EFFECTS OF HOT FAT TRIMMING OF CARCASSES ON SENSORY CHARACTERISTICS AND CALPASTATIN ACTIVITY OF BEEF LONGISSIMUS MUSCLE

By

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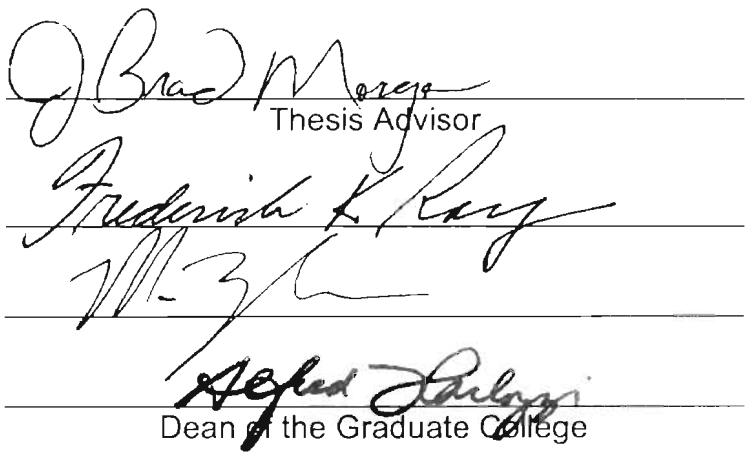

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FORMAT OF THESIS

This Thesis is presented in the Journal of Animal Science style format, as outlined by the Oklahoma State University Graduate College Style Manual. The use of this format allows for independent chapters to be prepared suitable for submission to scientific journals.

CHAPTER I

INTRODUCTION

In the preliminary stages of the hot fat trimming process, the concept was to improve production efficiency and realign the value based system for carcass pricing as outlined by Savell et al. (1989). One benefit of hot fat trimming is the reduction of variation in boneless, lean meat yields attributed to differences in carcass fatness (Savell et al., 1989; Williams et al., 1989; Ahmed et al., 1992). By using hot fat trimmed beef carcass weight as a pricing basis, producer's would be inclined to produce trimmer, heavier muscled market cattle (Savell et al., 1989). This would be especially beneficial at a time when consumers are demanding for leaner, healthier meat products from the packing and retail industries. Also at this time, approximately $112 was being lost per market steer/heifer carcass due to excessive external fat according to the findings in the National Beef Quality Audit (Smith et al., 1992). The recommendation of Savell et al. (1989) was that hot fat trimming should be encouraged as a way to reduce carcass variation in the short-term. Currently, according to the most recent National Beef Quality Audit (Smith et al., 1995) the top three quality concerns include 1) low overall uniformity and consistency of beef products, 2) inadequate tenderness, and 3) low overall palatability.

Certainly, a great deal of potential exists for hot fat trimming. However, potential problems have not been addressed regarding hot fat trimming and its impact on sensory characteristics of beef products. Therefore, the current study

addresses the effect of hot fat trimming on the early postmortem changes of muscle and the overall palatability of the product.

CHAPTER II

LITERATURE REVIEW

Hot Fat Trimming of Beef Carcasses

In the past, health conscious consumers' demand for closely trimmed cuts of beef at the retail level has caused a shift in the beef processing industry. To meet this demand of trimmer beef from consumers, many retailers have practiced trimming beef cuts to 0.64 cm or less of external fat trim (Cross et al., 1986). This demand also caused many beef processors to shift toward producing special lines of boxed beef that targeted this specific segment of the industry.

This recent method of marketing beef to purveyors and retailers has raised some questions regarding maximizing efficiency throughout the harvest and fabrication processes. An alternative to trimming chilled fat from subprimals during carcass fabrication is hot fat trimming. Hot fat trimming involves removing all subcutaneous fat in excess of 0.64 cm, all trimmable kidney, pelvic, and heart fat, and all cod or udder fat before chilling (Savell et al., 1989; Williams et al., 1989; Ahmed et al., 1992; Miller et al., 1995). This procedure of removing hot fat versus chilled fat allows packers to produce closely trimmed subprimals more efficiently and reduces labor costs due to the soft, pliable nature of hot fat (Ahmed et al., 1991; Miller et al., 1995). Miller and co-workers (1995) reported that the total subprimal cut yield was approximately 5% higher for hot fat trimmed sides than for untrimmed sides, and the total fat trim during fabrication was more than 6% less than their untrimmed counterparts. When the value of the sides

was calculated from unadjusted weights, the untrimmed sides were approximately $26.50 more valuable than hot fat trimmed sides. However, when the carcass weight was adjusted to a constant, the hot fat trimmed sides were approximately $40.00 more valuable than untrimmed sides. This study showed that hot fat trimming would be more beneficial to the beef processing industry if the product were sold on a subprimal cut yield basis with 0.6 cm of fat. Savell et al. (1989) reported the percentage adjusted hot fat trim from carcasses of different yield grades and sex classes were: 1.0 = 5.48% for steers and 6.82% for heifers, 1.5 = 6.75% for steers and 8.09% for heifers, 2.0 = 8.03% for steers and 9.37% for heifers, 2.5 = 9.30% for steers and 10.64% for heifers, 3.0 = 10.57% for steers and 11.91% for heifers, 3.5 = 11.85% for steers and 13.19% for heifers, 4.0 = 13.12% for steers and 14.46% for heifers, and 4.5 = 14.40% for steers and 15.74% for heifers.

This procedure of hot fat trimming would negate the USDA Yield Grade system of identifying carcasses that differ in cutability due to fatness. However, it has been reported that hot fat trimming reduces the variation in cutability across different cattle types (Savell et al., 1989; Williams et al., 1989; Miller et al., 1995) and yield grades (Ahmed et al., 1992). The uncoupling of the yield and quality grades (USDA/AMS, 1989), still allow quality grades to be assigned to carcasses that have been hot fat trimmed. This may possibly allow the potential for a more accurate value based pricing system that could be established from trimmed, hot carcass weight in which could redefine dressing percentage so that it would no longer include excess, trimmable fat.

However, there should be some concern with hot fat trimming relative to several published reports. According to Bowling et al. (1977), Smith et al. (1976), and Tatum et al, (1982), the amount of subcutaneous fat on beef and lamb carcasses plays an important role in overall palatability of the meat. The insulatory effect of subcutaneous fat, which slows temperature decline, has been shown to increase and/or extend postmortem enzyme activity, and reduce or eliminate shortening of myofibrils in beef carcasses, and subsequently increasing palatability of cooked beef (Bowling et al., 1977). Tatum et al. (1982) reported for carcasses with at least a "slight" degree of marbling and 7.62 mm of subcutaneous fat was sufficient to ensure acceptable beef palatability.

Association of Calpain and Calpastatin to Beef Palatability

The association of calpain and calpastatin to beef palatability in postmortem muscle tissue has been known for quite some time among the scientific community. A calcium dependent protease, μ-calpain, was first extracted from rat brain in 1964 and the inhibitor of this protease, calpastatin, was isolated in bovine cardiac muscle in 1978 (Murachi, 1983). Murachi and others determined the cause for degradation of myofibrillar and neurofilament proteins were calpains. Davey and Gilbert (1969) demonstrated that in situ levels of calpains could be implicated, and indicated degradation of troponin T (an indicator of tenderness) was lower in psoas major than in semitendinosus or longissimus dorsi muscle and was correlated to its lower content of calpains.

Dayton et al. (1976) were the first to purify calpain and since then there have been many experiments conducted to determine how to isolate these

important enzymes and their impact on meat palatability. Koohmaraie (1987) was one of the first researchers to identify the calpain system's effects on meat tenderness under typical postmortem aging conditions. Direct evidence for the involvement of calpains and calpastatin to tenderness was later shown with the addition of calpains and/or calcium ions to raw meat resulting in enhanced tenderness of cooked meat (Koohmaraie, 1988a). Accordingly, Koohmaraie (1990) developed a procedure, involving hydrophobic and ion-exchange chromatography that independently isolated m-calpain, μ-calpain, and calpastatin. Shackelford et al. (1994b) then modified a shorter procedure for the measure of calpastatin activity and Doumit et al. (1996) developed the first ELISA to quantify bovine skeletal muscle calpastatin.

The calpains, m-calpain and μ-calpain, are differed by the amount of calcium required for activation. Endogenous to skeletal muscle cell, μ-calpain requires micromolar order of calcium (~10m*M*) and m-calpain requires millimolar order of calcium (200-300 μ*M*) for activation. The calpains are inhibited by an endogenous substrate, known 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. 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 (Koohmaraie, 1992a). When μ and m-calpain are activated in the presence of sufficient calcium, improved meat tenderness is observed.

Skeletal muscle is composed of three classes of proteins: sarcoplasmic, connective tissue, and myofibrillar. Proteolysis of myofibrillar protein fraction is the principle mechanism of postmortem tenderization. Proteases are the cause behind the improvement in meat tenderness over time. To be classified as a protease, it should have the following characteristics: 1) located within the skeletal muscle, 2) have access to the substrate, and 3) have the ability to hydrolyze the same proteins in an in vitro system that are degraded during postmortem storage (Koohmaraie, 1988a; Koohmaraie, 1992a). There are many proteases found in skeletal muscle, however, only calpains and some lysosomal enzymes have been shown to cause degradation of myofibrillar proteins (Koohmaraie, 1988b; Goll et al., 1992; Thomson et al., 1997). Also, it is assumed that during postmortem storage, lysosomes are ruptured and cathepsins are released into the cytosol, however, little research supports this theory. Consequently, there has been a great deal of research to support the theory that calpains are the primary enzymes attributing to postmortem proteolysis, resulting in tenderization of meat. (Koohmaraie, 1988a; Goll et al., 1992).

During the postmortem storage of carcasses there are several changes that takes place within skeletal muscle that causes a decline in myofibrillar integrity and/or an increase in tenderness: Z-disk degradation, disappearance of troponin-T and simultaneous appearance of polypeptides with molecular weights

of 28 to 32 kDa, degradation of desmin, degradation of titin and nebulin, and removal of α-actinin (Elgasim et al., 1985; Koohmaraie, 1988a; Koohmaraie, 1992a; Kendall et al., 1993).

Since the discovery of calpain and calpastatin and the procedures to isolate and quantify their activity, many studies have been done to determine their effects on tenderness. It was found that postmortem aging decreased calpastatin activity and μ-calpain while m-calpain levels stayed relatively constant (Koohmaraie, 1987). Koohmaraie concluded that postmortem tenderness is a calcium-mediated process and calcium effects tenderness through the activation of calcium dependent proteases. Furthermore, one study reported initial shear force value was correlated ($r = -0.71$) to μ-calpain activity (Calkins et al., 1988), whereas, Johnson et al. (1990) showed calpastatin level was correlated ($r = 0.41$) to Warner-Bratzler shear force measurements at 1 d of aging. Also, prediction equations have shown 24-h calpastatin activity and 0-h μ-calpain activity account for 41% of the variation in Warner-Bratzler shear force measurements of beef aged 14 d (Shackelford et al., 1991). Research conducted thus far indicates that the proteolytic roles of calpain and calpastatin in relation to muscle have a wide array of activity concerning degradation. Unfortunately, there is more research needed in determining the precise mechanism of action.

Effect of Muscle Temperature and pH Decline on Tenderness

It has been demonstrated that extremely cold temperature exposure to beef carcasses can have a negative effect on tenderness. A study by Locker (1960) showed a close relationship between muscle shortening and meat

tenderness. Locker and Hagyard (1963) demonstrated the effect of temperature on muscle shortening. They reported minimal muscle shortening (less than 10 percent) occurred in the temperature range of 14 to 19 °C while muscle exposed to 0°C shortened to 47.7 percent of their original length. Many researchers have examined the effect of altered chilling rates on meat tenderness (Davey and Gilbert, 1975; Locker et al., 1975; Moeller et al., 1976; Bowling et al., 1977; Lochner et al., 1980; and Marsh et al., 1981). These studies associate the improvement in tenderness to the retardation of cold induced toughening. Research also indicates that if muscle pH is not below approximately 6.0 before muscle temperature reaches 10 to 12 °C or lower, cold-induced shortening may occur. The basic myofibrillar unit of muscle, the sarcomere, is commonly utilized to measure muscle shortening. While several studies have indicated a high correlation between sarcomere length and tenderness (Smith et al., 1971; Yu and Lee, 1986; May et al., 1992;), other studies have found little or no relationship (Field et al., 1970; Lochner et al., 1980) particularly in well-finished beef. Studies have suggested that the effect of muscle shortening on tenderness may be more obvious in rapidly chilled, lightweight carcasses with little external fat.

The rate of pH decline is influenced by temperature (Marsh, 1954; Cassens and Newbold, 1967). Cassens and Newbold (1967) determined with use of sternomandibularis muscle that pH decreases more rapidly at higher temperatures when the temperatures range is from 5 to 37 °C. Also, muscles within the same animal have different rates of pH decline (Bendall, 1978).

Collectively, pH in normal beef muscle drops at the rate of 0.27 to 0.40 units per hour (Bechtel, 1986).

The final pH of muscle that has achieved rigor mortis is termed ultimate pH (Callow, 1937). The ultimate pH of muscle, approximately 5.4 to 5.5, is dependent on the glycogen supply within the muscle prior to slaughter (Lawrie, 1979). Ultimate pH is related to muscle tenderness (Bouton et al., 1972, 1973), and when the ultimate pH was 5.8 to 6.0, tenderness values were the lowest in relation to those readings, however when pH values were shifted from 6.0, tenderness values increased (Yu and Lee, 1986).

Yu and Lee (1986) also reported that steaks from beef carcasses with high ultimate pH values (> than 6.3) were most tender, followed by steaks with pH less than 5.8, and then steaks with intermediate pH readings, 5.8 to 6.3. Marsh (1983) reported that high early postmortem pH was related to tenderness. Yu and Lee (1986) stated, meat with high initial (pH within 24 h) pH values became tender within 24 h postmortem. These studies suggest that high muscle temperature and high muscle pH are related to improvements in tenderness. Yu and Lee (1986) revealed histological examinations of the myofibril showed the degradation of the Z-lines and formation of a 30,000 Dalton unit. The pH range under these conditions would allow calpains to maintain activity. Accordingly, Koohmaraie et al. (1986) suggested that the mechanism for improving tenderness in this situation could be attributed to calpains or other neutral proteases.

Dutson (1983) indicated that meat during early postmortem with low pH values resulted in more tender meat compared to those with higher pH values. In the study by Yu and Lee (1986) mentioned previously, meat with low pH values, improved in tenderness with increased aging (1 to 7 d), also it had myosin and actin partially degraded. With low pH values and the degradation of the contractile proteins, the researchers contributed the improved tenderness to lysosomal enzymes. Meat with intermediate pH values, appear to fall in the range in which enzymatic activity is low, therefore, steaks within this range tend to be toughest (Yu and Lee, 1986).

The conversion of muscle to meat is complex and involves metabolic, physical, and structural changes. The oxygen supply is ceased when blood to the tissues is ceased. The products of metabolism (glycolysis) cannot be removed resulting in lactic acid accumulation. This in turn causes a gradual decline in pH of the tissue from 7.0 to 5.5 over a 24 h period. In conjunction, temperature of the carcass falls and elevation of free calcium concentration increases due to its release from the mitochondria and sarcoplasmic reticulum will occur. These three changes, 1) gradual decline in pH, 2) decreased temperature, 3) and an increase in calcium/calpains have a dramatic effect on the endogenous proteolytic systems in regards to postmortem tenderization (Koohmaraie et al., 1992b).

As pH declines free calcium concentration rises and activates μ-calpain with most of it being bound to calpastatin. As pH further declines, the binding of activated μ-calpain to calpastatin is decreased and the level of free activated μ-

calpain increases causing increased tenderization (Dransfield, 1993). The same type of process occurs with m-calpain only at higher free calcium ion concentrations, which occur as further pH declines. The level of free activated calpains is determined by the balance between their decay and their release from calpastatin as well as their inactivation in the presence of a sufficient concentration of calcium ions. The balance between inhibition, inactivation, and activity of calpains and their decrease as the pH declines, maintains the proteolytic activity of calpains and gradually produces the process of tenderization (Koohmaraie, 1988a; Dransfield, 1993).

The pH of muscle has long been measured as an indicator of glycolytic rate. During the course of these investigations, muscle pH as a predictor of tenderness has been thoroughly examined with varied results. Pike et al. (1993) revealed that a pH measurement at 3 h postmortem gave the best estimate of Warner-Bratzler shear force measurements over four aging periods (2, 4, 8, 16 d). However, contradictory results have been published suggesting pH at 3 h postmortem is not a good indicator of meat tenderness (Shackelford et al., 1994b). In another study, Jones and Tatum (1994), who attempted to find predictors of beef tenderness among carcasses produced under commercial conditions, marbling score was the most effective factor evaluated for classifying carcasses into tenderness. Use of a minimum fat thickness constraint of 0.5 cm was effective for identifying tenderness differences among Select grade carcasses, but was less effective within the Choice grade. Compared with marbling and subcutaneous fat thickness, pH_3 (pH taken at 3 h) was less

effective for use in classifying carcasses into tenderness groups, however, pH_3 values below 6.2 were associated with a reduction in tenderness variation. Measurements of early postmortem longissimus muscle temperature were not effective for use in identifying differences in tenderness. Although, a study by Wulf and Page (2000) indicated that pH measurements alone explained 15 to 23 percent of the variation in beef palatability, and when pH values are coupled with color score measurements, they explain 36 to 46 percent of the variation in beef palatability. Klont et al. (2000) reported that veal carcasses with a higher rate of pH fall over a 24 or 48 h period, produced meat with lower shear force values than carcasses with low rates of pH decline. O'Halloran et al., (1995) found similar results, in that faster glycolyzing muscle produced more tender meat than slow glycolyzing muscles.

Nevertheless, Marsh et al. (1988) stated that minimal progress has been made toward improving tenderness since Lehmann reported his studies on toughness in relation to connective tissue and fiber diameter in 1907. Marsh et al. (1988) also reported that changes in meat-industry practices such as the gradual reduction of postmortem delay between harvest and chilling, faster and faster chilling rates, and the trend toward leaner beef have had a detrimental effect on tenderness. They pointed to a lack of appreciation of the highly dynamic nature of pre-rigor muscle, stating, "the brief period between life and death of the musculature presents us with a unique and still much under-utilized opportunity to influence the tissue's ultimate structure and properties, for muscle at this time is a highly sensitive and vulnerable material." This hypothecation

from Marsh et al. (1988) can be supported from the two National Beef Quality Audits. In 1991, researches conducted the first National Beef Quality Audit (Smith et al., 1992) from which it was found that the top concern of beef purveyors, restaurateurs, purveyors, and retailers was excessive external fat. Between the time this information was disseminated to the industry and the 1995 National Beef Quality Audit (Smith et al., 1995), excessive external fat levels had been diminished out as the top quality concern. However, currently the top three quality concerns include 1) low overall uniformity and consistency of beef products, 2) inadequate tenderness, and 3) low overall palatability. Production practices between the two audits had effectively reduced the amount of external fat on carcasses, but also decreased the degree of intramuscular marbling, giving rise to inadequate eating quality.

Beef Palatability

Beef palatability consists of several traits in which consumers consider important to overall eating satisfaction. These traits are tenderness, texture, juiciness, and flavor (intensity, beef like, and off-flavor). Currently, measuring palatability attributes or overall acceptability can be very difficult and quite costly. Trained or untrained, and consumer type sensory panels are typically used to determine overall palatability differences when conducting research.

Tenderness has been considered the most important palatability attribute to consumers for decades and accordingly has received the most attention among all of the other palatability characteristics (Neely et al., 1998, 1999; Savell et al., 1999; Lorenzen et al., 1999). Moreover, other investigations have

suggested that consumers are willing to pay more for guaranteed tender beef products (Boleman et al., 1997). Measurements of tenderness by trained or consumer panels offer the advantage of approximating the actual assessment of tenderness encountered under normal eating conditions. However, the subjectivity of any panel, makes quantitative measurements difficult. Perceiving tenderness is a complicated physical process as chewing involves cutting, grinding, squeezing, shearing, and tearing from vertical and lateral movements of the jaw. Weir (1960) described the impression of tenderness in meat as being derived from three factors: 1) ease of initial teeth penetration; 2) ease of fragmentation; and 3) residue remaining after chewing. Further, the complexity of impressions sent to the brain from neurons in the tongue, teeth, gums, lips, and cheeks must be translated into measurable and repeatable descriptive terms. Sensory or taste panels have been utilized extensively in meat research. Lowe (1949) stated that it is was preferable to have a small panel of highly sensitive people rather than a large, less sensitive group.

The consumer impression of eating meat is not determined from the individual palatability factors, but in concert, more of an overall perception of all factors, unless there are one or two characteristics that overwhelm the others. Therefore, meat that is very juicy and flavorful, may seem more tender than Warner-Bratzler shear force would indicate compared to dry, bland meat. Owens and Gardner (1999) report correlations in most studies between tenderness, juiciness, flavor, and overall acceptability, in both trained and consumer panels supports this conclusion.

The Beef Customer Satisfaction Study (Neely et al., 1998, 1999; Savell et al., 1999; Lorenzen et al., 1999) revealed that beef cut plated a very important role, accounting for 66.8% of the explained variation in customer satisfaction, and the most important thing the beef industry should do is assure that consumers are using the appropriate cuts for the appropriate meals. Other factors affecting customer satisfaction were: geographical location of the consumer, method of cookery, and USDA quality grade. Degree of doneness and production practices/management practices showed little effect of the finished product, accounting for only 1.5% of the variation in customer satisfaction. Only fed cattle were used in this study, meaning there were no bulls, cows, or older cattle subjected to other than normal management practices included in this study. However, the results do indicate that the use of anabolic implants containing androgens or a combination of androgens and estrogens slightly decreased beef customer satisfaction.

The 1990 National Beef Tenderness Survey (Morgan et al., 1991) reported that nearly 30% of beef steaks produced in the United States were rated less than acceptable for tenderness. This finding has been alluded to fact of lost market share among different meat industries other than beef. However, when compared to the most recent National Beef Tenderness Survey, (Brooks et al., 2000) tenderness was improved. Yet, retail cuts from the round still require close observation in processing and preparation to assure acceptable tenderness. Brooks et al., (2000) implicated reducing the number of cuts that are not

adequately aged before consumption may help reduce beef tenderness problems.

This review of literature has indicated that there are several carcass, production, and postmortem muscle traits which affect beef palatability. The practical implication of how these traits, or combination of traits, could be utilized to predict overall beef palatability are still quite challenging. Certainly, additional means of improving beef palatability, in particular tenderness, would prove beneficial to the meat industry.

CHAPTER III

EFFECTS OF HOT FAT TRIMMING OF CARCASSES ON SENSORY CHARACTERISTICS AND CALPASTATIN ACTIVITY OF BEEF LONGISSIMUS MUSCLE

J.C. Mafi, J.B. Morgan, J.E. Cannon, J.D. Tatum, G. Whipple, and G.C. Smith

ABSTRACT

Paired sides from Gelbvieh steer carcasses (n = 74) were used to determine the effect of hot fat trimming (HFT) on longissimus calpastatin activity and sensory panel characteristics. After carcass splitting, sides from each carcass were randomly assigned to either a untrimmed control group (CON) or the HFT group. All external fat in excess of 0.64 cm and all internal fat were removed from HFT sides prior to chilling at 1.1°C. During the chilling process, longissimus muscle pH and temperature measurements were taken at 0, 3, 6, 12, and 24 h. After a 24 h chilling period, USDA Yield and Quality grade factors were collected from CON sides. Longissimus muscle sections were taken from all sides and calpastatin activity, Warner-Bratzler shear force values (WBS), and sensory panel characteristics were determined. HFT increased ($P < 0.05$) the rate of longissimus temperature decline and decreased ($P < 0.05$) the rate of pH decline compared to CON sides. HFT increased ($P < 0.05$) WBS values by 0.22 kg and calpastatin activity by 0.20 units / gram of muscle, and subsequently decreased ($P < 0.05$) sensory tenderness and overall palatability scores compared to CON sides. Differences in calpastatin activity and sensory panel characteristics of longissimus muscle from HFT carcasses at different 12th rib fat

thickness levels were determined by dividing the carcasses into four subclasses (< 0.50 cm; 0.50 - 0.63 cm; 0.64 – 0.75 cm; > 0.75 cm) based on actual 12th rib fat thickness. At < 0.50 cm fat thickness, no differences existed for longissimus sensory properties or calpastatin activity between CON and HFT sides. However, differences for tenderness and overall palatability scores, WBS values, and calpastatin activity became significantly evident as fat thickness differences increased. These results indicate that HFT increases calpastatin activity and decreases beef longissimus muscle tenderness. Also, the magnitude of increased calpastatin activity or decreased tenderness becomes more evident as actual 12th rib fat thickness increases.

Key words: Beef, tenderness, fat, calpastatin

Introduction

In the preliminary stages of the HFT process, the concept was to improve production efficiency and realign the value based system for carcass pricing as outlined by Savell et al. (1989). One benefit of HFT is the reduction of variation in boneless, lean meat yields attributed to differences in carcass fatness (Savell et al., 1989; Williams et al., 1989; Ahmed et al., 1992). By using HFT beef carcass weight as a pricing basis, producers would be inclined to produce trimmer, heavier muscled market cattle (Savell et al., 1989). This would be especially beneficial at a time when consumer's demanding for leaner, healthier meat products has induced the packing and retail industries to generate closely trimmed products. Also at this time, approximately $112 was being lost per market steer/heifer due to excessive external fat according to the findings in the

National Beef Quality Audit (Smith et al., 1992). The recommendation of Savell et al. (1989) was that hot fat trimming should be encouraged as a way to reduce carcass variation in the short-term. Currently, according to the most recent National Beef Quality Audit (Smith et al., 1995) the top three quality concerns include 1) low overall uniformity and consistency of beef products, 2) inadequate tenderness, and 3) low overall palatability.

Certainly, a great deal of potential exists for HFT. However, potential problems have not been addressed regarding HFT and its potential negative impact on sensory characteristics of beef products. Therefore, the current study addresses the effect of hot fat trimming on the early postmortem changes of muscle and the overall palatability of the product.

Experimental Procedures

Trimming Procedures

Gelbvieh steers (n = 74) were fed a high-energy diet and harvested at the Monfort/ConAgra beef packing facility in Greeley, Colorado. After carcass splitting, one side of each carcass was randomly assigned to be hot fat trimmed (HFT) and the other side served as an untrimmed control (CON). Prior to chilling at 1.1°C, HFT sides were trimmed of external fat in excess of 0.64 cm as well as all cod, flank, and internal fat. Additionally, the *Cutaneus trunci* muscle was removed and the superficial fat in excess of 0.64 cm was removed. All HFT sides were trimmed using a Bettcher whizard knife, (Bettcher Industries, Vermillion, OH) or hand-held knives.

Early-Postmortem Muscle Temperature and pH

Internal temperature of the longissimus muscle (LM) was measured at 0, 3, 6, 12, and 24 h postmortem, using a stainless steel thermometer. Temperature measurements were taken on both sides of each carcass, at approximately in the geometric center of the LM, 10 cm posterior to the 13th rib. For measuring muscle pH, tissue samples (1 g) were excised from the center of the LM, posterior to the 13th rib at 0, 3, 6, 12, and 24 h on both sides of each carcass. Individual samples were homogenized for approximately 30 s in 10 mL of neutralized 5 mM sodium iodoacetate/150 mM potassium chloride solution using a VirTis ("VirTishear") homogenizer (Gardiner, NY) at a speed setting of 70. The pH of the homogenate was measured using an Accumet pH Meter 50 (Fisher Scientific, Pittsburgh, PA).

Carcass Evaluation

All sides were chilled for 24 h at 1.1°C. CON sides were ribbed between the 12th and 13th ribs, and factors used to determine USDA quality grade (carcass maturity and marbling score) and yield grade (adjusted fat thickness, ribeye area, hot carcass weight, and percentage of kidney, pelvic, and heart fat) were recorded for each carcass (USDA, 1989) using trained personnel.

Sample Fabrication

At 24 h postmortem a 10 cm longissimus muscle (LM) section was removed from each side. LM sections from CON sides were trimmed to no more than 0.64 cm of external fat. A 2.54 cm sample was taken from each LM to be assayed for calpastatin activity. LM sections were aged under vacuum for 7 d at approximately 1.1°C and then frozen and fabricated into 2.54 cm steaks, which

were used for sensory evaluation and Warner-Bratzler shear force (WBS) determination.

Calpastatin Activity

Calpastatin activity was determined using techniques similar to the procedures described by Shackelford et al. (1994b). Samples (10.0 g) were extracted at 24 and 28 h postmortem in 25 ml of 150 mM Tris, 10 mM EDTA, 7 mM β-mercaptoethanol (pH 8.3; 3.6 to 4°C) by homogenizing for three, 30 s intervals with polytron. Samples were allowed to cool for 30 s between each homogenization. The homogenate was then centrifuged at 35,000 X g for 30 min and the supernatant was filtered through glass wool and cheesecloth. Following dialysis, the supernatant was heated (95°C water bath) for 15 min to denature the calpain. Immediately after heating, samples were chilled for 15 min in an ice water bath. The coagulated protein was scrambled to facilitate separation of pellet and supernatant during configuration. Samples were centrifuged for 30 min at 1500 X g. After centrifugation, supernatant volume was determined and used for calculation of calpastatin activity. Calpastatin activity was calculated and reported as activity / gram of muscle, using the same procedures of Koohmaraie (1990).

Warner-Bratzler Shear Force and Sensory Panel Evaluation

Steaks for sensory evaluation and WBS measurements were cooked on Faberware open hearth grills (Walter Kidde, Bronx, NY) to an internal temperature of 70°C monitored by a thermocouple thermometer (Atkins Technical Inc., Gainesville, FL). 2.54 cm cubed portions from each steak were

served warm with water to an 8-member trained sensory panel for evaluation of tenderness, juiciness, flavor desirability, flavor intensity, and overall palatability. Panelist used an 8-point scale (1 = extremely tough, dry, bland, undesirable flavor, and undesirable overall palatability; 8 = extremely tender, juicy, intense beef flavor, desirable flavor, and desirable palatability) to describe each characteristic (Cross et al., 1978). Steaks used for WBS determination were cooled to room temperature, and six 1.25 cm diameter cores were removed from each steak parallel to the muscle fiber orientation. Shear force was measured using a Warner-Bratzler shear machine.

Statistical Analysis

Data were analyzed as Randomized Complete Block Design using the Mixed procedure of SAS Institute, Inc. (1999). Means were separated using a least significant difference procedure. To examine differences between trimming procedures at different 12^{th} rib fat thickness levels, carcasses were divided into subclasses based on 12^{th} rib fat thickness: <0.50 cm; 0.50 – 0.63 cm; 0.64 – 0.75 cm; > 0.75 cm. Data within each subclass were analyzed using a least significant difference procedure as previously mentioned.

Results and Discussion

Carcass Evaluation

Carcass characteristics for the Gelbvieh steers used in this study are presented in Table 1. Means for hot carcass weight and ribeye area of the steers were typical of current industry values. However, mean values for fat thickness, kidney, pelvic, and heart fat, USDA Yield Grade, and marbling score were substantially lower for steers in the present study compared with industry averages determined in the National Beef Quality Audit (Smith et al., 1995). In fact, approximately 97% of all carcasses were a Yield Grade 2.9 or better in this study.

Effect of Early Postmortem Muscle Temperature on Tenderness

There was a significant interaction between treatment and chilling time (Figure 1). Initial temperature measurements taken at 0 h from both sides was not affected by the HFT process. However, at 3, 6, 12, and 24 h, temperature from HFT sides declined more rapidly, when compared to CON sides. These findings suggest that carcass fatness altered the chilling rate. These results are consistent with Smith et al. (1976) who demonstrated with lamb carcasses, which differed in the degree of finish, that carcass fatness had an isulatory effect and slowed the rate of temperature decline compared to trimmer carcasses. These findings are also in agreement with May et al. (1992), who reported HFT sides chilled more rapidly ($P < 0.05$) and had lower ($P < 0.05$) 2.5 h LM temperatures and lower ($P < 0.05$) sensory tenderness ratings than untrimmed sides.

Several studies have looked at the effect of altering carcass chilling rate and its affect on tenderness (Moeller et al., 1976; Smith et al., 1980; and Lochner et al., 1980). These investigations have attributed the changes in tenderness to either cold toughening (cold-induced muscle contraction prior to rigor) or endogenous proteolytic enzyme activity. May et al. (1992) reported trimming external fat from carcasses resulted in lower 2.5 h LM temperature measurements, higher 24 h pH values, shorter sarcomere lengths, and lower sensory tenderness scores compared to conventionally harvested carcasses

Effect of Early Postmortem Muscle pH on Tenderness

There was a significant interaction between treatment and chilling time for muscle pH decline. The pH measurements from CON sides reacted differently over time, decreasing more rapidly when compared to HFT sides. These results suggest that carcass fatness and rapid cooling temperatures altered the chilling rate, influencing normal pH decline between HFT and CON sides. Results of the current study are similar to findings by Marsh et al. (1954), Cassens and Newbold (1967), and May et al. (1992), in which pH declines more rapidly relative to higher muscle temperatures. Also, Dutson (1983) found that meat within the first 24 h postmortem with low pH values resulted in more tender meat compared to early postmortem meat with higher pH values. Yu and Lee (1986) found similar results, in that meat with low pH values, improved in tenderness with increased aging (1 to 7 d). With low pH values and the degradation of contractile proteins, the researchers contributed the improved tenderness to lysosomal enzymes. This theory is also supported by Eilers et al. (1996), who

used electrical stimulation to rapidly decrease early postmortem pH decline which, effectively influenced tenderness, decreasing Warner-Bratzler shear in LM steaks. The pH of muscle has long been measured as an indicator of glycolytic rate. During the course of these investigations, muscle pH as a predictor of tenderness has been thoroughly examined with varied results. Pike et al. (1993) revealed that a pH measurement at 3 h postmortem gave the best estimate of Warner-Bratzler shear force measurements over four aging periods (2, 4, 8, 16 d). However, contradictory results have been published suggesting pH at 3 h postmortem is not a good indicator of meat tenderness (Shackelford et al., 1994b). Jones and Tatum (1994) found pH taken at 3 hour, was less effective than marbling score and subcutaneous fat thickness in classifying carcasses into tenderness groups. However, pH_3 values below 6.2 were associated with a reduction in tenderness variation. As pH declines, free calcium concentration rises and activates μ-calpain with most of it being bound to calpastatin. As further pH declines the binding of activated μ-calpain to calpastatin is decreased and the level of free activated μ-calpain increases causing increased tenderization (Koohmaraie, 1988a; Dransfield, 1993; Kendall et al., 1993). Marsh et al. (1988) reported that changes in meat industry practices such as the gradual reduction of postmortem delay between harvest and chilling, faster chilling rates, and the trend toward leaner beef have had a detrimental effect on tenderness.

Calpastatin Activity

Calpastatin activity was 0.20 units of activity / gram of muscle higher ($P < 0.05$) in LM samples from HFT sides compared to those from CON sides (Table

2). Calpastatin inhibits the activity of calpain, an important enzyme involved in myofibrillar protein degradation. Researchers have found that the amount of myofibrillar protein degradation directly influences meat tenderness (Koohmaraie, 1988a). Higher calpastatin activity would indicate inhibition of calplain, which could be associated with the observed reduction tenderness.

A gradual decline in pH, temperature, and an increase in calcium/calpains have a dramatic effect on the endogenous proteolytic systems relative to postmortem tenderization (Koohmaraie et al., 1992b). The activity of tenderization in meat due to the proteolytic activity is correlated to the amount of free unbound μ-calpain and m-calpain. As pH declines, free calcium concentration rises and activates μ-calpain with most of it being bound to calpastatin. As pH further declines, the binding of activated μ-calpain to calpastatin is decreased and the level of free activated μ-calpain increases, causing increased tenderization (Dransfield, 1993). The same process occurs with m-capain, only at higher free calcium ion concentrations, which occur as pH further declines. The level of free activated calpains is determined by the balance between their decay and their releases from calpastatin as well as their inactivation in the presence of a sufficient concentration of calcium ions. The balance between inhibition, inactivation, and activity of calpains and their decrease as the pH declines, maintains the proteolytic activity of calpains and gradually produces the process of tenderization (Koohmaraie, 1988a; Dransfield, 1993).

In the case of this study, rapid temperature decline obviously had a negative impact on pH decline, not allowing pH to decline conventionally. This possibly did not allow for adequate increases in calcium concentration in the HFT sides, resulting in a higher concentration of calpastatin, low levels of free activated μ-calpain, and decreased tenderization compared CON sides. These findings, in the case of pH decline are similar to the results of Klont et al. (2000). Their results showed meat with faster rates of pH decline from 0 to 24 h or from 0 to 48 h resulted in lower shear force values than meat with slower rates of pH decline.

Warner-Bratzler Shear Force and Sensory Panel Evaluation

HFT significantly decreased sensory panel tenderness and overall palatability scores of longissimus steaks, and increased ($P < 0.05$) WBS measurements 0.22 kg compared to CON steaks (Table 2). This is possibly the result of rapid temperature decline impacting pH, and elevating calpastatin activity inhibiting traditional proteolysis in the HFT sides in relation to CON sides. There were no differences were observed for sensory juiciness, flavor intensity, or flavor desirability scores among treatment groups.

Effects of Hot Fat Trimming Associated with Differing Subcutaneous Fat Levels

Additional comparisons were made between steaks from CON and HFT carcass sides that increased in external fat thickness levels (Figures 3 – 6). At fat levels of < 0.50 cm, no differences were observed in WBS measurements, sensory characteristics or calpastatin activity among treatments. However, as external fat increased, steaks from HFT carcasses had decreased ($P < 0.05$)

sensory tenderness scores for > 0.75 cm, and decreased ($P < 0.05$) overall palatability scores at 0.50 to 0.63 cm and > 0.75 cm fat thickness. Also, increased ($P < 0.05$) WBS measurements and calpastatin activity were observed at all external fat levels except < 0.50 cm, when compared to CON steaks. However, carcass fat level did not impact juiciness, flavor intensity, or flavor desirability between HFT and CON carcasses (data not in tabular form). The lack of differences observed at the lower external fat thickness levels were expected, because the two sides had similar fat covering. Consequently, as little or no fat trimming was required in which both carcass sides cooled and responded to the 24 h chill in the same manner. These results indicate that HFT effects temperature decline, causing pH decline to be affected. Accordingly HFT increases calpastatin activity and ultimately decreases beef longissimus muscle tenderness. Also the magnitude of increased calpastatin activity or decreased tenderness becomes more evident as actual 12^{th} rib fat thickness increases.

Implications

HFT can be a useful tool to improve production efficiency and decrease carcass variation. However, the possibility to experience detrimental effects on beef palatability exists due to rapid chilling rates. HFT may be most beneficial when used during a longer postmortem chill versus a 24 h chill. Unfortunately, in this study only 24 h chilling was investigated. Also, HFT could be used primarily on end cuts of the carcass that are expected to be slightly less desirable when compared to middle meats, which would possibly allow for improved production efficiency with a minimal effect on carcass quality under current conditions.

Table 1. Means, standard deviations, minimum, and maximum values of steers used to compare fat trimming methods[a].

Trait	Mean	SD[b]	Min[b]	Max[b]
Hot carcass weight, kg	329.5	23.7	269.0	374.2
Fat thickness, cm	0.66	0.23	0.25	1.14
Longissimus muscle area, cm^2	89.9	7.1	69.6	101.9
KPH fat, %	1.7	0.49	1.0	3.0
Yield Grade	2.09	0.53	0.77	3.49
Marbling score[c]	257.0	52.0	150.0	390.0

[a]N = 74
[b]SD = Standard deviation; Min = minimum value; Max = maximum value.
[c]Marbling scores are based on the USDA Standards for Beef Grades (USDA, 1989) where 300 = $Small^{00}$; 200 = $Slight^{00}$; 100 = $Traces^{00}$.

Table 2. Effects of hot-fat trimming on calpastatin activity, Warner-Bratzler shear force measurements, and sensory characteristics of beef longissimus muscle.

	CON[a]	HFT[a]	SE[b]
Calpastatin activity[c]	2.71[f]	2.91[g]	0.03
Warner Bratzler shear force, kg[d]	2.84[f]	3.06[g]	0.04
Tenderness[e]	5.21[f]	5.02[g]	0.05
Juiciness[e]	4.96	4.88	0.04
Flavor intensity[e]	5.03	5.03	0.02
Flavor desirability[e]	5.07	5.06	0.03
Overall palatability[e]	5.14[f]	5.00[g]	0.03

[a]CON = conventional trimming 24 h postmortem; HFT = hot fat trimmed, immediately postmortem.
[b]Standard error of difference of means.
[c]Expressed as units of activity / gram of muscle.
[d]Warner-Bratzler shear force measurements taken on 1.25 cm meat cores.
[e]8 = extremely tender, juicy, intense beef flavor, desirable beef flavor, and desirable in overall palatability; 1 = extremely tough, dry, bland, undesirable in beef flavor, and undesirable in overall palatability.
[f,g]Means within the same row with a different superscript differ ($P < 0.05$).

Figure 1. Treatment by time interaction for Longissimus muscle temperature ($P < 0.05$).

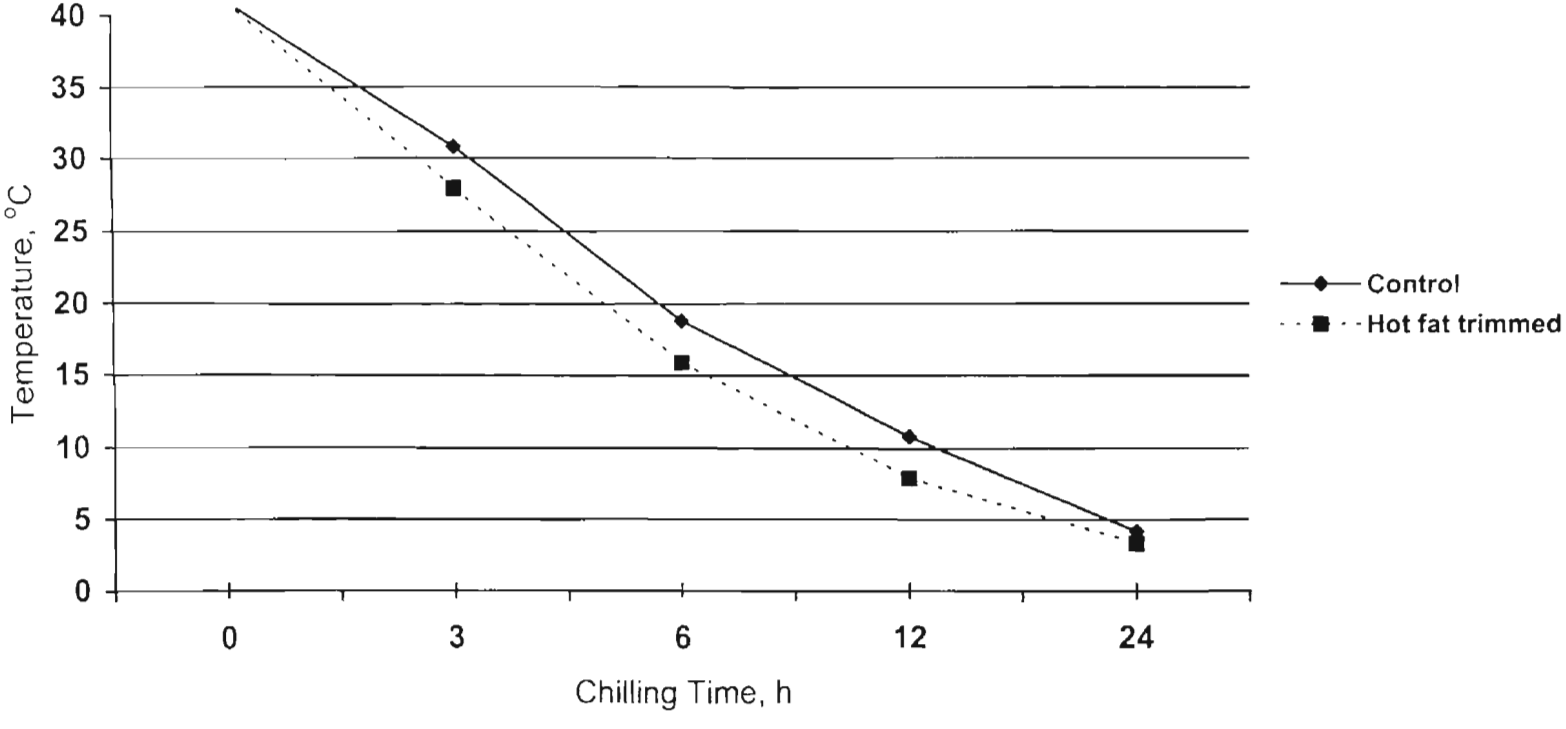


Figure 2. Treatment by time interaction for Longissimus muscle pH ($P < 0.05$).

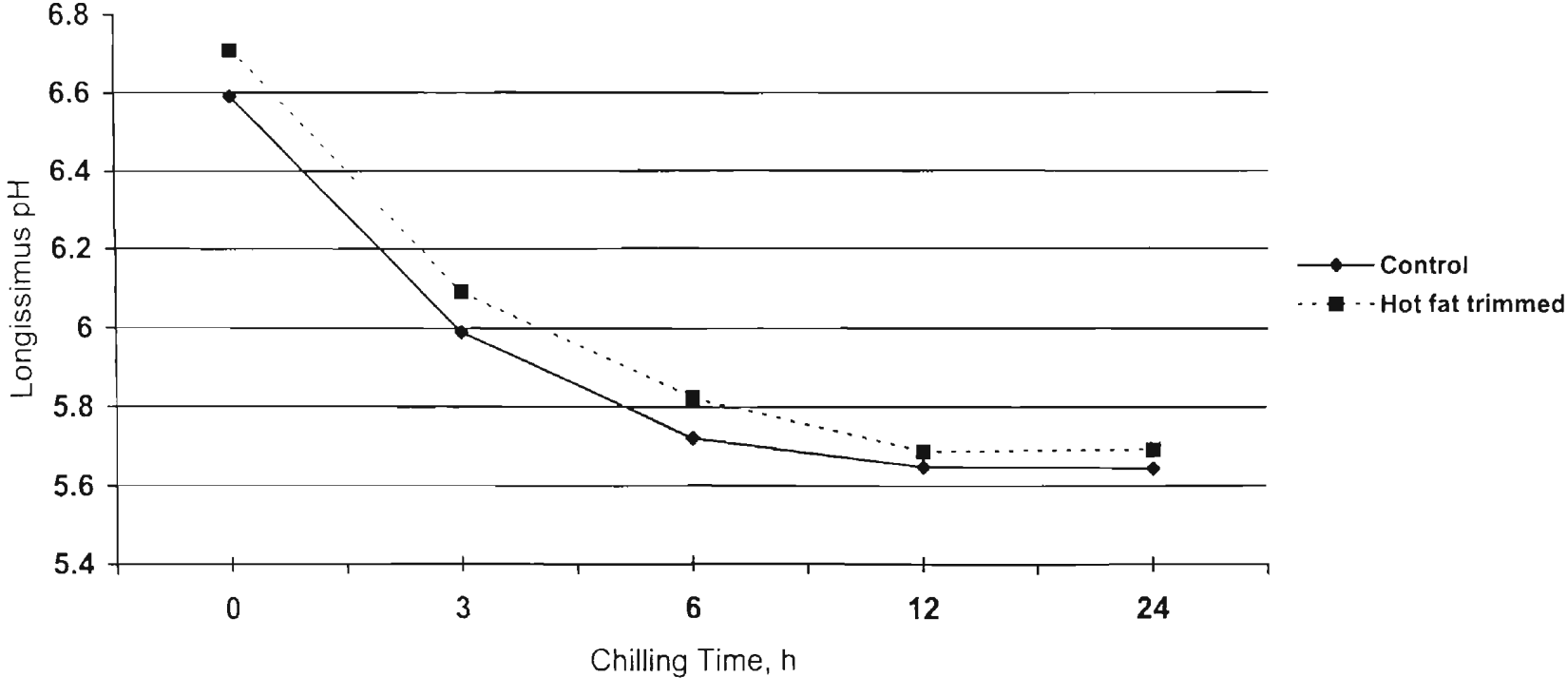


Figure 3. Comparison of calpastatin activity in longissimus muscle from CON and HFT beef sides subdivided into external fat thickness groups. Columns within each external fat group with different superscripts differ (P < 0.05).

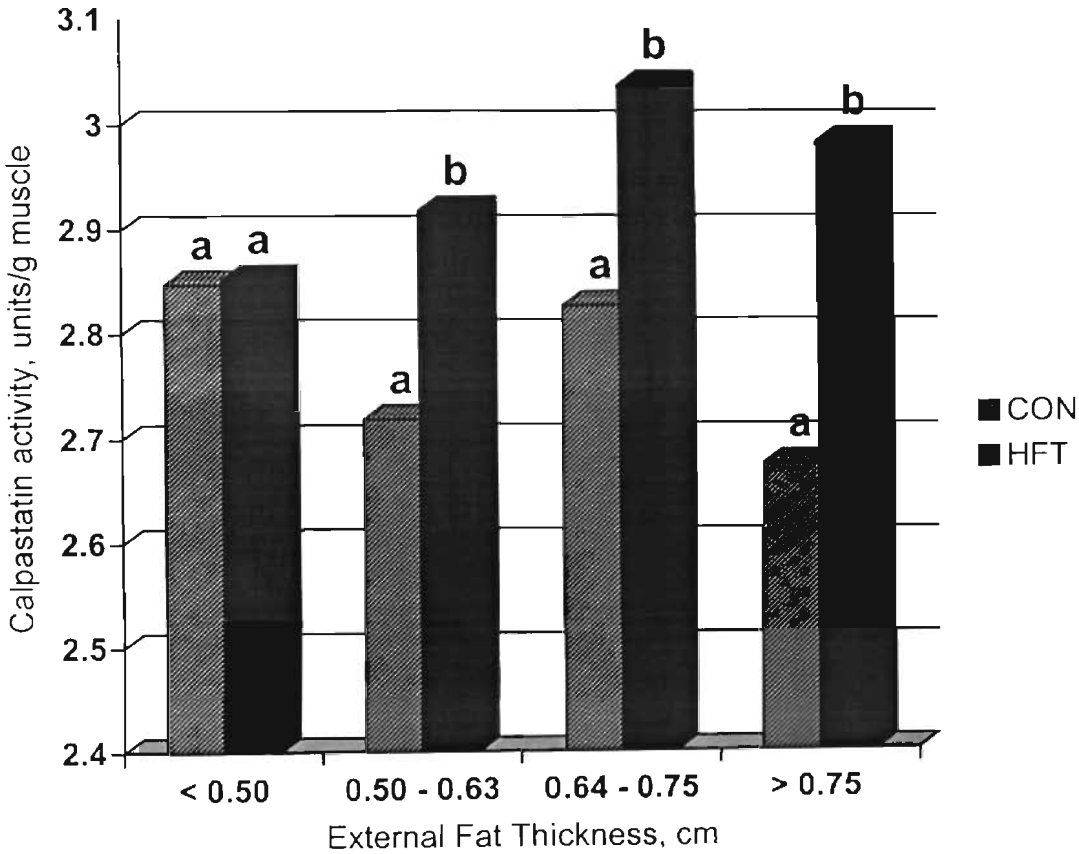


Figure 4. Comparison of WBS measurements of longissimus steaks from CON and HFT beef sides subdivided into external fat thickness groups. Columns within each external fat group with different superscripts differ ($P < 0.05$).

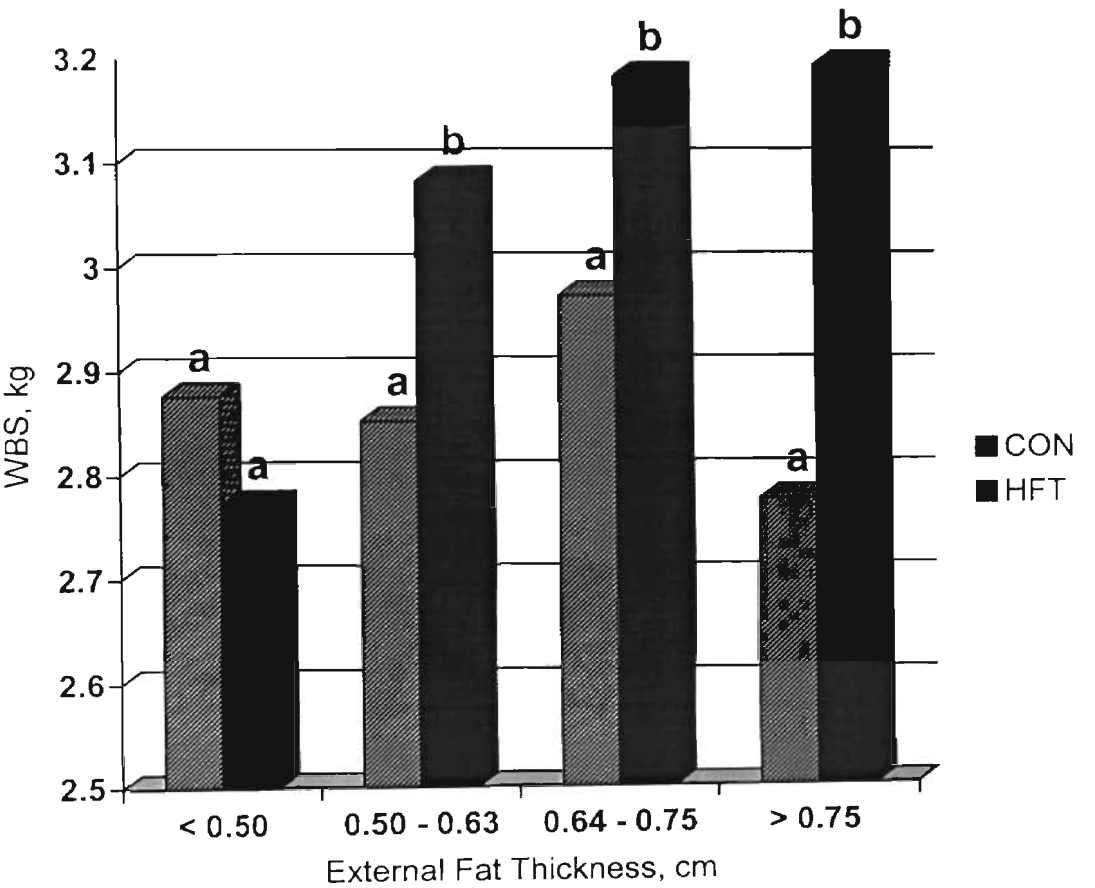


Figure 5. Comparison of sensory panel tenderness scores for longissimus steaks from CON and HFT beef sides subdivided into external fat thickness groups. Columns within each external fat group with different superscripts differ (P < 0.05).

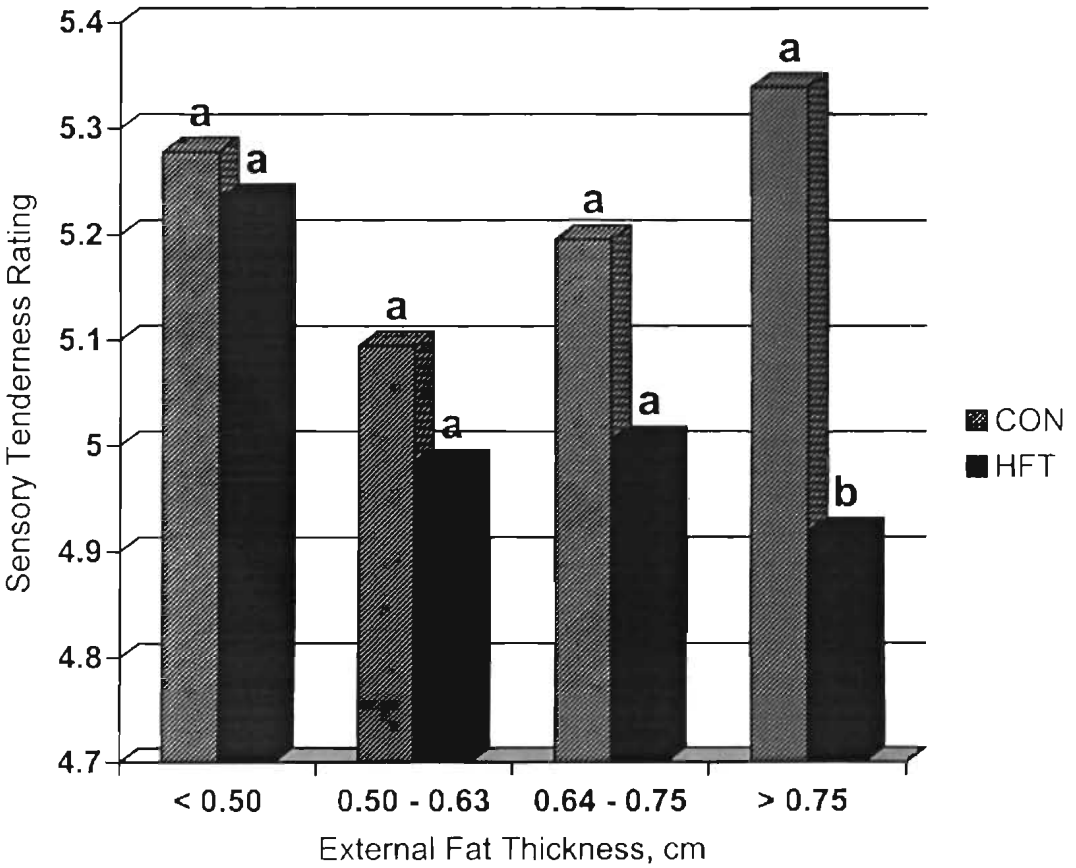
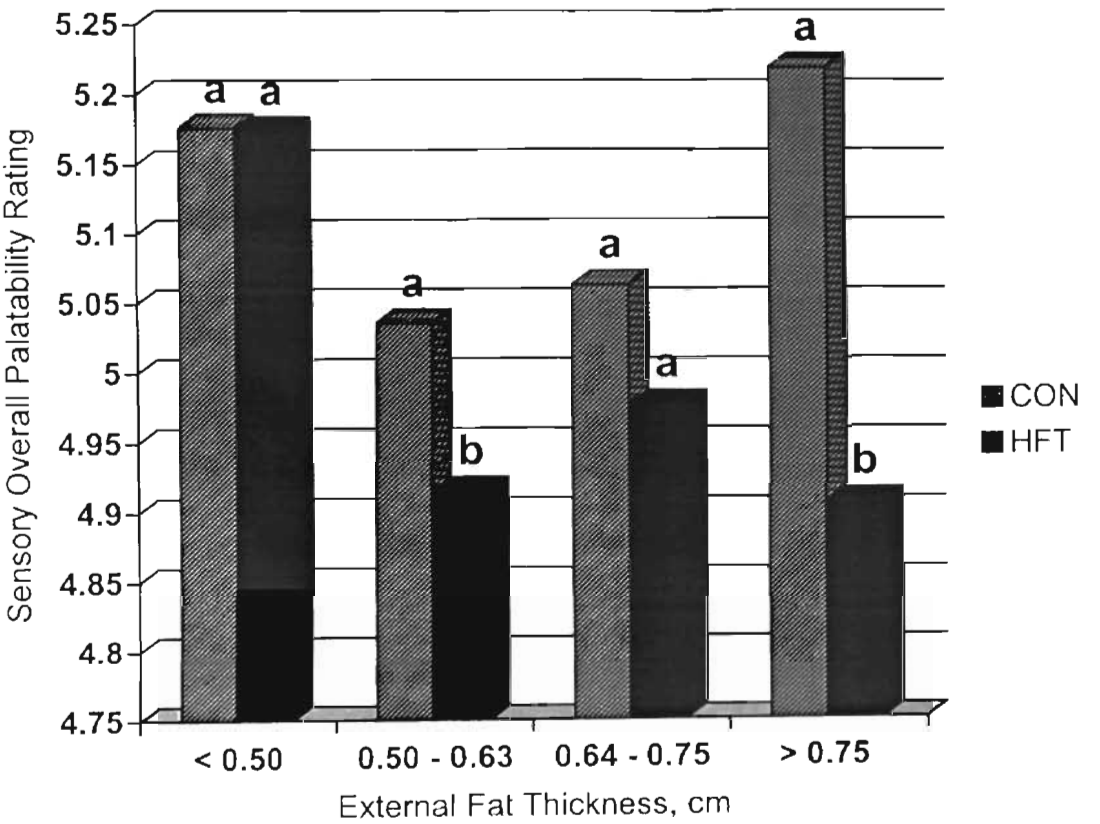


Figure 6. Comparison of sensory overall palatability scores for longissimus steaks from CON and HFT beef sides subdivided into external fat thickness groups. Columns within each external fat group with different superscripts differ ($P < 0.05$).



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