Although infusion with calcium chloride was found to increase lipid oxidation and warmed over flavor, the use of antioxidants solved this problem without harming the positive effect of calcium infusion or interfering with tenderness (St. Angelo et al., 1991). One negative effect of calcium chloride injections was reported by Weiner and Pearson (1969) in that calcium chloride infusions resulted in extensive muscle shortening, though they did not report on subsequent effects on tenderness.

The process of salting has been considered for its effect on water holding capacity and tenderness. Honikel et al. (1981b) found that the addition of NaCl reduced cooking losses by raising the water holding capacity, but only if salted prerigor. The onset of rigor mortis caused a large decrease in the water holding capacity of salted muscle, but not in unsalted, though the unsalted muscle still had higher cooking losses (Honikel et al., 1981b). Salting had the positive effect of causing a much higher solubility of proteins, especially myosin (Honikel et al., 1981b). Fischer et al. (1982) found that salting of prerigor beef maintains the high water holding capacity of prerigor muscle for several days,

while the water holding capacity of unsalted muscle after 24 hr was poor.

### **2.6.3 Calcium chelators**

The effect of calcium chelators has also been studied. The purpose of chelators is to bind trace amounts of calcium in the actomyosin complex (Weiner and Pearson, 1969). Chelators form a complex with free calcium and therefore inhibit shortening (Weiner and Pearson, 1969). The removal of free calcium is associated with increased tenderness (Weiner and Pearson, 1969). Injection of EDTA, EGTA, or CDTA (calcium chelators) significantly inhibited shortening of the semitendinosus muscle of rabbit (Weiner and Pearson, 1969). Injection of EDTA did not cause a significant difference in shear values, or the association between pH and ATP levels (Weiner and Pearson, 1969).

### **2.6.4 Organic acids**

Organic acid treatments may act to tenderize meat by affecting the properties of collagen (Arganosa and Marriott, 1989). Some research has been done on the effects of lactic acid injection. Stanton and Light (1990) stated that a more rapid decrease in pH (the result of lactic acid injection) should speed activation of muscle cathepsins, and possibly

enhance collagen degradation, therefore causing meat to become tender in less time. The lactic acid injections have not been shown to affect the rate of development of rigor mortis (Stanton and Light, 1990). Whiting and Strange (1990) found that immersion in organic acids (acetic acid 0.5M, lactic acid 0.5M and citrate 0.15M) reduced shear force. Arganosa and Marriott (1989), however, found that treatments using the same three acids resulted in shear values that were not significantly different than those of the controls. They attributed this to the hardening of other muscle proteins, due to the acid. The acid treatments resulted in denaturation of collagen (Arganosa and Marriott, 1989). Although the collagen solubility of the lactic acid treatment was like the unconditioned control, acetic acid and citrate treatments had higher collagen solubilities than conditioned and unconditioned controls (Arganosa and Marriott, 1989). The amount of myofibrillar protein was also decreased by the acid treatments (Arganosa and Marriott, 1989). While Whiting and Strange (1990) found that the samples which had been treated with the acids and then dipped into lactic acid became swollen and translucent as compared to the opaque controls, they found no change in the appearance of cooked steaks. Arganosa and Marriott (1989) found that the acid treatment caused a brownish appearance in uncooked patties and a whiter appearance when cooked as compared to controls. While lactic acid treated steaks had higher cooking losses (Whiting and

Strange, 1990), acetic acid treatments lowered cooking losses (Kotula and Heath, 1986).

## CHAPTER III

### MATERIALS AND METHODS

Five steers were sacrificed at the Virginia Polytechnic Institute and State University abattoir yielding two U.S. Standard and Select carcasses, and one U.S. Choice grade carcass. Approximately 45 min after exsanguination, a cut was made through the 12th thoracic vertebrae, on one randomly selected side of each carcass. The adipose tissue and epimysium on the dorsal side of the *M. longissimus thoracis* (LM) were sliced. A cut was made between the 12th and 13th costal bones, extending 12.5 cm laterally from the lateral side of the LM. The intercostal muscles and connective tissues located ventral to the LM were severed and the *Multifidus dorsi* was cut away from the longissimus to increase the tension on the latter muscle. After completion of the cut, the LM represented the only dorsal attachment that held the posterior portion of the carcass to the anterior portion. This procedure created the Tendercut<sup>TM</sup> treatment, while the other side, processed conventionally, served as the control.

#### 3.1 Sampling and storage

One core from the LM was removed at the time of treatment. Glycolytic activity in this core was halted with

an iodoacetate solution (5mM sodium iodoacetate, 150mM potassium chloride), prepared according to Bendall (1973), and pH was measured on homogenized samples using a Corning deep vessel combination electrode (Corning model 576570, Corning, Inc., Corning, N.Y.) and an Accumet pH meter (Model 925, Fisher Scientific Co., Medford, Maryland). The carcasses were stored in a 4°C cooler for 24 hr postmortem, prior to determining ribeye area and yield grade, and quality grade of the carcass. Percentage stretch of each side was determined by comparing the distance the muscle stretched with the length of the control side from the posterior end of the 6th lumbar vertebrae to the bottom of the 6th thoracic vertebrae. Average percentage stretch among the five carcasses was 7.2 cm (8.7%). After 48 hr postmortem, the short loin region was separated from the carcass by cuts between the 5th and 6th lumbar vertebrae and between the 12th and 13th thoracic vertebrae. The rib was separated from the chuck at the 5th-6th thoracic vertebrae. The short loin region and rib were cut with a Hobart meat saw (Model 5216, The Hobart Manufacturing Company, Troy, Ohio) into 28 steaks and labeled according to Fig. 1. One steak from the Tendercut<sup>TM</sup> side and the control side were removed between the 12th and 13th thoracic vertebrae, immediately vacuum packaged to prevent color differences due to time of processing of the two sides, stored in a 4°C cooler for 30 min to 1 hr. These steaks were then opened, wrapped in polyvinyl chloride (PVC) film and allowed

to oxygenate for 45 min at 4°C prior to visual and instrumental color evaluations. The remainder of the steaks were individually vacuum packaged and stored in a 4°C cooler for 3 or 10 days prior to being frozen (-29°C for 4 hr) and stored (-26°C) until subsequent analysis.

### **3.2 Sensory and Instrumental Color Determinations**

Steaks allotted for visual and instrumental color determinations were placed in a 4°C display case (Tyler Commercial Refrigerator and/or Freezer CGS8M, Tyler Refrigeration Corp., Niles, Mich.) and evaluated under 1076 lux of cold white fluorescent light (30 watts) for color, texture [appearance of cut surface of muscle bundles], and overall appearance by a trained panel of seven using an eight-point scale with 1=least desirable and 8=most desirable. Panelists were trained in three 30 min sessions using PVC wrapped samples under the aforementioned lighting and temperature conditions and were allowed to use color guides when evaluating. PVC wrapped steaks were evaluated for color instrumentally with a Minolta CR-200 Chroma Meter (Minolta Camera Co., Ltd., Osaka, Japan) and a UV-2101 PC scanning spectrophotometer (Shimadzu Scientific Instruments, Inc., Kyoto, Japan). The Chroma Meter was referenced against a standard Minolta calibration plate (No. 20933026; CIE L\* 97.91, a\* -0.70, b\* +2.44). CIE L\* a\* b\* values on three areas

per steak were obtained, using the average of the three readings as a representation of the whole steak. An average from three reflectance readings per steak in the visible light region (400-700 nm) were obtained from the scanning spectrophotometer, which was first baselined with the same reference plate used with the Minolta Chroma Meter. The instrument was used with a sampling interval of 1.0 nm, slit width of 1.0 nm, and a medium scanning speed. Reduced myoglobin, metmyoglobin, and oxymyoglobin were determined by using the ratios of the reflectances at 474/525 nm, 630/525 nm, and the difference between 630-580 nm, respectively (Hunt, 1980).

### **3.3 Thaw and Cooking Loss Evaluation**

Thaw loss was measured by weighing 80 steaks before vacuum packaging and after thawing (36 hr at 4°C) and blotting with a paper towel. Percentage thaw loss was determined by calculating the difference between the two weights and comparing this to the original weight of the steak. Cooking loss was measured by weighing the same steaks after thawing and blotting and after cooking to an internal temperature of 70°C following AMSA cookery guidelines (AMSA, 1978). Cooked steaks were cooled to room temperature, blotted with a paper towel and weighed. Percentage cooking loss was determined by calculating the difference between the two weights and

comparing this to the weight of the steak after thawing.

### **3.4 Warner-Bratzler Shear Determinations**

Steaks were thawed in a 4°C cooler for 36 hr and then cooked according to AMSA cookery guidelines (AMSA, 1978) to an internal temperature of 70°C. Temperature was monitored using an Omega Digital Thermometer (Model #2160-A-7, Omega Engineering, Inc., Stamford Conn.) with copper-constantan thermocouple wires. The thermocouple was placed in the geometric center of each steak. Seven 1.27 cm cores were taken from each steak (cooled to room temperature) in designated locations (Fig. 2). Peak force required to shear through the center of each core was measured and recorded using an Instron (Model 1011, Instron Corp., Canton, Mass.) with a Warner-Bratzler shear attachment.

### **3.5 Sensory Evaluation**

Seven panel members were trained following general guidelines of AMSA (1978) to evaluate steaks treated or selected to have various levels of tenderness (myofibrillar and overall), juiciness, and connective tissue during five 1 hr long sessions in which the panelists were given samples of meat varying in tenderness, juiciness, and connective tissue.

Eighty steaks (8 per session) were thawed in a 4°C cooler for 24 hr and cooked according to AMSA Cookery Guidelines (AMSA, 1978) to an internal temperature of 70°C (temperature monitored as for Warner-Bratzler shear determinations). One 1.27 cm core from each of the 7 designated locations (Fig. 2) was removed and stored in a sealed plastic bag which was placed in a 54°C water bath (30 min) until evaluation. Steaks were evaluated in sensory booths under red light on an 8 point hedonic scale with 1=extremely tough and 8=extremely tender for myofibrillar and overall tenderness; 1=extremely dry and 8=extremely juicy for juiciness; and 1=abundant and 8=none for connective tissue.

### **3.6 Laser Sarcomere Measurements**

Forty steaks were thawed for 24 hr in a 4°C cooler and then 1.27 cm cores were removed from the seven designated locations (Fig. 2). The remainder of each steak was ground and used for subsequent chemical determinations. Fibers were prepared according to the fixed tissue method of Cross et al. (1981). Three fibers were removed from each core, and from each fiber, three sarcomeres were measured. Calculations were made according to the formula presented by Cross et al. (1981).

### **3.7 Fragmentation Index**

Methods followed are those by Davis et al. (1980) with one exception. A Virtishear mechanical homogenizer (Virtis Co., Inc., Gardiner, N.Y.) at 30,000 RPM (full power) was used in place of the Model 45 Macro Homogenizer at 45,000 RPM. A 200 mL fluted glass sample flask (Model No. 16 171413, Virtis Co., Inc., Gardiner, N.Y.) with two standard macro blades aligned and set in reverse position were used in the homogenization (40 sec). Three repeated measurements per steak were taken and the average was calculated for each steak.

### **3.8 Chemical Determinations**

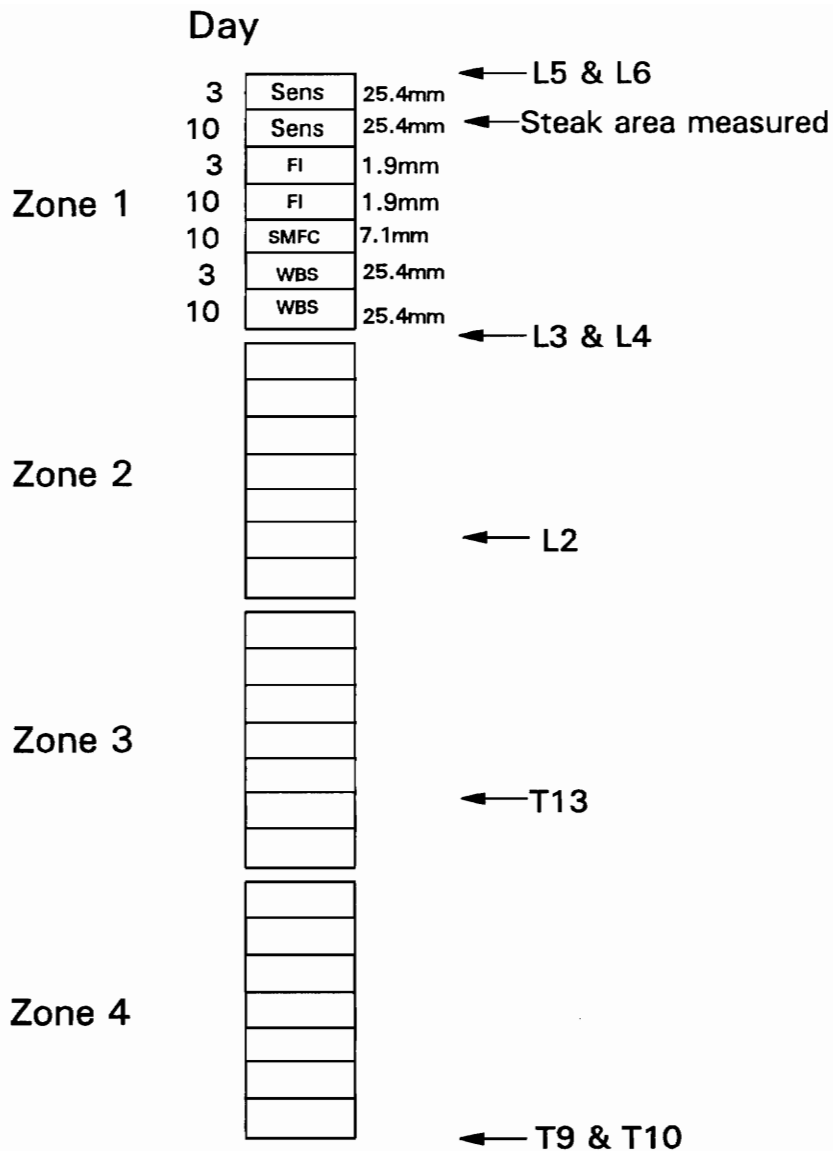
Percentage moisture was determined by measuring the weight loss of duplicate 10 g samples from each steak following AOAC (1990) procedures. Percentage fat was determined on duplicate samples by extracting with petroleum ether in a Goldfish extraction apparatus (AOAC, 1990). Duplicate samples were measured for protein using a Micro-Kjeldahl method (AOAC, 1990). Insoluble, soluble, and total collagen amounts were determined on duplicate samples following the procedures of Hill (1966) and Bergman and Loxley (1963) with slight modifications. Ground samples were thawed slightly in a 4°C cooler, cut into small chunks, refrozen, and

lyophilized for 48 hr. Samples were then analyzed according to Hill and Bergman and Loxley procedures. In the clarifying step, 450 mg and 800 mg of carbon decolorizing agent were added to the supernatants and residuals, respectively. In the following step, 5N NaOH was added until the endpoint color was near, and then 2.5N NaOH was used to achieve the yellow endpoint (pH 6-7). In the next step, the filtrate was washed prior to filtering with 5-10 mL of distilled water.

### **3.9 Statistical Analysis**

Results were analyzed using SAS (1987) as a split-split-split plot design, with the whole plot being the prerigor treatment (Control, Tendercut<sup>TM</sup>) with subsequent splits of zone (1,2,3,4), day (3,10) and core (1-7) (Fig. 3). The split-split-split design was used for analyzing data from Warner-Bratzler shear determinations and sensory panel ratings. A split-split plot (prerigor treatment, zone, core) was used to analyze differences between sarcomere lengths. A split-split design consisting of prerigor treatment, zone, and day was used to evaluate fragmentation index. A split-split design consisting of treatment, zone, and day was used for fragmentation index. A split design consisting of treatment and zone was used for analysis of differences in chemical composition. Differences between treatments in area, visual and instrumental color ratings, and yield grade were analyzed

as a completely randomized block design. Analysis of variance was performed on all data except for the total energy component of the Warner-Bratzler shear force determinations, in which the General Linear Model (GLM) procedure was performed. When there were significant differences in means of the independent variable, dependent variable means were separated using the least significant difference test (SAS, 1987; Lentner and Bishop, 1986) at  $P < 0.05$ . For total energy, means were separated by the standard error predicted difference procedure of GLM (SAS, 1989). Significance of all results was determined at  $P < 0.05$ .



Sens = Sensory Panel  
 FI = Fragmentation Index  
 SMFC = Sarcomeres, Moisture, Fat, Collagen  
 WBS = Warner-Bratzler Shear  
 L = Lumbar Vertebrae  
 T = Thoracic Vertebrae

Figure 1. Location and allotment of steaks for specific analyses

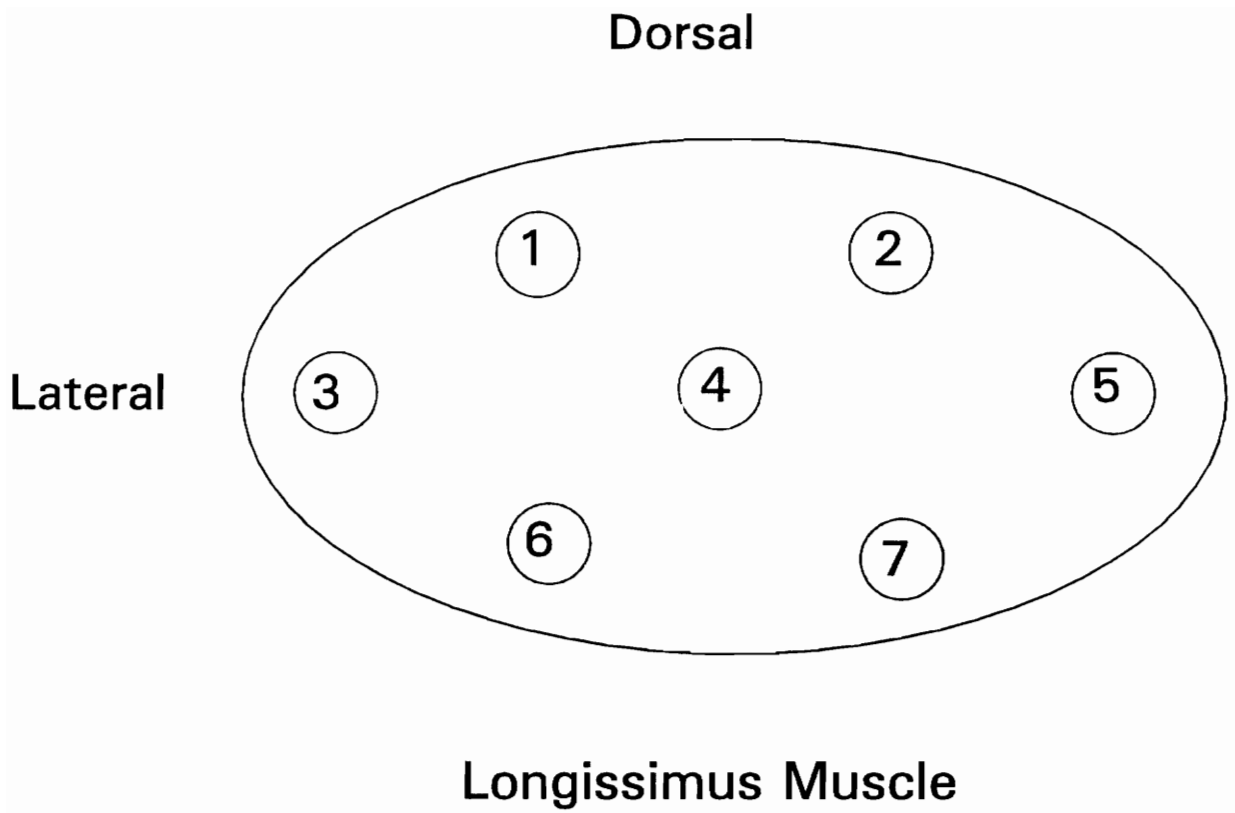


Figure 2. Core locations within a steak

<u>CLASS</u>	<u>LEVEL</u>	<u>VALUE</u>
Prerigor cut	2	Control, Tendercut
Zone	4	1,2,3,4
Storage time	2	3 and 10 days
Core location	7	1,2,3,4,5,6,7
Replication	5	1,2,3,4,5

**Figure 3. Experimental Design**

## CHAPTER IV

### RESULTS AND DISCUSSION

#### 4.1 Treatment effects on carcass physical characteristics

No difference ( $P>0.05$ ) in yield grade between controls and Tendercut<sup>TM</sup> was observed (Table 1), although the controls tended to have a numerically superior yield grade, (1.85) than Tendercut<sup>TM</sup> (2.02). This discrepancy was most likely due to the fact that the treated sides had a slightly, though not significantly smaller rib eye area, with the control having a mean area of 90.19 cm<sup>2</sup> and Tendercut<sup>TM</sup> having a mean area of 86.75 cm<sup>2</sup>. The treated sides were allowed to stretch in such a way that the steaks became somewhat elongated, therefore decreasing the surface area. Steak surface area in each zone (Fig. 1) did not differ significantly by treatment, though zone did have an effect ( $P<0.05$ ) on area (Table 2), with zone 3 having the largest area. This can be explained by differences in muscle shape along the length of the *Longissimus* muscle (Judge et al. 1989).

Steaks removed from the 12th-13th rib interface did not differ ( $P>0.05$ ) due to prerigor treatment in visual sensory panel rating of texture (Table 1). Color and overall appearance ratings, however, were significantly affected by treatment. In both cases, the control had higher ratings

**Table 1 - Effects of prerigor treatment on various physical and sensory traits of beef longissimus muscle**

	Prerigor Treatment		Standard Error
	Control	Tendercut <sup>TM</sup>	
<b>Tenderness indices</b>			
Sarcomere length ( $\mu\text{m}$ ) <sup>c</sup>	1.75 <sup>b</sup>	2.19 <sup>a</sup>	0.06
Fragmentation index	116.89 <sup>a</sup>	106.31 <sup>a</sup>	12.69
Peak force (kg)	3.96 <sup>a</sup>	3.50 <sup>a</sup>	0.30
Total energy (kg*mm)	30.89 <sup>a</sup>	27.45 <sup>a</sup>	2.43
<b>Instrumental color<sup>d</sup></b>			
L <sup>*</sup>	40.85 <sup>a</sup>	40.27 <sup>a</sup>	0.88
a <sup>*</sup>	17.07 <sup>a</sup>	16.62 <sup>a</sup>	1.12
b <sup>*</sup>	6.73 <sup>a</sup>	6.62 <sup>a</sup>	0.78
Oxymyoglobin	19.09 <sup>a</sup>	18.47 <sup>a</sup>	0.83
Metmyoglobin	2.60 <sup>a</sup>	2.38 <sup>a</sup>	0.09
Reduced myoglobin	1.16 <sup>a</sup>	1.14 <sup>a</sup>	0.01
<b>Sensory traits<sup>e</sup></b>			
Color	5.97 <sup>a</sup>	5.40 <sup>b</sup>	0.19
Texture	6.49 <sup>a</sup>	6.20 <sup>a</sup>	0.05
Overall appearance	6.23 <sup>a</sup>	5.57 <sup>b</sup>	0.22
<b>Thaw and cooking loss</b>			
Thaw loss (%)	4.69 <sup>a</sup>	4.34 <sup>a</sup>	0.45
Cooking loss (%)	22.05 <sup>a</sup>	21.90 <sup>a</sup>	0.76
<b>Chemical analyses</b>			
Moisture (%)	73.12 <sup>a</sup>	73.62 <sup>a</sup>	0.29
Fat (%)	2.87 <sup>a</sup>	2.82 <sup>a</sup>	0.15
Protein (%)	24.16 <sup>a</sup>	23.76 <sup>a</sup>	0.48
Total collagen (mg/g)	5.78 <sup>a</sup>	5.67 <sup>a</sup>	0.66
<b>Physical characteristics</b>			
Ribeye area (cm <sup>2</sup> )	90.19 <sup>a</sup>	86.75 <sup>a</sup>	1.54
Overall area (cm <sup>2</sup> ) <sup>f</sup>	80.66 <sup>a</sup>	78.77 <sup>a</sup>	1.87
Yield grade	2.02 <sup>a</sup>	1.85 <sup>a</sup>	0.07

<sup>ab</sup> means within the same row bearing identical superscripts are not different (P>0.05)

<sup>c</sup> Treatment\*zone interaction (P<0.05)

<sup>d</sup> Instrumental color: oxymyoglobin (%R 630 nm - %R 580 nm); metmyoglobin (%R 630 nm / %R 525 nm); reduced myoglobin (%R 474 nm / %R 525 nm)

<sup>e</sup> Sensory trait: 1=extremely undesirable; 8=extremely desirable

<sup>f</sup> Average of the cross-sectional area of longissimus muscle in four zones

( $P < 0.05$ ) than Tendercut<sup>TM</sup>. Judge et al. (1989) stated that a condition known as heat-ring can occur in the longissimus muscle of beef carcasses that have minimal finish and are chilled rapidly. Since the longissimus muscle in this area of the Tendercut<sup>TM</sup> carcasses was exposed, this may have resulted in a darker appearance, although no classical "heat ring" was observed. CIE L\* a\* b\* values, oxymyoglobin, metmyoglobin, and reduced myoglobin values did not support the sensory results as these values were not different ( $P < 0.05$ ) between treatments.

#### **4.2 Chemical analyses**

There were no significant differences in moisture, fat and protein between treatments or zones. Total collagen did not differ between treatments ( $P > 0.05$ ), although zone 3 had significantly more collagen than the other three zones (Table 2).

#### **4.3 Thaw and cooking loss evaluation**

There was no significant difference in thaw loss between treatments, though Tendercut<sup>TM</sup> steaks tended to have a lower thaw loss compared to the control by an average of 0.35% (Table 1). Thaw loss was less in zones 3 and 4 than in zones 1 and 2 (Table 2). This trend was exhibited in both control

**Table 2 - Effects of zone on various physical and sensory traits<sup>d</sup> of beef longissimus muscle**

	Zone				Std. Error
	1	2	3	4	
<b>Instrumental tenderness</b>					
Sarcomere length ( $\mu\text{m}$ ) <sup>ef</sup>	1.83 <sup>c</sup>	1.81 <sup>c</sup>	2.03 <sup>b</sup>	2.21 <sup>a</sup>	0.04
Fragmentation index	118.05 <sup>a</sup>	111.76 <sup>a</sup>	107.85 <sup>a</sup>	108.73 <sup>a</sup>	13.21
Peak force (kg) <sup>f</sup>	3.82 <sup>a</sup>	3.83 <sup>a</sup>	3.93 <sup>a</sup>	3.35 <sup>b</sup>	0.18
Total energy (kg*mm) <sup>f</sup>	29.28 <sup>ab</sup>	29.13 <sup>ab</sup>	31.65 <sup>a</sup>	26.62 <sup>b</sup>	1.55
<b>Sensory trait<sup>g</sup></b>					
Myofibrillar tenderness <sup>f</sup>	6.71 <sup>a</sup>	6.02 <sup>b</sup>	6.16 <sup>b</sup>	6.40 <sup>ab</sup>	0.19
Juiciness	6.29 <sup>a</sup>	6.32 <sup>a</sup>	6.22 <sup>a</sup>	6.26 <sup>a</sup>	0.14
Connective tissue <sup>f</sup>	6.38 <sup>a</sup>	6.15 <sup>a</sup>	6.39 <sup>a</sup>	6.48 <sup>a</sup>	0.20
Overall tenderness <sup>f</sup>	6.46 <sup>a</sup>	6.06 <sup>a</sup>	6.19 <sup>a</sup>	6.38 <sup>a</sup>	0.19
<b>Thaw and cooking loss</b>					
Thaw loss (%)	5.60 <sup>a</sup>	4.82 <sup>ab</sup>	3.73 <sup>c</sup>	3.92 <sup>bc</sup>	0.51
Cooking loss (%)	21.82 <sup>a</sup>	22.12 <sup>a</sup>	21.95 <sup>a</sup>	22.00 <sup>a</sup>	0.85
<b>Chemical analyses</b>					
Moisture (%)	73.09 <sup>a</sup>	73.41 <sup>a</sup>	73.07 <sup>a</sup>	73.90 <sup>a</sup>	0.42
Fat (%)	3.00 <sup>a</sup>	2.58 <sup>a</sup>	3.14 <sup>a</sup>	2.65 <sup>a</sup>	0.36
Protein (%)	23.78 <sup>a</sup>	24.28 <sup>a</sup>	23.58 <sup>a</sup>	24.20 <sup>a</sup>	0.46
Total collagen (mg/g)	5.64 <sup>b</sup>	5.44 <sup>b</sup>	6.72 <sup>a</sup>	5.10 <sup>b</sup>	0.53
<b>Physical characteristic</b>					
Overall area (cm <sup>2</sup> ) <sup>h</sup>	75.50 <sup>b</sup>	77.50 <sup>b</sup>	89.83 <sup>a</sup>	76.02 <sup>b</sup>	2.64

<sup>a-c</sup> means within the same row bearing identical superscripts are not different (P>0.05)

<sup>d</sup> Traits determined on cooked products include: Warner-Bratzler peak force, total energy, and cooking loss

<sup>e</sup> Treatment\*zone interaction (P<0.05)

<sup>f</sup> Zone\*core interaction (P<0.05)

<sup>g</sup> Sensory trait: Myofibrillar and overall tenderness:

1=extremely tough, 8=extremely tender; Juiciness: 1=extremely dry, 8=extremely juicy; Connective tissue: 1=abundant, 8=none

<sup>h</sup> Overall area is the cross-sectional area of longissimus muscle

and Tendercut<sup>TM</sup>, suggesting that water holding capacity of steaks was dependent upon location within the carcass. It appeared that as the anterior end was approached, more stretching of the muscle occurred, allowing for greater space within the sarcomere to hold water. This greater space has been reported to increase water holding capacity (Judge et al., 1989). Aging time did not significantly affect thaw loss (Table 3). There were no significant differences in cooking loss between treatments, among zones, or between days.

#### **4.4 Tenderness evaluation**

##### **4.4.1 Warner-Bratzler Shear**

Peak force and total energy were not different ( $P>0.05$ ) between treatments, possibly because means were pooled across zone, day and core location (Table 1). In addition, the five beef carcasses were not the same quality grade (U.S. Standard to U.S. Choice). This variability may have detracted from the ability to measure differences in tenderness between prerigor treatments. Higher quality grades are known to result in more tender product (Judge et al., 1989). Nevertheless, treated sides tended to have lower peak force (3.50 vs 3.96 kg) and total energy values (27.45 vs 30.79 kg) than control counterparts (Fig. 4). This improvement in tenderness may not have been due only to the fact that stretching resulted in

**Table 3 - Effects of aging on various physical and sensory traits<sup>c</sup> of beef longissimus muscle**

	Day		Standard Error
	3	10	
<b>Instrumental tenderness</b>			
Fragmentation index	126.28 <sup>a</sup>	96.91 <sup>b</sup>	11.26
Peak force (kg)	4.10 <sup>a</sup>	3.36 <sup>b</sup>	0.10
Total energy (kg*mm)	31.50 <sup>a</sup>	26.83 <sup>b</sup>	0.80
<b>Sensory trait<sup>d</sup></b>			
Myofibrillar tenderness <sup>e</sup>	6.05 <sup>b</sup>	6.60 <sup>a</sup>	0.10
Juiciness	6.27 <sup>a</sup>	6.28 <sup>a</sup>	0.08
Connective tissue	6.10 <sup>b</sup>	6.60 <sup>a</sup>	0.12
Overall tenderness <sup>e</sup>	6.04 <sup>b</sup>	6.51 <sup>a</sup>	0.10
<b>Thaw and cooking loss</b>			
Thaw loss (%)	4.35 <sup>a</sup>	4.68 <sup>a</sup>	0.64
Cooking loss (%)	22.40 <sup>a</sup>	21.55 <sup>a</sup>	0.60

<sup>a</sup><sub>b</sub> means within the same row bearing identical superscripts are not different (P>0.05)

<sup>c</sup> Traits determined on cooked product include: Warner-Bratzler peak force, total energy, cooking loss

<sup>d</sup> Sensory trait: Myofibrillar and overall tenderness:

1=extremely tough, 8=extremely tender; Juiciness: 1=extremely dry, 2=extremely tender; Connective tissue: 1=abundant, 8=none

<sup>e</sup> Treatment\*day interaction (P<0.05)

**Table 4 - Effects of aging on various physical and sensory traits<sup>c</sup> of beef longissimus muscle by treatment**

	Day				Standard Error
	3		10		
	Ctrl	TC	Ctrl	TC	
<b>Instrumental tenderness</b>					
Fragmentation index	137.63 <sup>a</sup>	114.93 <sup>a</sup>	96.15 <sup>a</sup>	97.68 <sup>a</sup>	28.39
Peak force (kg)	4.39 <sup>a</sup>	3.81 <sup>a</sup>	3.53 <sup>a</sup>	3.20 <sup>a</sup>	0.30
Total energy (kg*mm)	33.44 <sup>a</sup>	29.57 <sup>a</sup>	28.34 <sup>a</sup>	25.33 <sup>a</sup>	2.43
<b>Sensory trait<sup>d</sup></b>					
Myofibrillar tenderness <sup>e</sup>	5.61 <sup>b</sup>	6.48 <sup>a</sup>	6.46 <sup>a</sup>	6.75 <sup>a</sup>	0.17
Juiciness	6.14 <sup>b</sup>	6.39 <sup>a</sup>	6.31 <sup>a</sup>	6.24 <sup>a</sup>	0.05
Connective tissue	5.72 <sup>b</sup>	6.47 <sup>a</sup>	6.39 <sup>b</sup>	6.81 <sup>a</sup>	0.12
Overall tenderness <sup>e</sup>	5.59 <sup>b</sup>	6.48 <sup>a</sup>	6.33 <sup>a</sup>	6.69 <sup>a</sup>	0.16
<b>Thaw and cooking loss</b>					
Thaw loss (%)	4.13 <sup>a</sup>	4.57 <sup>a</sup>	5.25 <sup>a</sup>	4.11 <sup>b</sup>	0.21
Cooking loss (%)	22.37 <sup>a</sup>	22.43 <sup>a</sup>	21.42 <sup>a</sup>	21.67 <sup>a</sup>	0.15

<sup>a</sup><sup>b</sup> means within the same row and day bearing identical superscripts are not different (P>0.05)

<sup>c</sup> Traits determined on cooked product include: Warner-Bratzler shear and total energy, sensory traits, cooking loss

<sup>d</sup> Sensory trait: Myofibrillar and overall tenderness:

1=extremely tough, 8=extremely tender; Juiciness: 1=extremely dry, 8=extremely juicy; Connective tissue: 1=abundant, 8=none

<sup>e</sup> Treatment\*day interaction (P<0.05)

longer sarcomeres, but perhaps the Tendercut™ treatment stretched the connective tissue also, making it more susceptible to breakage during cooking. Significant differences in peak force and total energy were found among zones (Table 2). Peak force and total energy tended to be the highest in zone 3, which also had significantly higher total collagen, and also more insoluble collagen than the other zones (Table 2). This finding is in accordance with the fact that increased solubility results in improved tenderness (Judge et al., 1989; Cross et al., 1973). In addition, control peak force and total energy values were higher, but not statistically different than Tendercut™ peak force values in every zone (Fig. 4). Zone 4 had the lowest ( $P < 0.05$ ) peak force (Table 2). Although not statistically the lowest zone in total collagen, the lower collagen may have contributed to the smaller peak force value. In addition, there may have been differences in collagen quality. Increased collagen solubility results in more tender meat (Herring et al., 1967; Cross et al., 1973). Furthermore, since the longissimus muscle in zone 4 was more anterior, in this location, the muscle is deeper within the carcass. Therefore, this section may have been chilled more slowly than other zones. Delayed chilling has been shown to be effective at improving tenderness (Dransfield et al., 1981; McCrae et al., 1971; Marsh et al., 1968).

Peak force and total energy were affected ( $P < 0.05$ ) by

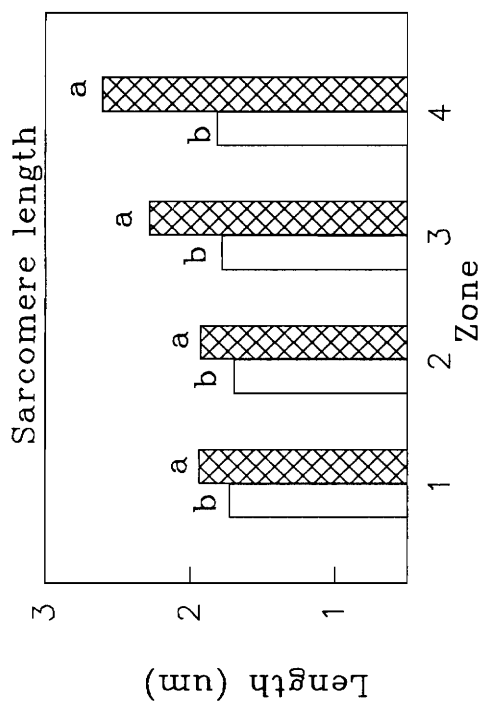
**Table 5 - Effects of core location on various physical and sensory traits of beef longissimus muscle**

	Core location							Standard Error
	1	2	3	4	5	6	7	
<b>Instrumental tenderness</b>								
Sarcomere length ( $\mu\text{m}$ ) <sup>e</sup>	2.05 <sup>a</sup>	2.02 <sup>ab</sup>	2.05 <sup>a</sup>	1.97 <sup>bc</sup>	1.87 <sup>d</sup>	1.95 <sup>c</sup>	1.91 <sup>cd</sup>	0.04
Peak force (kg) <sup>e</sup>	3.75 <sup>b</sup>	3.66 <sup>b</sup>	3.72 <sup>b</sup>	3.15 <sup>c</sup>	4.65 <sup>a</sup>	3.40 <sup>c</sup>	3.81 <sup>b</sup>	0.13
Total energy (kg*mm) <sup>e</sup>	29.26 <sup>c</sup>	29.31 <sup>c</sup>	31.54 <sup>b</sup>	25.10 <sup>d</sup>	35.08 <sup>a</sup>	25.85 <sup>d</sup>	28.02 <sup>c</sup>	0.98
<b>Sensory trait<sup>f</sup></b>								
Myofibrillar tenderness <sup>e</sup>	6.04 <sup>c</sup>	6.48 <sup>b</sup>	6.46 <sup>b</sup>	6.84 <sup>a</sup>	5.40 <sup>d</sup>	6.48 <sup>b</sup>	6.59 <sup>ab</sup>	0.17
Juiciness	6.23 <sup>a</sup>	6.45 <sup>a</sup>	6.35 <sup>a</sup>	6.20 <sup>a</sup>	6.09 <sup>a</sup>	6.21 <sup>a</sup>	6.39 <sup>a</sup>	0.15
Connective tissue <sup>e</sup>	6.38 <sup>ab</sup>	6.46 <sup>ab</sup>	6.33 <sup>b</sup>	6.58 <sup>ab</sup>	5.48 <sup>c</sup>	6.48 <sup>ab</sup>	6.75 <sup>a</sup>	0.20
Overall tenderness <sup>e</sup>	6.14 <sup>b</sup>	6.46 <sup>ab</sup>	6.39 <sup>ab</sup>	6.58 <sup>a</sup>	5.36 <sup>c</sup>	6.44 <sup>ab</sup>	6.55 <sup>a</sup>	0.17

<sup>a-d</sup> means within the same row bearing identical superscripts are not different (P>0.05)

<sup>e</sup> Zone\*core interaction (P<0.05)

<sup>f</sup> Sensory trait: Myofibrillar and overall tenderness: 1=extremely tough, 8=extremely tender; Juiciness: 1=extremely dry, 8=extremely juicy; Connective tissue: 1=abundant, 8=none



Control  
Tendercut™

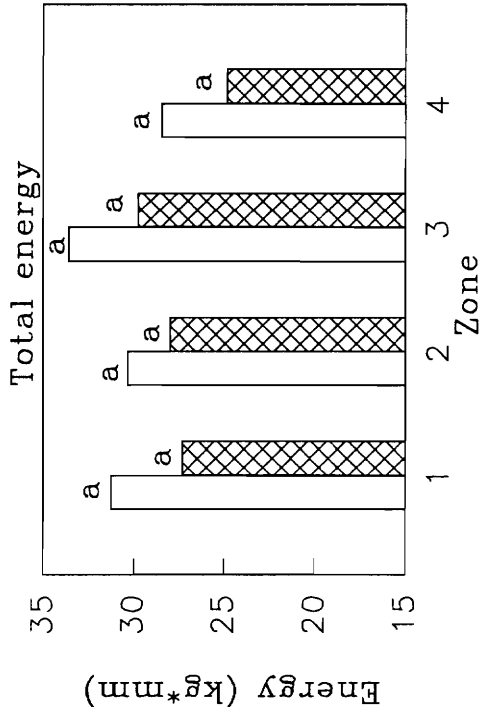
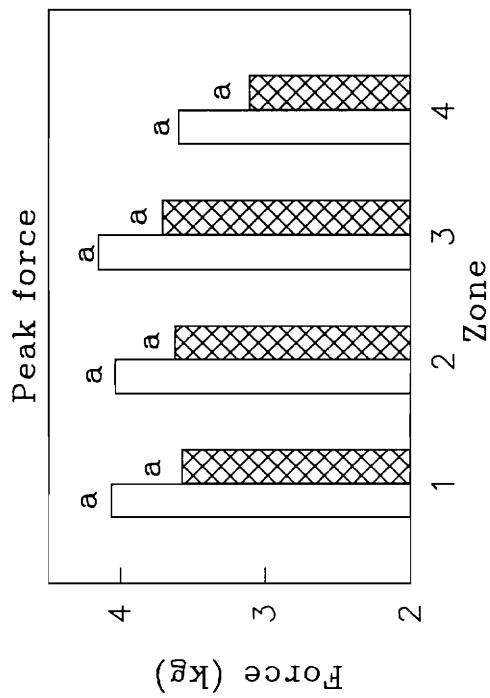


Figure 4. Effect of zone upon Warner–Bratzler peak force and total energy values and sarcomere length on beef steaks by treatment

aging time (Table 3), which was expected due to the postmortem degradation of myofibrillar proteins (Olson and Parrish, 1977). Within day (Table 4), peak force was consistently, though not significantly, less (13.2 and 9.4%) for Tendercut<sup>TM</sup> as compared to the control. The Tendercut<sup>TM</sup> process combined with aging appears to be an additive effect since peak force values for this treatment from 10-day aged steaks were still lower than 10-day aged control steaks. However, the Tendercut<sup>TM</sup> process appears to be more effective at improving tenderness, as measured by all indices of tenderness (Table 4), on day 3 than on day 10, perhaps due to the tenderizing effect of proteolytic enzymes on the 10 day aged steaks.

Peak force and total energy differed significantly according to core location (Table 5). In both cases, core 5 required the greatest amount of force to shear, which is logical since it was the most medially located core. Therefore, this area would have been stretched the least because it was more closely anchored to the vertebrae by connective tissue. These results agree with Crouse et al. (1989) and Howard and Judge (1968) who found that shear force was higher for medially located cores than for laterally located cores, though others have found the opposite results (Tuma et al., 1962; Williams et al., 1983). For peak force and total energy, cores 4 and 6 were significantly lower than the other cores. Core 4, the central core, may have been the least cooked portion of the steak, perhaps resulting in

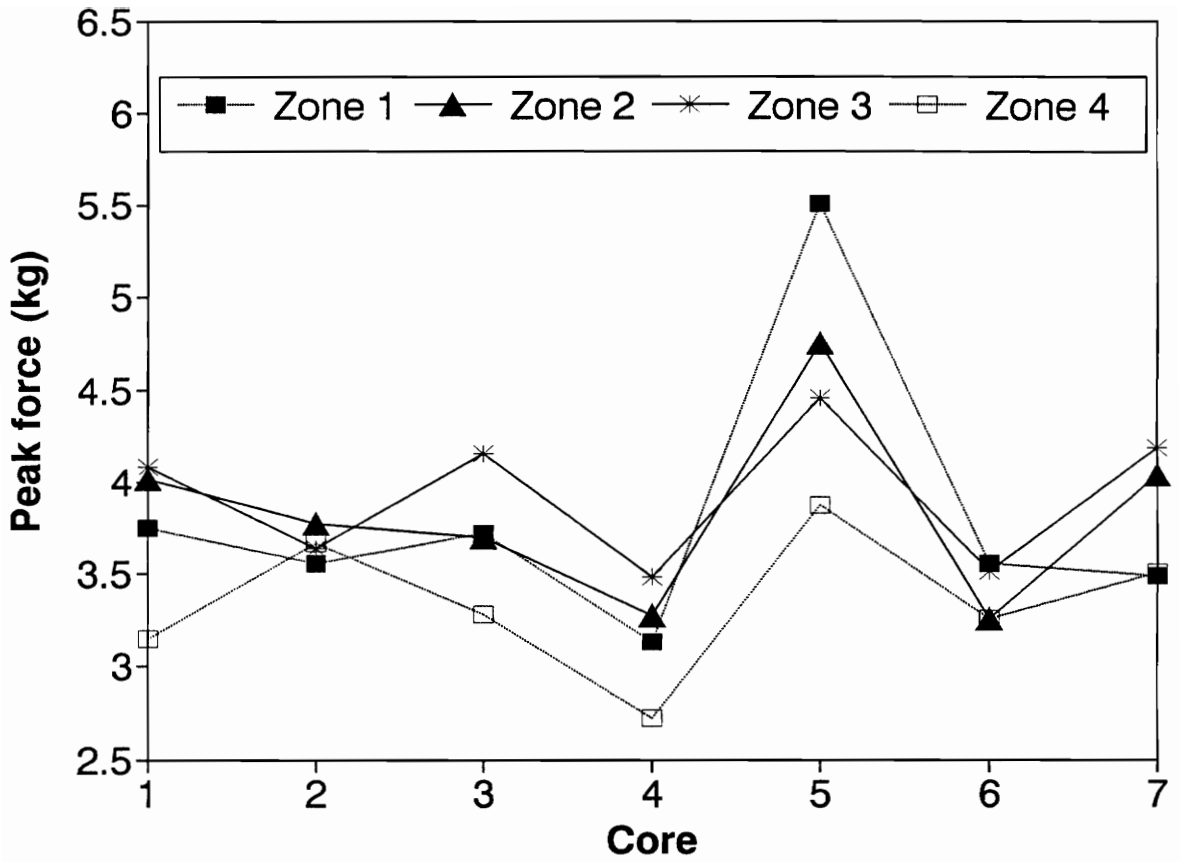


Figure 5. Peak force values by zone and core location for beef steaks

greater tenderness. Sensory data support this result, as core 4 was given the highest, although not significant, rating for myofibrillar and overall tenderness (Table 5).

There was a significant zone by core interaction for both peak force (Fig. 5) and total energy (Appendix A, Fig. 11). In both Tendercut<sup>TM</sup> and control, in all zones, core 5 had the highest shear force values, which is in accordance with aforementioned research showing the medial area to be less tender than the lateral. The interactions between zone and core that occurred could be due to physical differences along the length of the longissimus muscle, for example, amount of connective tissue and deposition of fat (Garipey et al., 1990). The interaction illustrates that total energy and peak force differed across zones and among cores.

#### **4.4.2 Fragmentation Index**

Fragmentation index was not different ( $P>0.05$ ) between treatments (Table 1) or among zones (Table 2). The only significant difference found was between days (Table 3), with day 10 being more fragmentable. While Davis et al. (1980) obtained fragmentation values ranging from 100 to 600, most of our values were less than 100. Our fragmentation procedure differed in the homogenizer used. The fragmentation index was highly susceptible to influence by connective tissue and the ability of the liquid to be properly filtered through the

**Table 6 - Pearson correlation coefficients of sensory ratings with instrumental measures of tenderness**

Instrumental Measurement	Sensory Trait				Overall Tenderness
	Myofibrillar Tenderness	Juiciness	Connective Tissue		
Peak force (kg)	-0.64**	-0.18	-0.73**		-0.71**
Total energy (kg*mm)	-0.60**	-0.21	-0.66**		-0.65**
Sarcomere length ( $\mu\text{m}$ )	0.34*	0.17	0.46**		0.41*
Fragmentation index	0.14	0.16	0.02		0.08

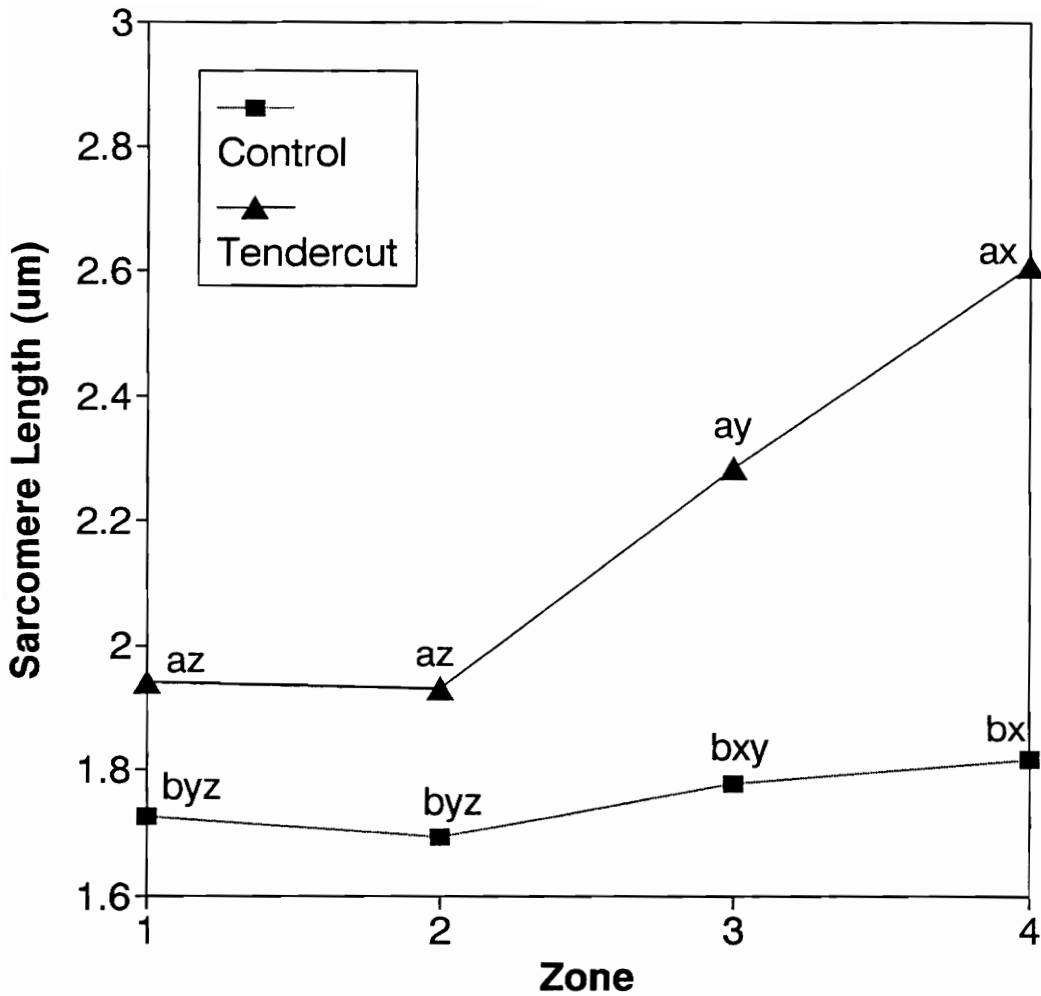
\*\* significant at  $P < 0.0001$

\* significant at  $P < 0.05$

nylon screen varied among samples. Some samples would immediately plug the screen before the free fluid could flow through, while others would be completely filtered in a matter of seconds. This measurement of tenderness did not correlate ( $P>0.05$ ) with any other measurements of tenderness (Table 6).

#### **4.4.3 Sarcomere length**

Tendercut<sup>TM</sup> steaks had longer ( $P<0.05$ ) sarcomeres (Table 1) than control steaks (2.19 vs 1.75  $\mu\text{m}$ , respectively). Stretching prerigor muscle and maintaining that stretched state until rigor is complete is known to result in longer sarcomeres (Herring et al., 1965; Hostetler et al., 1970; Hostetler et al., 1972). Furthermore, sarcomere length has a significant positive relationship to tenderness (Locker, 1960). There was a difference ( $P<0.05$ ) in sarcomere length by zone (Table 2). Zones 1 and 2 had the shortest sarcomeres (1.83  $\mu\text{m}$  and 1.81  $\mu\text{m}$ , respectively), thereby suggesting less tender beef compared to zones 3 and 4. This agrees with peak force data as these zones required higher shear force than zone 4 (Table 2). The mean sarcomere length of zone 4 was 2.21  $\mu\text{m}$ , significantly longer than all other zones. According to sarcomere length data, the Tendercut<sup>TM</sup> treatment caused the greatest improvement in zones 3 and 4. Conclusions made concerning differences among zones in sarcomere length are somewhat limited as a significant treatment by zone



**Figure 6. Effect of zone on sarcomere lengths by treatment of beef steaks**

<sup>ab</sup> means within the same zone having identical letters are not different ( $P>0.05$ )

<sup>xyz</sup> means within the same prerigor treatment having identical letters are not different ( $P>0.05$ )

interaction (Fig. 6) was found. While the sarcomere lengths of the control generally remained consistent across zones, those of the Tendercut<sup>TM</sup> treatment increased dramatically in zones 3 and 4. Sarcomeres from the Tendercut<sup>TM</sup> treatment were consistently higher than those of the control (Fig. 6) relating to an improvement in tenderness as measured by increases in sarcomere length in all zones. This observation is in agreement with the sensory results which indicated significant improvement in overall tenderness among all zones (Fig. 7).

There was a significant difference in sarcomere length between cores (Table 5). Core 5 had the shortest sarcomeres, although not significantly different from those of core 7. Since core 5 was close to the vertebral column, it may not have stretched as much in both the control side and the Tendercut<sup>TM</sup> side possibly because of greater tendonous attachments. Core 5 contained the shortest sarcomeres for both the control and the Tendercut<sup>TM</sup> samples. This data supports previous research that indicated that the medial portions are less tender than lateral portions (Crouse et al., 1989; Howard and Judge, 1968). Core 7 was also located in the medial portion. Cores 1 and 3, both located in the lateral portion, were among the cores with the longest sarcomeres, again supporting the idea that the lateral portion of a steak was more tender than medial portions.

There was a significant zone by core interaction

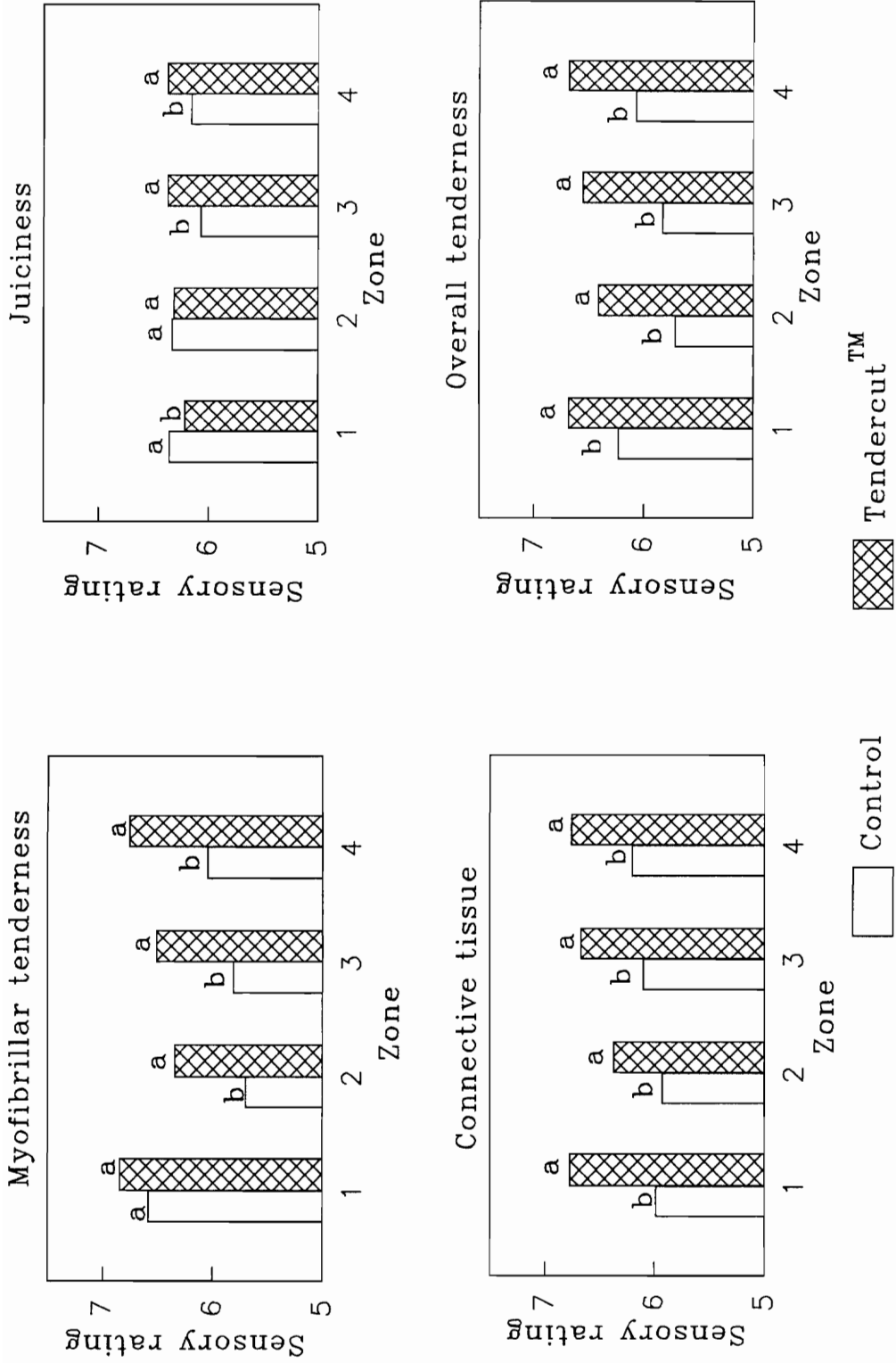
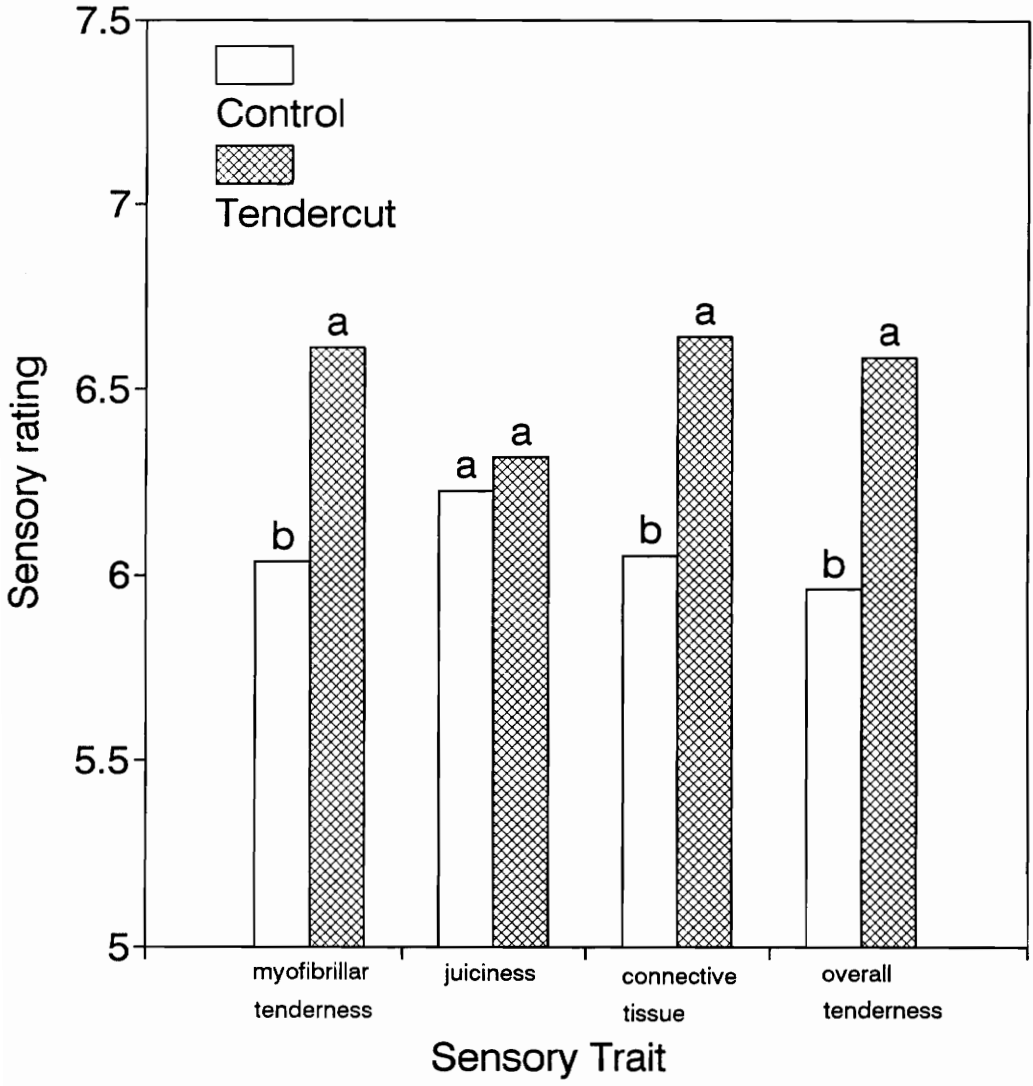


Figure 7. Effect of zone upon sensory panel ratings of beef steaks. Bars within a given trait and zone with the same letter are not different ( $P > 0.05$ )

(Appendix A, Fig. 12). With the exception of core 6, zone 4 had the longest sarcomeres, which is in accordance with shear values (Table 2). For sarcomere lengths, zones 1 and 2 generally followed the same pattern, while zones 3 and 4 were quite dissimilar (Appendix A, Fig. 12). This trend was most likely due to the effects of the treatment.

#### 4.4.4 Sensory panel

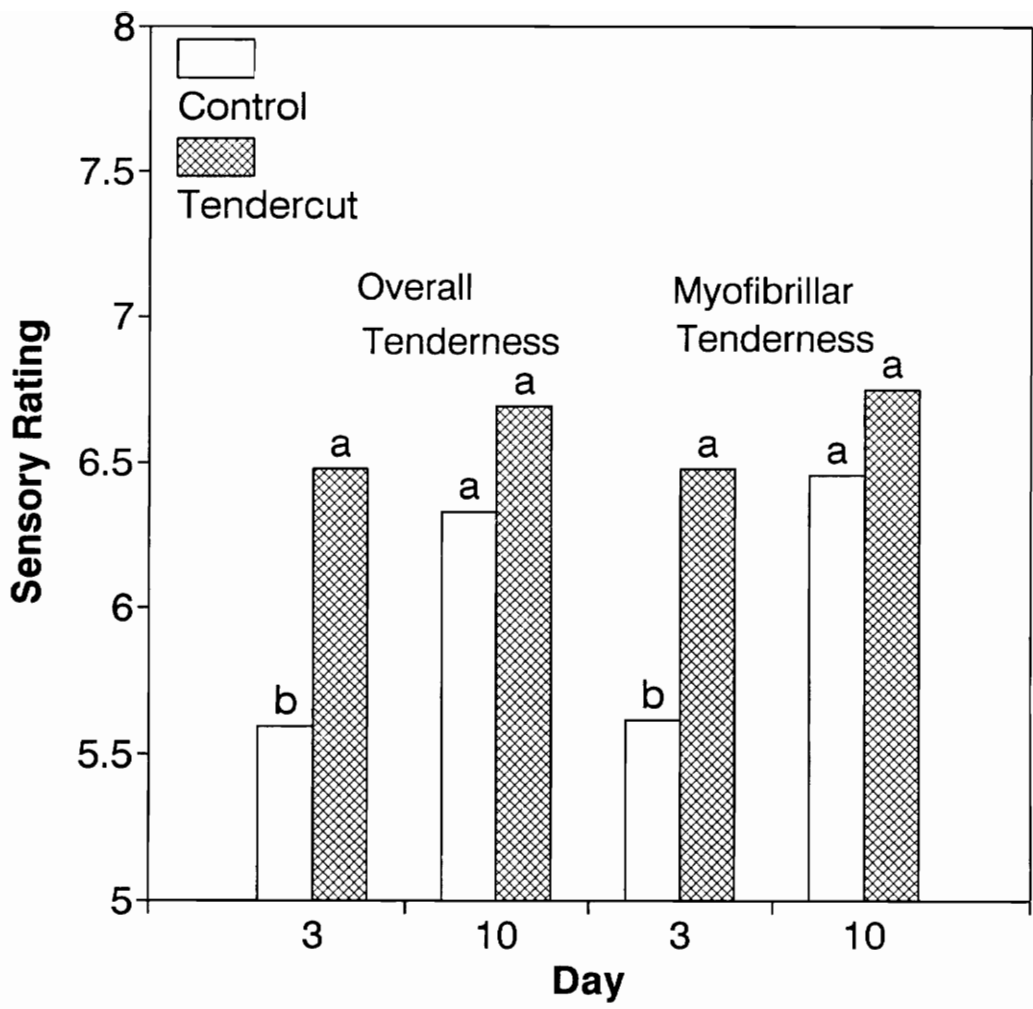
Steaks were rated for myofibrillar tenderness, juiciness, connective tissue and overall tenderness. For juiciness, no significant differences due to treatment, zone, day or core were found. There were significant differences between treatments in sensory panel ratings of myofibrillar tenderness, connective tissue, and overall tenderness (Fig. 8). For all three traits, the rating for Tendercut<sup>TM</sup> steaks was higher ( $P < 0.05$ ) than for the controls. Instrumental measures of tenderness (Table 6) correlated very well with the sensory ratings of these three traits, especially with peak force. For peak force, the correlations ( $P < 0.0001$ ) for myofibrillar tenderness, connective tissue, and overall tenderness were  $r = -0.64$ ,  $r = -0.73$ , and  $r = -0.71$ , respectively. The fact that the Tendercut<sup>TM</sup> treatment improved tenderness may not only be the result of stretching the muscle, but could also be due to the fact that shortening of the muscle was prevented. Herring et al. (1967) found it



**Figure 8. Effect of treatment on sensory panel ratings of beef steaks**  
<sup>ab</sup> bars within an individual sensory trait with unlike letters are different (P<0.05)

was more important to tenderness improvement to prevent postmortem shortening than to ensure maximum stretch. No significant differences in ratings were observed for juiciness, connective tissue and overall tenderness by zone, although there was a significant difference in myofibrillar tenderness by zone (Table 2). Zone 1 had the highest mean myofibrillar tenderness rating at 6.71, though this rating was not significantly different from that of zone 4. The high ratings obtained for zone 1 were partly due to the evaluations received for day 3 steaks. The ratings for day 3 steaks in zone 1, at 6.5 were much higher than those of day 3 steaks in zones 2, 3, and 4, which were 5.84, 5.81 and 6.03, respectively.

For myofibrillar tenderness, connective tissue, and overall tenderness ratings were higher ( $P < 0.05$ ) for day 3 than day 10 (Table 3). This was expected due to the action of cathepsins and calcium activated factors [calpains] on myofibrillar proteins (Olson and Parrish, 1977). There was a significant treatment by day interaction for myofibrillar tenderness and overall tenderness (Fig. 9). Both plots illustrate that the difference in tenderness between the control and Tendercut<sup>TM</sup> was much greater at day 3 than at day 10. In both examples, the rating for Tendercut<sup>TM</sup> steaks at day 3 was higher than for control steaks after 10 days of aging (Table 4). This has important implications for the meat industry. With the Tendercut<sup>TM</sup> treatment, in just 3 days, it



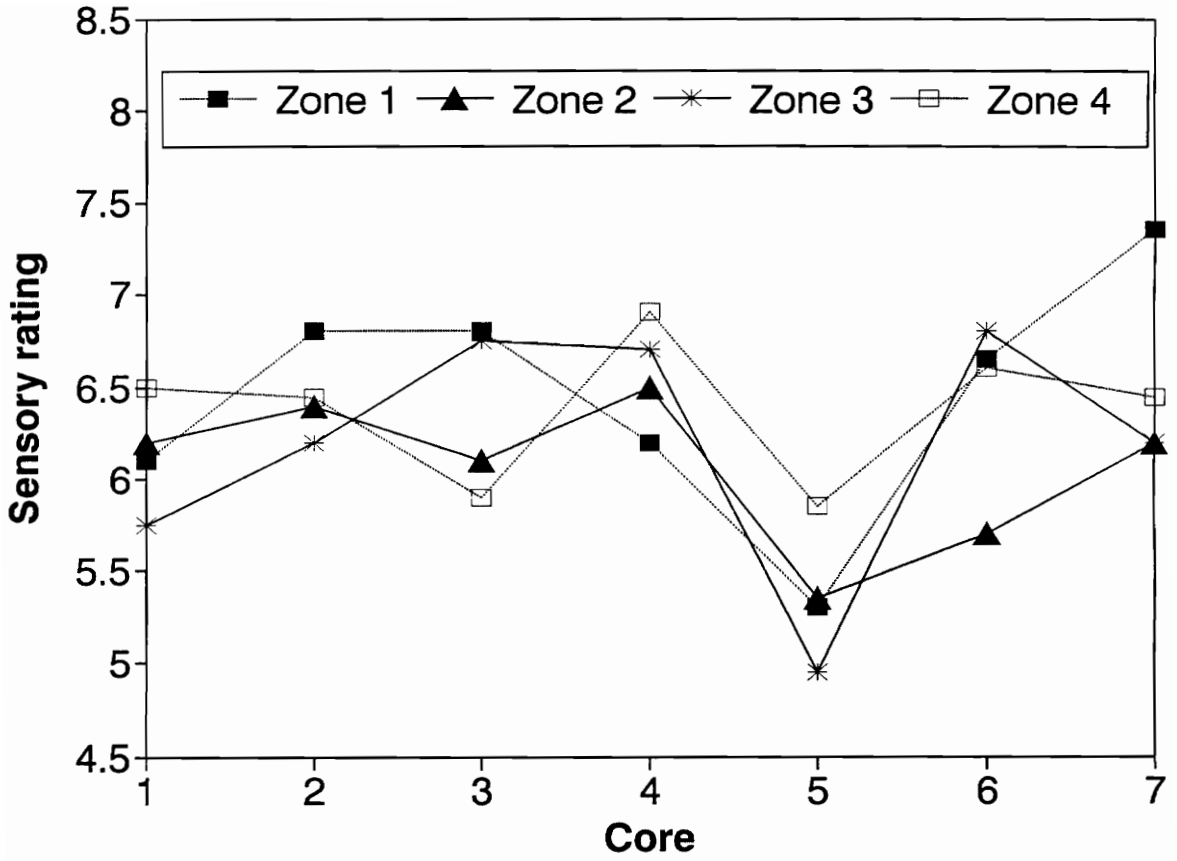
**Figure 9. Effect of aging time upon sensory panel ratings of overall tenderness and myofibrillar tenderness of beef steaks by treatment**  
<sup>ab</sup> bars within an individual sensory trait and day with unlike letters are different (P<0.05)

would be possible to market tender steaks.

For myofibrillar tenderness, connective tissue and overall tenderness, significant differences were found among cores (Table 5). Myofibrillar ratings for core 4 were highest ( $P < 0.05$ ), though not significantly different from core 7. Core 4, the center core, may have been the least cooked and therefore expected to have higher juiciness and tenderness ratings. Ratings for myofibrillar and overall tenderness indicated that core 5 was significantly less tender than the other cores. This agrees with the work of Crouse et al. (1989) and Howard and Judge (1968) who found that medial locations were less tender than lateral locations. Core 5 also had the highest ( $P < 0.05$ ) connective tissue rating, which could adversely affect sensory tenderness ratings. All measurements rank core 5 as being the least tender (Table 5).

For myofibrillar tenderness, connective tissue, and overall tenderness, there was a significant zone by core interaction. Myofibrillar tenderness (Appendix A, Fig. 13) of samples from zone 1 exhibited the highest sensory rating in all cores except core 5. This was in disagreement with sarcomere length and peak force data (Table 2), which indicated that zone 4 was the most tender. This observation could have been due to the fact that these were subjective measurements that can be more influenced by juiciness and fat content than instrumental measures of tenderness. Overall tenderness, however, did not show this trend (Fig. 10) since

it was not uniform across zones or among cores. Connective tissue amount was highest in core 5 in all zones (Appendix A, Fig. 14). Like overall tenderness, connective tissue amount was not uniform across zones or among cores.



**Figure 10. Overall tenderness ratings by zone and core location**

## CHAPTER V

### SUMMARY AND CONCLUSIONS

The Tendercut<sup>TM</sup> treatment did not have any significant adverse effects on  $L^*$   $a^*$   $b^*$  values and oxymyoglobin, metmyoglobin, and reduced myoglobin values, though color and overall appearance ratings by the sensory panel were significantly lower in these steaks than in the control counterparts. However, visual sensory ratings for the Tendercut<sup>TM</sup> steaks were in the slightly desirable to moderately desirable range. There were no significant differences in thaw loss between treatments, although Tendercut<sup>TM</sup> steaks tended to have a lower amount of thaw loss. Cooking loss did not differ significantly between treatments.

Warner-Bratzler shear force values for Tendercut<sup>TM</sup> steaks tended to be numerically lower than control steaks, although not significantly less, perhaps due to the confounding factors of zone, day and core. There were significant differences in tenderness by zone, with zone 4, the area most affected by the treatment, being the most tender. Shear force also differed significantly depending on core location. Results generally indicated that the least tender core location was the medial location and the most tender was the lateral portion. Variations in core shear force values could have been

dependent on zone, connective tissue, fat deposition, and fiber diameter and orientation. There was a significant zone by core interaction, which illustrated that tenderness improvement was not uniform among cores and across zones.

The fragmentation procedure was highly variable. Significant differences between Tendercut<sup>TM</sup> and control were not found. Nevertheless, the procedure did identify differences in fragmentation between aging days 3 and 10, with day 10 steaks being more fragmented than day 3 steaks.

Treated steaks had significantly longer sarcomeres than control steaks, indicating that the former were more tender. There was also a significant difference in sarcomere length by zone. As with shear force values, zone 4 was considered to be most tender as indicated by the longest sarcomeres. Zone 3 had the next longest sarcomeres, which indicated that, according to the sarcomere measurement of tenderness, the treatment also positively affected tenderness in zone 3. The control had sarcomere lengths that were similar across all four zones. There was also a significant treatment by zone interaction which reinforced the fact that the greatest improvement occurred in zone 4, followed by zone 3. Sarcomere lengths differed significantly among cores, with the laterally located cores being more tender than the medially located cores. There was also a significant zone by core interaction which indicated that sarcomere lengths did not increase uniformly across zones or among cores.

Tendercut<sup>TM</sup> steaks received significantly higher sensory ratings for myofibrillar tenderness, amount of connective tissue and overall tenderness than did the control samples. For myofibrillar tenderness, zone 1 had the highest rating, followed by zone 4,3, and 2 respectively. This was in disagreement with shear force values and sarcomere lengths which rated zone 4 as being more tender than zone 1. This difference may have been due to the fact that the sensory ratings were affected by juiciness, doneness, or connective tissue of the steaks, factors which may not alter instrumental measures of tenderness in the same manner. Overall tenderness did not differ significantly among zones.

Myofibrillar tenderness, connective tissue, and overall tenderness differed significantly by day, with ratings for day 10 being higher than those for day 3. This was attributable to the postmortem action of calpains and cathepsins. For both ratings of tenderness, there was a significant treatment by day interaction which illustrated that the treatment had a much greater effect on tenderness at day 3 than at day 10. For both myofibrillar and overall tenderness, the rating for the Tendercut<sup>TM</sup> steaks was higher at day 3 than the rating for the control steaks at day 10, an observation which has important implications to the meat industry.

There were significant differences in myofibrillar tenderness, connective tissue and overall tenderness due to core location. The general pattern was that tenderness

increased from medial to lateral, with the exception of the center core, which was the most tender.

The main conclusions that can be drawn are: 1) the Tendercut™ treatment resulted in improved tenderness as measured by shear force, sarcomere length, and sensory panel, in all zones (i.e. along the length of the *longissimus* muscle) 2) tenderness increase (according to shear force and sarcomere length) was most noticeable in zone 4 (between the 9th and 13th thoracic vertebrae) with sarcomere lengths indicating significant improvements in zone 3 (13th thoracic vertebrae to 2nd lumbar vertebrae) and 3) tenderness differed due to core location in both control and Tendercut™ samples with the most medially located core being the least tender according to sensory ratings, sarcomere lengths and shear values; the center core being the most tender; and the lateral cores being generally more tender than the medial cores.

More research should be considered to evaluate the optimum locations for skeletal alterations to improve tenderness. Furthermore, additional study is needed to determine the effect of skeletal alterations on the different quality grades of beef, aging times, and endpoint cooking temperatures. Tendercut™ is a simple and effective method of improving tenderness. It requires no new equipment and could be implemented in industry today.

## References

- AOAC. 1990. *Official Methods of Analysis*, 15th ed. Association of Official Analytical Chemists, Washington, D.C.
- AMSA. 1978. Guidelines for Cookery and Sensory Evaluation of Meat. Am. Meat Science Assoc. Nat'l. Live Stock and Meat Board. Chicago, Ill.
- Arganosa, G.C. and Marriott, N.G. 1989. Organic acids as tenderizers of collagen in restructured beef. *J. Food Sci.* 54:1173.
- Babiker, SA. and Lawrie, R.A. 1983. Post-mortem electrical stimulation and high temperature ageing of hot-deboned beef. *Meat Sci.* 8:1.
- Bailey, A.J. 1972. The basis of meat texture. *J. Sci. Food Agric.* 23:995.
- Bandman, E. 1987. Chemistry of animal tissues. Ch. 3. Part 1. Proteins. In *The Science of Meat and Meat Products*. J.F. Price and B.S. Schweigert (Eds.). p. 61. Third edition. Food & Nutrition Press, Inc., Westport, Conn.
- Bechtel, P.J. (Ed.). 1986. *Muscle As Food*. Academic Press Inc., San Diego, Ca.
- Bechtel, P.J. and Parrish, Jr., F.C. 1983. Effects of post-mortem storage and temperature on muscle protein degradation : Analysis by SDS gel electrophoresis. *J. Food Sci.* 48:294.
- Beilken, S.L., Bouton, P.E. and Harris, P.V. 1986. Some effects on the mechanical properties of meat produced by cooking temperatures between 50 and 60°C. *J. Food Sci.* 51:791.
- Bendall, J.R. 1973. *Structure and Function of Muscle*. Vol. 2. G.H. Bourne, Ed. Academic Press, New York.
- Bentley, D.S., Reagan, J.O. and Miller, M.F. 1989. Effects of gas atmosphere, storage temperature and storage time on the shelflife and sensory attributes of vacuum packaged ground beef patties. *J. Food Sci.* 54:284.

- Bergman, I. and Loxley, R. 1963. Two improved and simplified methods for the spectrophotometric determination of hydroxyproline. *Anal. Chem.* 35:1961.
- Berry, B.W. and Leddy, K.F. 1990. Influence of steak temperature at the beginning of broiling on palatability, shear and cooking properties of beef loin steaks differing in marbling. *J. Foodservice Syst.* 5:287.
- Bouton, P.E. and Harris, P.V. 1972. The effects of cooking temperature and time on some mechanical properties of meat. *J. Food Sci.* 37:140.
- Bouton, P.E. and Harris, P.V. 1981. Changes in the tenderness of meat cooked at 50-65°C. *J. Food Sci.* 46:475.
- Bouton, P.E., Harris, P.V., Shorthose, W.R. and Ratcliff, D. 1974. Changes in the mechanical properties of veal muscles produced by myofibrillar contraction state, cooking temperature and cooking time. *J. Food Sci.* 39:869.
- Bruce, H.L. and Ball, R.O. 1990. Postmortem interactions of muscle temperature, pH and extension on beef quality. *J. Anim. Sci.* 68:4167.
- Buege, D.R. and Stouffer, J.R. 1974a. Effects of pre-rigor tension on tenderness of intact bovine and ovine muscle. *J. Food Sci.* 39:396.
- Buege, D.R. and Stouffer, J.R. 1974b. Tenderness variation in ovine longissimus muscle. *J. Food Sci.* 39:402.
- Busch, W.A., Parrish, Jr., F.C., and Goll, D.E. 1967. Molecular properties of post-mortem muscle. 4. Effect of temperature on adenosine triphosphate degradation, isometric tension parameters, and shear resistance of bovine muscle. *J. Food Sci.* 32:390.
- Cassens, R.G. 1987. Structure of Muscle. Ch. 2., In *The Science of Meat and Meat Products*, J.F. Price and B.S. Schweigert (Ed.), 3rd ed. Food & Nutrition Press, Inc. Westport, Conn.

- Cecchi, L.A., Huffman, D.L., Egbert, W.R. and Jones, W.R. 1988. Chemical and physical characteristics of beef chuck muscles: Effect of electrical stimulation, hot boning and high temperature conditioning. *J. Food Sci.* 53:411.
- Cia, G. and Marsh, B.B. 1976. Properties of beef cooked before rigor onset. *J. Food Sci.* 41:1259.
- Coleman, M.E., Rhee, K.S. and Cross, H.R. 1988. Sensory and cooking properties of beef steaks and roasts cooked with and without external fat. *J. Food Sci.* 53:34.
- Cross, H.R. 1977. Effects of amount, distribution and texture of marbling on cooking properties of beef longissimus. *J. Food Sci.* 42:182.
- Cross, H.R. 1987. Sensory characteristics of meat. Ch. 8. Part 1. Sensory factors and evaluation. In *The Science of Meat and Meat Products*. J.F. Price and B.S. Schweigert (Ed.), 3rd ed. Food & Nutrition Press, Inc. Westport, Conn.
- Cross, H.R. and Tennent, I. 1980. Accelerated processing systems for USDA choice and good beef carcasses. *J. Food Sci.* 45:765.
- Cross, H.R., Carpenter, Z.L. and Smith, G.C. 1973. Effects of intramuscular collagen and elastin on bovine muscle tenderness. *J. Food Sci.* 38:998.
- Cross, H.R., West, R.L. and Dutson, T.R. 1981. Comparison of methods for measuring sarcomere length in beef semitendinosus muscle. *Meat Sci.* 5:261.
- Crouse, J.D., Theer, L.K. and Seideman, S.C. 1989. The measurement of shear force by core location in *longissimus dorsi* beef steaks from four tenderness groups. *J. Food Qual.* 11:341.
- Culler, R.D., Parrish, Jr., F.C., Smith, G.C., and Cross, H.R. 1978. Relationship of myofibril fragmentation index to certain chemical, physical and sensory characteristics of bovine longissimus muscle. *J. Food Sci.* 43:1177.
- Davis, G.W., Dutson, T.R., Smith, G.C. and Carpenter, Z.L. 1980. Fragmentation procedure for bovine longissimus muscle as an index of cooked steak tenderness. *J. Food Sci.* 45:880.

- Dransfield, E., Brown, A.J. and Rhodes, D.N. 1976. Eating quality of hot deboned beef. *J. Food Technol.* 11:401.
- Dransfield, E., Jones, R.C.D. and MacFie, H.J.H. 1981. Tenderising in *M. longissimus dorsi* of beef, veal, rabbit, lamb, pork. *Meat Sci.* 5:139.
- Eisenhut, R.C., Cassens, R.G., Bray, R.W. and Briskey, E.J. 1965. Fiber arrangement and microstructure of bovine longissimus dorsi muscle. *J. Food Sci.* 30:955.
- Fischer, C., Honikel, K.O. and Hamm, R. 1982. Influence of sodium chloride on biochemical changes, water-holding capacity and sarcomere length of slaughter-fresh beef muscle. *Lebensmittel-Untersuchung und-Forschung.* 174:447.
- Flores, H.A., Kastner, C.L., Kropf, D.H. and Hunt, M.C. 1986. Effects of blade tenderization and trimming of connective tissue on hot-boned, restructured, pre-cooked roasts from cows. *J. Food Sci.* 51:1176.
- Gariepy, C., Jones, S.D.M., and Robertson, W.M. 1990. Variation in meat quality at three sites along the length of the beef longissimus muscle. *J. Anim. Sci.* 70:707.
- Gillis, W.A. and Henrickson, R.L. 1969. The influence of tension on pre-rigor excised bovine muscle. *J. Food Sci.* 34:375.
- Herring, H.K., Cassens, R.G., and Briskey, E.J. 1965. Further studies on bovine muscle tenderness as influenced by carcass position, sarcomere length, and fiber diameter. *J. Food Sci.* 30:1049.
- Herring, H.K., Cassens, R.G., Suess, G.G., Brungardt, V.H. and Briskey, E.J. 1967. Tenderness and associated characteristics of stretched and contracted bovine muscles. *J. Food Sci.* 32:317.
- Hill, F. 1966. The solubility of intramuscular collagen in meat animals of various ages. *J. Food Sci.* 31:161.
- Hodges, J.H., Cahill, V.R. and Ockerman, H.W. 1974. Effect of vacuum packaging on weight loss, microbial growth and palatability of fresh beef wholesale cuts. *J. Food Sci.* 39:143.

- Honikel, K.O. 1989. The meat aspects of water and food quality. Ch. 8. In *Water and Food Quality*. T.M. Hardman (Ed.). p. 277. Elsevier Science Publishers Ltd. New York, N.Y.
- Honikel, K.O., Fischer, C., Hamid, A. and Hamm, R. 1981a. Influence of postmortem changes in bovine muscle on the water-holding capacity of beef. Postmortem storage of muscle at 20C. *J. Food Sci.* 46:1.
- Honikel, K.O., Hamid, A., Fischer, C. and Hamm, R. 1981b. Influence of postmortem changes in bovine muscle on the water-holding capacity of beef. Postmortem storage of muscle at various temperatures between 0 and 30C. *J. Food Sci.* 46:23.
- Hostetler, R.L., Landmann, W.A., Link, B.A., and Fitzhugh, Jr., H.A. 1970. Influence of carcass position during rigor mortis on tenderness of beef muscles: Comparison of two treatments. *J. Anim. Sci.* 31:47.
- Hostetler, R.L., Link, B.A., Landmann, W.A. and Fitzhugh, Jr., H.A. 1972. Effect of carcass suspension on sarcomere length and shear force of some major bovine muscles. *J. Food Sci.* 37:132.
- Howard, R.D. and Judge, M.D. 1968. Comparison of sarcomere length to other predictors of beef tenderness. *J. Food Sci.* 33:456.
- Hunt, M.C. 1980. Meat color measurements. *Proc. Recip. Meat Conf.* 33:41.
- Johnson, M.H., Calkins, C.R., Huffman, R.D., Johnson, D.D. and Hargrove, D.D. 1990. Differences in cathepsin B & L and calcium dependent protease activities among breed type and their relationship to beef tenderness. *J. Anim. Sci.* 68:2371.
- Judge, M.D., Aberle, E.D., Forrest, J.C., Hedrick, H.B. and Merkel, R.A. 1989. *Principles of Meat Science*. 2nd ed. Kendall/Hunt Publishing Co., Dubuque, Iowa.
- Kastner, C.L. and Russell, T.S. 1975. Characteristics of conventionally and hot-boned bovine muscle excised at various conditioning periods. *J. Food Sci.* 40:747.
- Koh, K.C., Bidner, T.D., McMillin, K.W. and Hill, G.M. 1987. Effects of electrical stimulation and temperature on beef quality and tenderness. *Meat Sci.* 21:189.

- Koohmaraie, M., Babiker, A.S., Schroeder, A.L., Merkel, K.A. and Dutson, T.R. 1988a. Acceleration of post-mortem tenderization in ovine carcasses through activation of calcium dependent proteases. *J. Food Sci.* 53:1638.
- Koohmaraie, M., Seideman, S.C., Schollmeyer, J.E., Dutson, T.R. and Babiker, A.S. 1988b. Factors associated with the tenderness of three bovine muscles. *J. Food Sci.* 53:407.
- Koolmees, P.A., Korteknie, F., Smulders, J.M. 1986. Accuracy and utility of sarcomere length assessment by laser diffraction. *Food Microstruct.* 5:71.
- Kotula, K.L. and Heath, J.L. 1986. Precook tumbling of hot-boned broiler breasts in sodium chloride and acetic acid. *Poultry Sci.* 65:929.
- Leander, R.C., Hedrick, H.B., Brown, M.F., White, J.A. 1980. Comparison of structural changes in bovine longissimus and semitendinosus muscles during cooking. *J. Food Sci.* 45:1.
- Lee, L.M., Hawrysh, Z.J., Jeremiah, L.E., Hardin, R.T. 1990. Shrouding, spray-chilling, and vacuum-packaged aging effects on processing and eating quality attributes of beef. *J. Food Sci.* 55:1270.
- Lee, Y.B. and Ashmore, C.R. 1985. Effect of early post-mortem temperature on beef tenderness. *J. Anim. Sci.* 60:1588.
- Lentner, M. and Bishop, T. 1986. *Experimental Design and Analysis*. Valley Book Company, Blacksburg, Va.
- Locker, R.H. 1960. Degree of muscular contraction as a factor in tenderness of beef. *Food Res.* 25:304.
- Locker, R.H. and Daines, G.J. 1975. Rigor mortis in beef sternomandibularis muscle at 37C. *J. Sci. Food Agric.* 26:1721.
- Locker, R.H. and Hagyard, C.J. 1963. A cold shortening effect in beef muscles. *J. Sci. Food Agric.* 14:787.
- Lusby, M.L., Ridpath, J.F., Parrish, Jr., F.C. and Robson, R.M. 1983. Effect of postmortem storage on degradation of the myofibrillar protein titin in bovine longissimus muscle. *J. Food Sci.* 48:1787.

- Marsh, B.B. 1981. Properties and behavior of prerigor meat. 34th Annual Reciprocal Meat Conference, p.75.
- Marsh, B.B. and Leet, N.G. 1966. Studies in meat tenderness. III. The effects of cold shortening on tenderness. J. Food Sci. 31:450.
- Marsh, B.B. and Thompson, J.F. 1958. Rigor mortis and thaw rigor in lamb. J. Sci. Food Agric. 9:417.
- Marsh, B.B., Woodhams, P.R. and Leet, N.G. 1968. Studies in meat tenderness. 5. The effects of tenderness of carcass cooling and freezing before the completion of rigor mortis. J. Food Sci. 33:12.
- Martin, A.H., Fredeen, H.T. and Weiss, G.M. 1971. Tenderness of beef longissimus dorsi muscle from steers, heifers and bulls as influenced by source, post-mortem aging and carcass characteristics. J. Food Sci. 36:619.
- McCrae, S.E., Seccombe, C.G., Marsh, B.B. and Carse, W.A. 1971. Studies in meat tenderness. 9. The tenderness of various lamb muscles in relation to their skeletal restraint and delay before freezing. J. Food Sci. 36:566.
- Miyada, D.S. and Tappel, A.L. 1956. The hydrolysis of beef proteins by various proteolytic enzymes. Food Res. 21:217.
- Moller, A.J., Vestergaard, T. and Wismer-Pedersen, J. 1973. Myofibril fragmentation in bovine longissimus dorsi as an index of tenderness. J. Food Sci. 38:824.
- Morgan, J.B., Miller, R.K., Mendez, F.M., Hale, D.S. and Savell, J.W. 1991. Using calcium chloride injection to improve tenderness of beef from mature cows. J. Anim. Sci. 69:4469.
- Noble, J.M., McMahon, P.S., Seman, D.L., Moody, W.G. and Douglass, L.W. 1990. Effect of institutional heating methods of cold and hot-boned restructured beef roasts. J. Food Sci. 55:658.
- Okitani, A., Matsukura, U., Kato, H. and Fujimaki, M. 1980. Purification and some properties of a myofibrillar protein-degrading protease, cathepsin L, from rabbit skeletal muscle. J. Biochem. 87:1133.

- Olson, D.G. and Parrish, Jr., F.C. 1977. Relationship of myofibril fragmentation index to measures of beef-steak tenderness. *J. Food Sci.* 42:506.
- Olson, D.G., Parrish, Jr., F.C. and Stromer, M.H. 1976. Myofibril fragmentation and shear resistance of three bovine muscles during postmortem storage. *J. Food Sci.* 41:1036.
- Ouali, A., Garrel, N., Obled, A., Deval, C., and Valin, C. 1987. Comparative action of cathepsins D, B, H, L and of a new lysosomal cysteine proteinase on rabbit myofibrils. *Meat Sci.* 19:83.
- Pearson, A.M. 1987. Muscle function and postmortem changes. Ch. 4. In *The Science of Meat and Meat Products*. J.F. Price and B.S. Schweigert (Ed.), p. 155. 3rd ed. Food & Nutrition Press, Inc., Westport, Conn.
- Purchas, R.W. 1990. An assessment of the role of pH differences in determining the relative tenderness of meat from bulls and steers. *Meat Sci.* 27:129.
- Quarrier, E., Carpenter, Z.L. and Smith, G.C. 1972. A physical method to increase tenderness in lamb carcasses. *J. Food Sci.* 37:130.
- Ray, E.E., Stiffler, D.M. and Berry, B.W. 1980. Effects of hot-boning and cooking method upon physical changes, cooking time and losses, and tenderness of beef roasts. *J. Food Sci.* 45:769.
- Robbins, F.M., Walker, J.E., Cohen, S.H. and Chatterjee, S. 1979. Action of proteolytic enzymes on bovine myofibrils. *J. Food Sci.* 44:1672.
- Rolan, T.L., Davis, G.W., Seideman, S.C., Wheeler, T.L. and Miller, M.F. 1988. Effects of blade tenderization and proteolytic enzymes on restructured steaks from beef bullock chucks. *J. Food Sci.* 53:1062.
- Romans, J.R., Jones, K.W. Costello, W.J., Carlson, C.W. and Ziegler, P.T. (Ed.). 1985. *The Meat We Eat*. 12th ed. The Interstate Printers & Publishers, Inc. Danville, Ill.
- SAS. 1987. SAS/STAT User's Guide (Release 6.04). SAS Inst., Inc., Cary, N.C.
- SAS. 1989. SAS/STAT User's Guide (Release 6.06.01). SAS Inst., Inc., Cary, N.C.

- St. Angelo, A.J., Koohmaraie, M., Crippen, K.L. and Crouse, J. 1991. Acceleration of tenderization/inhibition of warmed-over flavor by calcium chloride-antioxidant infusion with lamb carcasses. *J. Food Sci.* 56:359.
- Sanderson, M. and Vail, G.C. 1963. Fluid content and tenderness of three muscles of beef cooked to three internal temperatures. *J. Food Sci.* 28:590.
- Savell, J.W., Branson, R.E., Cross, H.R., Stiffler, D.M., Wise, J.W., Griffin, D.B. and Smith, G.C. 1987. National consumer retail beef study: Palatability evaluations of beef loin steaks that differed in marbling. *J. Food Sci.* 52:517.
- Savell, J.W., Cross, H.R. and Smith, G.C. 1986. Percentage ether extractable fat and moisture content of beef longissimus muscle as related to USDA marbling score. *J. Food Sci.* 51:3.
- Savell, J.W., McKeith, F.K., Murphey, C.E., Smith, G.C. and Carpenter, Z.L. 1982. Singular and combined effects of electrical stimulation, postmortem ageing and blade tenderisation on the palatability attributes of beef from young bulls. *Meat Sci.* 6:97.
- Schmidt, G.R. and Gilbert, K.V. 1970. The effect of muscle excision before the onset of rigor mortis on the palatability of beef. *J. Food Technol.* 5:331.
- Schmidt, G.R. and Keman, S. 1974. Hot boning and vacuum packaging of eight major bovine muscles. *J. Food Sci.* 39:140.
- Smith, G.C., Culp, G.R. and Carpenter, Z.L. 1978. Postmortem aging of beef carcasses. *J. Food Sci.* 43:823.
- Smith, G.C., Dutson, T.R., Hostetler, R.L. and Carpenter, A.L. 1976. Fatness, rate of chilling and tenderness of lamb. *J. Food Sci.* 41:748.
- Stanton, C. and Light, N. 1990. The effects of conditioning on meat collagen: Part 4 - The use of pre-rigor lactic acid injection to accelerate conditioning in bovine meat. *Meat Sci.* 27:141.
- Stouffer, J.R. 1975. A method for improving tenderness of carcasses. *New York's Food and Life Sciences.* 8:14.

- Stouffer, J.R., Buege, D.R., and Gillis, W.A. 1971. Meat tenderizing method. U.S. patent 801,483. May 25.
- Swanson, L.A., Kline, E.A. and Goll, D.E. 1965. Variability of muscle fiber size in bovine longissimus dorsi. J. Anim. Sci. 24:97.
- Swatland, H.J. 1982. The challenges of improving meat quality. Can. J. Anim. Sci. 62:15.
- Sweeten, M.K., Cross, H.R., Savell, J.W. and Smith, S.B. 1990. Lean Beef: Impetus for lipid modifications. J. Am. Diet Assoc. 90:87.
- Tatum, J.D., Smith, G.C. and Carpenter, Z.L. 1982. Interrelationships between marbling, subcutaneous fat thickness and cooked beef palatability. J. Anim. Sci. 54:777.
- Tsen, C.C. and Tappel, A.L. 1959. Meat tenderization. III. Hydrolysis of actomyosin, actin and collagen by papain. Food Res. 24:362.
- Tuma, H.J., Venable, J.H., Wuthier, P.R. and Hendrickson, R.I. 1962. Relationship of fiber diameter to tenderness and meatiness as influenced by bovine age. J. Anim. Sci. 21:33.
- Wang, H., Weir, C.E., Birkner, M.L. and Ginger, B. 1957. Studies on enzymatic tenderization of meat. III. Histological and panel analyses of enzyme preparations from three distinct sources. Food Res. 23:423.
- Weiner, P.D. and Pearson, A.M. 1969. Calcium chelators influence some physical and chemical properties of rabbit and pig muscle. J. Food Sci. 34:592.
- West, R.L. 1983. Functional characteristics of hot-boned meat. Food Technol. 37:57.
- Whiting, R.C. and Strange, E.D. 1990. Effects of lactic acid on epimysial connective tissues of muscles used for restructured beef steaks. J. Food Sci. 55:859.
- Williams, J.C., Field, R.A. and Riley, M.L. 1983. Influence of storage times after cooking on Warner-Bratzler shear values of beef roasts. J. Food Sci. 48:309.

- Wismer-Pedersen, J. 1987. Chemistry of animal tissues. Ch. 3. Part 5. Water. *In The Science of Meat and Meat Products*. J.F. Price and B.S. Schweigert (Ed.). p. 141. 3rd ed. Food & Nutrition Press Inc., Westport, Conn.
- Yates, L.D., Dutson, T.R., Caldwell, J. and Carpenter, Z.L. 1983. Effect of temperature and pH on the postmortem degradation of myofibrillar proteins. *Meat Sci.* 9:157.
- Zeece, M.G. and Katoh, K. 1989. Cathepsin D and its effect on myofibrillar proteins: A review. *J. Food Biochem.* 13:157.
- Zeece, M.G., Katoh, K., Robson, R.M. and Parrish, Jr., F.C. 1986b. Effect of cathepsin D on bovine myofibrils under different conditions of pH and temperature. *J. Food Sci.* 51:769.
- Zeece, M.G., Robson, R.M., Lusby, M.L. and Parrish, Jr., F.C. 1986a. Effect of calcium activated protease (CAF) on bovine myofibrils under different conditions of pH and temperature. *J. Food Sci.* 51:797.

**APPENDIX A**

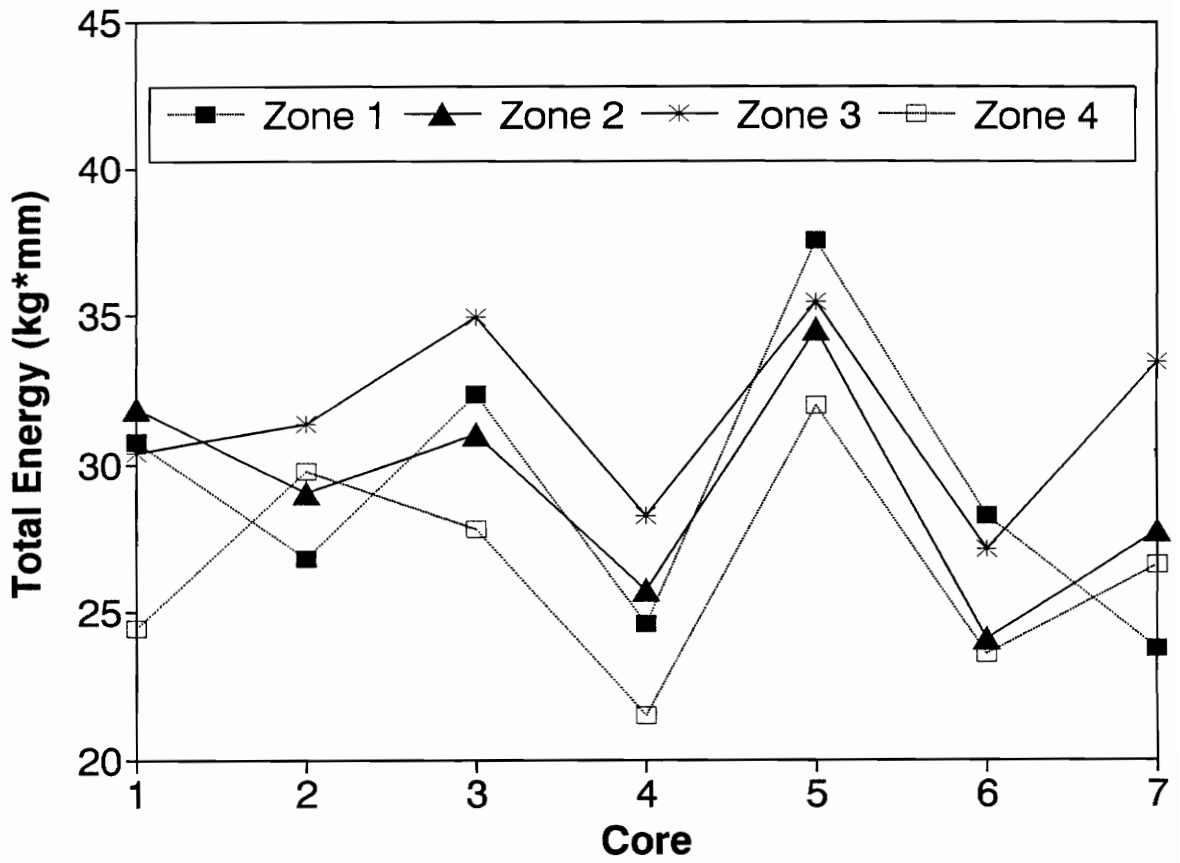


Figure 11. Total energy values by zone and core location

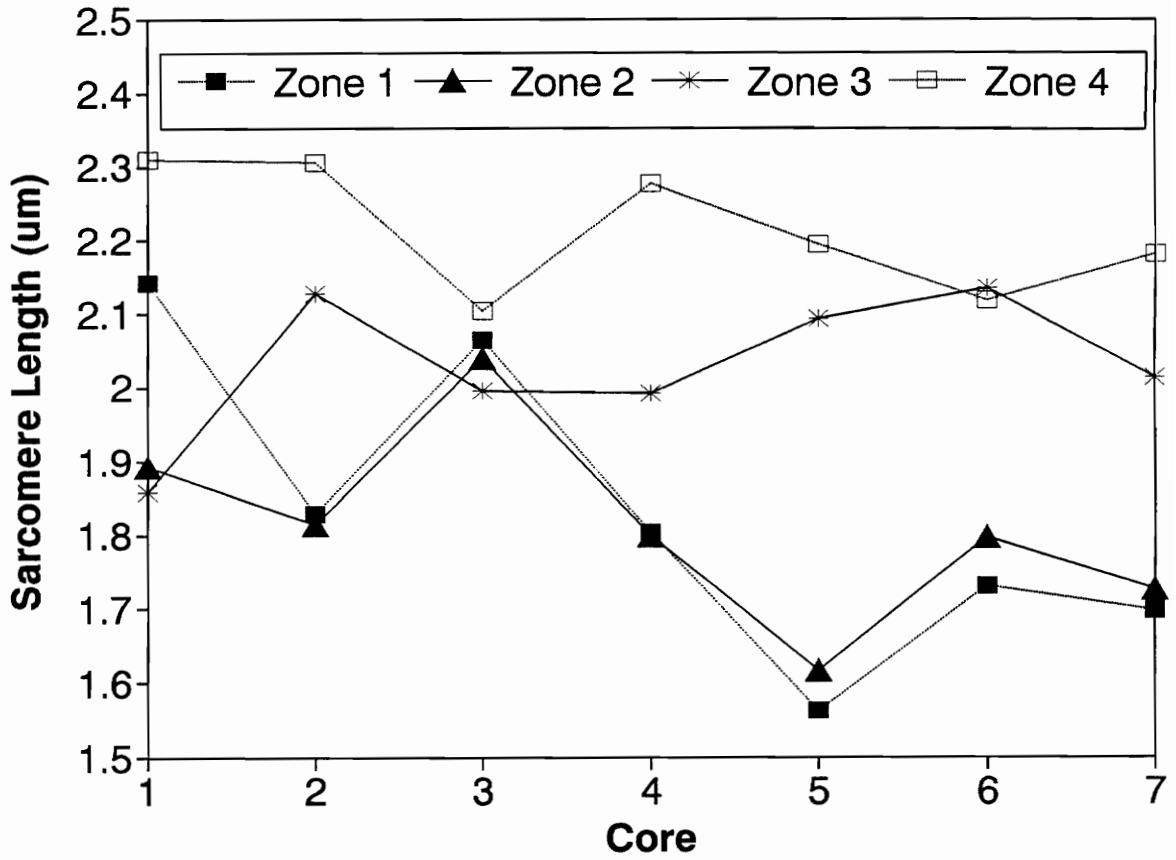
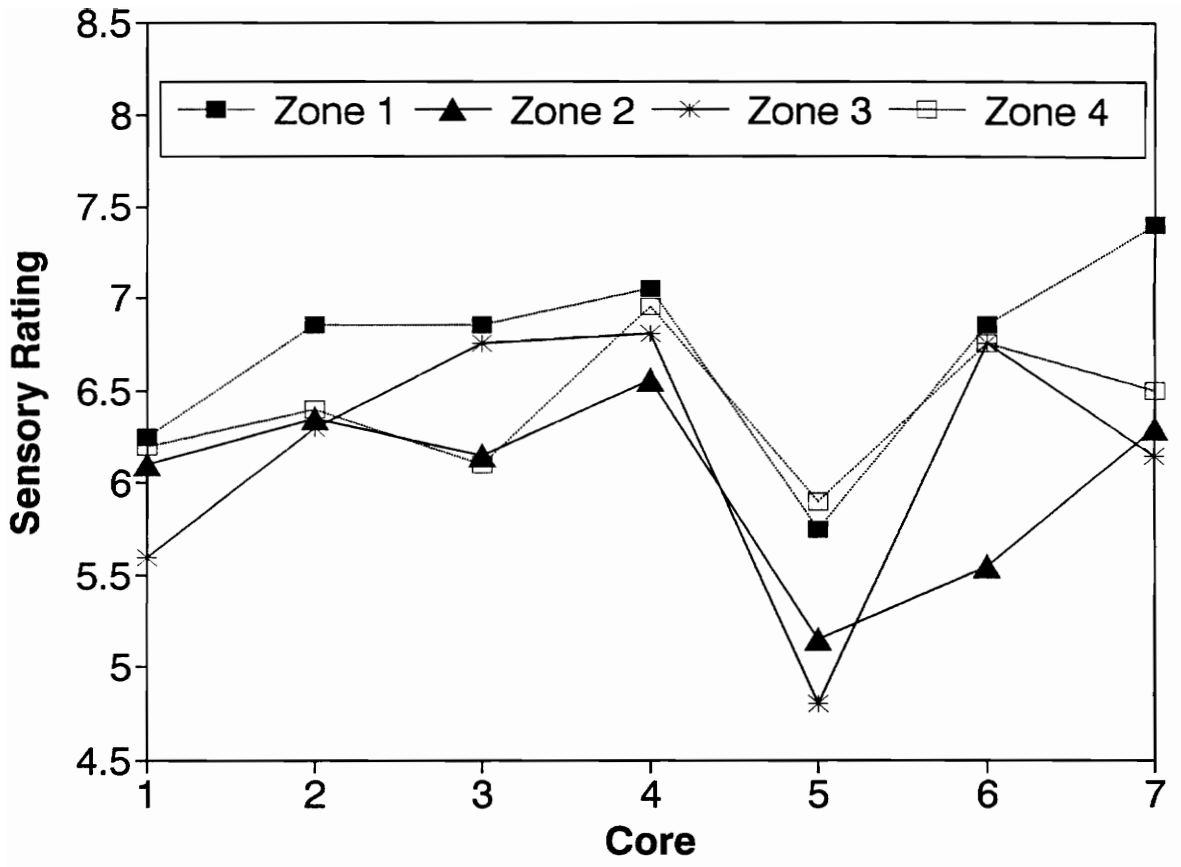


Figure 12. Sarcomere lengths by zone and core location



**Figure 13. Myofibrillar tenderness ratings by zone and core location**

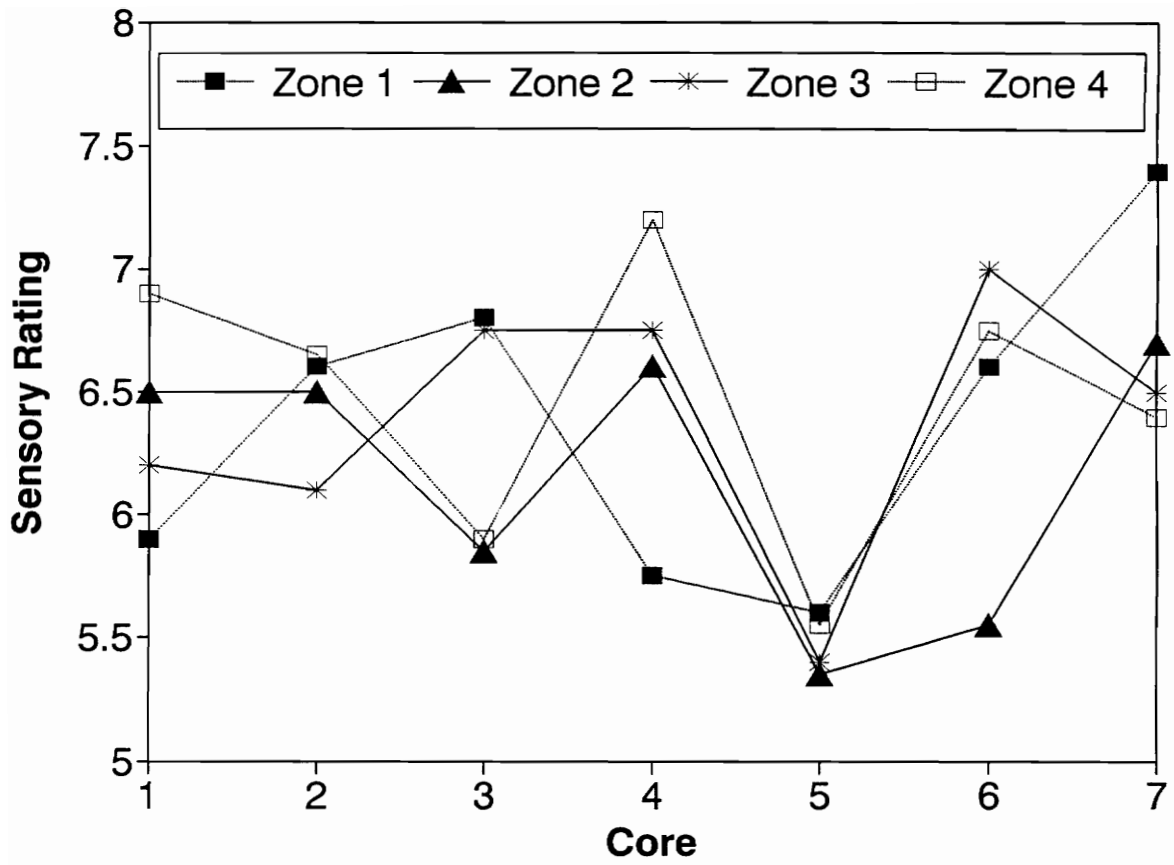


Figure 14. Connective tissue ratings by zone and core location

## **APPENDIX B**

### Iodoacetate-KCl Solution

**FOR:** Blending of prerigor muscle samples for pH determination.

**PROCEDURE:** Blend muscle sample with 10 volumes of 5 mM NaIAC in 150mM KCl. Measure pH after standardization of pH meter (1 to 2 g of tissue is often used).

**SOLUTION:** Dissolve 1.04 g NaIAC in 1000 mL of 150 mM KCl, or if free acid is used, dissolve 0.9805 g IAC free acid in 1000 mL of 150 mM KCl.

150 mM KCl = 11.184 g/L

Adjust pH to 7.0 with HCl or .1N NaOH.

(MW NaIAC = 207.93 KCl = 74.56)

This solution should be reasonably fresh and the pH checked before use. The buffering capacity is rather low and seems to change with age.

**PRINCIPLE:** The NaIAC arrests glycolytic activity in prerigor muscle and prevents pH decline while preparing sample for pH determination. The KCl adjusts the ionic strength of the solution to that of mammalian muscle and thus allows the blending of samples with up to 30 volumes of solution without affecting pH.

\*\*\*Using surface or spear type electrodes for pH

does not always agree with blended determinations.  
Differences are muscle dependent.

**SOURCE:** Bendall, J.R. 1973. In Structure and Function of Muscle. Vol. 2. p. 243. G.H. Bourne, Ed. Academic Press, New York.

### Modified Fragmentation Procedure

- 1) Thaw frozen meat (7 mm thick) approximately 15 min in a 4°C cooler. Remove epimysial tissue and subcutaneous fat.
- 2) Cut the frozen slice into 7 mm x 7 mm cubes and weigh 10 g of the cubes into a Virtis 200 mL fluted glass homogenization flask (Model No. 16 171413) in triplicate.
- 3) Add 50 mL cold solution of 0.25M sucrose, 0.02M KCl to each glass and allow 5 min for cubes to thaw.
- 4) With two Virtis micro stainless steel blades aligned and position 1 mm below the surface of the solution (Calkins and Davis, 1978) and set in a reverse position, homogenize in a Virtishear Homogenizer at full speed (30,000 RPM) for 40 sec.
- 5) By use of an adapted 115 mL Nalgene Filter Unit attached to an aspirator, filter the homogenate through a 250  $\mu$ m nylon monofilament cloth screen. Rapid stirring will expedite the filtering process.
- 6) The resulting fraction (muscle fragments greater than 250  $\mu$ m in size) with screen is blotted on Whatman No. 3 filter paper and allowed to dry at 25°C.

7) After a 40 min drying period, the net fraction weight is determined. Fragmentation index = net fraction weight (in grams) x 100. The fragmentation index will vary from approximately 10 (very tender) to 700 (very tough).

#### References

- Davis, G.W., Dutson, T.R., Smith, G.C. and Carpenter, Z.L. 1980. Fragmentation procedure for bovine longissimus muscle as an index of cooked steak tenderness. J. Food Sci. 45:880.
- Calkins, C.R. and Davis, G.W. 1978. Refinement of a muscle fragmentation procedure for predicting cooked meat tenderness. Tennessee Farm & Home Science, No. 108, p. 14. Univ. of Tennessee Agricultural Experiment Station.

## Determination of residual, soluble, and total collagen

### Modified Hill Procedure

1. Thaw frozen meat approximately 15 min at 4°C. Cut into small pieces and weigh duplicate 4.0 g portions into plastic centrifuge tubes. Refreeze and lyophilize 48 hr. Grind each sample with mortar and pestle and place in centrifuge tubes.
2. Pipette 12 mL of 1/4 strength Ringer's solution into each centrifuge tube. With separate stirring rods for each tube, stir 20 revolutions to suspend meat sample. Leave stirring rods in tubes.
3. Place centrifuge tubes in a 77°C water bath. Heat for 70 min, stirring 10 revolutions every 10 min, including at the end of 70 min.
4. Remove from water bath and allow to cool for 30 min at room temperature (17-21°C).
5. Place tubes in centrifuge cooled to 2°C running temperature. Centrifuge at 6000 x G for 10 min.
6. Remove tubes from centrifuge and pipette 8 ml of

supernatant into 50 mL screw top test tubes with teflon coated screw caps.

7. Add 8 mL of 1/4 strength Ringer's solution to each centrifuge tube and resuspend pellet using separate stirring rods for each tube.
8. Centrifuge again for 10 min.
9. Remove tubes and pipette 8 mL of supernatant into respective test tubes as in step 6.
10. Add 8 mL of dd H<sub>2</sub>O to residuals, stir to suspend (using separate stirring rods for each test tube) and pour into 50 ml screw top test tubes with teflon coated caps. Add 10 ml of concentrated (12 N) HCl to centrifuge tubes to rinse and pour into respective test tubes (conduct under fume hood).
11. Add 16 mL of concentrated HCl to each supernatant tube.
12. Screw caps on loosely and autoclave (hydrolyze) for 12 hr at 20 lbs of pressure (120-127°C).
13. Allow slow exhaust of pressure to prevent overflowing, remove tubes and cool to room temperature.

14. Add 450 ( $\pm 40$ ) mg carbon decolorizing agent to clarify supernatants, and 800 ( $\pm 40$ ) mg for residuals, mix and filter samples through Whatman No. 1 filter paper into 250 mL Erlenmeyer flasks. Aspirate to speed process. Follow with a rinse (5-10 mL of dd H<sub>2</sub>O).
  
15. Add 7 drops of methyl red indicator, mix, then slowly pipette 5N NaOH. When nearing yellow endpoint, pipette 2.5N NaOH to achieve yellow endpoint (pH 6-7).
  
16. Filter (with aspiration) samples through Whatman No. 1 filter paper into a graduated cylinder, follow with a rinse (5-10 mL) of dd H<sub>2</sub>O. Dilute supernatants to 100 mL and residuals to 500 mL with dd H<sub>2</sub>O.
  
17. Mix dilution well and store a sample of it at 4°C. Samples are now ready to analyze for hydroxyproline and are stable for a few days.

#### Reagents

1. Ringer's solution: 7.0 g NaCl, 0.026 g CaCl<sub>2</sub>, 0.35 g KCl brought to 1000 mL with dd H<sub>2</sub>O. Prepare 1/4 strength Ringer's by mixing 1 part of Ringer's solution with 3 parts dd H<sub>2</sub>O.

2. 12 N HCl
3. 5 N NaOH: 200 g NaOH brought to 1000 mL with dd H<sub>2</sub>O (highly exothermic reaction).
4. Methyl red indicator: 0.02 g methyl red granules dissolved in 100 g 95% methanol.

**Determination of hydroxyproline by a modified  
Bergman and Loxley rapid procedure**

1. Pipette duplicate 1 mL aliquots of each sample into 15 mL screw top tubes, 1 mL dd H<sub>2</sub>O into each blank, and 1 mL aliquots of each standard (2-12 ug/mL).
2. Pipette 2 mL of isopropanol and vortex for about 10 seconds.
3. Pipette 1 mL of oxidant solution, vortex, and allow to stand 4 min at room temperature (17-21°C)
4. Pipette 2 mL Erlich's reagent and vortex well, screw on caps to limit evaporation.
5. Heat tubes for 25 min (±15 sec) in a 60°C water bath.

6. Cool tubes for 4.5 min in running tap water.
7. Mix and measure absorbance at 558 nm against a 0 ug/mL blank immediately (20 min maximum).

## Reagents

1. Isopropanol
2. Oxidant solution
  - A. 3.5 g chloramine T dissolved in 50 mL dd H<sub>2</sub>O, store in refrigerator, discard after 1 month.
  - B. Acetate/citrate buffer: 34.4 g sodium acetate anhydrous, 37.5 g trisodium citrate dihydrous, 5.5 g citric acid monohydrous and 385 mL isopropanol. Check pH before addition of isopropanol, it should be close to 6, if not, adjust down with concentrated acetic acid. Bring to 1000 mL with dd H<sub>2</sub>O, store at room temperature and discard after 1 month.
  - C. Oxidant solution: Mix 1 volume of A with 4 volumes of B. Make fresh daily before use.
3. Erlich's Reagent
  - A. Dissolve 2.0 g p-dimethylaminobenzaldehyde (DABA) in 2.5 mL of 70% perchloric acid (mix under perchloric

acid hood). Store in refrigerator up to 1 month; if green color appears, it is inactive.

B. Isopropanol

C. Erlich's reagent: Mix 3 volumes of A with 13 volumes of B (mix under perchloric acid hood). Make daily before use.

#### 4. Stock hydroxyproline and standards

A. Stock: Dissolve 0.1 g hydroxyproline per liter of 0.001 N HCl and store in refrigerator. Discard after 1 month.

B. Working standards: 2-12 ug/mL, example: 2 mL stock hydroxyproline brought to a 100 mL volume=2 ug/mL. Store in refrigerator, good for 1 month.

#### Calculations for Collagen Analysis

1. From absorbances for standards, prepare a regression of ug/mL on the x-axis and absorbance on the y-axis. Use absorbance to obtain ug/mL for each sample.
2. Multiply ug/mL by the total volume to which the sample was diluted (100 for supernatants and 500 for residual). Divide this value by the grams of sample (4.0) to get ug hydroxyproline/g sample.

3. Convert hydroxyproline to collagen by multiplying the supernatants by 7.52 (Cross et al., 1973) and the residuals by 7.25 to get ug collagen/g of sample.
4. Divide by 1000 to convert ug collagen/g to mg collagen/g.
5. Report as soluble collagen (supernatant), insoluble collagen (residual), total collagen (soluble+insoluble) and % soluble collagen (soluble/total x 100).

#### References

- Bergman, I. and Loxley, R. 1963. Two improved and simplified methods for the spectrophotometric determination of hydroxyproline. *Anal. Chem.* 35:1961.
- Cross, H.R., Carpenter, Z.L. and Smith, G.C. 1973. Effects of intramuscular collagen and elastin on bovine muscle tenderness. *J. Food Sci.* 38:998.
- Hill, F. 1966. The solubility of intramuscular collagen in meat animals of various ages. *J. Food Sci.* 31:161.

## Sarcomere length measurements

### Laser Method

Preparation for fixed tissue:

SOLUTION A:

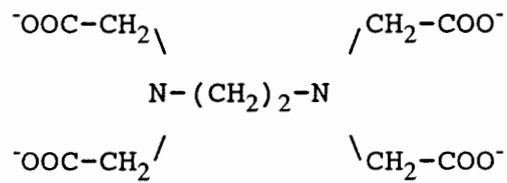
0.1M KCL, .039M Boric acid, and 5 mM EDTA in a 2.5% glutaraldehyde solution

Potassium Chloride; Fwt. = 74.56

Boric acid;  $H_3BO_3$ , Fwt. = 61.83

Disodium Ethylenediaminetetraacetic acid  
dihydrate Fwt. = 370.238

Disodium EDTA dihydrate,  $Na_2C_{10}H_{14}N_2O_8 \cdot 2H_2O$



Glutaraldehyde; Fwt. = 100.12

SOLUTION B:

0.25M KCL, 0.29 M Boric acid, and 5 mM EDTA in a 2.5% glutaraldehyde solution

1. Excise small pieces of muscle. (3 X 2 X 2 cm)
2. Place the sample in a vial and cover with Solution A for two hours.
3. Transfer the sample to a fresh vial and cover with Solution B.
4. Soak overnight (17-19 hr) and measure sarcomere lengths within two days.
5. Turn laser on to warm up for at least 15 min. before making measurements. The following Safety statement is quoted from VPI's LASER SAFETY TRAINING MANUAL:

Class 3a lasers cannot damage the eye within the duration of the blink or aversion response. However, injury is possible if the beam is viewed through binoculars or similar optical devices, or by staring at the direct beam. Power outputs for CW lasers operating in the visible range are between 1-5mW.

6. Tease out individual fibers from the sample and place on a microscope slide with a drop of Solution B.
  
7. Place the slide on the laser so that the light goes through the fiber. Care should be taken to prevent the sample from drying out. Start at one edge and move the slide until you find diffraction patterns.
  
8. Measure and record the distance between the origin and the first order diffraction band, or measure the distance between the two first order diffraction bands and divide by two.

		GLUTARALDEHYDE			
		KCL	BORIC	EDTA	DILUTE TO 50%
	<u>mL</u>	<u>(g)</u>	<u>(g)</u>	<u>(g)</u>	<u>(mL)</u>
Solution A	100	0.7456	0.241	0.185	5.0
Solution B	100	1.8640	1.793	0.185	5.0

CALCULATIONS:

(Cross et al. 1981)

$$\text{Sarcomere Length, } \mu\text{m} = \frac{.6328 \times D \sqrt{(T/D)^2 + 1}}{T}$$

Where, D = distance in mm from specimen to the diffraction screen. This should be set at 100 mm before the measurements are taken.

T = distance in mm from the origin to the first order diffraction band or the distance between the two first order diffraction bands divided by two.

.6328 = wavelength of the He-Ne laser light.

OR

Physics Formula

Sarcomere length  $\mu =$

$$\tan \phi = x/y$$

$$\phi = \tan^{-1}$$

$$d = .6328/\sin \phi$$

$$d = \text{length in } \mu$$

where x = distance in mm from the origin to the first order diffraction band or the distance between the two first order diffraction bands divided by two.

y = distance in mm from specimen to the diffraction screen. This should be set at 100 mm before measurements are taken.

.6328 = wavelength of the He-Ne laser light

NOTE: procedure takes 40-45 min. when collecting 15 sarcomere lengths per sample.

#### REFERENCES

Cross, H.R., R.L. West, T.R. Dutson. 1981. Comparison of Methods for Measuring Sarcomere Length in Beef Semitendinosus Muscle. Meat Science 5:261.

Koolmees, Peter A., Frank Korteknie and Frans J.M. Smulders. 1986. Accuracy and Utility of Sarcomere

Length Assessment by Laser Diffraction. Food  
Microstructure 5:71.

Locker, R.H. and C.J. Hagyard. 1963. A Cold Shortening  
Effect in Beef Muscles. J. Sci. Fd. Agric., 14:787.

Orcutt, M.W., T.R. Dutson, D.P. Cornforth and G.C. Smith.  
1984. Factors Affecting the Formation of a Dark,  
Coarse Band ("Heat Ring") in Bovine Longissimus  
Muscle. J. Anim. Sci. 58:6.

Ruddick, J.E. and J.F. Richards. 1975. Comparison of  
Sarcomere Length Measurement of Cooked Chicken  
Pectoralis Muscle by Laser Diffraction and Oil  
Immersion Microscopy. J. Food Sci. 40:500.

Schrock, Calvin. 1987. Effects of Spray Chilling on  
Beef Carcass Yields and Traits, Cut-Out Yields,  
Vacuum Aging Purge Losses and Warner-Bratzler Shear  
Force Values. Masters Thesis. KSU.  
Manhattan, Kansas.

Voyle, C.A. 1971. Sarcomere Length and Meat Quality.  
Proc. 17th European Meeting of Meat Research  
Workers, Bristol, England. p. 95.

Weber A., K. Wassilev, A.J. Moller, B. Schmidt-Nielsen,  
and F. Glad. 1988. Computerized laser  
diffractometer for routine measurements of sarcomere  
lengths in meat. J. Food Sci. 53:3.

Mean Sarcomere Lengths Obtained From Muscles  
Taken 2-3 Days Postmortem<sup>a</sup>

---

Muscle	Sarcomere length ( $\mu$ )
Semimembranosus	1.70
Gluteus medius	1.68
Longissimus	1.80
Biceps femoris	1.78
Semitendinosus	2.30
Infraspinatus	2.10
Psoas major	3.20

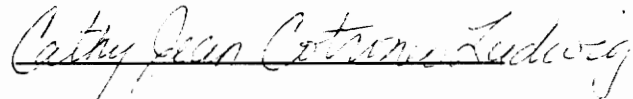
---

<sup>a</sup>Bouton, P.E. et al. 1973. J. Food Technol. 8:39

Notes: Plan on 40 fibers per hour for analysis

## VITA

Cathy Jean Cotroneo Ludwig was born on December 30, 1968 in Rome, New York. She is the daughter of James and Florence Cotroneo. Cathy received her high school diploma from Rome Free Academy in June, 1987 and her Bachelor's degree in Biology from State University of New York at Geneseo in December, 1990. She married Dale Richard Ludwig on June 20, 1992. She received a Master of Science degree in Food Science and Technology at Virginia Polytechnic Institute and State University in July, 1992. Cathy has accepted a position as Packaging Specialist with Cryovac Division of W.R. Grace & Co. in Duncan, South Carolina.

  
Cathy Jean Cotroneo Ludwig