OPTIMAL UTILIZATION OF THE BEEF CHUCK

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(ABSTRACT)

The effects of a prerigor $CaCl_2$ injection and blade tenderization on several sensory and physical characteristics of beef *infraspinatus* and *longissimus* muscles were determined. Blade tenderization resulted in increased tenderness (P<0.05) of postrigor *infraspinatus* muscle as measured by sensory panel, and numerically higher, though insignificant (P>0.05), sensory scores for postrigor *longissimus* muscle. However, no improvements (P>0.05) in tenderness of prerigor $CaCl_2$ injected muscles were observed due to blade tenderization. Objective and subjective measurements revealed that prerigor $CaCl_2$ injected muscles were less tender (P<0.05) than postrigor muscles independent of blade tenderization. Furthermore, steaks from prerigor $CaCl_2$ injected cuts had lower (P<0.05) texture scores for both muscles and flavor scores for *infraspinatus* samples, as well as higher microbial counts (P<0.05) than samples from postrigor muscles. Increased aging from 3 days to 7 days resulted in improved (P<0.05) tenderness, but also resulted in decreased (P<0.05) flavor, texture, color, and overall appearance scores, as well as increased (P<0.05) TBA values and microbial load.

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CHAPTER I

INTRODUCTION

During the past decade, the attitude of consumers toward meat has changed. Contemporary health concerns over saturated fat and cholesterol intakes have caused a reduction in the consumption of meat (Anonymous, 1990). In previous years, consumers perceived meat as a nutritious and flavorful product and purchases were dictated by the availability of product marketed by meat processors (Jeremiah, 1982). However, contemporary consumers are very discriminating and demand tasty, appealing, low-fat meat at affordable prices. Beef in particular has suffered from association of its image with high fat, expensive products. Meat processors must now cater to the consumers' demands and market products that will maximize consumer appeal.

The amount of disposable income has traditionally been the main determinant of the foods purchased (Jeremiah, 1982). In addition, tenderness has been identified as the most important factor in the perception of meat by consumers (Savell et al., 1989; Whipple et al., 1990). Consumers have also long preferred steaks over ground beef and roasts. However, those portions of the beef carcasses that produce the most tender steaks are limited and comprise less than 25% of the total carcass weight. This limitation in product availability results in high prices for these cuts (Mandigo, 1982a). Alternative use of other carcass portions such as the beef chuck to produce consistently tender steaks has not been successful. This problem is attributable to the inherent differences in tenderness found within muscles from this cut (Morgan et al., 1991b). The beef chuck is traditionally marketed as low priced steaks or roasts and has been described as the most underutilized wholesale cut from the beef carcass (Cecchi et al., 1988). Use of treatments to improve the tenderness of the chuck muscles will add value to the whole carcass by enabling processors to market consistently tender steaks. The intent is to increase returns for the beef processor and satisfy the consumers' demand for tender, affordable steaks. A variety of treatments have been evaluated to improve tenderness.

Electrical stimulation can increase tenderness. The mechanism of tenderization is reported to consist of three parts: hastening of rigor with less rigor and cold shortening; activation of proteolytic enzymes at a high pH and high temperature; and disruption of the muscle structure as a result of extensive contraction (Dutson et al., 1980). However, adverse effects on water holding capacity due to electrical stimulation have also been reported (Judge et al., 1989).

Blade tenderization causes improved tenderness by disrupting the muscle structure with thin blades which penetrate the muscles. While a convenient and effective method for improving tenderness, blade tenderization has been reported to result in higher drip losses during storage and reduced storage case life (Davis et al., 1977).

The effect of different methods of carcass suspension has also been investigated. Conventional suspension methods may release tension and cause shortening in some muscles including the *longissimus* muscle (Hostetler et al., 1970). Recently, it has been reported that skeletal alterations through a prerigor cut through the 12th thoracic vertebrae results in improved tenderness (Cotroneo, 1992).

Chemical methods for improving tenderness have been proposed by some researchers. Whiting and Strange (1990) reported that improved tenderness results from immersing meat in lactic, citric, and acetic acids.

Use of proteolytic enzymes, including papain, bromelin, and ficin, to hydrolyze collagen in meat have been reported to improve tenderness. However, this treatment may also result in overtenderized meat as well as development of off-flavors (Smith et al., 1979).

It has been reported that calcium chloride ($CaCl_2$) injections result in tenderness improvements and reductions in the postmortem storage time necessary to produce more tender meat. Tenderization is due to the increased calpain activity caused by the increase in the calcium ion concentration (Koohmaraie et al., 1991, St.Angelo et al., 1991). However, increased bitter and salty flavor notes in injected meat may also result from $CaCl_2$ injection treatments (St.Angelo et al., 1991).

These treatments may or may not be practical for large scale production applications. Possible applications of tenderizing treatments to improve the acceptability of beef chuck muscles will be beneficial for meat processors as well as the consumers. Thus, the objective of this study was to determine the effect of a prerigor $CaCl_2$ injection as well as blade tenderization on physical and sensory properties of beef chuck steaks from the *infraspinatus* and *longissimus* muscles.

CHAPTER II

REVIEW OF LITERATURE

2.1. Beef Chuck Utilization.

During the past decade consumer health and budget concerns have caused a decrease in the consumption of beef and other meats. Beef, in particular, suffered a loss of 7% to fish and poultry between 1985 and 1986 (Anonymous, 1990). Price has traditionally been a major factor influencing meat consumption. In previous years the nutritional and taste qualities of meat made it very appealing to consumers who essentially purchased whatever products were available (Jeremiah, 1982). However, contemporary consumers are very discriminating in their meat purchases and their preferences dictate the kind of retail cuts that will be produced. Consumers want tasty, appealing, low-fat meat at a reasonable price. Health and weight concerns regarding fat intake in meat consumption are attributable for the decrease in meat consumption, especially red meat, in the past years (Jeremiah, 1982). In order to reverse this trend beef processors must market products which emphasize factors that will make this commodity more appealing to consumers.

Many factors are involved in the consumers acceptance of red meat. Among these are color, flavor, tenderness, and price. The amount of disposable income has been identified as the main determinant of the type of food purchased (Jeremiah, 1982). Furthermore, beef tenderness has been identified as the most important factor in consumers' perception of meat (Calkins and Seideman, 1988; Savell et al., 1989; Whipple et al., 1990). The National Consumer Retail Beef Study (Savell et al., 1989) has identified tenderness as the single most important factor which affects the taste perception in meat consumption. Meat processors must be able to market tender retail cuts that will appeal to the customer.

Meat processors have experienced difficulty in producing consistently tender cuts from the chuck due to the inherent differences in tenderness found within muscles from this cut (Morgan et al, 1991b). This difficulty may reduce the possible uses of cuts from the chuck, especially those muscles which are not tender enough to be marketed as steaks or roasts. Extensive research has been focused on methods to increase the tenderness of meat. Through the use of innovative methods that will improve the tenderness of chuck muscles, whole muscle cuts that will appeal to the consumer may be produced. The typical wholesale beef chuck represents about 26% of the carcass. This cut is traditionally marketed as low priced steaks and roasts or ground beef (Paterson and Parrish, 1986). The chuck has been described as perhaps the most under utilized wholesale cut of beef carcasses (Cecchi et al., 1988). Any treatment that may increase the tenderness of the chuck will add value to the whole carcass and increase returns for the producer.

Previous studies have shown that a variety of treatments may improve tenderness including electrical stimulation (Hostetler et al., 1982; Eikelenboom et al., 1985), blade tenderization (Savell et al., 1982; Medeiros et al., 1989), and calcium chloride infusion (Koohmaraie et al.,1988b, 1989, 1990, 1991). These treatments may or may not be practical for large scale production.

2.2 Tenderness and Texture Considerations.

The ability to produce meat with consistent tenderness is extremely important in order to market a product that will appeal to consumers. A high correlation (r=0.904) between consumer acceptance of meat and tenderness has been reported (Means and King, 1959). However, the perception of tenderness is very difficult to define or duplicate using objective methods. The tenderness of muscle can be affected by connective tissue and muscle fibers (Price and Schweigert, 1987). Tenderness has been described as the following (Judge et al, 1989):

1- Softness to tongue and cheek

2- Resistance to tooth pressure

3- Ease of fragmentation

4- Adhesion

5- Residue after chewing

Tenderness can be measured by objective and subjective methods. Objective methods can be divided into chemical and mechanical. Chemical techniques concentrate on the measurement of connective tissue content. Mechanical methods are divided into shear force and penetration force determinations, and biting devices (Price and Schweigert, 1987). The instrument which is typically used is the Warner-Bratzler shear device. It measures the amount of force needed to shear through a cylindrical core of meat with a 1 mm. blade. Less force is required to shear tender samples. According to Stanley et al. (1971) this instrument has been shown to correlate well with tenderness measurements conducted with trained sensory panels. Another measurement of tenderness involves subjective evaluation by a consumer panel. These panelists are able to integrate all of the perceptions which affect tenderness, such as juiciness, texture, and flavor, to give an accurate measurement of tenderness (Price and Schweigert, 1987).

There are several variables which influence tenderness of meat. These can be divided into pre- and post-slaughter factors.

2.3 Pre-Slaughter Factors.

Pre-slaughter factors are generally inherent to the animal being slaughtered and determine the starting quality of meat. These factors will affect the type and distribution of connective tissue, intramuscular fat, and other factors which will affect tenderness.

2.3.1. Yield and Quality Grades.

Meat grading is a procedure of product segregation on the basis of expected palatability or yield attributes, or other economically important traits (Judge et al., 1989). Grading of beef is administered by the Agricultural Marketing Service of the USDA. There are two grades assigned to beef. Quality grades segment beef into groups with similar expected palatability attributes. Yield grades (numerical grades) categorize carcasses on the basis of quantity determined by the expected yield of trimmed retail cuts (Judge et al., 1989).

Since quality grades are designed to separate beef into expected palatability groups, researchers have investigated the relationship of quality grades to tenderness. The literature provides conflicting evidence in this area. While some researchers have discovered close associations between quality grades and palatability (Jennings et al., 1978; Davis et al., 1979), others have reported that an assigned quality grade or marbling scores may not be good predictors of beef palatability (Campion et al., 1975). Research has demonstrated that even when all grading factors are the same there exists tenderness variations among carcasses of animals with the same quality grade (Davis et al., 1979).

Alternative methods of quality grading have been proposed by researchers where subcutaneous fat thickness is considered a more precise predictor of beef palatability (Dolezal et al., 1982). Some of the factors considered in grading, such as marbling and maturity, appear to be more closely associated with palatability and tenderness than others. Marbling results in a juicy and flavorful product while little or no intramuscular fat results in a dry, flavorless, and tougher product (Price and Schweigert, 1987). Smith et al. (1984) reported that marbling explained 33% and 7% of the variation in palatability of beef loins and top rounds, respectively. Loin steaks with moderately abundant marbling have been reported to be more likely to have lower shear force values than loin steaks with practically devoid marbling (Smith et al., 1984). However, Wheeler et al. (1990) reported that differences in marbling degrees within top sirloin steaks of U.S. choice grade did not have a significant effect on shear force values and sensory tenderness ratings.

Maturity is considered to be more closely related to tenderness than any other factor used in grading (Judge et al., 1989). Physiologically mature animals tend to produce meat that is less tender than that from immature animals. This difference results from the contribution of connective tissue to the toughness of meat. Older animals have collagen which is more heat stable, hence tougher, than younger animals due to the increase in covalent crosslinks which occur with age (Shimokomaki et al., 1972).

2.3.2. Connective Tissue.

Meat tenderness is strongly related to its structural components. Toughness has been shown to depend on the extent of muscle fiber contraction (Herring et al., 1967) as well as background toughness caused by the presence of connective tissue (Burson and Hunt, 1986). In living tissue these proteins support the structure of the animal's body. The tensile strength and resistance to stress of these proteins allow the efforts of muscle contraction to be transmitted to the tendons and bones ultimately resulting in action. It is this strength which is responsible for the toughness observed in meat. Even after denaturation connective tissue fibers retain tensile strength (Shimokomaki et al, 1972). Connective tissue in meat is composed mainly of different types of collagen and elastin. Elastin is found in smaller amounts than collagen in connective tissue hence its contribution to toughness is smaller. This is a protein with elastic properties found in tissues which undergo continual tension and deformation. Elastin is very stable and is not solubilized by cooking or enzymes present in the muscle. This is a result of desmosine cross-links and the high proportion of non-polar amino acids which are found in the molecules (Price and Schweigert., 1987)

Judge et al. (1989) identified collagen as the most abundant protein in the animal body comprising 20-25% of the total protein. This protein is the main structural component of connective tissue. The repeating unit of collagen has the following arrangement: Gly-X-Y, where X and Y are any amino acid but approximately one third of the time, X and Y are either proline or hydroxyproline (Nguyen and Zarkadas, 1989). Hydroxyproline occurs mainly in collagen and is not significantly present in other proteins. This allows hydroxyproline assays to be used for collagen determination in meat tissues. There are several genetically different species of collagen termed types I, II, III, IV, and V. Types I and III predominate in muscle (Light and Champion, 1984). Type I is found mainly in tendons and the epimysium. The perimysium has been shown to contain mainly type III while the endomysium contains a mixture of types I and III (Bailey and Sims, 1977;

Light and Champion, 1984). Some researchers have reported that more tender muscles have a lower proportion of Type III collagen than do tougher muscles (Bailey et al., 1979), while other studies reported that the proportion of type III collagen was not different in muscles varying in tenderness (Burson and Hunt, 1986).

Collagen is stabilized by intra and intermolecular cross links. Of these, intermolecular cross links result in a greater stabilization of the collagen fibers. Reducible intermolecular cross links are ultimately converted to stable non-reducible aldimine bonds through aldol condensations. These aldimine bonds stabilize the fiber (Shimokomaki et al, 1972). A higher proportion of stable cross links results in a less soluble collagen. Collagen undergoes changes during cooking that will affect meat quality. When the insoluble collagen is heated it undergoes shrinkage. The shrunken collagen may increase toughness by expelling water as well as compressing the myofibrillar matrix (Ledward, 1984). The number of stable cross links which occur in collagen increase with the age of the animal (Judge et al., 1989).

2.3.3. Age.

Animal age has a very important effect on meat tenderness. It is well known that older animals produce tougher meat than those that are younger. This difference is attributable to the different nature of connective tissue found in young and old animals. The total collagen content changes very little with age but the number of stable intermolecular cross links increases with animal age. As the animal becomes older, collagen stabilization increases and solubility decreases. Decreased solubility results in less tender meat as the collagen cannot be dissolved by cookery or protease action during aging. However, meat toughness increases are not strictly linear with age. Shimokomaki et al. (1972) reported that the most tender beef should occur at about 12-14 months of age. Since the amount of connective tissue changes little with age, this trend is likely due to the rapid increase in the size of muscle fibers which causes a dilution of the existing connective tissue (Judge et al., 1989)

2.3.4. Fiber Size and Sarcomere Length.

Fiber diameter has an important effect on tenderness. Larger fiber diameter is reported to result in decreased tenderness. A decrease in fiber diameter caused by application of tension on the muscle prior to rigor completion results in increased tenderness. Differences in sarcomere length were highly related to differences in shear force ($r=.73$, $P< 0.01$) (Herring et al., 1965). Muscle contraction causes fiber shortening, increased fiber diameter, and reduces sarcomere length (Judge et al., 1989), resulting in less tender meat (Herring et al., 1967),. Locker (1960) reported that short sarcomere length is associated with less tender meat. An increase in sarcomere length is reported to decrease shear force (Herring et al., 1965). The same study reported that strong correlation exists between sarcomere length and shear force values ($r=.80$, $P< 0.01$). The toughening effect of shortening measured as the correlation between panel tenderness and sarcomere length was reported to be higher in muscles with slow glycolysis rates ($r=0.84$) as compared with those of faster pH decline ($r=0.16$) (Smulders et al., 1990).

2.3.5. Muscle Origin.

The origin of a specific muscle within the carcass has an important effect on tenderness. Muscles which are used heavily in locomotion are less tender than those which are supportive and have minimal functional use. This is due to the differences in connective tissue amounts which are needed for function. Less tender meat results from muscles which are not tensioned during rigor mortis (Price and Schweigert, 1987). This may cause shortening of the muscle which in turn results in tougher meat. Researchers have reported that muscles from the round and chuck are generally rated as tougher than those from the loin or rib, with the exception of the *infraspinatus* (McKeith et al., 1985). The *longissimus* and *infraspinatus* muscles have been rated as slightly tender when compared to other muscles in beef (Judge et al., 1989). The *infraspinatus* and *longissimus* within the chuck have been ranked as the first and second most tender muscles respectively (Paterson and Parrish, 1986).

2.4 Post-Slaughter Factors.

Post-slaughter conditions and treatments may have a profound effect on the tenderness of meat. To some extent these conditions can be controlled by meat processors to maximize the tenderness and eating quality of meat. Considerable research has been focused on postmortem conditions to maintain and improve tenderness.

2.4.1. Rigor Mortis.

It has been known for centuries that rigor mortis causes stiffening and toughening of meat. The stiffening is produced by permanent crossbridges which form between actin and myosin filaments. Relaxation of the muscle is impossible as the ATP needed to relax the muscle is depleted (Judge et al., 1989). The increase in toughness associated with rigor mortis has been attributed to postmortem contraction of the muscle (Locker, 1960). Rigor contraction may occur due to the release of calcium ions during rigor development (Honikel et al., 1981). The degree of shortening caused by contraction will determine the loss of tenderness (Marsden and Henrickson, 1977). Inhibition of contraction during rigor mortis development will improve tenderness compared to uninhibited muscle. By treating the muscle with chelating agents that sequester calcium ions, formation of actomyosin and contraction are prevented, thus improving tenderness (Carpenter et al, 1961; Streitel et al, 1977).

2.4.2 Temperature and pH.

Post-rigor meat quality is affected by a series of events taking place during rigor development. These are rate of pH decline, ultimate pH, and temperature. All these factors are intimately interrelated. The pH decline is directly related to postmortem temperature (Dutson, 1983). Low postmortem storage temperatures will slow the rate of pH decline and may bring about cold shortening. Cold shortening is caused by a rapid release of calcium ions during chilling which results in contraction of the muscle (Judge et al, 1989). It is desirable to minimize the amount of time that prerigor muscle is exposed to excessively cold temperatures to minimize cold shortening.

Electrical stimulation in the industry is widely used to reduce cold shortening (Kastner, 1982; Romita et al., 1987). Elevated postmortem temperatures generally improve tenderness. This is due to the prevention of cold shortening and the increased rates of proteolysis which occur at higher temperatures, which are near the proteases' optimum temperature of 37 °C (Dutson, 1983). Elevated temperatures stimulate the release of lysosomal enzymes as well as increase their activity in degrading meat proteins and tenderizing meat during aging (Moeller et al., 1976).

Ultimate pH is that achieved by meat after completion of rigor mortis. This may be affected by the amount of glycogen present in the muscle. Glycogen is used as a source of energy for ATP production and is converted to lactic acid in the process. Lactic acid accumulates in the muscle due to the failure of the circulatory system after slaughter of the animal and lowers the pH of muscle. The pH continues to fall until nearly all the glycogen stores are depleted or the glycolytic enzymes are inactivated by the low pH (Judge et al., 1989). High initial glycogen levels may ultimately result in a lower final pH. Glycogen levels may be reduced by stress, exercise, and starvation and this may result in a higher ultimate pH as less glycogen is available to be converted to lactic acid. The typical ultimate pH in beef is 5.7 (Newbold and Harris, 1972). It has been reported that tenderness will be minimized at pH 6.0 and increased with an ultimate pH above or below 6.0. Above pH 6.0, meat may be more tender due to increased activity of calpains which have an optimum pH near neutral. However, high pH may result in dark cutting beef which is tender but is associated with low quality meat (Dutson, 1983). Below pH 6.0, meat is believed to be more tender through the action of lysosomal enzymes which have a pH optimum of 4.0-6.0. In addition, disruption of lysosomal membranes which release the enzymes is more prevalent in an acidic pH (Dutson, 1983).

The water holding capacity of meat is also affected by pH. A fall in pH reduces the number of protein groups which are available for water binding and reduces the water binding capacity of meat (Judge et al., 1989).

2.4.3. Chilling.

Traditional processing of meat in the U.S. involves refrigeration before cutting and subsequent aging in coolers for several days to improve tenderness. After slaughter, body heat must be removed. Chilling of beef carcasses in conventional coolers to an internal temperature of 5°C or less may take as long as 48 hours. Slow chilling rates may result in excessive evaporative and drip loss (Taylor, 1987). Chilling is also responsible for a large part of the cost of the meat industry operation. Any reduction of chilling time or cooler space requirements would represent substantial savings for the industry (Kastner, 1982). More efficient coolers with high air velocity may reduce this time. Recently, rapid chilling and spray chilling methods have received considerable attention from the industry as ways to reduce chilling time (Hippe et al., 1991). Rapid chilling has several advantages as it permits greater throughput and more rapid distribution of meat, reduces weight losses by forming a protective crust on the meat surface, and reduces drip loss. However, rapid chilling may also result in cold shortening and decrease in tenderness (Rosset, 1982). Hippe et al. (1991) reported that spray chilling reduced cooler shrink in beef but had no effect on purge loss from vacuum packaged cuts, cook loss or shear values.

2.4.4. Aging.

After chilling of beef to internal temperatures of 5 °C or lower, meat is usually kept at refrigeration temperatures for several days. This process, called aging, improves the tenderness of meat and is related to the resolution of rigor stiffening. Smith et al. (1978) reported that tenderization by aging was optimized at 11 days as further aging did not significantly improve tenderness. However, Savell et al.

(1978b) reported that aging for 21 days compared to 7 days did improve tenderness significantly. The resolution of rigor is brought about by protease activity which degrades the myofibrillar components. These proteases have been identified as calpains which act before acidification occurs and the release of cathepsins at a lower pH. Degradation by these proteases of Z-disk structure as well as troponin-T results in fragmentation of the myofibrillar structure into smaller fragments and is related to increased tenderness of aged meat (Olson et al., 1977). This degradation has been demonstrated by the appearance of a 30,000 dalton component previously non-existent in fresh muscle and is considered to be an indicator of muscle proteolysis (Olson et al., 1977). It has been reported that the 30,000 dalton component is present in tender but not in tough *longissimus* muscle from A maturity carcasses aged for 1 and 10-14 days (McBride and Parrish, 1977; Parrish et al., 1981) but was found in both tender and tough *longissimus* muscle from E maturity carcasses aged 10-14 days (Parrish et al., 1981).

2.4.5. Proteases Involved in Aging.

Two types of enzymes are known to be involved in the tenderness increase observed during aging. They are Cathepsins and Calpains (Etherington, 1987; Koohmaraie et al., 1988c).

Cathepsins are lysosomal enzymes which have acidic pH optimums (2.5-6.0). Cathepsins B,D,H, and L have been shown to degrade one or more of the following: troponin-T, myosin, Z-line structures, and gap filaments (Dutson, 1983). Disruption of the lysosomal membrane and release of cathepsins is more likely to occur at a higher temperature and low pH which would also optimize their activity.

Calpains have received many names including Calcium activated factor (CAF), Calcium dependent neutral protease, and calcium activated protease (Koohmaraie et al., 1988c). There are two forms of the enzyme found in muscle. One form requires millimolar amounts of calcium ions while the other requires only a micromolar amount. Both forms are found mainly in the cytosol (Koohmaraie et al., 1988c). Some researchers have stated that the low levels of free intracellular calcium (1-10 μM) make it likely that μM rather than mM calcium requiring calpain is responsible for postmortem proteolysis (Ducastaing et al., 1985, Koohmaraie et al., 1986). The μM calcium requiring form has been reported to retain 24-28% of its maximum activity postmortem at storage conditions (pH 5.5-5.8 and 5 °C) (Koohmaraie et al., 1986). Hathaway et al. (1981) reported that the mM requiring calcium activated protease in chicken smooth muscle may be converted to a lower calcium requiring form by limited autolysis.

It is generally accepted that both calpains and cathepsins act in combination to increase tenderness during postmortem aging (Judge et al., 1989). However, the relative importance of each type of enzyme in the tenderization associated with aging is supported by conflicting evidence. Koohmaraie et al. (1988a, 1988c) reported that most of the postmortem changes occurred within the first 24 hours and that those muscles with a higher response to aging had higher calpain activity than those which exhibited a lower response, suggesting that calpains are a best determinant of postmortem tenderization. In contrast with these results, Calkins and Seideman (1988) reported that most of the aging observed in their study occurred between day 3 and day 6 where cathepsins B and H are the predominant proteases. These results suggest that calpains may help in establishing initial tenderness but cathepsins B and H are responsible for later tenderization.

2.4.6. Accelerated Processing.

In contrast with traditional operations, accelerated processing of meat involves boning and cutting before chilling (Kastner, 1982). In traditional operations, carcasses are stored in uneconomical storage rooms instead of efficient facilities that cool only the edible meat (Henrickson, 1982). Accelerated processing has several advantages over conventional processing. Removal of bones and excess fat before chilling reduces chill time and energy costs as well as decreases the cost and space needed for transportation with no significant loss in meat quality or tenderness. Despite these theoretical advantages hot processing has not been widely used by the industry and hot boning seems to be concentrated towards rapid processing of prerigor pork, and beef to a lesser extent, for the sausage industry (Henrickson, 1982; Taylor, 1987). The main reason for the slow acceptance of accelerated processing is the high cost of building new facilities or re-designing conventional operations needed to incorporate hot processing (Henrickson, 1982). Accelerated processing of beef compared to pork represents a larger problem due to the potential tenderness problems associated with prerigor excision (Falk et al., 1975). There is conflicting evidence on the influence of hot boning on tenderness. It has been reported that hot boned muscles at one hour (Cecchi et al., 1988) and six hours postmortem (Kastner and Russell, 1975) had higher shear force values than conventionally processed muscles. However, Schmidt and Gilbert (1970) reported that muscle hot boned at two hours postmortem were equally or more tender than cold boned muscles.

Cold shortening and loss of tenderness may occur if prerigor meat with a high pH is chilled. This may occur due to the slower rate of glycolysis in beef compared to pork which takes longer to achieve its ultimate pH. Also, excised muscle may chill more rapidly due to the removal of fat as well as contract more freely due to the lack of carcass and skeletal restraints (Kastner, 1982). Cold shortening may be prevented in hot processed meat by the use of electrical stimulation and high temperature conditioning. Electrical stimulation speeds the rate of pH decline and allows earlier chilling by accelerating rigor development (Kastner, 1982).

High temperature conditioning involves storing the warm excised meat at high temperatures and delaying chilling until rigor develops. The higher holding temperature prevents development of cold shortening (Falk, 1975). Etherington (1987) reported that a reduction in the aging time can be obtained from high temperature conditioning due to the increased protease activity caused by the higher temperatures. Schmidt and Gilbert (1970) found that bovine muscles excised 2 hours postmortem and held at 15°C for 24 hours were as tender as the same muscles excised after a 24 hr chill at 9°C. When the conditioning period was extended to 24 hr at 15°C shear force values were significantly lower than those from conventionally chilled muscles. Babiker and Lawrie (1983) reported that storing hot boned electrically stimulated muscles for 5 hr at 30 or 40°C resulted in significantly more tender muscles. However, Cecchi et al. (1988) reported that storing hot boned chucks for 5 hr at 15°C decreased shear force values significantly when compared to hot boned counterparts but had significantly higher values when compared to cold boned counterparts.

2.4.7. Tenderization Treatments.

A variety of treatments have been investigated to improve tenderness. These include electrical stimulation, calcium chloride infusion, blade tenderization, and enzymatic treatments. Some of these have been shown to be more successful and practical than others.

2.4.7.1. Electrical Stimulation.

Electrical stimulation has been widely incorporated in the meat industry as an economical and efficient method to improve tenderness (Savell, 1982; Romita et al., 1987). There are two general types which are divided into low voltage (20-90 V) and high voltage (300-700 V). The voltage is usually applied in short impulses which cause violent contractions of the skeletal muscles. This contraction causes a depletion of ATP and accelerates glycolysis to replace the spent ATP. More rapid glycolysis results in faster rates of lactic acid accumulation and pH decline as well as acceleration of rigor onset (Savell, 1982). The mechanism of tenderization has been proposed to consist of three parts: hastening of rigor results in less fiber contraction during chilling, preventing cold shortening; a rapid pH drop combined with a high temperature favors the release and activation of lysosomal enzymes; and violent contractions produced by the voltage may cause structural damage which would make the muscle easier to fragment, hence more tender (Savell et al., 1978a; Dutson et al., 1980).

Other reported advantages of electrical stimulation include reduced aging time, frequent improvement of USDA quality grade (Savell et al., 1982), and improved flavor (Calkins et al., 1982). Tang and Henrickson (1980) reported higher myoglobin concentration and brighter lean color following stimulation.

Electrical stimulation has some limitations and disadvantages. The increases in tenderness are greater in meat that is originally tougher and will not significantly improve product that is inherently tender (Savell et al., 1982). In some studies, stimulation did not improve palatability of specific muscles from the chuck and round (McKeith et al., 1981) and did not affect meat from dark cutters (Dutson et al., 1982).

2.4.7.2. Blade Tenderization.

Blade tenderization treatments consist of conveying meat under a group of long metal needles with small blades that penetrate the tissues. The tenderizing effect is a result of disruption of the muscle structure by the needles which make the meat easier to fragment and more tender. The main advantage of blade tenderization is its ability to upgrade cuts not normally used for steaks, chops, or roasts (Miller,1975). The extensive use of this treatment in the HRI industry is justified by Miller (1975) because it: (1) assures acceptable tenderness of table-grade cuts; (2) equalizes tenderness in portions containing two or more muscles differing in tenderness; (3) upgrades cuts not normally used for steaks; and (4) effects tenderizing more uniformly and is more easily controlled than enzymatic treatments commonly used in the HRI trade. Blade tenderization appears to be more effective for cuts that are less tender because of high collagen content or the presence of more physiologically mature collagen (Miller, 1975).

Several researchers have reported significantly increased tenderness both in sensory scores and shear values when using blade tenderization as well as decreased amounts of sensory detectable connective tissue (Glover et al., 1977; Hayward et al., 1980; Seideman et al., 1986). Mechanical tenderization has also been reported to decrease cooking time (Glover et al., 1977; Smith et al., 1979). While more than one pass through the tenderizer has been reported to provide additional tenderness improvements (Seideman et al., 1986), varying conveyor speeds were reported as not significantly affecting tenderness (Schwartz and Mandigo, 1974).

Loucks et al. (1981) reported that mechanical tenderization improves tenderness of postrigor beef roasts as measured by trained panels and shear force values, but does not significantly affect tenderness of prerigor counterparts.

Blade tenderization can also be used to facilitate restructuring by reducing the influence of connective tissue in restructured, pre-cooked roasts. This process requires less labor and reduces losses when compared to hand trimming of connective tissue. Extractability of salt soluble proteins may also be improved by blade tenderization, thus aiding restructuring (Mandigo, 1982b).

Some reported disadvantages of blade tenderization include increased drip losses during storage and reduced storage case life (Davis et al., 1977). Over-tenderization may occur resulting in higher cooking losses and a dry cooked product (Miller, 1975). Smith et al. (1979) reported that blade tenderization will not improve meat of unsatisfactory quality, such as cow meat, to a level comparable to high quality beef.

2.4.7.3. Calcium Chloride Infusion.

Calcium chloride infusion treatment to increase tenderness is a relatively new concept. Calcium requiring proteases, also known as calpains, have been identified as responsible for at least part of the tenderness increase observed during aging especially in the first 24 hours postmortem (Ducastaing et al., 1985; Koohmaraie et al., 1986). Activation of calpains by added calcium may eliminate the requirement of aging beyond 24 hours (Koohmaraie et al., 1988a). Two methods of infusion into intact carcasses have been reported in the literature.

One infusion method used a ham pumping device to intraarterially infuse a 0.3M calcium chloride solution (10% of live weight) into ovine carcasses prior to evisceration. In some cases the carcasses were electrically stimulated as well prior to infusion using low frequency stimulation. The infusion resulted in increased levels of water extractable calcium , accelerated postmortem tenderization, and significantly reduced shear force values (Koohmaraie et al., 1988b, 1989, and 1990; Koohmaraie and Shackelford, 1991; St.Angelo et al., 1991). Koohmaraie et al. (1988b) determined that the volume of liquid injected had no significant tenderizing effect and that the best results were obtained using 0.3M calcium chloride solutions (Koohmaraie et al, 1989). These researchers also reported that infusion will increase tenderness with or without electrical stimulation (Koohmaraie et al., 1990). St.Angelo et al. (1991) reported that infusion of a calcium chloride-antioxidant solution into lamb carcasses using this method resulted in acceleration of postmortem tenderization, inhibition of lipid oxidation, and retardation of warmed over flavor.

The second method used involves needle injection of 400 to 500 ml. of 0.3M calcium chloride solution into a 38 cm section of the beef *longissimus* muscle with the carcass intact. The injected and control muscles were removed after 24 hours. The calcium chloride injection resulted in a definable effect on calpain activity and acceleration of postmortem tenderization (Koohmaraie et al., 1990).

The possible use of calcium chloride injection coupled with accelerated processing has also been studied. Injection of the excised muscle immediately following boning is reported to result in significant improvements on the tenderness of beef longissimus muscle. (Wheeler et al., 1992). The same study reported that injection at 24 hours after slaughter produces significant tenderness improvements, although to a smaller extent than injection at 0 hours after boning. Morgan et al. (1991a) reported that injection of $CaCl_2$ into hot boned subprimals improved tenderness as measured by sensory panel and shear force, and eliminated the requirement for extended postmortem storage. However, the same study reported that $CaCl_2$ injection coupled with hot boning resulted in significant contraction and shorter sarcomere lengths.

Calcium chloride infusion treatments have a potential to be used beneficially in the meat industry to reduce the aging time required for meat to reach acceptable tenderness (Koohmaraie et al., 1988b, 1989). However, the practicality and viability of this process when applied to large scale meat production still needs to be determined.

2.4.7.4 Enzymatic Treatments.

Enzymatic treatments to increase meat tenderness have been utilized in the foodservice industry. Enzymes break down structural components of muscle tissue, reducing toughness. This method has been used widely and effectively in the HRI industry but has also encountered numerous problems including development of off-flavors, and under and over-tenderization (Smith et al., 1979). These problems are mainly the result of a lack of knowledge of activity and specificity. Most commercial enzymes are non-specific and break down both connective tissue and muscle fibers. This breakdown can take two main forms: (1) complex peptides are hydrolyzed into a few smaller and more flavorful proteins through endopeptidase activity, and (2) small units are broken off chain ends through exopeptidase activity. These smaller units tend to produce off-flavors. Ideally, a suitable enzyme should be highly specific for the breakdown of collagen (Fawcett and McDowell, 1987).

Several enzymes have been reported to tenderize meat. Papain, ficin, bromelain, and actinidin are non-specific plant proteases which hydrolyze meat proteins but have limited collagenase activity (Rolan et al., 1988). Other suitable enzymes of fungal and bacterial origin have been identified (Fawcett and McDowell, 1987). It is thought that biotechnological engineering of these organisms may produce more specific enzymes and commercial preparations of known activity with excellent control characteristics (Fawcett and McDowell, 1987).

2.4.7.5. Organic Acids.

The effect of organic acids on tenderness has also been studied. It has been reported that the injection of fresh bovine muscle with lactic acid resulted in a more rapid pH decline and increased collagen solubility. These researchers proposed that a more rapid pH decline should accelerate activation of cathepsins which added to the collagen degradation produce more tender meat (Stanton and Light, 1990). Arganosa and Marriott (1989) reported that incorporation of lactic, citric, and acetic acid into restructured beef resulted in increased collagen solubility but produced no significant differences in shear force. This was attributed to the hardening effect of organic acids on other muscle proteins. However, Whiting and Strange (1990) reported that immersion in lactic, citric, and acetic acids significantly reduced shear force.

2.4.7.6. Carcass Suspension.

There is conflicting evidence on the literature on the effect of carcass suspension on tenderness. Locker (1960) found that the strain imposed by the hung carcass affected the state of contraction of the muscle. Herring et al. (1965) found that the conventional suspension method released tension in the *longissimus* muscle. However, Hostetler et al. (1970) found that conventional hanging caused shortening of the *longissimus* muscle. Cotroneo (1992) reported that a prerigor cut through the 12th thoracic vertebra resulted in significantly more desirable sensory panel ratings for myofibrillar tenderness and overall tenderness, although no significant differences in shear force were observed.

2.4.8. Cooking.

Heat from cooking may cause both tenderization and toughening of meat (Judge et al., 1989). The connective tissue and myofibrillar proteins are altered by heat which can significantly influence tenderness by enhancing or nullifying previous treatments. During cooking, muscle fibers become tougher while connective tissue shrinks and becomes more soluble (Price and Schweigert, 1987). At low temperatures (56-58°C), collagen shrinkage may occur accompanied by increased solubility and tenderness. However, this tenderization occurs slowly. Raising the temperature to 62-64°C will accelerate collagen shrinkage. At temperatures of 72-74°C rapid shrinkage of collagen occurs but is accompanied by protein hardening and toughening (Judge et al., 1989). Machlick and Draudt (1963) found that shear force values decreased upon raising meat temperature from 50°C to 54°C and reached a minimum at 60-64°C. Davey and Gilbert (1974) reported that increases from 60-75°C resulted in increasing shear force values as well as increased fiber shrinkage and weight loss.

2.4.9. Restructured Products.

Restructuring offers an intermediate value product where the portion size, shape, composition and texture can be closely controlled. This process permits alternative uses of less valuable products from the carcass to give an acceptable product as well as a more efficient utilization of the entire carcass. This process normally involves the subdivision of muscles to achieve tenderization of lesser value components, addition of salt, manipulation to extract salt soluble proteins, and reforming (Kastner, 1982). Consumers have long preferred steaks over ground meat and roasts but the high price of these cuts prevent many consumers from purchasing them. Furthermore, those portions of the carcass which produce the most desirable steaks are limited and comprise only a small fraction of the total carcass weight (Mandigo, 1982a).

Restructured products utilize less valuable portions of high quality carcasses or less valuable carcasses that may lack tenderness (Mandigo, 1982a). The particle size reduction and successful restructuring provide acceptable tenderness as well as texture which resembles intact cuts. There are several particle reduction methods which include grinding, flaking, chopping, chunking, and slicing. The most important step in the process is the binding of the meat pieces to give acceptable consistency and tenderness (Judge et al., 1989). Salt and phosphates are used to accelerate the solubilization of myofibrillar proteins. The solubilized proteins serve as the binder in the restructured steak. In addition, salt and phosphates are used to minimize cooking losses (Mandigo, 1982b). However, sodium chloride has been reported to contribute to raw color and flavor degradation of restructured muscle foods during frozen storage (Marriott et al., 1985b).

Forming and portioning is achieved before complete binding action occurs by forming, filling, or pressing equipment (Judge et al., 1989).

Accelerated processing and the use of prerigor beef in restructuring have been studied. Combining accelerated processing and restructuring would be an excellent method to upgrade low quality cuts of beef while reducing energy needs. Huffman et al (1984) reported that trained sensory panelists could not distinguish between hot boned and cold boned restructured steaks when compared for flavor, texture, and acceptability. Marriott et al (1985a) reported that the color and overall appearance of restructured beef steaks is affected more by storage time than by rigor state of the raw materials. Furthermore, the study indicated that prerigor beef may be utilized in restructured beef steaks without affecting texture and tenderness.

2.5. Color and Appearance.

Product appearance is the most important factor affecting consumer perception of meat and will influence purchase decisions (Faustman and Cassens, 1990). Consumers relate meat color to freshness and rely on this attribute as a criterion of quality and to discriminate against meat which lacks a fresh appearance (Seideman et al., 1984). Loss of fresh meat color is inevitable and may be interpreted as an indication of unwholesomeness. As the industry recognized the importance of maintaining fresh color, various methods of transportation and distribution of meat were devised. Today, the method of choice used is vacuum packaging of primals and subprimals to minimize color loss (Faustman and Cassens, 1990).

Meat color is primarily determined by the concentration and chemical form of myoglobin (Judge et al., 1989). Hemoglobin makes minor contributions to color as most hemoglobin is lost when the animal is slaughtered and bled. The concentration of myoglobin varies with species, sex, age , and even within muscles. Beef appears more red than pork due to higher concentrations of myoglobin. Males and older animals usually produce darker meat in addition to muscles which are heavily used in locomotion due to the higher myoglobin concentrations needed for activity (Seideman et al., 1984). The valence state of the iron atom and the ligand bond to the free binding site of the heme ring are responsible for the color of myoglobin and meat. There are three main forms of myoglobin which determine its color. Reduced myoglobin (purple color) predominates under in the absence of oxygen. Oxymyoglobin (red color) results when the muscle is exposed to oxygen. Metmyoglobin (brown color) predominates when low oxygen tension exists and is caused by oxidation of the heme iron. Usually two or more of the color forms coexist with the predominant form giving the overall color to meat (Seideman et al., 1984).

Pigment stability is variable and governed by a variety of factors which may cause meat discoloration including pH, light, and bacterial growth. Fresh meat color is maximized with conditions which delay myoglobin oxidation and/or reduce oxidized metmyoglobin formation (Faustman and Cassens, 1990).

Ultimate pH and the rate of pH decline affect pigment stability. In general ,a low pH favors oxidation of myoglobin. Meat with a pH over 5.8 is more color stable than with a pH of 5.6 (Ledward et al., 1986). A high ultimate pH will result in dark meat which will result in economic loss to the producer due to the decreased consumer acceptance of this color. A rapid rate of pH decline may result in PSE muscle in pork which is also undesirable for both consumers and producers (Faustman and Cassens, 1990).

The effects of light and various lighting types on meat color have received much attention. Marriott et al. (1967) reported that meat stored under light versus dark conditions underwent greater pigment oxidation. This may be due to the higher temperatures that occur under light conditions which may increase bacterial growth. Ultraviolet light is also very detrimental to meat color. This may be due to photooxidation caused by singlet oxygen production catalyzed by incident light (Faustman and Cassens, 1990).

Lack of fresh color is frequently perceived by the consumer as being spoiled even though this may not always be true (Seideman et al., 1984). The consumption of oxygen by bacteria lowers the oxygen tension favoring metmyoglobin formation. Reduced myoglobin formation may occur eventually if the oxygen tension is very low (Faustman and Cassens, 1990). Greening discoloration may also result from bacterial production of hydrogen peroxide and hydrogen sulfide. These compounds react with myoglobin to give green choleglobin and sulfmyoglobin, respectively (Faustman and Cassens, 1990).

Postmortem treatments may also affect the color of meat. Electrical stimulation has been reported to improve color of lean meat as well as increased myoglobin content (Tang and Henrickson, 1980). Davis et al (1977) reported that blade tenderization prior to storage caused discoloration and produced unattractive meat following storage. Huffman (1980) suggested that discoloration is more likely to form at the sites of blade injection. No results have been reported in the literature on the effects of calcium chloride injection on color.

2.6. Microbial Effects.

Growth of bacteria on meat may result in the production of off odors and flavors which are unappealing to the consumer as well as pathogen contamination which may cause foodborne infections (Gill and Newton, 1978).

Degradation of proteins, lipids, carbohydrates, and other complex molecules present in meat is accomplished by inherent hydrolytic enzymes and those produced by microorganisms. These products of hydrolysis are used by the microorganisms as nutrient sources for growth and activity.

Under aerobic conditions, protein hydrolysis results in simple peptides and amino acids. However, under anaerobic conditions proteins are degraded to sulfur containing compounds which impart off-flavors (Judge et al., 1989). Spoilage odors and flavors are due to the production of volatile substances as a result of microbial degradation of amino acids. In addition, amino acid degradation results in the release of ammonia (Gill and Newton, 1978).

Lipid oxidation may occur in meat in the absence of bacteria. However, hydrolysis of lipids by microbial lipases may accelerate this process due to the greater susceptibility of free fatty acids to oxidation and impart a tallowy or chalky flavor (Gill, 1982).

Carbohydrate levels in meat are usually very low. However, carbohydrates are preferred as a source of energy by microorganisms and are utilized readily when available. This may result in a variety of end products such as alcohols and organic acids which contribute distinctive flavors (Judge et al., 1989).

The microbial quality of meat is determined by the condition of the animal at slaughter, spread of contamination during slaughter and processing, and conditions of storage and distribution (Nottingham, 1982). At chill temperatures (-1 to 5°C) meat is spoiled by psychrotrophs. Under humid conditions, aerobic floras which originate largely from the hide of slaughter animals (ie. *Pseudomonas*) are predominant in oxygen permeable packaged meat. Deep tissues of healthy animals are generally believed to be free of bacteria (Gill and Newton, 1978). However, meat may be contaminated through intensive handling which occurs during slaughter, fabrication, and boning. Good hygiene practices can minimize this contamination by eliminating its source (Nottingham, 1982).

Specific treatments or conditions can reduce or increase the microbial load of meat. Prerigor boning has received special attention over possible health hazards that may arise from this type of operation. Hot boning increases the surface area to volume ratio when the meat is warm and sticky. Thus, freshly cut warm meat is exposed to contamination and may facilitate growth of microorganisms to a greater extent than conventionally processed meat (Nottingham, 1982). Hot boning coupled with high temperature conditioning in a range of 0°C to 35°C for 3 days is reported to significantly increase bacterial counts as can be expected from the higher bacterial growth at high temperatures (Smulders and Eikelenboom, 1987).

Blade tenderization treatments may affect the microbial quality of meat. Contamination of the needles from one tainted cut may spread spoilage organisms throughout clean tissue of that piece and to other products which are subsequently tenderized (Booren and Mandigo, 1987). Davis et al. (1977) reported that blade tenderizing before storage resulted in a product which had a very limited retail case life when compared to that which was not tenderized.

There are no reports in the literature on the effect of calcium chloride injection on the microbial quality of meat. As with any injection procedure, needle contamination may pose a serious risk of contamination spread which may be minimized with good sanitation and hygiene practices.

2.7 Packaging Effects.

Packaging can also influence the acceptability of meat. Consumer packaging of meat requires a thin transparent film with effective oxygen permeability to assure a bright display color. However, this kind of package may also encourage growth of aerobic microorganisms which limit the retail caselife of meat in display environments (Nottingham, 1982). The existing microbial load when meat is packaged determines the product shelf life. Bright color may be maintained 5-7 days under display conditions. Microorganisms compete for the available oxygen and reduce the amount available for oxymyoglobin formation (Judge et al., 1989). Another problem associated with high oxygen transmission rates is the enhanced opportunity for oxidative rancidity to develop (Judge et al., 1989).

Vacuum packaging of meat has gained considerable acceptance in the meat industry for shipping and storing. Sealing the meat in a relatively impermeable plastic wrap with the minimum volume of air prevents further oxygen transfer into the package (Taylor, 1982). Restricting oxygen from the meat extends shelf life by minimizing growth of *Pseudomonas* and allowing lactic acid bacteria to predominate (Foegeding et al., 1983). The small amount of residual oxygen may be rapidly consumed by meat and bacterial respiration. The low amounts of oxygen favor the formation of a layer of metmyoglobin brown pigment on the meat surface. This layer may not be thick enough to obscure the underlying purple-red myoglobin of the interior and this color may actually predominate. Under oxygen-free conditions metmyoglobin formation is minimal and color stability is maintained during storage. At the end of storage, vacuum packaged meat is often repacked in oxygen permeable films to allow the red color associated with fresh meat to return and minimize consumer rejection (Nottingham, 1982).

CHAPTER III

MATERIALS AND METHODS

Five steers were sacrificed and converted to carcasses according to established industry procedures at the Virginia Polytechnic Institute and State University facilities yielding one U.S. Choice and two U.S. Select and Standard grade carcasses . Carcass sides of each animal were randomly assigned to either prerigor and postrigor treatments. Prerigor sides were processed and treated within 1 hour after exsanguination. The M. *infraspinatus* and M. *longissimus thoracis* muscles were excised and injected with a solution of $CaCl_2$. The muscles were split longitudinally into two sections. One section was assigned for blade tenderization for blade tenderization (CABT) while the other was not blade tenderized (CANB). Treated muscles were cut into 2 cm steaks, packaged in high oxygen transmission rate (OTR) polyvinyl chloride (PVC) film and stored in a 4°C cooler for 3 and 7 days before analysis. After storage of the postrigor sides in a 4°C cooler for 24 hours, the *infraspinatus* and *longissimus* muscles were excised and split longitudinally into two sections. One section was assigned for blade tenderization treatment (BT) while the other was designated as the control

(C). These muscles were cut into 2 cm steaks, packaged in high OTR PVC film and stored in a 4°C cooler. One steak from each treatment was frozen and stored (-26°C) and identified for insoluble, soluble and total collagen determinations. The remainder of the packaged steaks were stored in the dark at 4°C for 3 and 7 days before subsequent analysis.

3.1 Treatments.

Excised prerigor muscles were injected 10% of meat weight with a 0.3 M solution (room temperature) of food grade calcium chloride (Dow Chemical U.S.A., Midland, Mich.) using a hand held pumping device. This equipment consisted of a 400 cc syringe with a 4.1 mm diameter needle with 16 radiating openings. The injection was performed at various sites along the muscle section until all the volume of solution was dispensed.

Each side of the muscle sections assigned to blade tenderization treatments was passed once through a blade tenderizer (Model TC700M, Ross Industries, Inc., Midland, Va.). $CaCl_2$ injection was performed prior to blade tenderization for CABT treatments.

3.2 Sensory Evaluation.

Sensory panelists were recruited among the faculty and students of the Food Science and Technology Department at V.P.I. & S.U.. The 7 member panel was trained during three 1-hour sessions to evaluate tenderness, texture, and flavor of the samples. Steaks were prepared according to AMSA cookery guidelines (AMSA 1978) (except for steak thickness), to an internal temperature of 70°C. A copper-constantan thermocouple was placed in the center of each steak and the temperature was recorded using a digital thermometer (Model #2160-A-7, Omega Engineering, Inc., Stamford, Conn.). Five-1.27 cm cores were obtained from each steak and stored for 20 minutes in pyrex containers embedded in sand preheated to 70°C until evaluation by the panelists. The samples were evaluated at the sensory facilities of the Food Science and Technology Department in individual booths under red lighting. An eight point hedonic scale was used with 1=extremely tough and 8=extremely tender for tenderness; and 1=extremely undesirable and 8=extremely desirable for texture and flavor.

3.3 Color Determination.

Instrumental color determination was performed with a Minolta Chroma Meter (Model CR-200, Minolta Camera Co., Ltd., Osaka, Japan). The Chroma Meter was referenced against a standard calibration plate (No. 20933026, CIE L* 97.91, a^* -0.70, b^* +2.44). Five readings of CIE L^* a^* b^* values were obtained for each steak. Average values and standard deviations were obtained using the statistics function of the Chroma Meter.

Visual color determination was performed by a trained panel. The seven panelists were recruited among the faculty and students of the Food Science and Technology Department, and trained according to AMSA (1978) in three 1 hour sessions using different steaks to represent a range of colors and textures, and incorporating a photographic guide (Anonymous). The samples assigned to visual color determination were evaluated in a 4°C display case (Tyler Commercial Refrigerator and/or Freezer Model CGS8M, Tyler Refrigeration Corp., Niles, Mich.) under 1076 lux of cold white fluorescent light (Model F30T12/CW/RS Cool White, N. American Philips Lighting Co., Sommerset, N.J.). Steaks were evaluated for color, texture, and overall appearance using an eight point hedonic scale with 1=extremely undesirable and 8=extremely desirable.

3.4 Warner-Bratzler Shear Determinations.

Steaks were prepared the same as for the sensory evaluation. Five-1.27 cm cores were obtained from each steak and cooled to room temperature. An Instron (Model 1011; Instron Corp., Canton, Mass.) with a Warner-Bratzler shear attachment was used to measure the force required to shear through each core. The Instron was run at 200 mm/min with a 50 kg load transducer and a load range of 10 kg. Averages of the five values obtained for each steak were obtained and used for subsequent analysis.

3.5 Microbial Analysis.

Samples were analyzed for microbial load at 0, 2, and 6 days using the Standard Plate Count (SPC) method. Eleven grams of product were aseptically transferred to 99 ml of sterile peptone water (Difco Bacto-Peptone, Difco Laboratories, Detroit, Mich.) in a sterile stomacher bag. The mixture was homogenized for 2 minutes in a stomacher (Lab Blender 200, Tekmar Company, Cincinatti, Ohio). This 1:10 dilution was used for subsequent dilutions with sterile peptone water and pour plates. Aerobic plate counts were conducted on duplicate pour plates with Standard Methods Agar (SMA, Difco Laboratories, Detroit, Mich.). and incubated at 25°C for 48 hours.

3.6 Chemical Analysis.

Insoluble, soluble, and total collagen amounts were determined on duplicate samples following the procedures of Hill (1966) and Bergman and Loxley (1963) with the following modifications: 4 grams of meat were frozen and freeze dried for 24 hours (Virtis Freeze drier, Model No. 10-145MR-BA; Gardiner, N.Y.); centrifuge tubes were heated for 70 minutes in a 77°C water bath stirring every 10 minutes; after centrifugation residuals and supernatants were hydrolyzed for 7 hours. In the preparation for spectrophotometric determination, 2 ml of Ehrlich's reagent were used.

Fat oxidation was determined using the Thiobarbituric Acid (TBA) method outlined by Ockerman (1985) with the modification proposed by Rhee (1978) which incorporates the addition of 5 ml of 0.5% propyl gallate/EDTA solution in the blending step. TBA measurements were performed after 2 and 6 days of storage at 4°C.

3.7 Statistical Analysis.

Data from five replications were analyzed using Statistical Analysis System (SAS, 1987). Analyses included the General Linear Models procedure. The least squares difference test was used to separate variable means when significant differences occurred. The effect of aging time on prerigor and postrigor muscles was analyzed using a split-split-plot design (replication, treatment, day). The effect of rigor state and treatment was analyzed using a split-split-plot design (replication, rigor, treatment). In addition, correlation between instrumental and chemical measurements with sensory measurements was determined using the Correlation procedure. Significance for all analyses was determined with $\alpha=0.05$.

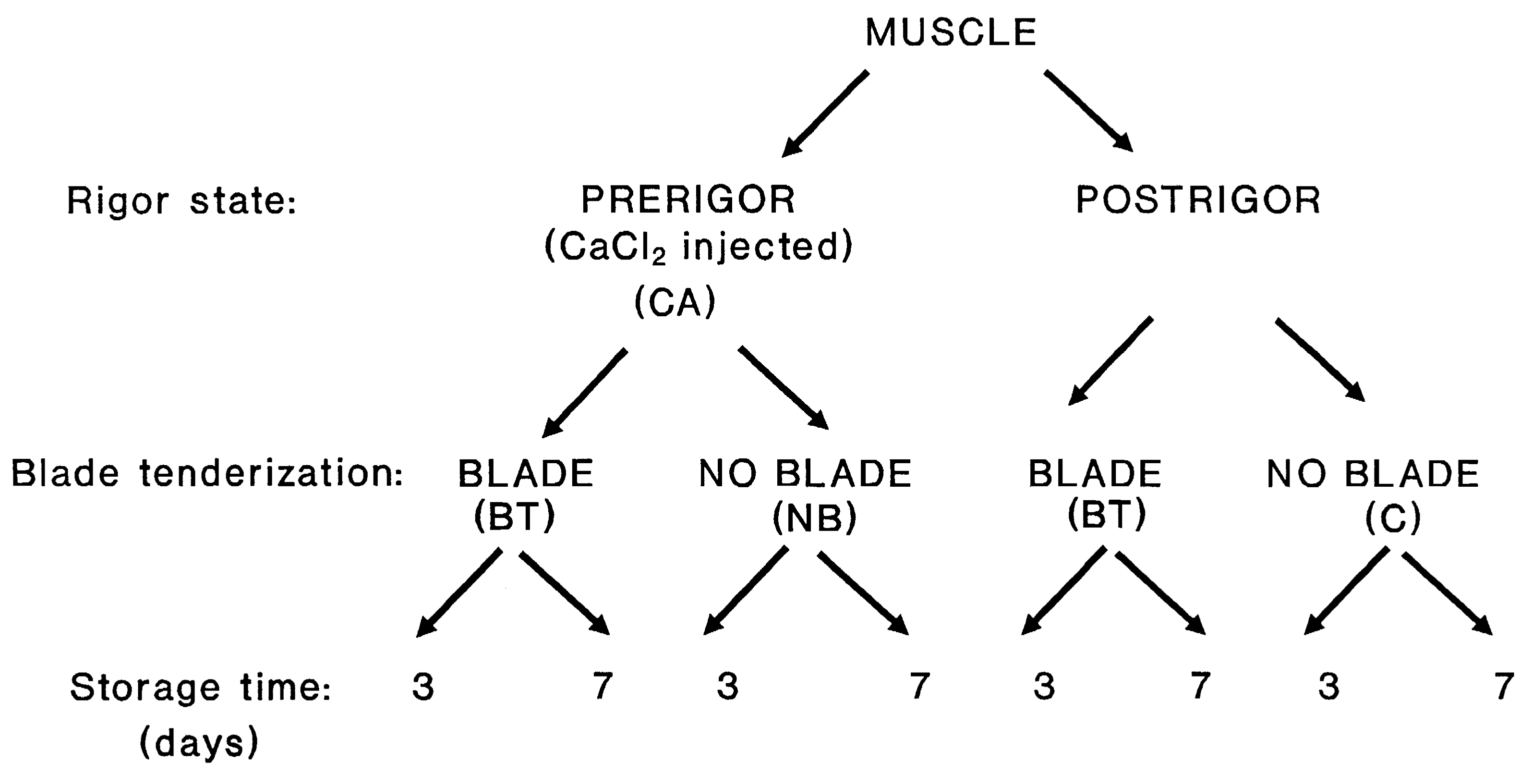

Figure 1 - Experimental flow chart for infraspinatus and longissimus muscles

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Visual Evaluations.

Color, texture, and overall appearance evaluations for all samples are presented in Tables 1 and 2. Blade tenderization did not affect (P>0.05) color scores for steaks from both muscles and overall appearance scores for *longissimus* steaks when compared to non-tenderized counterparts. However, the C steaks from the *infraspinatus* muscle samples were rated higher (P<0.05) than the BT counterparts for overall appearance and texture. In addition C steaks from the *longissimus* muscle had higher (P<0.05) scores for texture than BT counterparts.

The effect of rigor state and treatment on visual panel measurements produced mixed results for different traits. Among the *infraspinatus* muscle steaks, the CABT samples were rated higher (P<0.05) for color than their CANB counterparts and the BT steaks but were not different (P>0.05) from the C control steaks. However, the CABT steaks were rated lower (P<0.05) for texture than the CANB counterparts as well as the BT steaks. Postrigor C steaks received higher (P<0.05) texture ratings than any treatment. Postrigor C steaks also received

Table 1 - Effects of rigor state and blade tenderization on color and other appearance traits of beef steaks from infraspinatus muscle

	Prerigor ($CaCl_2$ injected)		Postrigor		
	Blade (CABT)	No Blade (CANB)	Blade (BT)	Control (C)	S.E.
Visual trait[a]					
Color[b]	4.8^{c}	4.5^{d}	4.3^{d}	4.6^{cd}	0.10
Texture	5.1^{e}	5.6^{d}	5.6^{d}	6.1^{c}	0.11
Overall appearance	4.8^{cd}	4.8^{cd}	4.6^{d}	4.9^{c}	0.10
Instrumental color					
L^* [b]	37.3^{e}	39.0^{d}	42.4^{c}	41.5^{c}	0.41
a^*	21.6^{c}	20.9^{c}	20.6^{c}	20.8^{c}	0.78
b^*	10.4^{d}	10.7^{d}	12.0^{c}	11.8^{c}	0.35
Microbial analysis					
SPC	5.3^{c}	5.0^{c}	4.5^{d}	4.3^{d}	0.14

[a] Color, texture, and overall appearance: 1=extremely undesirable, 8=extremely desirable

[b] Rigor*blade tenderization interaction (P<0.05)

[c-e] Means within the same row bearing identical superscripts are not different (P>0.05)

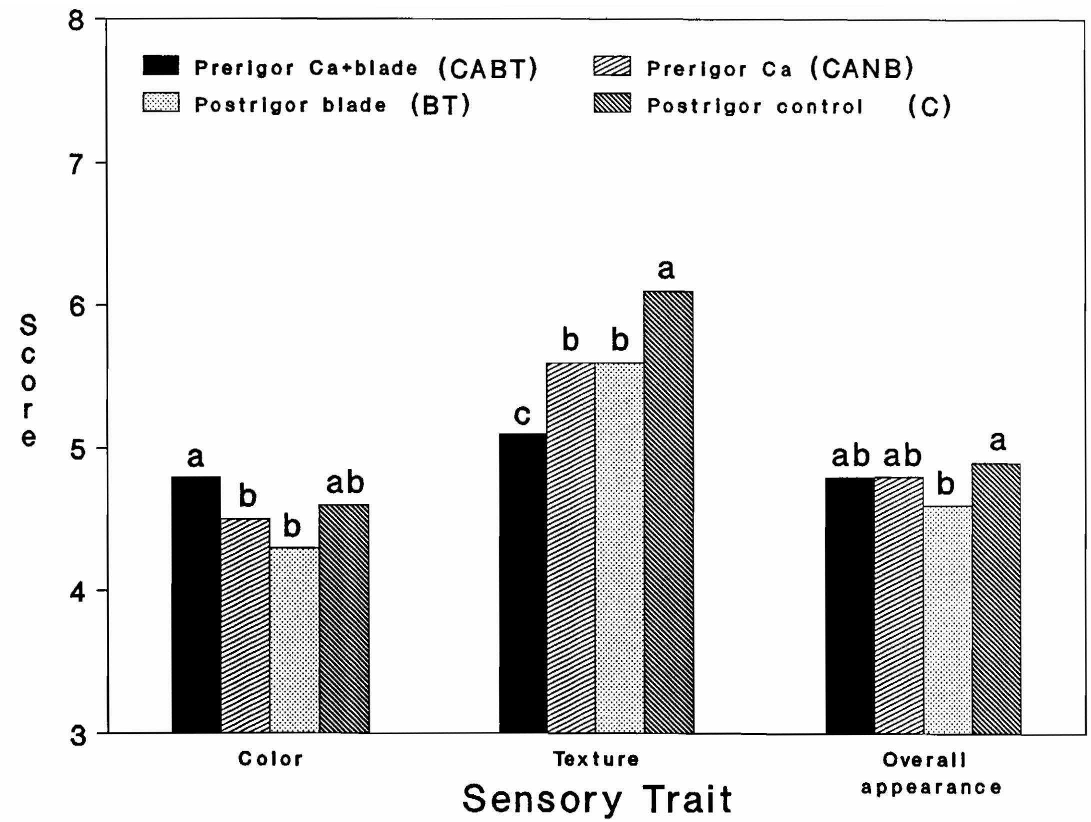

Figure 2 - Effect of rigor state and blade tenderization on visual panel scores for beef steaks from infraspinatus muscle

a-c bars within the same trait bearing identical letters are not different (P>0.05) higher (P<0.05) overall appearance ratings than BT counterpart steaks but were not different (P>0.05) from either the CANB or CABT steaks (Figure 2). There were no differences (P>0.05) due to rigor state and treatment for color ratings of *longissimus* muscle steaks. However, there was a significant interaction between rigor state and blade tenderization which may have detracted from the ability to measure differences. For texture, CABT steaks received lower (P<0.05) scores than the CANB counterparts, BT, and C steaks. In addition, C steaks were rated higher (P<0.05) for texture than the BT and CABT steaks (Figure 3). Panelists may have been influenced by the effect of the blade penetration on the steak surface. There were small but noticeable cuts on the surface which may have negatively influenced texture evaluation. For overall appearance of *infraspinatus* steaks, the C steaks were rated higher (P<0.05) than the BT counterparts, but were not different (P>0.05) from either the CANB or CABT steaks (Figure 2). For cuts from the *longissimus* muscle, the CABT steaks received lower (P<0.05) overall appearance than the postrigor C and BT steaks independent of blade tenderization, but were not different (P>0.05) from CANB counterparts (Figure 3). These results suggest that $CaCl_2$ prerigor injection and blade tenderization of postrigor muscles may significantly lower the acceptability of cuts from the *longissimus* and *infraspinatus*

Table 2 - Effects of rigor state and blade tenderization on color and other appearance traits of beef steaks from longissimus muscle

	Prerigor ($CaCl_2$ injected)		Postrigor		
	Blade (CABT)	No Blade (CANB)	Blade (BT)	Control (C)	S.E.
Visual trait[a]					
Color[b]	5.2^c	5.3^c	5.5^c	5.5^c	0.10
Texture	4.7^e	5.2^d	5.1^d	5.5^c	0.10
Overall appearance	5.0^d	5.2^{cd}	5.3^c	5.4^c	0.10
Instrumental color					
L^*	36.5^e	37.8^d	41.6^c	41.0^c	0.42
a^*	20.8^d	20.9^d	23.6^c	22.6^c	0.44
b^*	9.8^d	9.9^d	11.6^c	11.0^c	0.26
Microbial analysis					
SPC	5.2^c	NA[f]	4.2^d	4.6^d	0.21

[a] Color, texture, and overall appearance: 1=extremely undesirable, 8=extremely desirable
[b] Rigor*blade tenderization interaction ($P<0.05$)
[c-e] Means within the same row bearing identical superscripts are not different ($P>0.05$)
[f] Missing values due to contamination of plates

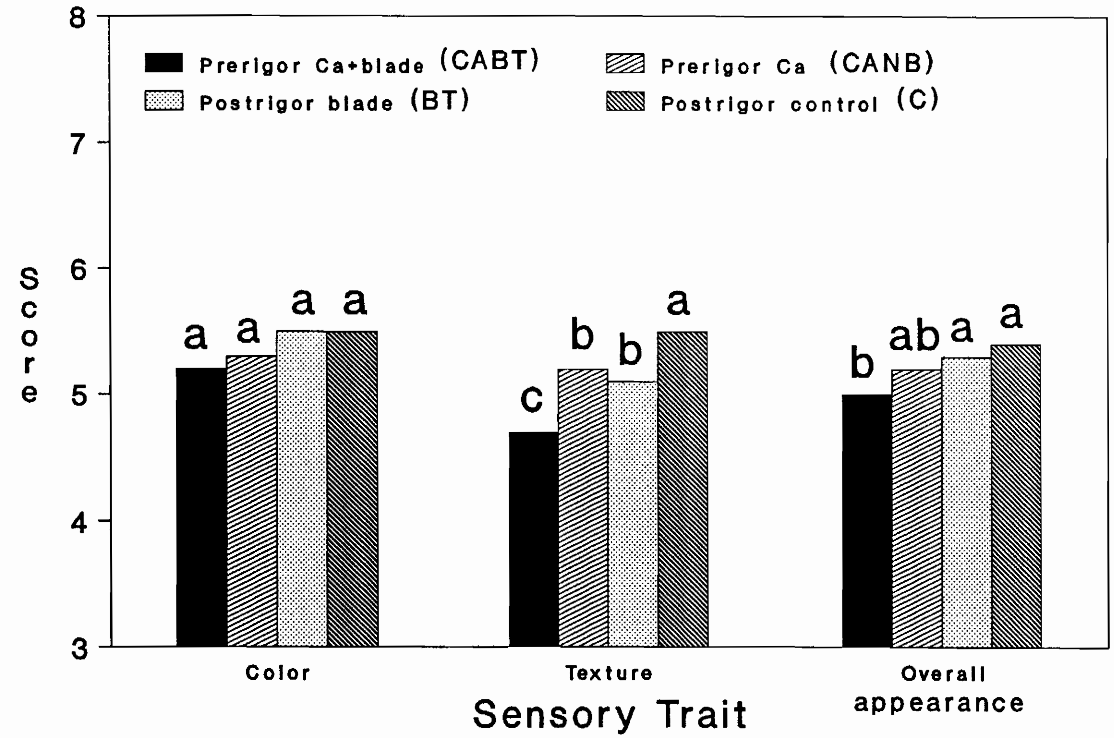

Figure 3 - Effect of rigor state and blade tenderization on visual panel scores for steaks from beef longissimus muscle

a-c bars within the same trait bearing identical letters are not different (P>0.05) *muscles* respectively, while conventionally processed steaks will yield the most acceptable steaks from either muscle.

Increased aging time (3 vs 7 days) resulted in lower ($P<0.05$) color and overall appearance scores for steaks from both muscles with the exception of postrigor *longissimus* muscle (Tables 3 and 4). Color deterioration has been reported to result from microbial consumption of oxygen resulting in a decrease in oxymyoglobin content, as well as byproducts of microbial metabolism which may cause greening discoloration (Faustmann and Cassens, 1990).

Increased aging did not have a significant effect on texture scores. The similar patterns observed between color and overall appearance scores indicate that color may have influenced overall appearance scores more than did texture scores.

4.2 Instrumental Color Measurements.

There were no differences ($P>0.05$) in CIE L^* a^* b^* values due to blade tenderization of muscles of the same rigor state, but CABT steaks from the *infraspinatus* and *longissimus* muscles had significantly lower L^* values than the CANB counterpart steaks (Tables 1 and 2). Steaks from postrigor treatments had higher ($P<0.05$) L^* a^* b^* values than those of prerigor treatments from both muscles, with the exception that a^* values were not different (P>0.05) for *infraspinatus* muscle steaks (Tables 1 and 2).

4.3 Microbial analysis.

No differences (P>0.05) in microbial counts were observed due to blade tenderization of muscles of the same rigor state (Tables 1 and 2), but blade tenderized muscles tended to have higher counts than non-tenderized counterparts (5.3 vs 5.0 and 4.5 vs 4.3). However, C steaks from the *longissimus* had numerically higher (P>0.05) counts than BT counterparts. In addition, plate contamination of $CaCl_2$ injected prerigor *longissimus* samples produced missing values so that no comparisons could be made to determine the effect of blade tenderization. However, the higher microbial counts observed in blade tenderized muscles agree with studies indicating that blade tenderization may reduce storage life of meat (Davis et al., 1977).

Postrigor C and BT samples had lower (P<0.05) counts than prerigor CANB and CABT samples for both muscles (Tables 1 and 2). It has been reported that the production of prerigor meat by accelerated processing may result in increased microbial counts (Kastner, 1982). This is due to the increased surface

Table 3 - Effect of aging on color and other appearance traits of prerigor $CaCl_2$ injected and postrigor beef steaks from infraspinatus muscle

	PRERIGOR ($CaCl_2$ injected)			POSTRIGOR		
	3 days	7 days	Standard error	3 days	7 days	Standard Error
Visual trait[a]						
Color	4.9^{b}	4.5^{c}	0.10	4.7^{b}	4.2^{c}	0.09
Texture	5.5^{b}	5.2^{b}	0.11	5.9^{b}	5.7^{b}	0.10
Overall appearance	5.0^{b}	4.6^{c}	0.10	5.0^{b}	4.5^{c}	0.11
Instrumental color						
L^*	38.7^{b}	37.6^{c}	0.29	42.3^{b}	41.6^{b}	0.42
a^*	22.4^{b}	20.1^{c}	0.22	22.6^{b}	18.8^{c}	0.50
b^*	11.1^{b}	10.1^{c}	0.17	12.4^{b}	11.4^{c}	0.18
SPC[d]	4.8^{c}	6.8^{b}	0.21	4.2^{c}	5.7^{b}	0.27

[a] Color, Texture, and Overall appearance: 1=extremely undesirable, 8=extremely desirable

[b-c] Means within the same row and rigor state bearing identical superscripts are not different (P>0.05)

[d] SPC measured at 2 and 6 days

Table 4 - Effect of aging on color and other appearance traits of prerigor $CaCl_2$ injected and postrigor beef steaks from longissimus muscle

	PRERIGOR ($CaCl_2$ injected)			POSTRIGOR		
	3 days	7 days	Standard Error	3 days	7 days	Standard Error
Color panel[a]						
Color	5.5^{b}	5.0^{c}	0.09	5.5^{b}	5.5^{b}	0.09
Texture	5.0^{b}	4.9^{b}	0.10	5.2^{b}	5.3^{b}	0.10
Overall appearance	5.3^{b}	4.9^{c}	0.11	5.4^{b}	5.3^{b}	0.09
Instrumental color						
L^*	37.9^{b}	36.4^{c}	0.29	41.2^{b}	41.3^{b}	0.36
a^*	21.8^{b}	19.9^{c}	0.22	23.9^{b}	22.4^{c}	0.30
b^*	10.2^{b}	9.3^{c}	0.17	11.5^{b}	11.0^{b}	0.26
SPC[d]	NA[e]	NA[e]		4.1^{c}	5.3^{b}	0.24

[a] Color, Texture, and Overall appearance: 1=extremely undesirable, 8=extremely desirable

[b-c] Means within the same row and rigor state bearing identical superscripts are not different ($P>0.05$)

[d] SPC and TBA measured at 2 and 6 days

[e] Not Available. Missing values due to plate contamination

area available and the high temperature of the meat which may facilitate contamination. In this study prerigor $CaCl_2$ injected muscles had higher counts ($P<0.05$) than postrigor muscles. However, it is unclear whether the higher counts were caused by accelerated processing or the $CaCl_2$ injection treatment, as no prerigor controls were used and there is not confirmatory data in the literature on the effect of $CaCl_2$ injection on microbial load.

Increased aging (2 days to 6 days) resulted in a significant increase in microbial counts for all rigor states and muscles as expected (Tables 3 and 4).

4.4 Sensory measurements.

In general, there were no significant differences in tenderness, texture and flavor scores due to treatment among muscles of the same rigor state (Tables 5 and 6). However, postrigor BT steaks from the *infraspinatus* muscle were rated as more tender ($P<0.05$) than C steaks, but BT steaks were not more tender ($P>0.05$) in postrigor *longissimus* muscle. Blade tenderization was responsible for numerically higher scores for postrigor *longissimus* BT steaks than C steaks (6.2 vs 6.0) although this difference was not significant ($P>0.05$). Blade tenderization of prerigor $CaCl_2$ injected muscles did not

(P>0.05) affect tenderness. The improvements in tenderness due to blade tenderization of postrigor muscles observed in this study agree with previous reports (Glover et al., 1977; Hayward et al., 1980; and Seideman et al., 1986). In addition, the prerigor muscle results agree with Loucks et al. (1981) in that no improvement in tenderness was observed when prerigor meat was blade tenderized. However, the prerigor $CaCl_2$ injection treatment may have introduced added changes in tenderness which may reduce the ability to measure differences. In addition, the carcasses used in this study were not the same quality grade, since the range was from U.S Standard to U.S. Choice. Higher quality grade carcasses produce a more tender product (Judge et al., 1989). The different carcass grades used in this study may have produced steaks with more variation in tenderness. This inconsistency may have introduced added variability, again reducing the ability to measure differences.

There were significant differences due to the effect of rigor state and treatment on tenderness, texture and flavor ratings (Tables 5 and 6). Postrigor C and BT *infraspinatus* steaks were rated higher (P<0.05) than CANB and CABT for all three traits (Figure 4). In addition, postrigor BT steaks were more tender (P<0.05) than the postrigor C steaks. Both postrigor C and BT *longissimus* steaks were more tender

Table 5 - Effects of rigor state and blade tenderization on physical and sensory traits of beef steaks from infraspinatus muscle

	Prerigor ($CaCl_2$ injected)		Postrigor		
	Blade (CABT)	No Blade (CANB)	Blade (BT)	Control (C)	S.E.
Sensory trait[a]					
Tenderness	5.6[d]	5.6[d]	6.6[b]	6.1[c]	0.16
Texture	5.2[c]	5.2[c]	6.3[b]	6.2[b]	0.14
Flavor	5.3[c]	5.2[c]	6.1[b]	6.0[b]	0.14
Shear force					
Total energy (kg*mm)	29.7[b]	27.9[b]	20.3[c]	23.0[c]	1.24
Peak force (kg)	3.8[b]	3.7[bc]	2.9[c]	3.3[c]	0.21
Connective tissue					
Total collagen[e]	14.5[b]	13.5[bc]	14.7[b]	12.1[c]	0.94
% soluble collagen[f]	23.3[d]	22.7[d]	28.0[c]	31.7[b]	1.01
Rancidity					
TBA value[f]	0.12[bc]	0.10[c]	0.15[bc]	0.17[b]	0.02

[a] Tenderness: 1=extremely tough, 8=extremely tender; Texture and flavor: 1=extremely undesirable, 8=extremely desirable

[b-d] Means within the same row bearing identical superscripts are not different ($P>0.05$)

[e] Total collagen measured in mg/gram of meat

[f] Rigor*blade tenderization interaction ($P<0.05$)

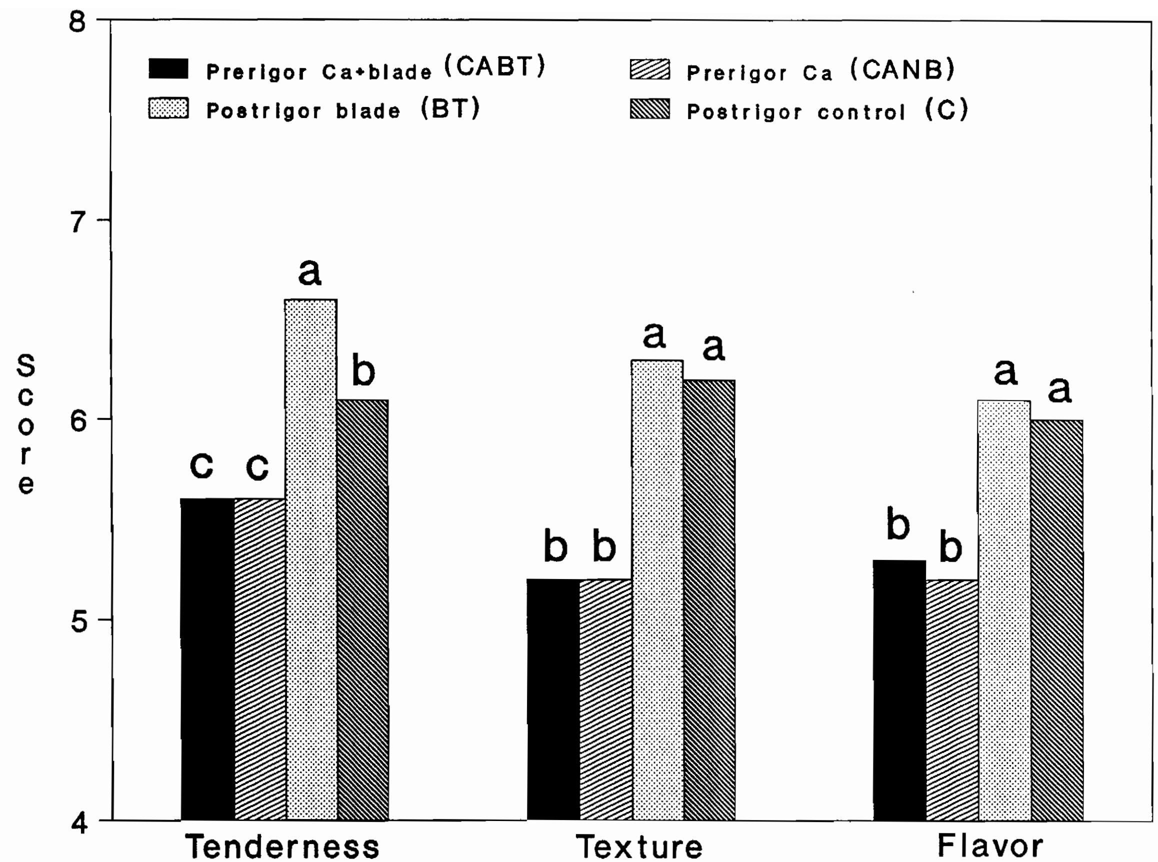

Figure 4 - Effect of rigor state and blade tenderization on sensory scores of steaks from beef infraspinatus muscle

a-c bars within the same trait bearing identical letters are not different ($P>0.05$)

($P<0.05$) than the prerigor muscle steaks regardless of treatment (Figure 5). These results are in agreement with some reports stating that postrigor meat is more tender than prerigor meat (Kastner and Russell, 1975 and Cecchi et al, 1988). However, the $CaCl_2$ injection treatment may have made significant contributions to this difference. It was expected that calcium chloride injection would improve tenderness as reported by Koohmaraie et al. (1988c, 1989, and 1990),St.Angelo et al. (1991), and Wheeler et al. (1992). These studies revealed that increased intramuscular calcium concentration as a result of $CaCl_2$ infusion increased the activity of calpains, which resulted in the acceleration of postmortem tenderization and improvements in tenderness. Infusion was performed on intact carcasses which were conventionally processed. In addition, Wheeler et al. (1992) reported that injecting $CaCl_2$ into excised prerigor and postrigor meat results in a more tender product. While in conflict with previous reports, the results from this study indicate that accelerated processing combined with $CaCl_2$ injection did not improve tenderness of prerigor muscles, and results in a less tender ($P<0.05$) product than that which is conventionally processed. In this study, accelerated processing was combined with injections performed on excised muscles. The increase in intramuscular calcium concentration

Table 6 - Effects of rigor state and blade tenderization on physical and sensory traits of beef steaks from longissimus muscle

	Prerigor ($CACl_2$ injected)		Postrigor		
	Blade (CABT)	No Blade (CANB)	Blade (BT)	Control (C)	S.E.
Sensory trait[a]					
Tenderness	5.3[c]	5.3[c]	6.2[b]	6.0[b]	0.16
Texture	5.4[c]	5.3[c]	5.81[bc]	5.84[b]	0.14
Flavor	5.5[b]	5.3[b]	5.6[b]	5.6[b]	0.13
Shear force					
Total energy (kg*mm)	42.4[b]	45.4[b]	33.2[c]	31.2[c]	1.57
Peak force (kg)	5.5[b]	5.9[b]	4.3[c]	4.2[c]	0.20
Connective tissue					
Total collagen[d]	8.2[b]	6.7[c]	6.6[c]	8.6[b]	0.30
% soluble collagen	17.2[b]	13.9[b]	17.3[b]	16.5[b]	1.56
Rancidity					
TBA value	0.23[b]	0.18[bc]	0.14[c]	0.13[c]	0.02

[a] Tenderness: 1=extremely tough, 8=extremely tender; Texture and flavor: 1=extremely undesirable, 8=extremely desirable

[b-c] Means within the same row bearing identical superscripts are not different ($P>0.05$)

[d] Total collagen measured as mg/gram of meat; rigor*blade tenderization interaction ($P<0.05$)

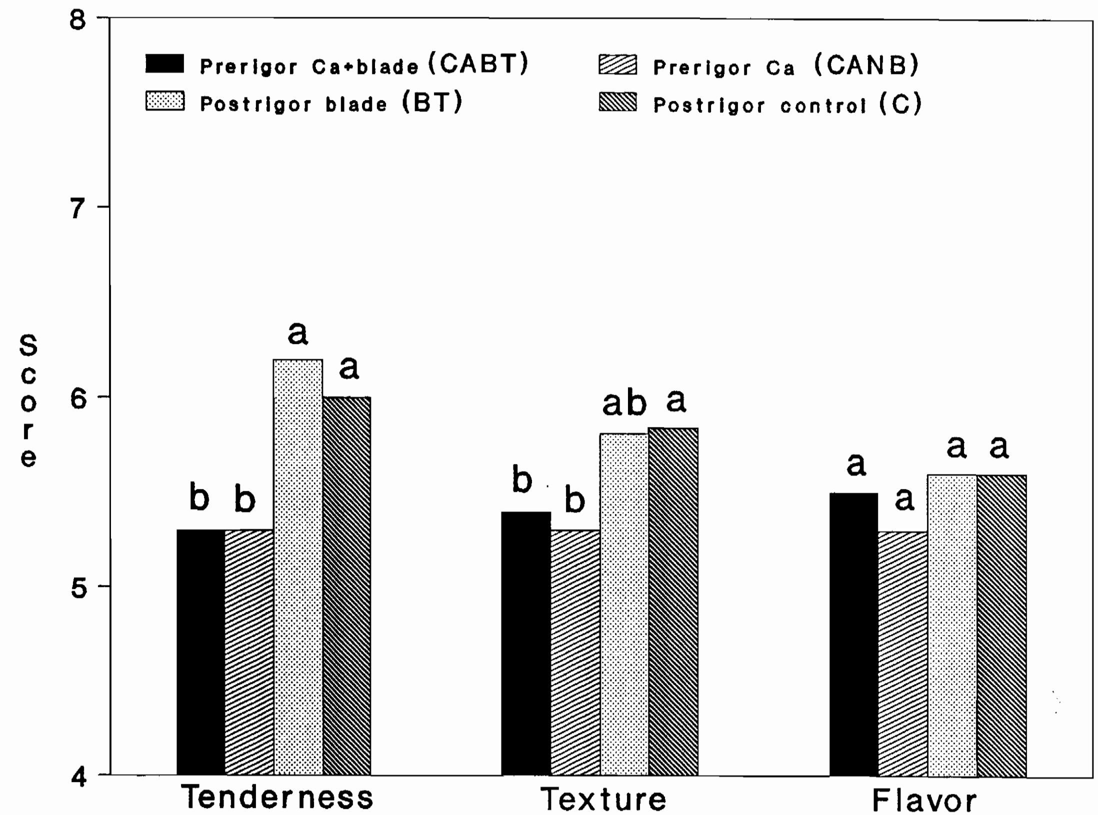

Figure 5 - Effect of rigor state and blade tenderization on sensory scores of steaks from beef longissimus muscle
a-b bars within the same trait bearing identical letters are not different (P>0.05)

and the lack of skeletal restraints may have caused an abnormal contraction of the excised muscle. If such a contraction occurred it would be associated with shorter sarcomere length, which has been reported to result in decreased tenderness (Locker, 1960). This contraction and subsequent toughening of the muscle may have masked any improvements in tenderness associated with increased proteolysis caused by the higher calcium concentration. Furthermore, the steaks from prerigor muscles were chilled within 1 hour of processing. It is known that cold shortening may occur when prerigor muscle with a high pH is chilled (Kastner, 1982). Added contraction from cold shortening may have occurred in this study, causing further decreases in the tenderness of prerigor meat. This hypotheses are supported by evidence from previous studies indicating that $CaCl_2$ injection of hot boned subprimals resulted in contraction of the muscles and significant decreases in sarcomere length (Morgan et al., 1991a). If this was true, electrical stimulation of the carcass prior to boning may alleviate tenderness decreases associated with muscle shortening. Kastner (1982) reported that electrical stimulation hastens rigor and prevents cold shortening. However, this hypothesis cannot be confirmed here as sarcomere length measurements were not performed. Further research on the possible uses of $CaCl_2$ injection combined with accelerated processing is needed to determine the causes of the loss of tenderness observed in this study.

No differences (P>0.05) in flavor scores were observed due to blade tenderization of muscles of the same rigor state. Postrigor C and BT steaks from the *infraspinatus* were rated as more flavorful (P<0.05) than the CANB and CABT prerigor steaks (Figure 4), but the postrigor C and BT *longissimus* steaks were not different (P>0.05) from the CANB and CABT prerigor steaks (Figure 5). Some researchers have reported increased salty and bitter flavor notes to be associated with injection of 0.3 M $CaCl_2$ at 10% muscle weight (St.Angelo et al., 1991). While no flavor descriptors were used in this study, the results indicate that $CaCl_2$ injection at these levels results in significantly undesirable flavor changes in some muscles.

The effects of aging (3 and 7 days) on taste panel measurements were determined in prerigor and postrigor muscles (Tables 7 and 8). Steaks aged for 7 days did not differ (P>0.05) in tenderness from those aged for 3 days. According to Calkins and Seideman (1988), an increase in tenderness should have occurred with increased aging time of 7 days. However, other researchers have reported that aging for 7 days did not improve tenderness (P>0.05) whereas benefits were achieved by storing for 21 days (Savell et al., 1978b).

Aging for 7 as compared to 3 days resulted in lower

Table 7 - Effect of aging on physical and sensory traits of prerigor $CaCl_2$ injected and postrigor beef steaks from infraspinatus muscle

	PRERIGOR ($CaCl_2$ injected)			POSTRIGOR		
	3 days	7 days	Standard Error	3 days	7 days	Standard Error
Sensory trait[a]						
Tenderness	5.8[b]	5.5[b]	0.15	6.3[b]	6.5[b]	0.16
Texture	5.5[b]	5.0[c]	0.13	6.4[b]	6.3[b]	0.13
Flavor	5.4[b]	5.1[b]	0.13	6.1[b]	6.0[b]	0.08
Shear force						
Total energy (kg*mm)	28.0[b]	28.8[b]	1.36	22.7[b]	20.8[b]	0.78
Peak force (kg)	3.8[b]	3.7[b]	0.25	3.3[b]	2.9[c]	0.10
TBA[d]	0.09[c]	0.16[b]	0.01	0.18[b]	0.19[b]	0.02

[a] Tenderness: 1=extremely tough, 8=extremely tender; Texture and flavor: 1=extremely undesirable, 8=extremely desirable

[b-c] Means within the same row and rigor state bearing identical superscripts are not different ($P>0.05$)

[d] TBA measured at 2 and 6 days

Table 8 - Effect of aging on physical and sensory traits of prerigor $CaCl_2$ injected and postrigor beef steaks from longissimus muscle

	PRERIGOR ($CaCl_2$ injected)			POSTRIGOR		
	3 days	7 days	Standard Error	3 days	7 days	Standard Error
Sensory trait[a]						
Tendernes	5.2^{b}	5.4^{b}	0.18	5.9^{b}	5.9^{b}	0.08
Texture	5.6^{b}	5.2^{b}	0.16	5.9^{b}	5.5^{c}	0.06
Flavor	5.6^{b}	5.3^{c}	0.11	5.7^{b}	5.5^{c}	0.06
Shear force						
Total energy (kg*mm)	43.9^{b}	44.0^{b}	1.74	34.8^{b}	29.5^{c}	1.08
Peak force (kg)	5.7^{b}	5.7^{b}	0.23	4.5^{b}	4.0^{c}	0.14
TBA[d]	0.18^{c}	0.28^{b}	0.02	0.15^{b}	0.18^{b}	0.01

[a] Tenderness: 1=extremely tough, 8=extremely tender; Texture and flavor: 1=extremely undesirable, 8=extremely desirable

[b-c] Means within the same row and rigor state bearing identical superscripts are not different ($P>0.05$)

[d] TBA measured at 2 and 6 days

(P<0.05) texture scores for prerigor *infraspinatus* and postrigor *longissimus* steaks and resulted in numerically but insignificantly lower (P>0.05) texture scores for other treatments. In addition, aging for 7 days resulted in less desirable (P<0.05) flavor in prerigor and postrigor *longissimus* steaks and numerically but insignificantly lower flavor scores for prerigor and postrigor *infraspinatus* steaks. These decreases in texture and flavor scores may be due to the degradation of the myofibrillar structure caused by endogenous and microbial proteases and the production of off-flavors by lipid oxidation and microbial metabolism (Judge et al.,1989). Steaks from *infraspinatus* muscles were rated as more tender (P<0.05) than those from *longissimus* muscles but no significant differences in texture and flavor between muscles were observed.

4.5 Objective Tenderness Measurements.

Peak force and total energy values of blade tenderized muscles were not different (P>0.05) among muscle of the same rigor state (Tables 5 and 6). However, steaks from BT postrigor *infraspinatus* muscles had numerically but insignificantly lower (P>0.05) total energy and peak force values (20.3 vs 23.3 and 2.9 vs 3.3, respectively). These results agree with the sensory tenderness scores suggesting

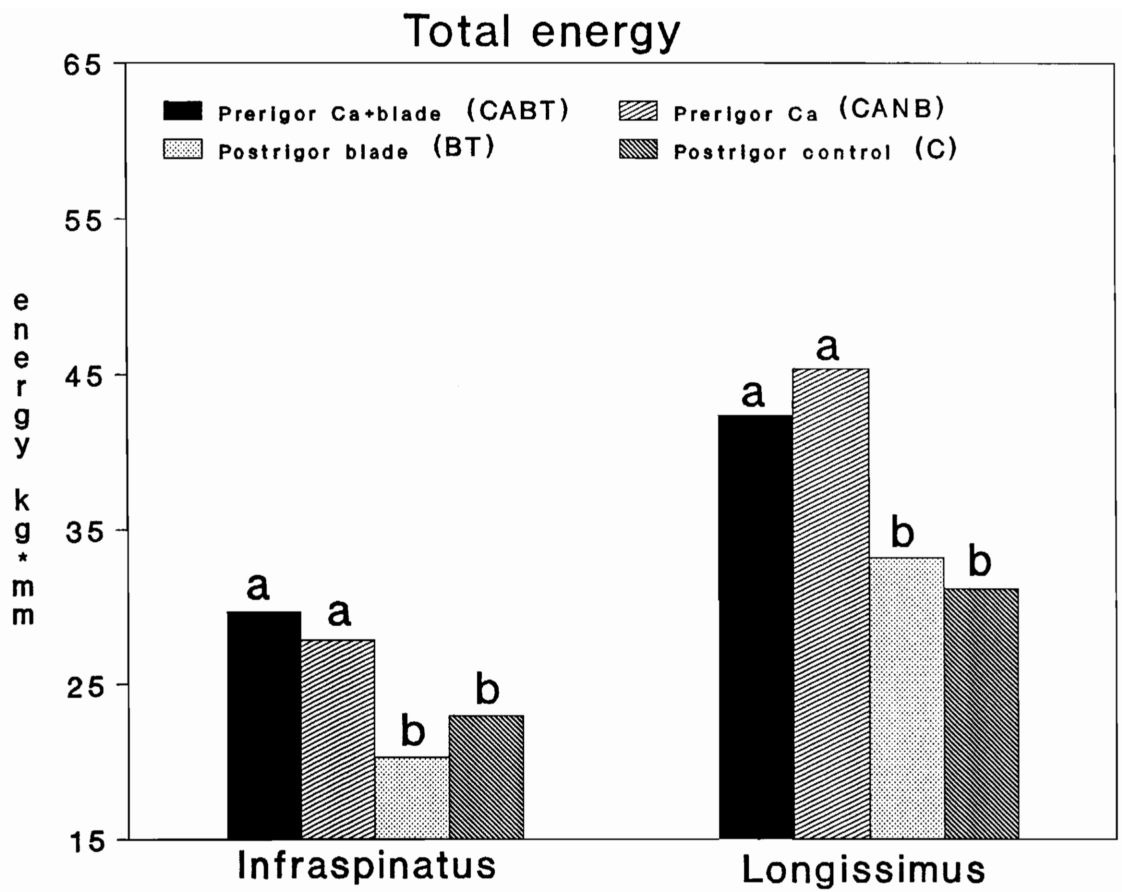

Figure 6 - Effect of rigor state and blade tenderization on total energy values of beef steaks from infraspinatus and longissimus muscles

a-b bars within the same muscle bearing identical letters are not different (P>0.05)

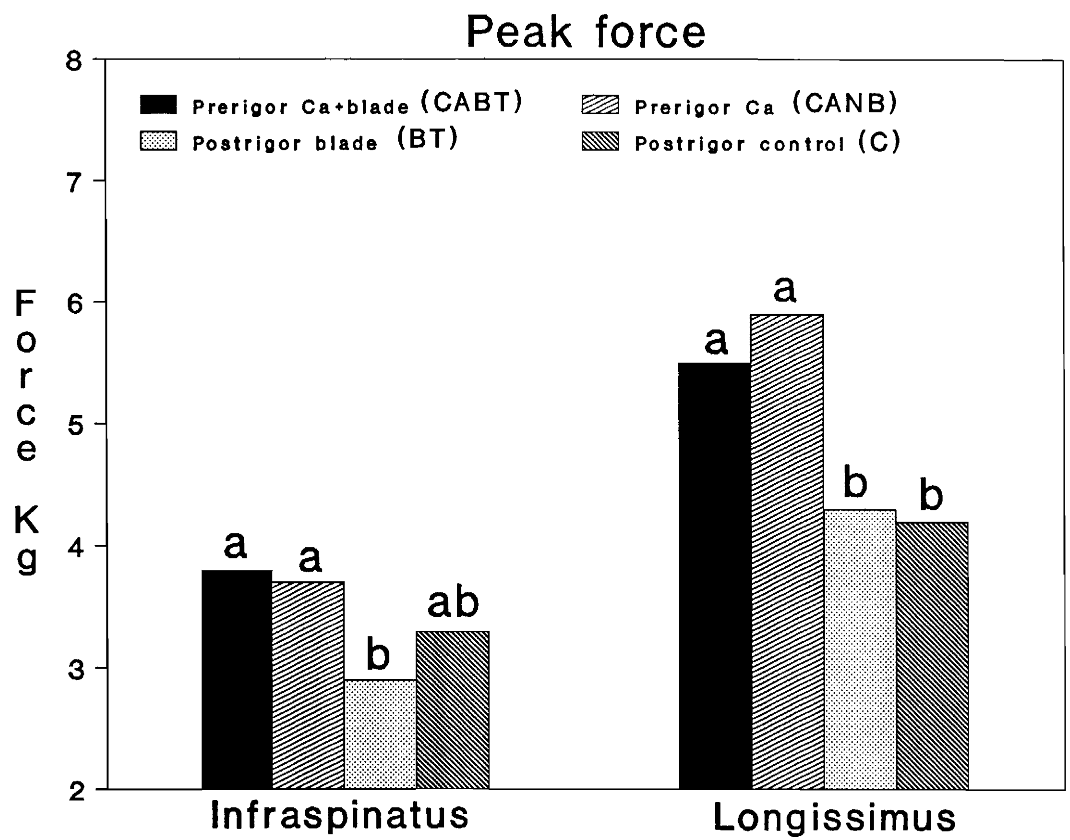

Figure 7 - Effect of rigor state and blade tenderization on peak force values of beef steaks from infraspinatus and longissimus muscles

a-b bars within the same muscle bearing identical letters are not different (P>0.05) that blade tenderization of $CaCl_2$ injected prerigor muscles does not improve tenderness. Again, the added treatment of $CaCl_2$ injection in prerigor muscle and the different quality grades of the carcasses used in this study may have reduced the ability to measure differences. Blade tenderization of postrigor muscles gave numerically lower shear values to support the sensory panel results, although improvements in tenderness as measured by shear force were not significant ($P>0.05$) and only tendencies were observed.

Postrigor BT and C steaks from both muscles had lower ($P<0.05$) total energy and peak force values than prerigor CANB and CABT steaks, but no significant difference due to blade tenderization within rigor treatments was observed (Tables 5 and 6). Only prerigor CANB *infraspinatus* steaks did not differ ($P>0.05$) for peak force values from either of the postrigor treatment steaks (Figures 6 and 7). Shear force values support the results obtained by the sensory panel indicating that postrigor beef is more tender ($P<0.05$) than $CaCl_2$ injected prerigor muscle independent of blade tenderization.

There were no differences ($P>0.05$) in shear force values due to increased aging time (3 days vs 7 days) within prerigor calcium chloride injected muscles (Tables 7 and 8). However, increased aging of postrigor steaks resulted in significant decreases in peak force values from both muscles and total energy values of postrigor *longissimus* steaks. Total energy values for postrigor *infraspinatus* steaks tended to be lower with increased aging (22.7 vs 20.8) although no significant difference (P>0.05) was observed.

4.6 Collagen Determination.

Total collagen as well as soluble and insoluble collagen contents were determined. Percentage soluble collagen was determined as a fraction of total collagen. There were no differences in total collagen and percentage soluble collagen due to blade tenderization in prerigor calcium chloride injected and postrigor steaks, although the postrigor C *longissimus* steaks had higher (P<0.05) total collagen content than the blade tenderized counterpart (Tables 5 and 6). This variation may have been due to sampling error as no differences in total collagen were expected. Also, the postrigor control steaks from the *infraspinatus* muscle had a higher (P<0.05) percentage of soluble collagen than the blade tenderized counterparts. The effect of rigor state and treatment was also determined. Postrigor BT and C steaks from the *infraspinatus* muscle had a higher (P<0.05) percentage of soluble collagen than prerigor CABT and CANB steaks, while no differences (P>0.05) among BT, C, CABT, and CANB steaks from *longissimus* was observed (Figure 8). There was a significant

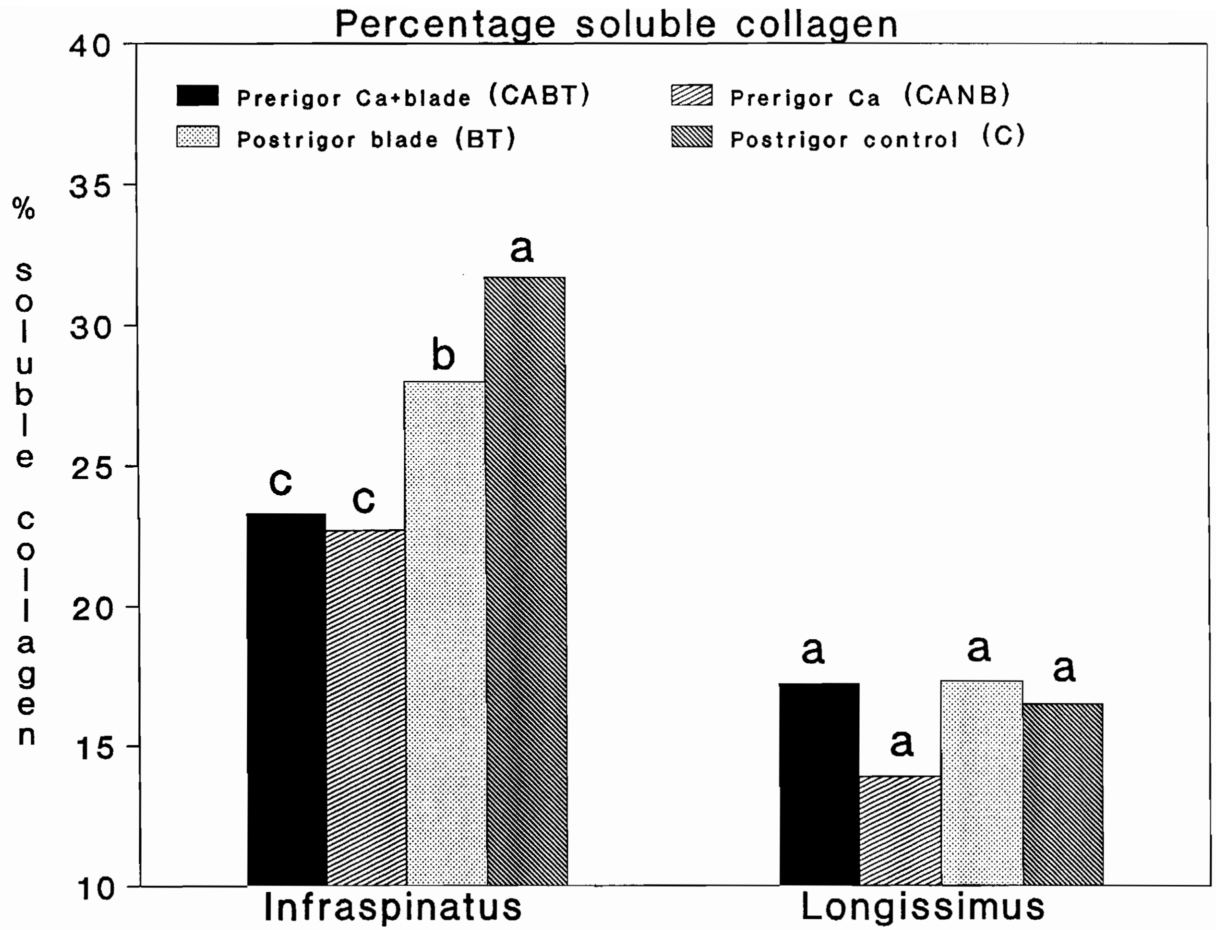

Figure 8 - Effect of rigor state and blade tenderization on percentage soluble collagen of beef steaks from infraspinatus and longissimus muscles

a-d bars within the same muscle bearing identical letters are not different (P>0.05) rigor by blade tenderization interaction for percentage soluble collagen in *infraspinatus* muscle samples as well as for total collagen in *longissimus* muscle samples. This may have detracted from the ability to correctly measure differences.

The results obtained from collagen determination as well as tenderness measurements by the sensory panel and shear force determinations indicate that no increase in tenderness may be obtained by blade tenderization of $CaCl_2$ injected prerigor muscles. However, blade tenderization of some postrigor muscles results in increased ($P<0.05$) tenderness. Furthermore, $CaCl_2$ injected prerigor muscles with and without blade tenderization were less tender ($P<0.05$) than postrigor muscles independent of this treatment.

4.7 Lipid Oxidation.

TBA values were measured at 2 and 6 days postmortem. No differences ($P>0.05$) in TBA values resulted from blade tenderization of muscles of the same rigor state (Tables 5 and 6). There were differences due to rigor states and treatments but the results for steaks from the *infraspinatus* and *longissimus* are in conflict. For *infraspinatus* muscle steaks, postrigor C samples had higher ($P<0.05$) TBA values than the

CANB prerigor samples, but neither of these were significantly different from BT and CABT samples (Table 5). However, for *longissimus* muscle steaks, CABT samples had higher (P<0.05) TBA values than both the BT and C samples but were not different (P>0.05) from the CANB samples (Table 6).

The effect of increased aging time (2 days vs 6 days) on TBA values was determined (Tables 7 and 8). Aging for 6 days caused a significant increase in TBA values for both *infraspinatus* and *longissimus* prerigor samples, but not for postrigor samples (P>0.05). However, there was a tendency toward higher values for 6 day postrigor samples compared to 2 day values (0.19 vs 0.18 for *infraspinatus* and 0.18 vs 0.15 for *longissimus* postrigor samples) although this difference was not significant (P>0.05).

4.8 Correlation Analysis.

Correlation coefficients between visual panel measurements and instrumental data as well as microbial counts were determined (Table 9). Sensory color and overall appearance ratings were significantly correlated (P<0.05) only with CIE a^* values, while texture ratings had weak but significant (P<0.05) correlation with CIE L^* and b^* values. It appears that panelists were more influenced for overall appearance evaluations by the redness of meat color as measured by CIE a^* than by the brightness of the meat surface as measured by CIE L^* values.

Microbial counts were not significantly correlated (P>0.05) to sensory panel ratings (Tables 9 and 10). However, a significant correlation with visual texture ratings was expected.

Correlations between sensory panel measurements and instrumental and chemical data were determined (Table 10). Sensory tenderness and texture measurements correlated (P<0.05) with total energy and peak force measurements to confirm the observations made by the sensory panel. Sensory tenderness measurements also correlated (P<0.05) with percentage soluble collagen not with total collagen (P>0.05). These data confirm reports stating that physiological state of collagen in meat, as measured by solubility, is a better indicator of tenderness than total collagen content (Shimokomaki et al., 1972; Judge et al., 1989).

Contrary to what was expected, sensory flavor determinations did not correlate well (P>0.05) with either TBA values or microbial counts. Even though flavor scores and TBA values were not significantly correlated, both measurements indicated similar significant differences and tendencies with regards to the effect of treatments and aging.

Table 9 - Correlation coefficients of visual panel ratings with instrumental and microbial measurements

	Sensory Trait		
	Color	Texture	Overall Appearance
Instrumental			
L^*	0.03	0.45*	0.15
a^*	0.53**	-0.02	0.47*
b^*	-0.19	0.36*	-0.10
Microbial			
SPC	-0.10	-0.31*	-0.21

** Significant at P<0.01
* Significant at P<0.05

Table 10 - Correlation coefficients of taste panel ratings with instrumental and chemical measurements

	Sensory Trait		
	Tenderness	Texture	Flavor
Instrumental			
Total energy (Kg*mm)	0.48*	0.38*	0.12
Peak Force (Kg)	-0.47*	0.34*	-0.25
Chemical			
Total Collagen	0.16	0.16	0.18
% Soluble Collagen	0.32*	0.25	0.31
TBA	-0.23	0.02	-0.16
SPC	-0.15	-0.23	-0.18

* Significant at $P<0.05$

CHAPTER V

SUMMARY AND CONCLUSIONS

Steaks from blade tenderized prerigor $CaCl_2$ injected muscles did not differ (P>0.05) in tenderness from non-tenderized counterparts as determined by the sensory panel, Warner-Bratzler shear force, and percentage soluble collagen content. However, steaks from blade tenderized postrigor muscles were more tender (P<0.05) than control steaks as verified by taste panel scores, while only tendencies (P>0.05) toward improved tenderness were observed as measured by shear force.

Postrigor steaks were more tender (P<0.05) than the prerigor $CaCl_2$ injected product independent of blade tenderization as measured by sensory panel ratings, Warner-Bratzler shear force, and percentage soluble collagen content. Percentage soluble collagen content for *longissimus* muscle was not different (P>0.05) when rigor states and treatments were compared.

Blade tenderization of prerigor $CaCl_2$ injected and postrigor muscles did not have significant effects (P>0.05) on texture, and flavor as measured by the sensory panel. Postrigor meat had superior texture (P<0.05) for both muscles, and higher flavor ($P<0.05$) for *infraspinatus* muscle ratings than $CaCl_2$ injected prerigor meat independent of blade tenderization. However, for *longissimus* muscle there were no differences ($P>0.05$) in flavor ratings due to rigor state and treatment.

Extent of lipid oxidation as measured by TBA values indicated that blade tenderization of $CaCl_2$ injected prerigor and postrigor meat did not affect ($P>0.05$) TBA values. Rigor state and treatment combinations produced mixed results since postrigor control samples from the *infraspinatus* muscle steaks had higher ($P<0.05$) TBA values than prerigor $CaCl_2$ injected samples. However, prerigor *longissimus* muscle $CaCl_2$ injected and blade tenderized samples had higher TBA values than steaks from prerigor muscles independent of blade tenderization.

Steaks from blade tenderized postrigor and prerigor $CaCl_2$ injected muscles did not differ ($P>0.05$) in color, texture, and overall appearance ratings from non-tenderized counterparts as evaluated by the visual panel. Rigor state and treatment combinations affected visual sensory traits differently. For texture, prerigor $CaCl_2$ injected and blade tenderized steaks had lower ratings ($P<0.05$) than prerigor $CaCl_2$ injected counterparts and postrigor blade tenderized steaks, while postrigor control steaks had higher ratings ($P<0.05$) than any treatment for both muscles. There were no differences ($P>0.05$) due to rigor state and treatment for color ratings of *longissimus* steaks. For *infraspinatus* muscle steaks, prerigor $CaCl_2$ injected and blade tenderized samples were rated higher ($P<0.05$) than $CaCl_2$ injected counterparts and postrigor blade tenderized steaks. For overall appearance ratings of *infraspinatus muscle,* postrigor control steaks were rated higher ($P<0.05$) than blade tenderized counterparts. However, for the *longissimus* muscle both postrigor treatment steaks were rated higher ($P<0.05$) than prerigor $CaCl_2$ injected and blade tenderized steaks.

Blade tenderization of prerigor $CaCl_2$ injected and postrigor steaks did not affect ($P>0.05$) CIE L^* a^* b^* values. However, postrigor blade tenderized steaks from *infraspinatus* muscle had higher ($P<0.05$) L^* values than control steaks. Postrigor steaks had higher ($P<0.05$) L^* a^* b^* values than prerigor $CaCl_2$ injected steaks regardless of blade tenderization but this trend did not apply ($P>0.05$) for a^* values of *infraspinatus* muscle steaks.

Blade tenderized samples tended ($P>0.05$) to have higher microbial counts than non tenderized muscles. In addition, postrigor samples had lower ($P<0.05$) microbial counts than prerigor $CaCl_2$ injected samples for both muscles.

Increased aging time (3 vs 7 days) resulted in improved tenderness ($P<0.05$) as measured by peak force and total energy values, but these improvements in tenderness were not significant ($P>0.05$) as measured by the taste panel. However, increased aging had detrimental effects on various meat characteristics. These include significant decreases ($P<0.05$) in sensory flavor, taste texture, color , and overall appearance ratings, as well as increased ($P<0.05$) TBA values and microbial counts.

The main conclusions that can be extracted are: 1) Accelerated processing combined with $CaCl_2$ injection yields less tender and less desirable texture for both *infraspinatus* and *longissimus* muscles than conventionally processed beef independent of blade tenderization. In addition, the accelerated processing and $CaCl_2$ injection treatment combination can have detrimental effects on flavor of steaks from *infraspinatus* muscle; 2) Blade tenderization can improve tenderness in postrigor muscles but not in prerigor $CaCl_2$ injected muscles; 3) Increased aging can improve tenderness as measured by shear force but can result in detrimental effects on sensory characteristics as well as increased microbial load and; 4) Postrigor muscles can exhibit lower microbial counts than prerigor $CaCl_2$ injected muscles.

$CaCl_2$ injection has been reported to be a simple and convenient method for improving tenderness of meat. However, further study on possible more precise and effective applications of this treatment in combination with accelerated processing is needed. Future research should be focused on determining the reason(s) for the decrease in tenderness caused by the prerigor $CaCl_2$ injection and possible methods that may alleviate this problem. In addition, the effect of $CaCl_2$ injection on microbial load needs to be determined to establish the viability of industrial applications in the future.

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APPENDIX

Table 12 - Effect of blade tenderization on beef steaks from the longissimus muscle analyzed separately by rigor state for color and other appearance traits

	PRERIGOR ($CaCl_2$ injected)			POSTRIGOR		
	Blade (CANB)	No Blade (CABT)	Standard Error	Blade (BT)	Control (C)	Standard Error
Visual trait[a]						
Color	5.3[b]	5.2[b]	0.09	5.5[b]	5.5[b]	0.05
Texture	5.1[b]	4.7[b]	0.16	5.1[b]	5.5[b]	0.14
Overall appearance	5.2[b]	5.0[b]	0.14	5.3[b]	5.4[b]	0.04
Instrumental color						
L^*	37.8[b]	36.5[b]	0.59	41.6[b]	41.0[b]	0.35
a^*	20.9[b]	20.8[b]	0.54	23.6[b]	22.6[b]	0.34
b^*	9.9[b]	9.8[b]	0.23	11.6[b]	11.0[b]	0.34
SPC	NA[d]	5.2	0.33	4.6[b]	4.2[b]	0.18

[a] Color, Texture, and Overall appearance: 1=extremely undesirable, 8=extremely desirable

[b-c] Means within the same row and rigor state bearing identical superscripts are not different ($p>0.05$)

[d] Missing values due to plate contamination

Table 13 - Effect of blade tenderization on beef steaks from the infraspinatus muscle analyzed separately by rigor state for physical and sensory traits

	PRERIGOR ($CaCl_2$ injected)			POSTRIGOR		
	Blade (CANB)	No Blade (CABT)	Standard Error	Blade (BT)	Control (C)	Standard Error
Sensory trait[a]						
Tenderness	5.7^b	5.6^b	0.18	6.6^b	6.1^c	0.11
Texture	5.2^b	5.2^b	0.16	6.3^b	6.2^b	0.13
Flavor	5.2^b	5.3^b	0.14	6.1^b	6.0^b	0.09
Shear force						
Total energy (kg*mm)	27.9^b	29.7^b	1.50	20.3^b	23.0^b	1.49
Peak force (kg)	3.7^b	3.8^b	0.28	2.9^b	3.3^b	0.16
Connective tissue						
Total collagen[d]	12.1^b	14.7^b	1.46	14.5^b	13.5^b	0.86
% soluble collagen	22.7^b	23.3^b	1.46	28.0^c	31.7^b	0.89
TBA	0.12^b	0.11^b	0.01	0.15^b	0.17^b	0.01

[a] Tenderness: 1=extremely tough, 8=extremely tender; Texture and flavor: 1=extremely undesirable, 8=extremely desirable

[b-c] Means within the same row and rigor state bearing identical superscripts are not different (P>0.05)

[d] Total collagen measured as mg/gram of meat

Table 14 - Effect of blade tenderization on beef steaks from the longissimus muscle analyzed separately by rigor state for physical and sensory traits

	PRERIGOR ($CaCl_2$ injected)			POSTRIGOR		
	Blade (CANB)	No Blade (CABT)	Standard Error	Blade (BT)	Control (C)	Standard Error
Sensory trait[a]						
Tenderness	5.3[b]	5.3[b]	0.14	6.2[b]	6.0[b]	0.18
Texture	5.3[b]	5.4[b]	0.11	5.8[b]	5.8[b]	0.14
Flavor	5.3[b]	5.5[b]	0.09	5.6[b]	5.7[b]	0.08
Shear force						
Total energy (kg*mm)	45.4[b]	42.4[b]	4.60	33.2[b]	31.2[b]	2.12
Peak force (kg)	5.9[b]	5.5[b]	0.60	4.3[b]	4.2[b]	0.32
Connective tissue						
Total collagen[d]	6.7[b]	8.2[b]	0.59	6.6[b]	8.6[c]	0.69
% soluble collagen	13.9[b]	17.2[b]	1.15	17.3[b]	16.5[b]	1.74
TBA	0.18[b]	0.23[b]	0.02	0.14[b]	0.13[b]	0.01

[a] Tenderness: 1=extremely tough, 8=extremely tender; Texture and flavor: 1=extremely undesirable, 8=extremely desirable

[b-c] Means within the same row and rigor state bearing identical superscripts are not different ($p>0.05$)

[d] Total collagen measured as mg/gram of meat

VITA

Julián Benito-Delgado Antúnez was born on May 25, 1967 in Madrid, Spain. He is the son of Julián Benito-Delgado Pérez and Margarita Antúnez Vidaurrazaga. In June 1985, he received his high school diploma from King's College in Madrid, Spain, and in September of that same year he entered the Center for International Studies in Madrid, Spain, where he completed the first two years of college. The author entered Rollins College in Winter Park, Florida, in 1987, where he obtained a Bachelor of Science degree in Biology with honors in February 1990. In September 1990, he began graduate study at the University of Vermont for a PhD. degree in Microbiology and Molecular Genetics until he realized that his vocation was in other fields. He entered the Virginia Polytechnic Institute and State University in August 1991, and began graduate study for a Master of Science degree in Food Science and Technology.

The author is a member of the Institute of Food Technologists and the Phi Sigma Honor Society for the Biological Sciences.

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