CHARACTERIZATION AND IMPROVEMENT OF

LONGISSIMUS MUSCLE PALATABILITY

FROM BOS INDICUS CATTLE

CARL DEAN WALLACE

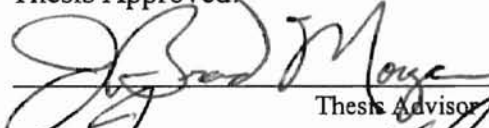Bachelor of Science

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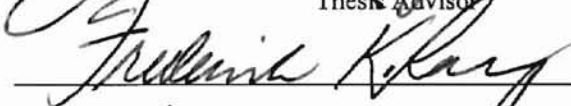
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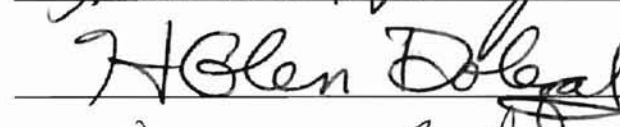the degree of
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Thesis Approved:

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I would like to thank my advisors, who served on my graduate committee; Dr. Brad Morgan, Dr. Glen Dolezal, and Dr. Fred Ray for their patience, knowledge, and words of encouragement. The opportunities and knowledge provided by these individuals was vast and very appreciated.

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My appreciation is beyond words to my parents, Harmon and Carolyn Wallace. The opportunities and backing they have provided is a shear expression of their love. Their encouraging words always come at the right time and I love them dearly.

This thesis is dedicated to my wife, Wendy and daughter, Maegen. Wendy's love and encouragement always helped me to strive onward and Maegen's smiles and energy was sure to lighten the mood. I truly cherish the years we have spent together and look forward to many to come.

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NOMENCLATURE

kDa	Kilodalton
%	Percent
h	Hour
d	Day
μM	Micromolar
$CaCl_2$	Calcium Chloride
M	Molar
mM	Millimolar
CDP	Calcium Dependent Protease
Ca^{2+}	Calcium ions
min	Minute
cm	Centimeters
°C	Degrees Celcius
F_1	First Cross
g	grams
x g	Unit of Speed
A	Absorbance

s	Second
μl	Microliter
EGTA	Ethyleneglycol Tetraacetic Acid
EDTA	Ethylenediamine Tetraacetic Acid
TCA	Trichloroacetic Acid
GB	Gray Brahman
RB	Red Brahman
A	Angus
N	Nelore
G	Gyr
IB	Indu Brazil
S	Simmental
H	Hereford
Trt	Treatment

CHAPTER I

INTRODUCTION

With the pork and poultry industries continuing to make strides, market share is becoming a major concern of the United States beef industry. The 1995 National Beef Quality Audit (NBQA) revealed consumers have a major concern for lack of consistency and toughness of the beef product purchased today (Boleman, 1998). In 1976 beef consumption per capita peaked at 42.8 kg (Savell 1994), on a retail weight basis, and since has been in a downward trend where in 1996 beef consumption resides at 31.2 kg per capita (USDA 1997). In contrast the poultry industry has climbed from 23.7 kg in 1976 (Savell 1994), to 41.4 kg in 1997 (USDA 1997). Chuck Schroeder, Chief Executive of the National Cattleman's Beef Association, stated "We have recognized for perhaps the first time, that people don't have to buy our product on whatever terms we choose to produce it" (NBQA 1995).

While strides have been made by the beef industry in some areas such as reducing external fat levels, eliminating injection sites, and improving cutability since the 1991 NBQA, positive stories associated with beef tenderness are few in number. Purveyors, restaurateurs, and retailers are concerned about the low overall eating quality of today's beef product due to the diminishing fat levels (NBQA 1995). In addition, they ranked beef tenderness as the number two problem within the beef industry. Tenderness or meat texture is the single most important factor affecting taste or consumer perception of taste

and in return acceptability (Savell 1987). The 1995 NBQA revealed that $137.82 was lost per steer or heifer due to quality defects. Of that $137.82, approximately 1/3 is attributed to taste and tenderness problems. Many researchers have documented a decline in beef tenderness associated with Bos indicus inheritance. Crouse and Koohmaraie (1990) reported freezing prior to postmortem aging enhanced beef steak tenderness due to increased proteolysis. Numerous researchers have reported dramatic improvements as a result of calcium chloride injection

Therefore this study was conducted to examine:

1) The effects of freezing and crust freezing in conjunction with calcium chloride injection and postmortem aging on beef Longissimus tenderness.

2) To identify differences in Longissimus tenderness of various *Bos indicus* breeds, and in return make recommendations as to which *Bos indicus* breed types can contribute to a positive palatability experience.

CHAPTER II

REVIEW OF LITERATURE

Aging Effects in Relation to Beef Tenderness. The conversion of muscle to meat has been an integral part of the human race for many decades. The challenge being producing an acceptable product in reference to consumer needs. Methods of manipulating beef tenderness are a commodity in demand. Currently, postmortem aging is the overall means for compensating for beef's lack of tenderness. Many researchers have documented improvements in beef tenderness following a 7 to 21 day aging period (Smith et al., 1978; Savell et al., 1978; Eilers et al. 1996). Pronounced improvements in beef tenderness, as a result of postmortem aging, are documented among both genders of beef cattle (Savell et al., 1978; Hopkinson et al., 1985). In addition, postmortem aging effects doesn't discriminate among various beef cuts or grades (Smith et al., 1978; Mitchell et al., 1991; Eilers et al., 1996).

Calpain Proteinase System and Beef Tenderness. Improvements in tenderness from aging beef muscle are well document, but understanding the mechanism behind the improvement has been the subject of considerable research. Koohmarie (1988a) documented aged muscle tissue produced a higher proportion of smaller fragments upon homogenization than unaged tissue. During postmortem storage there are several changes in skeletal muscle that result in a decline in myofibrillar integrity and/or an incre-

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ase in tenderness: 1) Z-disk degradation, 2) disappearance of troponin-T and simultaneous appearance of polypeptides with molecular weights of 28 to 32 kDa, 3) degradation of desmin, 4) degradation of titin, and 5) removal of α-actinin (Elgasim et al., 1985; Koohmaraie, 1988a; Koohmaraie, 1992; Kendall et al., 1993).

Proteolysis of key myofibrillar proteins is the principal reason for ultrastructural changes in skeletal muscle, resulting in the degradation of muscle cell integrity (Koohmaraie, 1992; Koohmaraie et al., 1987). Skeletal muscle is composed of three classes of proteins: sarcoplasmic, connective tissue, and myofibrillar. Koohmarie (1992) reported very few changes in sarcoplasmic and connective tissue proteins during postmortem aging, therefore the principle mode of postmortem tenderization is limited to myofibrillar proteins. To be a candidate in postmortem tenderization a protease must be located in skeletal muscle, must be able to reproduce postmortem changes in an *invitro* system, and must have access to the substrate (i.e. myofibrils)(Koohmaraie, 1988a; Koohmaraie, 1992). Calpains, endogenous to skeletal muscle cell, consist as two isoenzymes with different calcium requirements for activity; μ-calpain requires micromolar order of calcium ($\approx 10mM$) and m-calpain requires millimolar order of calcium (200-300 μM). The two proteases are additionally inhibited by a endogenous inhibitor refereed to as calpastatin (Koohmaraie, 1988a). One molecule of calpastatin inhibits about six molecules of protease, when greater quantities of protease are present the inhibitor is hydrolyzed (Shannon and Goll, 1985). Calcium from the sarcoplasmic reticulum and mitochondria activates the protease during rigor development (Busch et al.,

1972). Koohmarie (1992) found under normal postmortem conditions m-calpain is very stable as the system lacks sufficient calcium for activation, whereas a gradual decline in activity occurs with μ-calpain, and calpastatin loses activity very rapidly. When μ and m-calpain are activated in the presence of sufficient calcium improved meat tenderness is observed.

Calpain is the only proteolytic system that has all the characteristics that are necessary for bringing about postmortem changes associated with meat tenderness (Koohmaraie 1994). Calpains are proteases having an absolute calcium requirement for proteolytic activity. Using a myofibrillar index (MFI), Koohmaraie and his co-workers (1987) reported that 50% of the changes in myofibrils associated with postmortem aging occurred in the first 24 hr, but it took up to thirteen days for the changes to be complete. In later studies, MFI changes were accelerated when tissue slices were incubated with $CaCl_2$. In the presence of calcium chelators (i.e., EDTA, EGTA) the reaction was halted. Slices incubated with calcium had significantly less calpain activity indicating calcium-dependent protease activation and autolysis (Koohmaraie, 1988a; Koohmaraie, 1988b). The presence of the 30,000 kDa, or degradation product associated myofibrillar degradation, occurred in the first twenty-four hr, but not in the presence of calcium chelators. Calcium treated muscle slices had highly fragmented myofibrils that lacked Z-disks and desmin disappeared (Koohmaraie, 1988b). In the absence of calcium, the activity of the proteases was preserved. Koohmarie found that degradation of desmin leads to fragmentation of myofibrils (Koohmaraie, 1992). The majority if not all of postmortem improvements in tenderness are the result of proteolytic degradation by

proteases endogenous to the skeletal muscle cell (Koohmaraie et al., 1987; Koohmaraie, 1988b). Calkins and Seideman (1988) reported a 39.5% overall reduction in shear force occurred within the first three d of postmortem aging whereas pH is higher and calpain and calpastatin activity is greater. He reported an additional 41.6% reduction from d 3 to 6 and a 18% reduction from d 6 to 14. Since both μ and m-calpain undergo autolysis in the presence of sufficient calcium, but μ-calpain is the only protease to decline in activity during postmortem storage, μ-calpain is involved in postmortem tenderization and not m-calpain (Koohmaraie, 1988a).

Huff-Lonergan et al. (1996) incubated myofibrils with μ-calpain and noted many of the changes in proteins were simultaneous with postmortem changes in naturally aged beef. Degradation patterns of myofibrils prepared from aged samples were compared to gels of μ-calpain digested 0-d myofibril samples and it was evident that μ-calpain incubation at 4° C, pH 5.6 and 100 μM $CaCl_2$ caused many of the same changes associated with postmortem aging. Degradation products of titin, nebulin, filamin, desmin, and troponin-T appeared rapidly in the presence of μ-calpain, but lacked the initial presence in control samples. Additionally they reported degradation of the myofibrillar proteins: titin, nebulin, filamin, desmin, and troponin-T led to tenderization. Myofibrillar proteins are illustrated in figure 1. Degradation of titin, a large structural protein extending from Z-disk to near the m-line, results in weakening of the longitudinal structure of the myofibrillar structure. Degradation of nebulin, an additional structure protein expanding from the Z-disk to the free end of the thin filament, could result in weakening of the thin filament linkages at the Z-disk. Degradation of filamin, an actin

Figure 1. Diagram depicting the location of various myofibril proteins in the sarcomere.
Adapted from Pearson and Young (1989).
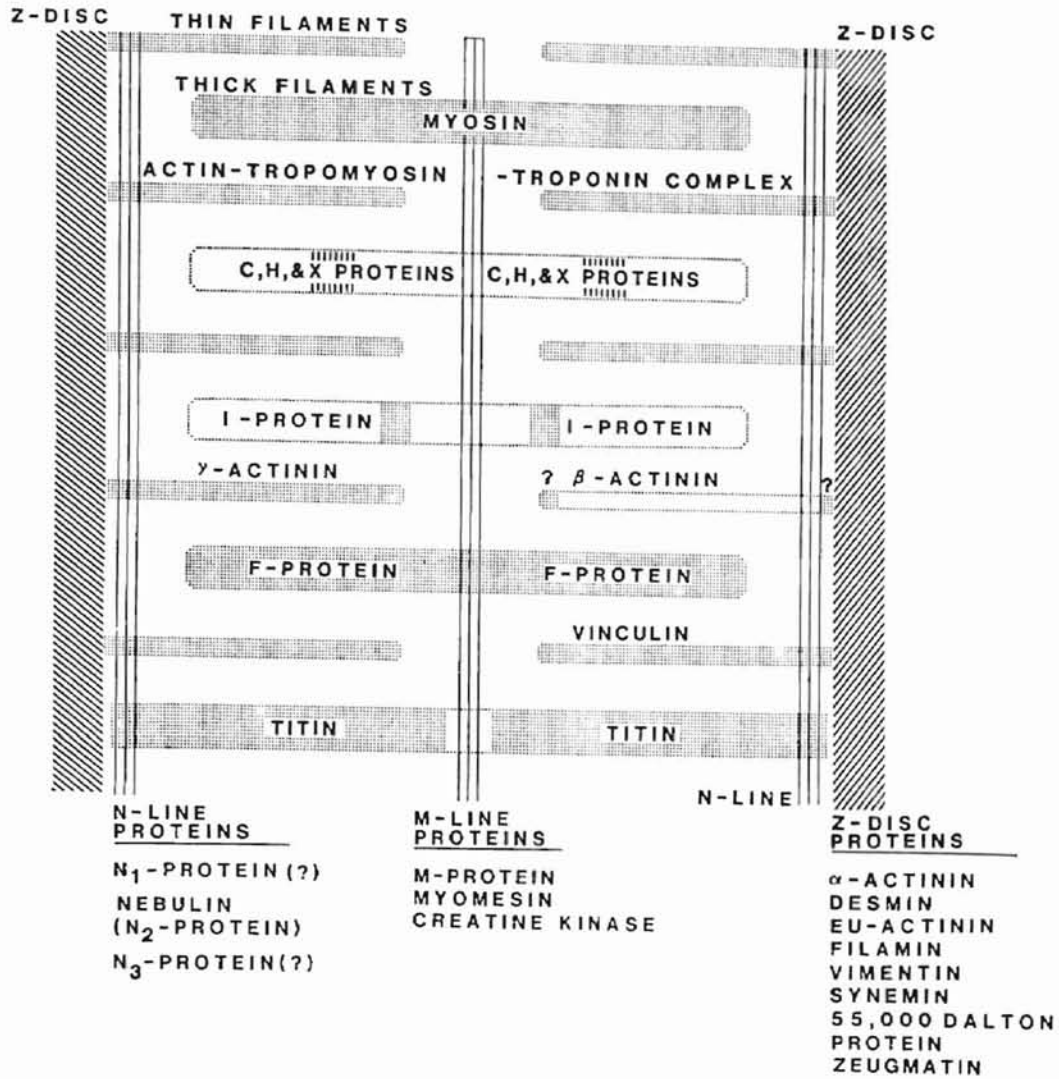
binding protein associated with the periphery of the Z-disk, may lead to disruption of key linkages that hold myofibrils in contingents. Degradation of desmin, an intermediate filament associated with the periphery of the myofibrillar Z-disk, may lead to enhanced tenderness due to lack of support of surrounding structures. Troponin-T degradation leads to appearance of the 30-kDa polypeptide associated with increase tenderness. Huff-Lonergan et al. (1996) deduced that postmortem tenderization, associated with μ-calpain does not depend on the degradation of a single protein, but rather the degradation and key structural changes of several proteins and regions of the muscle cell. Additionally, degradation of the key proteins associated with the myofibrils leads to overall weakening of the cell structure and therefor enhanced tenderness.

Effects of Calcium Chloride Injection on Beef Tenderness. Tenderness is the predominant quality determinant and probably the most variable organoleptic characteristic of meat (Lawrie 1979). Variability in tenderness is a major quality defect of the beef industry (Morgan et al., 1991). With this surmounting problem, $CaCl_2$ injection in prerigor and postmortem muscle was brought to the forefront in the late 80's by Koohmarie as a means of reducing tenderness variations. Koohmarie et al.(1988) incubated prerigor Longissimus tissue in a .3M $CaCl_2$ solution and discovered that most postmortem changes resulting in tenderization occurred in the first 24 h. Further research of the phenomenon found that the tenderizing process was blocked if the same samples were incubated in 10 mM EGTA or EDTA. Koohmarie conclude that postmortem tenderization was due to the calpain protease system .

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Numerous researchers found prerigor lamb Longissimus tissue injected with a 40 fold solution containing very high levels of Ca^{2+} had significant reductions in Warner-Bratzler shear force values within the first 24 h postmortem and did not significantly change with further postmortem aging (Koohmaraie et al., 1988; Koohmaraie et al., 1993; St. Angelo et al., 1991; Wheeler et al., 1992). Many studies have revealed similar results on various meat systems (Koohmaraie et al., 1990; Morgan et al., 1991; Wheeler et al., 1991, 1992, 1997). $CaCl_2$ has additionally shown to be very affective in improving tenderness in tougher cuts within the first 24 h postmortem. Morgan et al. (1991) found a 40-50 % reduction in Warner Bratzler Shear values in the first 24 h postmortem from cows having D and E maturity scores injected with a .3M $CaCl_2$ solution at 10 % by weight. Similar results were discovered with Brahman crossbred bull cuts (Wheeler et al., 1991) and cuts from β-Agonist fed steers (Whipple et al., 1992a).

Improvements in $CaCl_2$ injected meat can be detected by consumers as well Morgan et al. (1991) placed $CaCl_2$ injected striploins before a trained sensory panel. Panelist rated the 300mM $CaCl_2$ injected steaks as being more tender, having less connective tissue, and more juicy. However, panelist also perceived the injected steaks as being more metallic, bitter, strong and livery in flavor. Wheeler et al. (1993) adjusted the $CaCl_2$ injection concentration to a 5% by weight with a 200mM $CaCl_2$ solution and minimized all flavor problems and had minimal or no effect on tenderness or juiciness ratings. The 5% injection resulted in meat with higher beefy flavors and less off flavors when compared to controls. $CaCl_2$ injected steaks were placed in a white tablecloth

restaurant setting and patrons rated these steaks superior to noninjected controls (Lansdell et al., 1993; Hoover et al., 1993). In a supermarket or retail setting, consumers visually preferred $CaCl_2$ treated steaks 71 % of the time over control steaks based on packaging and labeling. The same consumers also perceived an increase in tenderness, juiciness, flavor, desirability and over all palatability of treated round steaks (Miller et al., 1995). These studies suggest $CaCl_2$ injection can help remove variations in tenderness without detrimental effects to other quality attributes.

Freezing Effects on Beef Tenderness. Freezing animal tissue is the predominant means of preserving meat for future consumption, but its effects on tenderness has been the question of considerable research (Berry et al., 1971; Crouse and Koohmaraie, 1990; Wheeler et al., 1990; Mitchell et al., 1991; Wheeler et al., 1992; Whipple and Koohmaraie, 1992b; Joseph, 1996). Joseph (1996) reported rapid chilled muscle (2.5 h to reach 0° C) was more tender than non chilled controls. Storing beef muscle at sub-zero temperatures allows rigor development to continue and produces some tenderization (Dransfield, 1996). Following a 27 d freezing period, aged Longissimus steaks were more tender than steaks aged prior to freezing (Crouse and Koohmarie, 1990). Whipple and Koohmarie (1992) reported freezing six weeks at -30° C tended (P=.07) to decrease Warner-Bratzler shear values and calpastatin activity. Their conclusions noted that rupturing of cell membranes as a result of freezing may have played an additive role in enhancing tenderness. Calpastatin activity was deminished between 45-66.6 % of its initial activity after 2, 4, and 6 weeks freezing at -70° C, whereas μ- and m-calpain remained very stable during freezing (Koohmarie, 1990). Nakai et al. (1995) found

proteolysis to be faster in samples frozen at -20° C. Calcium ions released during cold ambient temperatures from the sarcoplasmic reticulum in muscle fibers stimulates the calpain system and produces intense tenderization capable of overcoming cold shortening (Joseph, 1996). Solubility of myofibrilliar proteins extracted from frozen samples were higher compared to nonfrozen samples if frozen at rates greater than .22 cm/h (Petrovic et al., 1990). This resulted in improved Warner-Bratzler shear values and improved sensory panel tenderness ratings. Additional research investigating the impact of freezing rate on tenderness has highlighted that as freezing rate increased so did tenderness. In fact Berry and Leddy documented that soy patties frozen to -18° C in 96 h were tougher than patties frozen to -18° C in 24 h.

As with most research topics, conflicting results are generated. Wheeler et al. (1992) reported freezing alone did not reduce shear values and freezing treatments had more residual calpain and calpastatin activity than $CaCl_2$ injected treatments. Additional tenderization by freezing and $CaCl_2$ injection was due to calpain activation by the introduction of Ca^{2+} and not due to a decline in calpastatin activity. Warner-Bratzler shear force measurements and sensory panel tenderness ratings were less desirable for Longissimus and gluteus medius steaks that were frozen four to eight weeks at -30° C than similar chilled samples (Wheeler et al., 1990). Freezing at very rapid rates, associated with liquid nitrogen, had no effect on WBS (McFarlane and Unruh, 1996) or sensory panel tenderness ratings of pork Longissimus muscle (Berry et al., 1971). In contrast, Joseph (1996) reported very fast chilling rates, enhanced toughness in beef Longissimus muscle when compared to slower chilling rates. In addition, rapid chilling

rates in conjunction with a equilibration period produced severe cold shortening, higher calpastatin activity, and tougher steaks.

Bos Indicus Breed Effects on Beef Tenderness. About 25% of the United States beef population contains *Bos indicus* origins (Wheeler et al., 1990a). The advantages of Brahman crossing on reproduction and production are well noted among ranchers in subtropical and tropical climates. Productivity of F_1 Brahman females has been very beneficial in improving growth and performance traits in relation to Bos Taurus F_1 females (Wheeler et al., 1993).

As Brahman cross females have been considered the "Cadillac" of bovine females in many regions of the world, the carcass traits of their offspring have been less than desirable from a palatability stand point. Many researchers have found that Brahman steers are significantly ($P<.05$) less tender than conventional Bos Taurus breeds (Luckett et al., 1975; Shackelford et al., 1991; Wheeler et al., 1990a; Wheeler et al., 1990c; Johnson et al., 1990a). Several researchers have reported, as Bos Indicus inheritance increases tenderness decreases (Carpenter et al., 1961; Crouse et al., 1989; Hoover et al., 1990a). In a recent study, Sherbeck et al. (1996) revealed as phenotypic expression of Bos Indicus increased tenderness decreased regardless of actual Bos Indicus inheritance. While Brahman dams have shown to have a positive effect on retail yield and ribeye area (Peacock et al., 1979), they have had a negative effect on quality grade (Wheeler et al., 1990). However, Wheeler et al. (1990a) found that Brahman x Hereford F_1 steers produced steaks that were comparable in tenderness and variability as straight bred

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Hereford steers. Wheeler additionally found that meat from 1/2 blood Brahman x Hereford carcasses had similar trained sensory panel scores when compared to meat from Hereford steers. Crouse et al. (1989) countered by stating, as Bos Indicus inheritance increased, sensory panel scores for ease of fragmentation, amount of connective tissue, and juiciness were less desirable.

In an effort to understand the tenderness problem many researchers have tried to identify various differences in characteristics among the *Bos indicus* breed types (Wheeler et al., 1990; Shackelford et al., 1991 Sherbeck et al., 1996; Whipple et al., 1990). While no differences in μ and m-calpain have been noted among Bos Indicus and Bos Taurus breeds, significant differences (P<.05) in calpastatin activity have been reported (Shackelford et al., 1991; Wheeler et al., 1993; Whipple et al., 1990). Shackelford et al. (1991) found a 16.8% increase in calpastatin activity in 5/8 Brahman heifers when compared to conventional Bos taurus heifers. This difference in calpastatin activity is the primary factor in explaining the difference in tenderness among Bos Indicus and Taurus breeds.

CHAPTER III

MATERIALS & METHODS

Experiment I. Seventy-five steers of similar age and frame size were fed at a commercial feedyard in the Texas Panhandle. Breed type varied from 0 to 50% Bos indicus. At the time of harvest, steers were transported to a beef processing plant in Texas. Animal ear tags were transferred on the harvest floor to maintain individual identification. At 24 h postmortem, a 5g sample from the Longissimus muscle was obtained for calpastatin activity. Complete grade data was collected on all carcasses after a 48 h chilling period. At approximately 48 h postmortem, both striploins were removed from each carcasses during fabrication. Striploins were transported to the Oklahoma State University Food and Agriculture Products Processing Center where they were fabricated into individual steaks and assigned to individual treatments. The left striploin served as a non-injected control. The striploin was fabricated into ten, 2.54 cm steaks (Figure 2). Four steaks, were obtained for the control portion of the study. The steaks were vacuum packaged and placed into the various aging treatments in sequential order. Following fabrication, the steaks were aged in a 1°C Cooler for 3, 7, 14 and 21 d; once completed the steaks were placed in a -34.5° C blast freezer to await cooking. Three additional steaks were obtained for the 40-d freeze treatment. Steaks were tagged in sequential order for a 7, 14, and 21 day aging period. Prior to aging, the steaks were placed in a -34.5°C Blast Freezer for 40 d. The steaks were then aged for 7, 14, and 21 d

Figure 2. Flow chart depicting left striploin fabrication procedures.
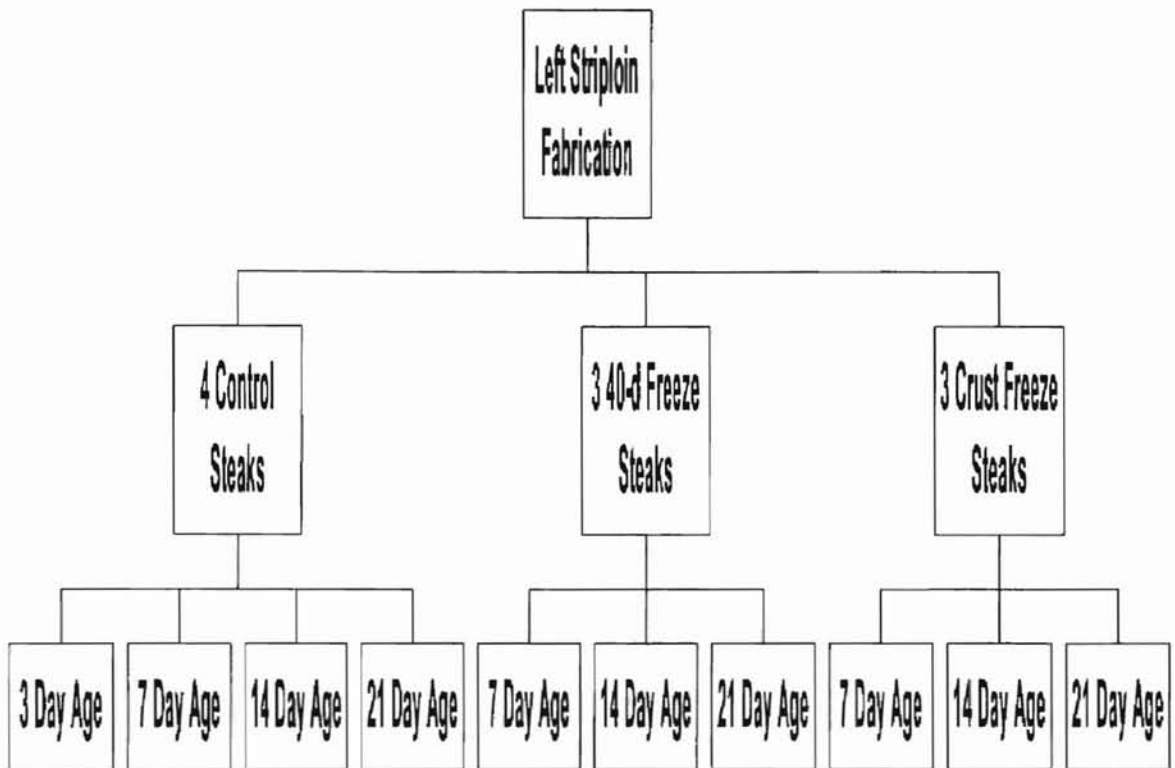
at 1°C. Steaks were then placed back in the blast freezer to await cooking. The remaining portion of the left striploin was placed in a -23.5°C freezer for 2.5 h. for the crust freeze treatments. The striploin was then fabricated into three one inch Longissimus steaks and tagged in sequential order for a 7, 14 and 21 d age. Steaks were then placed in a -2°C cooler to equilibrate for approximately 14 h. Once completed, the steaks were placed in a 1°C cooler for 7, 14 or 21 d. The steaks were then stored in the blast freezer.

The right striploin was fabricated into five 2.54 cm steaks (Figure 3). One steak was removed from the right striploin for sensory analysis and the remaining portion was injected with a 200 mM CaCl$_2$ solution using a Formaco Multi-needle injector at a 5 % injection by weight and allowed to equilibrate for 4 h. Four steaks were obtained from the right striploin to complete the calcium chloride injection portion of the study. One steak was removed for sensory analysis and one each for the control calcium, 40 d freeze, and crust freeze treatments. All calcium injected treatments were treated in the same manner as the aforementioned noninjected treatments except for being aged only 7 d.

Quantification of Calpastatin Activity. Samples were taken following 24 h postmortem, following crust freezing, and upon completion of the 40 d freezing period. All samples were analyzed for calpastatin activity prior to postmortem aging. A 1:5 ratio of sample to extraction buffer (25 mL of 100 mM Tris, 2mM EDTA, and 7mM β-mercaptoethanol, pH 8.3), was utilized. All visible fat was removed prior to homogenizing with a polytron for three 30-s bursts. The homogenate was centrifuged at 12,100 x g for 1 h and filtered through a prerinsed cheesecloth. A 20 mL aliquot was divided equally into four test tubes and placed in a preheated water bath at 95°C for 15

Figure 3. Flow chart depicting right striploin fabrication procedures.

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                    ┌─────────────────────┐
                    │   Right Striploin   │
                    │    Fabrication      │
                    └─────────────────────┘
                              │
                    ┌─────────────────────┐
                    │  1 Sensory Steak    │
                    │     7 Day Age       │
                    └─────────────────────┘
                              │
                    ┌─────────────────────┐
                    │  Calcium Chloride   │
                    │      Inject         │
                    └─────────────────────┘
                              │
        ┌────────────┬────────┴────────┬────────────┐
┌───────────────┐ ┌───────────────┐ ┌─────────────────┐ ┌──────────────────┐
│1 Control Calcium│ │1 Sensory Calcium│ │1 40-d Freeze Calcium│ │1 Crust Freeze Calcium│
│     Steak     │ │     Steak     │ │      Steak      │ │      Steak       │
│   7 Day Age   │ │   7 Day Age   │ │    7 Day Age    │ │    7 Day Age     │
└───────────────┘ └───────────────┘ └─────────────────┘ └──────────────────┘
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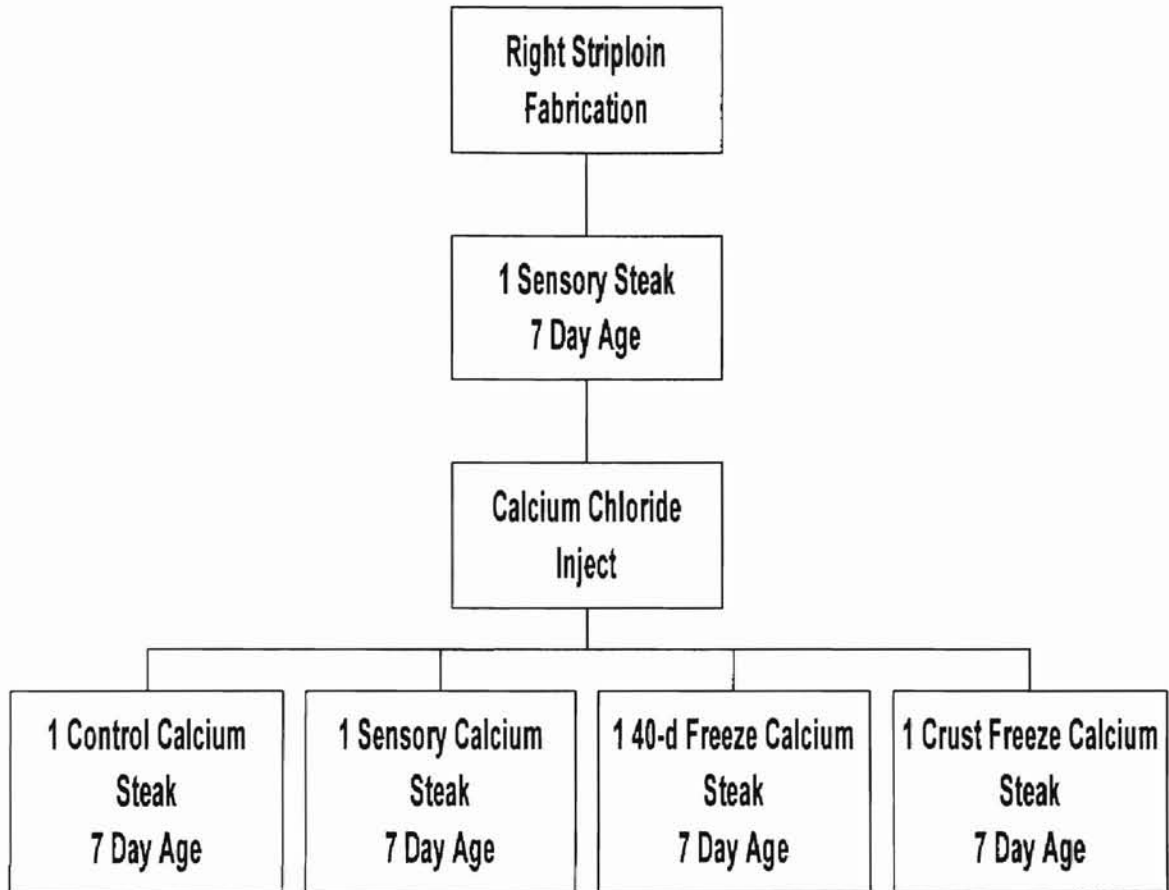
min. After heating, samples were placed in an ice water bath and stirred with a glass rod to agitate the coagulated protein. Samples were then centrifuged at 1,900 x g for 30 min The supernatant was then filtered a second time through prerinsed cheesecloth into 50-mL centrifuge tubes and the weight of the supernatant was recorded. The samples were then assayed by the same procedure as described by Shackelford et al. (1994). Following centrifugation, samples were read on a Beckman DU 7500 spectrophotometer at A_{278} nm. Calpastatin activity was then calculated and reported as activity / gram of muscle. Quantification of calpastatin activity for the calcium chloride injected treatments is the same as the aforementioned procedure (Shackelford et al., 1994) with the following slight modifications: EGTA was substituted for EDTA in the extraction buffer, the samples were dialyzed against a 20mM Tris, 5 mM EDTA, and 7mM β-mercaptoethanol for approximately 24 h.

Warner-Bratzler Shear Force and Cooking Loss Determination. Steaks treatment for shear force and cooking loss determination were thawed at 4°C for 24 h and the precooked weight was recorded. Steaks were broiled on a Lincoln Impinger oven with the temperature set at 176°C for 16.5 min to reach a final internal temperature of 21°C or a medium degree of doneness. Final temperatures were monitored using an Omega 202 Temperature Logger. Steaks were allowed to equilibrate to room temperature and the cooked weight was recorded. Six to eight cores, 1.27 cm in diameter, were removed parallel to the muscle fiber orientation using a drill type coring device. Cores were sheared using a Universal Instron testing device with a Warner-Bratzler shearing attachment with a crosshead speed of 200 mm/min. The average for each steak was used

for statistical analysis. Percentage cooking loss was determined by the following equation:

$$\frac{\text{Precooked Weight-Cooked Weight}}{\text{Precooked Weight}} \quad X \quad 100$$

Sensory Panel. One calcium chloride injected and one control steak were obtained from each carcass for sensory panel analysis. Steaks were cooked in the same manner as described for Warner-Bratzler shear force determinations. A seven to ten member trained sensory panel rated warm duplicate samples according to the procedures outlined by AMSA (1995). Steaks were characterized for overall tenderness, cooked beef fat flavor, juiciness, off-flavor, flavor intensity, and connective tissue amount. An 8-point scale was used for overall tenderness, juiciness, flavor intensity and connective tissue amount (8 = extremely tender, extremely juicy, extremely intense and no connective tissue; 1 = extremely tough, extremely dry, extremely bland and abundant connective tissue). Cook beef fat flavor was scored on a 3-point scale (2 = very strong; 0 = none detected) and off-flavor on a 4-point scale (4 = none; 1 = intense).

Statistical Analysis. Means for carcass data were separated using Fisher's LSD. Effects of storage treatment and calcium chloride injection on Warner-Bratzler shear force measures and cooking loss were analyzed by analysis of variance using a split block procedure (SAS, 1994). Treatment served as the whole plot where as postmortem aging time was the split plot. Treatment tests of significance for noninjected treatments were

computed utilizing the postmortem aging-treatment as the appropriate error term. Means were separated by Fisher's LSD procedures. Effect of calcium chloride injection on sensory panel profiles were also analyzed by analysis of variance (SAS, 1994) and means were separated by Fisher's LSD procedures.

CHAPTER IV

RESULTS & DISCUSSION

Experiment 1

Carcass Characteristics. Least squares means and ranges for carcass characteristics are presented in Table 1. Hot carcass weight averaged 351.6 kg, fat thickness 1.19 cm, ribeye area 78.90 cm^2, kidney, pelvic, and heart fat 2.76 %, yield grade 3.24, Maturity A, and quality grade was low choice.

Effect of storage treatment on Longissimus muscle tenderness. Least squares means for effect of storage treatment on Longissimus muscle tenderness are presented in Table 2. Warner-Bratzler shear force was effected by a storage treatment by postmortem aging time interaction (see figure 4). Crust freeze and control steaks aged in a similar pattern with a decline in WBS throughout the 21 d aging period. However, 40-d freeze steaks did not respond to postmortem aging throughout the aging period. Whipple and Koohmarie (1992) reported freezing six weeks at - 30°C tended to decrease WBS (P = .07). Calcium dependent protease activity declined 45 to 66.6% after six weeks freezing. Crust freezing increased WBS by approximately 1 kg at d 7. Results from this study, showed 40-d frozen steaks had no effect on improving tenderness when compared to control steaks (P >. 05). However, crust freezing showed a negative

Table 1. Carcass characteristics of steers used in Experiment 1.

Trait	Mean	Min.	Max.
Hot Carcass Weight[a], kg	351.6	243.0	415.4
Fat Thickness, cm	1.16	.51	2.94
Ribeye area, cm^2	78.90	52.71	101.94
Kidney, Pelvic, and Heart fat[b], %	2.76	1.00	5.00
Yield Grade	3.24	2.07	5.37
Overall Maturity[c]	157.4	260.0	199.0
Marbling Score[d]	403.38	260.00	680.00

[a]Hot carcass weight.
[b]Kidney, pelvic, and heart fat percentage.
[c]100-199 = A, 200-299 = B.
[d]200-299 = Standard, 400-499 = choice⁻, 600-699 = choice⁺ (USDA, 1997).

Table 2. Effect of storage treatment on Warner-Bratzler shear force (WBS) and cooking loss of steer Longissimus muscle at 7, 14, and 21 days postmortem aging, Experiment 1.

Item	WBS, kg	Cooking Loss, %
Treatment[a]	N= 225	N= 225
Crust Freeze	5.13	26.83
40-d Freeze	4.57	25.95
Control	4.25	23.74
Days Postmortem		
7	5.14	25.39
14	4.57	25.64
21	4.26	25.50
RMSE[b]	.83	2.34
Treatment X Days Postmortem	N= 75	N= 75
Probability Level	<.01	<.01
Control / 7d age	4.92^v	23.96^y
Control / 14d age	4.13^y	24.40^y
Control / 21d age	3.73^z	22.85^z
Crust Freeze / 7d age	5.84^u	26.78^w
Crust Freeze / 14d age	4.87^{vw}	25.72^x
Crust Freeze / 21d age	4.67^{vw}	28.01^v
40-d Freeze / 7d age	4.65^{wx}	25.43^x
40-d Freeze / 14d age	4.69^{vw}	26.80^w
40-d Freeze / 21d age	4.39^x	25.64^x

[a]See materials and methods for details of treatment.

[b]Standard errors for means can be calculated using the following equation: $SE = RMSE/\sqrt{n}$.

[u,v,w,x,y,z]Means within a column lacking a common superscript letter differ (P<.05).

Figure 4: Interaction plot showing the effect of storage treatment and aging on Warner Bratzler shear force values.
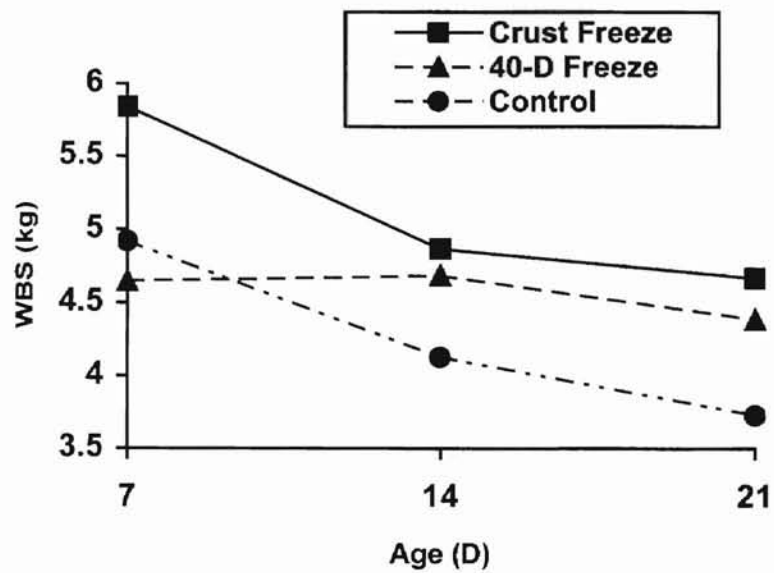
effect on WBS values. Crust freezing increased WBS by approximately 1 kg at d 7. Control steaks averaged 4.92 kg, while crust freeze averaged 5.84 kg. Joseph (1996) reported similar results with rapid chilling rates along with an equilibration period, but attributed their findings to severe cold shortening. Due to the completion of rigor, in the current study, similar conclusions are doubtful. Crust freezing resulted in a pronounced increases in WBS at all aging periods. Joseph (1996) reported an increase in calcium released from the sarcoplasmic reticulum in cold ambient temperatures resulted in increased tenderness due to stimulation of the calpain system. Results from the current study, lacked any increase in tenderness attributed to the cold ambient temperatures of crust or 40-d freezing at -30°C.

Cooking loss. Least squares means showing the effect of storage treatment and postmortem aging on Longissimus muscle cooking loss are summarized in Table 2 and Figure 5. Cooking loss was affected by a storage treatment by postmortem aging time interaction in that steaks from crust and 40-d freeze treatments were higher in cooking loss percentage than all corresponding control treatments (P < .05). Freezing rates in both frozen treatments (crust and 40 d) may be vindictive to extracellular ice crystal formation. Anon and Cavelo (1980) reported freezing rates beyond 20 min leads to the formation of extracellular ice crystals that grow at the expense of intracellular water that is potentially released upon thawing and cooking. Crust freezing may result in more extracellular ice that doesn't allow for reabsorbtion upon thawing. While similar results are noted with

Figure 5. Interaction plot showing the effect storage treatment and postmortem aging on cooking loss percent.
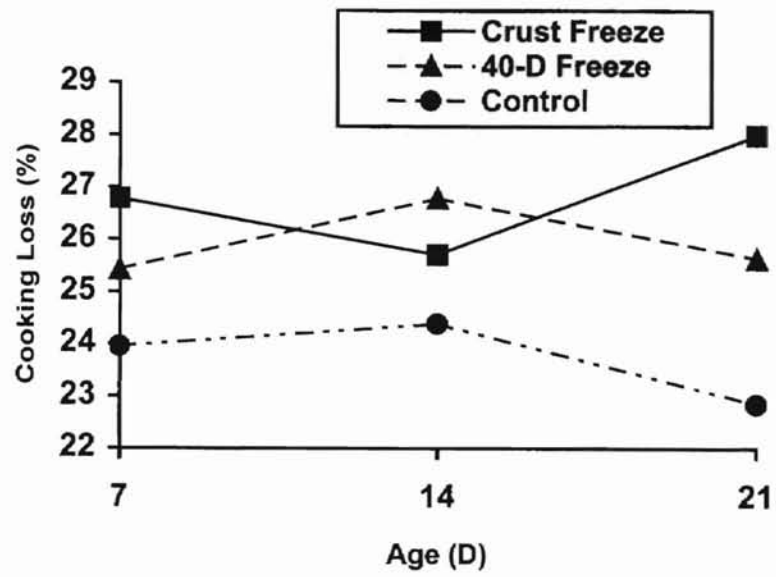
the 40-d freeze treatments, some intracellular ice may have formed that wasn't allowed in the short freezing time occurred by crust freezing.

Effect of storage treatment and calcium chloride injection on Longissimus tenderness. Least squares means for storage treatment and calcium chloride effects on WBS are summarized in table 3. Koohmarie et al. (1989) reported freezing decreased WBS and calpastatin activity. Results from the current study found no improvements in tenderness associated with a 40-d freeze with or without calcium chloride injection. Calcium chloride injected control steaks were more tender than non-injected control steaks. Warner-Bratzler shear force was significantly reduced in the calcium chloride injected crust freeze treatments if compared to the noninjected crust freeze treatment. However, both crust freeze treatments were still drastically tougher than any other treatment.

Effect of calcium chloride injection and storage treatment on calpastatin activity. Least square means for effect of calcium chloride injection and storage treatment are presented in Table 3. Calcium chloride injection decreased calpastatin activity in all corresponding treatments. Compared to control steaks, all storage and calcium injected treatments had reduced (P < .05) calpastatin activities. However, it should be noted that a confounding effect due to sampling time could have attributed to these results. Calpastatin activity was higher in steaks 40-d freeze and crust freeze than the other storage treatments. Higher calpastatin activities in the crust freeze treatments are in agreement with WBS. Wheeler et al. (1992) reported freezing reduced WBS values and

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Table 3. Effect of storage treatment and calcium chloride injection on calpastatin activity, Warner-Bratzler shear force values (WBS) and cooking loss.

Treatment[a]	Calpastatin Activity, Units / g of Muscle	WBS[1], kg N = 75	Cooking Loss[1], % N = 75
Control[2]	2.87[a]	4.92[c]	23.96[b]
Calcium / Control[3]	1.38[c]	4.58[b]	24.45[b]
40-d Freeze[3]	1.84[b]	4.65[bc]	26.32[c]
Calcium / 40-d Freeze[3]	1.02[d]	4.67[bc]	26.82[cd]
Crust Freeze[4]	1.92[b]	5.84[e]	26.78[cd]
Calcium / Crust Freeze[4]	1.22[cd]	5.47[d]	27.62[d]
RMSE[f]	.61	.94	3.62

[a]See materials and methods for details of treatment.

[b,c,d,e]Means within a column lacking a common superscript letter differ (P<.05).

[f]Standard errors for means can be calculated using the following equation: $SE = RMSE/\sqrt{n}$.

[1]WBS and cooking loss were taken after 7 days postmortem aging.

[2]Calpastatin activity at 24 hr postmortem.

[3]Calpastatin activity after 3 days postmortem aging and completion of storage treatment.

[4]Calpastatin activity after 4 days postmortem aging and completion of storage treatment.

calpastatin activities. Calpastatin activities decreased in the freeze treatments, when compared to 24 h calpastatin activity, however both treatments had 3 to 4 d of postmortem aging prior to starting the storage treatments and subsequent calpastatin assays.

Effect of calcium chloride injection and storage treatment on cook loss at 7 d postmortem. Least squares means for effect of calcium chloride injection and storage treatment on cooking loss at 7 d postmortem are presented in Table 3. Once again, cuts which were subjected to either freeze treatments resulted in greater cooking losses as compared to control cuts. However, calcium chloride injection had no effect on cooking loss when compared to their non-injected controls.

Effect of calcium chloride injection on sensory panel characteristics. Calcium chloride injection has improved sensory panel tenderness ratings in numerous research projects (Hoover et al., 1993; Lansdell et al., 1993; Morgan et al., 1991). In the present study calcium chloride injected steaks were rated as being slightly tender compared to control steaks which received slightly tough tenderness scores (Table 4.). Additionally, sensory panelist noted 20% more calcium chloride injected steaks as being slightly tender (5.0 or >) when compared to non-injected control steaks (Figure 9.). Results from this study showed no differences in juiciness, cooked beef fat flavor and connective tissue. However, calcium chloride injection did result in increased off flavors $(P < .05)$ when compared to non injected controls. Salty flavors were reported by taste panelist for calcium chloride injected steaks. In addition, calcium chloride injected steaks resulted in an increase in flavor intensity $(P < .05)$. These results are in agreement with Morgan et

al. (1991) which an increase of metallic, bitter, strong, and livery flavors in calcium chloride injected cow subprimals.

Table 4. Effect of calcium chloride injection on sensory panel ratings of Longissimus muscle following seven day postmortem aging.

Item	N	Juiciness[a]	Cooked Beef Fat[b] Flavor	Off Flavor[c]	CT[d]	Flavor Intensity[e]	Tenderness[f]
Treatment							
Control	75	4.80	.45	3.95^y	5.25	5.03^y	4.96
Calcium-injected	75	4.77	.45	3.87^z	5.35	5.14^z	5.10
P-value		.68	.91	<.01	.17	.02	.13
RMSE[h]		.43	.22	.14	.46	.28	.59

[a]8 = extremely juicy, 1 = extremely dry.

[b]2 = very strong, 0 = none detectable.

[c]4 = none, 1 = intense.

[d]Connective tissue. 8 = none, 1= abundant.

[f]8 = extremely intense, 1 = extremely bland.

[g]8 = extremely tender, 1 = extremely tough.

[h]Standard errors can be calculated using the following equation: $SE = RMSE/\sqrt{N}$.

[y,z]Means with in the same row followed by a common subscript do not differ (P>.05).

Figure 6. Effect of calcium chloride injection on sensory tenderness ratings of longissimus steaks aged 7 days.

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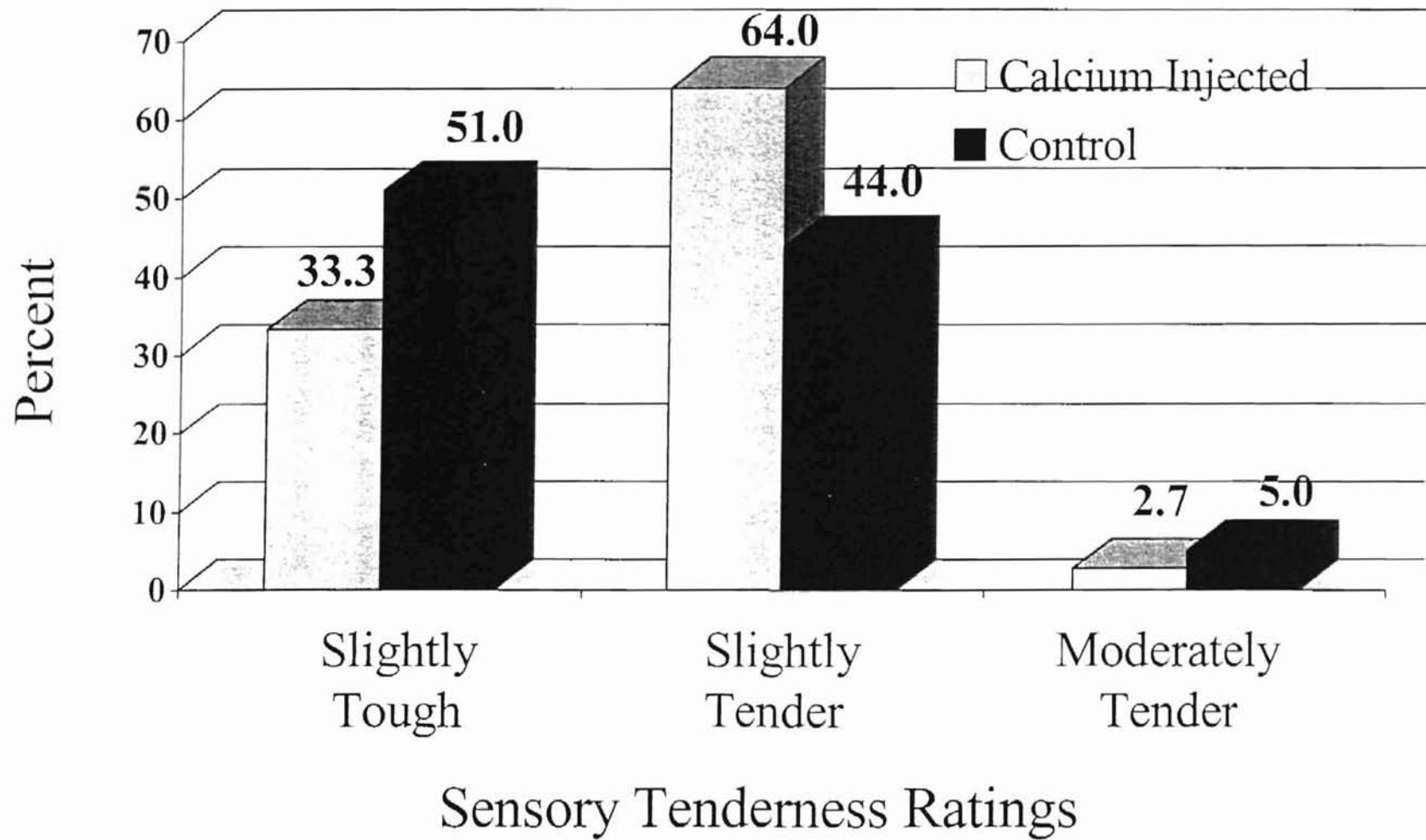
IMPLICATIONS

Results from this investigation suggest that calcium chloride injection improves Warner-Bratzler shear and sensory tenderness ratings. Additionally, crust freezing of beef should be eliminated from daily processing due to it's detrimental effects on tenderness and cooking loss properties. It appears that extensive freezing should be used for preservation purposes and not as a technique to improve palatability.

CHAPTER V

MATERIALS & METHODS

Experiment II. Fifty-five steers from experiment one were utilized for a breed type comparison study. Five Bos indicus breed types: 1/2 Gray Brahman x 1/4 Angus x 1/4 Hereford (Gray Brahman, GB), 1/4 Nelore x 1/4 Gray Brahman x 1/4 Angus x 1/4 Hereford (Nelore, N), 1/4 Red Brahman x 1/4 Gray Brahman x 1/4 Angus x 1/4 Hereford (Red Brahman, RB), 1/4 Gyr x 1/4 Gray Brahman x 1/4 Angus x 1/4 Hereford (Gyr, G), 1/4 Indu Brazil x 1/4 Gray Brahman x 1/4 Angus x 1/4 Hereford (Indu Brazil, IB). Angus x Simmental F_1 steers served as experimental controls. Pictorial examples of each of the various breed types are included in Figures 7, 8, and 9. Steaks were analyzed for Warner-Bratzler shear force, calpastatin activity, and sensory profiles.

Statistical Analysis. Means for carcass data, WBS, and sensory characteristics, stratified by breed type, were computed utilizing Fisher's LSD. Effects of breed type on carcass characteristics, WBS, and sensory panel profiles were analyzed by analysis of variance (SAS 1994). Treatment tests of significance for on Warner-Bratzler shear force measures were computed utilizing the postmortem aging-treatment as the appropriate error term. Means were separated by Fisher's LSD procedures.

Figure 7. (Top) A Simmental Angus F_1 steer (Controls).

(Bottom) A 1/2 Gray Brahman x 1/4 Angus x 1/4 Hereford steer

Figure 8. (Top) A 1/4 Nelore x 1/4 Gray Brahman x 1/4 Angus x 1/4 Hereford steer.

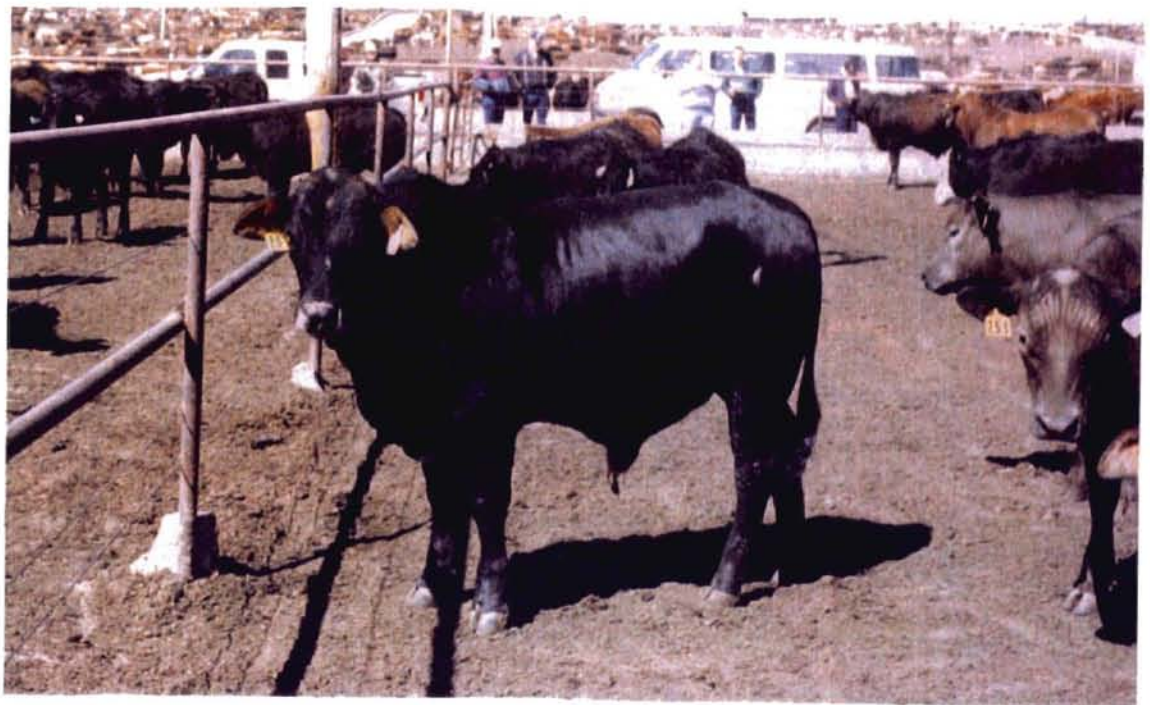(Bottom) A 1/4 Red Brahman x 1/4 Gray Brahman x 1/4 Angus x 1/4 Hereford steer.

Figure 9. (Top) A 1/4 Gyr x 1/4 Gray Brahman x 1/4 Angus x 1/4 Hereford steer.

(Bottom) A 1/4 Indu Brazil x 1/4 Gray Brahman x 1/4 Angus x 1/4 Hereford steer.

CHAPTER VI

RESULTS & DISCUSSION

Experiment 2

Carcass grade traits stratified by breed types. Results summarizing least squares

means for breed effects on carcass characteristics are shown in Table 5. Crouse et al.

(1989) found as Bos indicus inheritance increased HCW decreased. Our results agree in

that (N), (RB), and (G) cross bred steers produced lighter carcasses compared to controls.

Gyr steers, being a smaller framed Bos indicus breed, had significantly (P < .05) lighter

HCW than all breed types. Peacock et al. (1979) reported Brahman breeding had a

negative effect on carcass quality grade and ribeye area. Pringle et al. (1997) suggests as

Brahman inheritance increase marbling score decreased. Similar findings were reported

for ribeye area Results from this study indicated that carcasses from (GB), (RB), (G),

and (IB) graded significantly (P < .05) lower (U.S. select[+]) than (N) and control

carcasses (U.S. Choice[-]). Even though all steers were within sixty d of chronological age,

differences in overall maturity were observed. Both (N) and control steers had higher (P

< .05) overall maturity scores than (RB), (G) and (IB) Steers. Fat thickness, percent KPH,

and yield grades were similar among all breed types.

Table 5. Carcass data stratified by breed types, Experiment 2.

Breed[a]	N	HCW, kg	Fat tk, cm	REA[b], cm^2	KPH[c], %	YG[d]	Overall maturity[e]	Marbling Score[f]
50A:50S (Con)	25	371.65^z	1.09	83.68^z	2.76	3.09	161.20^{yz}	421.60^x
50GB:25A:25H (GB)	6	348.60^{yz}	1.65	74.71^{xy}	2.50	3.55	150.83^{xy}	363.33^w
25N:25GB:25A:25H (N)	6	345.31^y	.97	78.00^{yz}	3.20	3.18	166.80^z	430.00^x
25RB:25GB:25A:25H (RB)	9	335.15^y	.91	76.13^y	2.67	2.97	150.56^x	351.11^w
25G:25GB:25A:25H (G)	7	298.73^x	1.09	68.06^x	3.14	3.34	148.57^x	360.00^w
25IB:25GB:25A:25H (IB)	2	319.73^{yz}	1.22	74.45^{xy}	3.00	3.29	147.50^x	370.00^{wx}
P-value		< .01	.33	<.01	.80	.43	.04	.03
RMSE[g]		60.61	.22	1.08	.90	.53	12.64	61.64

[a]A = Angus, H = Hereford, GB = Gray Brahman, N = Nelore, RB = Red Brahman, G = Gyr, IB = Indu Brazil, S = Simmental.

[b]REA = Ribeye area, cm^2.

[c]KPH = Kidney, Pelvic, and Heart.

[d]YG = Yield Grade.

[e]Mat. = Maturity, 100-199=A.

[f]Marbling score, 300-349 = Select$^-$, 350-399 = Select$^+$, 400 -433 = Choice$^-$ (USDA, 1997).

[g]Standard errors can be calculated using the following equation: SE = RMSE/√N.

[x,y,z]Means with in the same row followed by a common subscript do not differ (P>.05).

Effect of Breed type and postmortem aging on longissimus muscle tenderness.
Least squares means for effect of breed type and postmortem aging on longissimus tenderness is summarized in Table 6. All breed types were affected by postmortem aging in a similar pattern, in that WBS declined throughout the 21 d aging period. At 7 d postmortem aging, control and (GB) steers produced steaks that were more tender than (RB) and (IB) crossbred steers. Meat from (IB) steers were tougher than control, (GB) and (G) crossbred steak samples. At 14 d postmortem aging, steaks from control steers were numerically more tender than all other breed types. However, they were only significantly different than steaks from (RB) and (IB) steers. Longissimus steaks from (IB) steers remained tougher than control, and (GB) crossbred steers. At 21 d postmortem aging variation in tenderness among all breed groups tightened considerably. Gray Brahman steers were more tender than all breed types but only significantly different than Indu Brazil steers. Numerous researchers have reported that meat from Brahman crossbred steers were significantly (P < .05) less tender than conventional Bos taurus steers (Luckett et al., 1975; Shackelford et al., 1991; Wheeler et al., 1990a). The current study showed that steaks from (GB) steers were comparable to control steers throughout a 21 d aging period. If aged at least 14 d postmortem, steaks from (N) and (G) crossbred steers were additionally comparable in tenderness. All Bos indicus breed groups were acceptable in tenderness if aged at least 21 d postmortem.

Sensory Panel. Effect of breed type on longissimus sensory panel ratings at 7 d postmortem aging is summarized in Table 7. With the exception of (GB), Angus Simmental (i.e. controls) steers produced steaks which were rated numerically higher

Table 6. Least square means and RMSE showing the effect of breed and postmortem aging on Warner-Bratzler shears force values (kg).

Breed[a]	N	Days Aged		
		7	14	21
50A:50S, (Con)	25	4.51^{bX}	3.76^{bXY}	3.49^{bY}
50GB:25A:25H, (GB)	6	4.77^{bcX}	4.00^{bcXY}	3.39^{bY}
25N:25GB:25A:25H, (N)	6	5.40^{cdeX}	4.40^{bcdY}	3.96^{bcY}
25RB:25GB:25A:25H, (RB)	9	5.63^{deX}	4.40^{cdY}	3.75^{bcY}
25G:25GB:25A:25H, (G)	7	5.01^{bcdX}	4.27^{bcdXY}	3.86^{bcY}
25IB:25GB:25A:25H,(IB)	2	6.16^{eX}	5.34^{dX}	4.62^{cXY}
RMSE[f]		.74		

[a]A = Angus, H = Hereford, GB = Gray Brahman, N = Nelore, RB = Red Brahman, G = Gyr, IB = Indu Brazil, S = Simmental.
[b,c,d,e] Means with in the same column followed by a common subscript do not differ (P>.05).
[f]Standard errors can be calculated using the following equation: SE = RMSE/√N.
[X,Y]Means with in the same row followed by a common subscript do not differ (P>.05).

Table 7. Effect of breed type on sensory panel ratings of Longissimus muscle at seven days postmortem aging.

Breed[a]	N	Juiciness[b]	Cooked Beef fat Flavor[c]	Off Flavor[d]	CT[e]	Flavor Intensity[f]	Tenderness[g]
50A:50S (con)	25	4.82	.48	3.94	5.42	5.10	5.32^y
50GB:25A:25H (GB)	5	4.70	.37	3.97	5.30	4.82	4.85^{yz}
25N:25GB:25A:25H (N)	5	4.82	.52	3.94	5.06	5.25	4.61^z
25RB:25GB:25A:25H (RB)	8	4.81	.33	3.96	4.98	4.92	4.54^z
25G:25GB:25A:25H (G)	7	4.74	.36	3.97	5.28	4.94	4.71^z
25IB:25GB:25A:25H (IB)	1	4.64	.64	3.86	4.79	5.21	4.57^z
P-value		.99	.38	.84	.25	.12	<.01
RMSE[h]		.49	.20	.08	.46	.27	.55

[a] A = Angus, H = Hereford, GB = Gray Brahman, N = Nelore, RB = Red Brahman, G = Gyr, IB = Indu Brazil, S = Simmental.

[b] 8 = extremely juicy, 1 = Extremely dry.

[c] 2 = very strong, 0= none detectable.

[d] 4 = none, 1 = intense.

[e] Connective tissue - 8 = none, 1 = abundant.

[f] 8 = extremely intense, 1 = extremely bland.

[g] 8 = extremely tender, 1 = extremely tough.

[h] Standard errors can be calculated using the following equation: $SE = RMSE/\sqrt{N}$.

[x,y,z] Means with in the same row followed by a common subscript do not differ (P>.05).

than all other breed types. Results are in agreement with Wheeler et al. (1990a), in that steaks from Brahman halfblood steers were not different form straightbred Hereford steers in reference to sensory panel tenderness ratings (P > .05). Sensory panel scores for flavor intensity approached significance (P = .12) in that (N), (IB) and Angus Simmental F_1 steaks were more intense in flavor than (GB), (RB), and (G) crossbred steer's longissimus steaks. Significant differences was not detected in connective tissue amount, off flavor, cooked beef fat flavor, and juiciness.

Effect of breed on calpastatin activity. Calpastatin activity and corresponding 3 d WBS are presented in Table 8. Longissimus samples from control steers had significantly lower (P < .05) 24 h calpastatin activities than all of the Bos indicus crossbred steers. Results are in agreement with Wheeler et al. (1990c), Whipple et al. (1990), Shackelford et al. (1991), Pringle et al. (1997). The samples from Bos indicus crossbred steers had approximately 65% higher calpastatin activities than control steers. Corresponding WBS at 3 d postmortem confirm the aforementioned calpastatin activities. Pringle et al. (1997) reported a 31 % increase in calpastatin activity as steers went from 0 to 100 % Brahman. However, calpain activity was higher for 37 % Brahman steers than all other steers.

Table 8. Effect of breed on calpastatin activity and Warner-Bratzler shears (WBS) force values of longissimus muscle.

Breed[a]	n	Calpastatin Activity, 24 h	n	WBS[c], kg
50A:50S, (Con)	9	2.31^y	25	5.01^y
50GB:25A:25H, (GB)	3	3.53^z	6	5.97^z
25N:25GB:25A:25H, (N)	2	3.53^z	6	6.52^z
25RB:25GB:25A:25H, (RB)	2	3.51^z	9	6.45^z
25G:25GB:25A:25H, (G)	3	3.54^z	7	6.02^z
RMSE[b]		.74		.85

[a]A = Angus, H = Hereford, GB = Gray Brahman, N = Nelore, RB = Red Brahman, G = Gyr, IB = Indu Brazil, S = Simmental.
[b]Standard errors can be calculated using the following equation: $SE = RMSE/\sqrt{N}$.
[c]WBS values were obtained after 3 days postmortem aging.
[y,z]Means with in the same row followed by a common subscript do not differ (P > .05).

IMPLICATIONS

Results from the present study reveal that Nelore crossbred steers graded as well as control steers. However, Gray Brahman, Red Brahman, Gyr, and Indu Brazil steers graded lower than control steers, all averaging select. In contrast to other publications, results from this study showed that Gray Brahman and Gyr steers were comparable in tenderness to control steers throughout a 7, 14, and 21 d aging period. Steaks from Red Brahman and Nelore steers were acceptable in tenderness if aged at least 14 days postmortem. However, steaks from Indu Brazil steers need 21 d of postmortem aging to meet the needs of the average consumer. The current study suggests, Gray Brahman steers confront the tenderness issue better than the other four Bos indicus breeds, but carcass traits were more desirable from the Nelore crossbred steers.

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APPENDIX

Table 9. Experimental Model (postmortem aging and storage treatment effects on WBS force), experiment 1.

Source	Degrees of freedom	WBS
Postmortem days aged (A)[a]	2	NS
Storage treatment (T)[a]	2	NS
A X T	4	**
Error	669	

[a] Appropriate error term = A x T
**$P < .05$

Table 10. Experimental Model (Calcium chloride injection and storage treatment effects on WBS force), experiment 1.

Source	Degrees of freedom	WBS
Storage treatment (T)	5	**
Error	443	

**$P < .05$

VITA

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