

EFFECT OF DEGREE OF DARK-CUTTING ON TENDERNESS AND FLAVOR
ATTRIBUTES OF BEEF

A Dissertation

by

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ABSTRACT

The objective of this study was to determine the effect of degree of dark-cutting (DC) on the tenderness, juiciness and flavor descriptive attributes of beef. During carcass grading at a large U.S. commercial beef harvesting facility, DC carcasses and matching normal cohort (NC) carcasses (n=160) were selected. Longissimus lumborum (LL) pH was determined online and DC carcasses were classified as severe (SEDC; mean pH=6.9, n=40), moderate (MODC; mean pH=6.6, n=40), mild (MIDC; mean pH=6.4, n=40) or shady (SHDC; mean pH=6.1, n=40). Vacuum-packaged strip loins (LL) were obtained from the left side of each carcass and aged for 14 d postmortem at 2°C. One steak (2.54 cm) was collected for fresh slice shear force (SSF). A 6 cm section was frozen and used for trained descriptive analysis of tenderness, juiciness and flavor. Cooked SSF pieces were frozen and utilized for western blotting of desmin to determine extent of postmortem proteolysis. Thaw and cook loss decreased as intensity of DC increased with SEDC having the lowest loss (1.83% and 10.1%, respectively) compared to NC (3.37% and 14.9%, respectively). Slice shear force was higher ($P<0.05$) for SHDC (25.6 kg) and MIDC (22.9 kg) compared to SEDC (16.8 kg), MODC (19.4 kg) and NC (17.8 kg). Sarcomere length was shorter ($P<0.05$) between DC class and NC (1.66, 1.67, 1.71 and 1.73 μm for SEDC, MODC, MIDC and SHDC) and NC (1.86 μm). Postmortem proteolysis of desmin was greater ($P<0.05$) for NC compared to all DC classes (59.83% vs. 49.20%, 40.31%, 42.07% and 43.30% for SEDC, MODC, MIDC and SHDC, respectively). Trained sensory panel ratings for tenderness differed ($P<0.05$) among DC class with SEDC (6.51) the most tender, followed by MODC

(6.04), then MIDC (5.19) while SHDC (4.66) and NC (4.93) were the toughest. Juiciness ratings differed ($P < 0.05$) among each DC class (5.9, 5.7, 5.4 and 5.2 for SEDC, MODC, MIDC and SHDC, respectively), with no difference between MIDC or SHDC compared to NC (5.23). Fat-like, rancid, heated oil, chemical and musty/earthy/hummus flavors increased ($P < 0.05$) while metallic, sour and salty flavors decreased as severity of DC increased. This study showed DC and NC differed in LL tenderness, juiciness and flavor. The direction and/or magnitude of those differences were greatly dependent of severity of DC. Steaks from intermediate pH (SHDC) are most likely to be tough, yet are regularly included in U.S. Select and U.S. Choice product lines.

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1. INTRODUCTION

According to the 2011 National Beef Quality Audit, 3.2 percent of cattle sampled were determined to be dark-cutters, which is an increase over the 2000 (2.3%) and 2005 (1.9%) National Beef Quality Audits (Garcia et al., 2008; McKenna et al., 2002; Moore et al., 2012). Carcasses that exhibit dark-cutting are known to have differences in tenderness (Bouton et al., 1973; Dransfield, 1981; Dutson, 1983; Purchas, 1990) and flavor (Wulf et al., 2002), in addition to lean color that is undesirable to the consumer. Currently, cattle that are sold using carcass grid-based pricing are discounted on average \$34.15/cwt (USDA-AMS, 2013) when classified a severe dark-cutter. Carcasses with slightly darker lean color may be downgraded one full quality grade level to either U.S. Choice or U.S. Select, instead of being discounted severely for being a dark-cutter. Although research has been conducted on the effects of dark-cutting on tenderness, data addressing varying degrees of dark-cutting across a large pH range is lacking. In addition, little research regarding sensory characteristics of dark-cutting beef has been conducted. The increase in percentages of dark-cutters and the potential detrimental effects on meat quality warrant further examination.

2. REVIEW OF LITERATURE

2.1 Endogenous factors that affect tenderness

There are many endogenous factors that affect tenderness in beef. Elements such as sarcomere length, collagen, amount of marbling, pH and extent of proteolysis are known factors that can have a great impact upon tenderness. Throughout historic meat science research, much emphasis has been placed upon the desire to increase tenderness. Consumers demand a product that is not only wholesome, but also palatable. Palatability consists of tenderness, juiciness and flavor, with tenderness and flavor as equal contributors to the overall like of beef (Neely et al., 1998). This literature review will examine prior research conducted on what affects tenderness and impact of dark-cutting on beef palatability.

2.1.1 Sarcomere length

Sarcomeres are the basic contractile unit of muscle. Sarcomeres connect end-to-end to make myofibrils, which are organized into muscle bundles. Muscle bundles combine to make muscles. A connective tissue hierarchy of epimysium, perimysium and endomysium helps maintain muscle structure. Skeletal muscle appears striated and this is caused by banding patterns within sarcomeres from the thick and thin filaments. Contraction and relaxation of sarcomeres result in the ability of muscles to move the body. The contractile state of sarcomeres after rigor mortis plays a role in the tenderness of meat, in addition to other factors. Severe contraction of sarcomeres postmortem has been shown to result in tougher meat (Locker, 1960; Herring et al., 1965b; Marsh and Leet, 1966), whereas stretching has been shown to result in more tender meat (Herring et

al., 1965a, b and 1967a; Hoestetler et al., 1970). Sarcomere length is defined as the distance from Z-disk to Z-disk. Sarcomeres are composed of many different proteins that are classified as contractile, regulatory or structural. Actin and myosin are contractile proteins. Troponin (I, C, and T) and tropomyosin are regulatory proteins. Structural proteins that keep the sarcomere structurally sound and capable of organized muscle contraction include: Desmin, titin, nebulin, filamin, skelemin, C-proteins, vinculin, talin, actinins (α and β), paxillin, myomesin, M-protein, and cap Z. Sarcomeres can range in length from less than 1.5 μm in a contracted state to more than 3.5 μm in a relaxed, stretched state. There is variation in postmortem sarcomere lengths between muscles in beef due to suspension method. During suspension from the Achilles tendon, various muscles are either stretched (psoas major) or free to contract (biceps femoris; Herring et al., 1965a; Rhee et al., 2004). The density and fiber size of a sarcomere is less when stretched, therefore would require less force to shear through, making it more tender whereas sarcomeres that are shorter are denser due to a larger overlap of actin and myosin, have larger fiber size and tend to be less tender (Hiner et al., 1953). This results in the need for a greater amount of force needed to shear through the cooked meat and therefore tougher meat.

Locker (1960) conducted research on how the degree of muscle contraction affected tenderness. He used the psoas major excised at three days postmortem to minimize the effects of aging and connective tissue on tenderness. Psoas major muscles were either left intact on the carcass or were cut pre-rigor and allowed to shorten 20-30% during chilling and rigor mortis development. The muscles were evaluated by sensory

tests and those allowed to shorten were determined to be tougher than muscles that had been left intact. The psoas major had very low connective tissue making any differences in tenderness due to other factors. Locker (1960) therefore determined that muscles that were relaxed were more tender compared to muscles that were contracted or shortened. Herring et al. (1965b) also examined tenderness in relation to the sarcomere length. They determined that muscles with sarcomere lengths that were shortened due to being excised pre-rigor without being fixed were the toughest. In comparison, muscles that had been excised and stretched had longer sarcomere lengths and were most tender. These were among some of the first results indicating that sarcomere length plays a role in meat tenderness. Other research confirmed that sarcomere length has an effect on tenderness and that sarcomere length is an important factor in explaining the variation in tenderness (Dutson et al., 1976; Herring et al., 1967a; Howard and Judge, 1968; Hoestetler et al., 1970, 1972; Smulders et al., 1990; Wheeler and Koohmaraie, 1994; Wheeler et al., 2000; Rhee et al., 2004).

Sarcomere length can be affected by different factors including temperature and rate of chilling and carcass hanging method. Rate of chilling was shown to affect the amount of shortening that occurred and was later termed cold-shortening (Locker and Hagyard, 1963). Carcasses that were chilled very rapidly after slaughter were susceptible to cold-shortening. During this rapid chilling, ATP is not depleted and calcium is not bound in the sarcoplasmic reticulum. The result of this is a bathing of the sarcomere in calcium with the energy from ATP to cause severe contractions of

sarcomeres. The energy provided from ATP is depleted during the contraction, resulting in muscles that are unable to relax.

Locker and Hagyard (1963) in their cold-shortening research determined that carcasses chilled at 0°C had a greater amount of shortening and, therefore, were less tender compared to those chilled at 14-19°C. They also determined that shortening was greater in carcasses that were chilled above 19°C, but the extent of shortening was less compared to carcasses chilled at 0°C. Marsh and Leet (1966) further researched cold-shortening to determine how cold-shortening affected tenderness in meat. They found that there was an increase in toughness when the percentage of shortening in the muscle ranged from 20-40%. Peak toughness was found at approximately 40% shortening of the muscle. When the muscle was shortened in the range of 40-60% there was a considerable decrease in toughness. They hypothesized that there was minimal structural damage done when shortening was 20-40%, but higher percentages caused structural damage that would reflect the decrease in toughness. Marsh et al. (1974) later examined histological muscle samples that were shortened 43%, 48.5% and 55%. The samples that were 43% shortened were the toughest while those samples that were 55% shortened were the most tender. The muscles that were shortened 43% were very similar to other shortened muscle samples with sarcomere lengths being very uniform and Z-disks remaining organized. As the percentage of shortening increased, patches of super-contracted sarcomeres began to appear in greater quantities. The super-contracted sarcomeres caused damage to the Z-disks and aided in the disorganization of the sarcomeres. Marsh et al. (1974) determined that this disorganization and super-

contraction of sarcomeres was the cause of the increase in tenderness that is seen when cold shortened muscles are shortened more than 50%.

Another condition that also could affect sarcomere length and tenderness is thaw rigor. Thaw rigor occurs when meat is frozen prior to the onset of rigor mortis. Since the muscle is frozen prior to the onset of rigor mortis, ATP is still present also. During the freezing, ice crystal formation causes damage to the sarcoplasmic reticulum. During thawing, the damaged sarcoplasmic reticulum releases very large amounts of calcium into the sarcoplasm, which bathes over the muscle fiber and in the presence of ATP causing severe contractions. The severe contraction that occurs uses the ATP that is left and the muscle is unable to relax, causing a permanent shortening. The majority of shortening in thaw rigor does not occur prior to freezing, but during the thawing of the meat. In order to minimize the shortening that occurs from thaw rigor, a slow, controlled thawing protocol must be implemented (Marsh and Thompson, 1958; Wheeler and Koohmaraie, 1994).

2.1.2 Connective tissue

Connective tissue is one of the components that can greatly affect meat tenderness. There are two types of connective tissue: supportive tissue (bones and cartilage) and connective tissue proper (ground substance and fibrous connective tissue). Connective tissue proper is the portion that is of concern with regards to meat tenderness. Connective tissue proper is made up of ground substance and various types of fibers. Ground substance is structure-less mass made up of soluble glycoproteins and has minimal impact upon meat tenderness.

Fibrous connective tissue is made up of collagen, elastin and reticulin. Collagen is the most abundant protein in the body and is composed of amino acids, mainly glycine (most abundant), hydroxyproline and proline. Many different fiber types of collagen have been identified and named. Collagen fiber types I and III are the fibers that primarily compose the three layers of connective tissue in muscle. The three layers of connective tissue associated with muscle are the epimysium, perimysium and endomysium. The endomysium is a layer of connective tissue that surrounds the individual muscle fiber. The perimysium is a connective tissue layer that surrounds the muscle bundle. The epimysium surrounds muscle bundles to provide support to the whole muscle. The epimysium can be trimmed off prior to consumption and would have a smaller role in cooked meat tenderness. The perimysium and endomysium (intramuscular connective tissue) are integral parts of the muscle/meat and play a larger role in cooked meat tenderness compared to the epimysium.

The effects of intramuscular connective tissue on tenderness have been thoroughly examined. Many different factors that cause variation in connective tissue are related to differences in tenderness. Factors such as muscle, animal age, gender, breed, feeding regime, and aging have been considered when determining how intramuscular connective tissue affects tenderness. Muscles have one of two functions in the body: locomotion or support. Locomotive muscles (i.e. semimembranosus or semitendinosus) are used when any part of the animal needs to move, whether it be the limbs to walk or the jaw to masticate feed. Supportive muscles (i.e. psoas major or longissimus) support the skeleton of the animal. These differences in function also

translate to a difference in amount of connective tissue present. Connective tissue amount, in addition to connective tissue solubility in individual muscles can play a role in cooked meat tenderness for that muscle. Ramsbottom et al. (1945) examined 25 muscles from the carcasses of three different heifers that were graded as U.S. Good. Histological examination of the raw muscles found that there were differing amounts of collagen present in different muscles. The latissimus dorsi had greater amounts of collagen fibers present compared to the psoas major. Histological examination of cooked muscle samples also showed a difference in the collagen fibers present. Collagen fibers present in the cooked muscle samples had indistinct and irregular borders, indicating that collagen fibers were solubilized somewhat by the cooking process. They also determined that muscles with higher amounts of connective tissue were tougher, while those with lower amounts of connective tissue were more tender, indicating that some variation between muscle tenderness scores could be attributed to differences in connective tissue amounts.

As animal age increases, decreases in tenderness also may occur. A portion of this decrease in tenderness has been attributed to differences in intramuscular connective tissue. Herring et al. (1967b) conducted an experiment that addressed factors that affected collagen solubility in bovine muscles. Cattle were selected based upon maturity and assigned to maturity groups (A, B, and E maturity) according to USDA standards. Fifteen longissimus dorsi and semimembranosus muscles (five muscles per maturity group) were collected after a 7 d postmortem aging time. Collagen solubility and collagen content were measured on the muscles while a trained sensory panel measured

tenderness. Collagen content for the longissimus dorsi was not different among maturity groups. In contrast, the collagen content was higher ($P < 0.05$) in the E maturity group compared to the A and B maturity groups for the semimembranosus. Collagen solubility differed for both muscles. As animal age or maturity group increases from A to E, percentage of collagen solubility decreased. Panel tenderness scores were similar for maturity groups A and B, however, carcasses in the E maturity group were tougher, which would indicate that as collagen solubility decreases, so does tenderness of muscles.

Cross et al. (1973) examined differences between muscles and ages with regard to collagen content and solubility. They looked at differences between the biceps femoris, longissimus dorsi, rectus femoris, semimembranosus and semitendinosus. They also examined differences between three different age groups (483.4 days of age; 1376.6 days of age; and 4087.6 days of age). Differences between muscles were found for both collagen content and collagen solubility. Collagen content was highest for the biceps femoris and lowest for the longissimus dorsi and semimembranosus. The only difference found in collagen solubility was that the longissimus dorsi was higher than all other muscles. The longissimus dorsi also was the most tender when compared to all other muscles, which indicated that higher collagen solubility influenced tenderness when comparing different muscles. In regards to age, there was no difference between age groups for whole tissue basis connective tissue amounts. However, there were age differences between the collagen solubility. Cattle from the youngest had much higher collagen solubility compared to the two older groups (10.66% vs 3.45% and 2.21%,

respectively). These differences were somewhat reflected in the shear force values. The youngest group had lower shear force values compared to the oldest group. They determined that collagen solubility was closely related to tenderness differences and that variation in tenderness between different muscles could be attributed to differences in collagen content and solubility differences.

Results from both Herring et al. (1967b) and Cross et al. (1973) indicated that as animal age increased, the solubility of collagen was reduced, due to greater ketoamine cross-linkages. In addition, Ramsbottom et al. (1945), Cross et al. (1973) and Dutson et al. (1976) have shown that different muscles have differing amounts of collagen that result in differences in tenderness. Others also have shown that as animal age increased, the amount of soluble collagen decreased and is one of the causes of meat from older animals to be tougher (Goll, et al., 1964; Hill, 1966; Cross, et al., 1984; Shorthose and Harris, 1990).

Cross et al. (1984) examined how gender and breed affected collagen content and solubility. Twenty bulls and steers representing four breeds (7/8 Charolais, 7/8 Simmental, Hereford, and Angus) were used to determine the effects of gender and breed. They determined that collagen solubility was affected by breed ($P < 0.05$) and gender ($P < 0.10$), with no interaction between breed and gender. Total amount of collagen was affected by gender ($P < 0.01$). They determined that bulls had higher amounts of total and soluble collagen compared to steers. In regards to breed differences, Simmental also had the highest percent of soluble collagen compared to all other breeds. They determined that earlier maturing breeds (Hereford) had lower collagen solubility compared to later

maturing breeds (Charolais) when compared at the same chronological age. Differences found in soluble collagen within gender were hypothesized to be related to testosterone levels, which also were affected by breed ($P < 0.05$).

Another aspect that affects collagen solubility is plane of nutrition prior to slaughter. Aberle et al. (1981) examined the effects of high-energy diet on muscle characteristics. They found that in cattle that had higher planes of nutrition prior to slaughter increased tenderness in comparison to cattle fed a low-energy diet. The conclusion was that those animals that had rapid growth prior to slaughter had not only a higher rate of protein synthesis, but also a higher rate of collagen synthesis. Higher collagen synthesis rates would result in a higher proportion of immature collagen that could positively affect tenderness. Others found similar results when studying the effect of nutrition on collagen solubility and tenderness (Miller et al., 1983; Miller et al., 1987).

Intramuscular connective tissue is an important component when trying to explain variation in tenderness across a wide range in animal age or among different muscles. Although there is not a large difference in the amount of connective tissue between genders or breeds, differences may be large enough to contribute to the differences in tenderness that are seen. Muscles that are responsible for repetitive motions such as walking or chewing have a higher percentage of intramuscular connective tissue present to accomplish movement, compared to support muscles which explains some of the variation in tenderness among these muscles. Finally, as animal age increases, the increase in amounts of heat-stable crosslinks has an important role in the decrease in tenderness that has been observed.

2.1.3 Postmortem proteolysis

Postmortem proteolysis of structural proteins has been determined to be one of the main causes for the increase in tenderness postmortem (Koochmaraie and Geesink, 2006). Meat can remain either as on the carcass, or cut into primals, subprimals or steaks and be “aged” by storage at refrigeration temperatures for a certain amount of time. Aging can be achieved by two methods: wet aging or dry aging. Wet aging means placing a cut of meat in a vacuum package, and then storing at refrigeration temperature for some amount of time. Wet aging is the most conventional method of aging due to ease and product loss is minimized in comparison to dry aging. In contrast, dry aging is typically conducted in wholesale cuts such as ribs or short loins. These unpackaged cuts are placed in an area specifically designed for dry aging with controlled temperature, relative humidity and air velocity and allowed to “age” for approximately 28 days. During the dry aging time, the outside surfaces dry out and become moldy due to exposure to air and high humidity and must be trimmed away at the conclusion of the aging period. Dry aging is used less often mainly due to cost. Cost of dry aging has a large overhead cost due to maintenance of facility, amount of product needed to keep on hand that is aging and the amount of loss due to moisture loss or trimming. High-temperature conditioning is a variation of aging that was researched. Temperatures during the aging process were elevated. Researchers determined that high-temperature conditioning increased tenderness (Fields et al., 1976; Olson et al., 1977), however food safety concerns made use of high temperature conditioning not viable.

During either type of aging, postmortem proteolysis of myofibrillar proteins occurs. The breakdown of structural proteins makes shearing through the muscle fibers easier and therefore would increase tenderness. Davey and Gilbert (1969) noted that there were disruptions in the Z-disk that led to structural weakness of myofibrils and increased tenderness, indicating a breakdown of muscle structural proteins. Koohmaraie (1992) reviewed the key changes that occur during postmortem proteolysis to muscle structure. These differences include: Z-disk weakening/degradation that resulted in higher amounts of fragmentation of myofibrils; disappearance of troponin-t, desmin, nebulin and titin (which all resulted in increased fragmentation of myofibrils); and the appearance of a 95,000 kDa polypeptide (did not have a known origin other than breakdown of a myofibrillar protein with a weight greater than 95,000 kDa).

Multiple causes have been hypothesized and researched to determine the cause of breakdown of myofibrillar proteins during postmortem aging. Lysosomal enzymes (cathepsins), calcium-dependent proteases (calpains) and caspases have all been investigated for their roles in postmortem tenderization of meat. Requirements were determined by Goll et al. (1983) and Koohmaraie (1988) in order for a system to be viable to explain differences in postmortem proteolysis of proteins. These requirements were that the proteinases had to be present in the muscle tissue, have access to the substrate needed to activate the proteinase and the production of similar degradation patterns found in aged muscle samples. Cathepsins are acidic enzymes that are stored in the lysosome and were purposed to aid in postmortem tenderization (Calkins and Seideman, 1988); however, cathepsins have not been shown to be released from the

lysosomes postmortem or do not affect postmortem proteolysis (Koohmaraie et al., 1988a,b; 1992). The targets of cathepsins are the contractile proteins, actin and myosin. Koohmaraie (1992) found no degradation in actin or myosin after 56 days of aging. These results indicated that cathepsins were not involved in postmortem tenderization.

Calcium-dependant proteases were found to be the primary cause for the structural protein degradation that caused the increase in postmortem tenderization (Olson et al., 1977; Koohmaraie et al., 1986, 1988abc, 1991b; Koohmaraie, 1994, 1996; Koohmaraie and Geesink, 2006). These calcium-dependant proteases are known as calpains. Calpains were classified according to the amount of calcium required for activations: m-calpain (mM amount of calcium needed) and μ -calpain (μ M amount of calcium needed). Calpains meet the requirements for being considered for postmortem proteolysis related to tenderization (Koohmaraie, 1992). Calpains cause the breakdown in structural proteins, especially those related to the area of the Z-disk. Calpains also have an endogenous inhibitor, calpastatin. Calpastatin regulates calpain activity. Instances where calpastatin was increased typically resulted in lower postmortem proteolysis of proteins and a decrease in tenderness (Wheeler and Koohmaraie, 1992; Koohmaraie et al., 1995). Calpastatin has been shown to be sensitive to freezing temperatures (Koohmaraie, 1990), providing a possible method to promote postmortem proteolysis by lowering calpastatin levels.

There is variation in the amount and rate of postmortem proteolysis that occurs in meat. The ratio of calpain to calpastatin has been shown to regulate not only the rate of proteolysis, but the extent of proteolysis as well (Geesink and Koohmaraie, 1999).

Differences in calpain and calpastatin levels or calpain/calpastatin ratio have been shown to differ across species (Koochmaraie et al., 1991c), breed (Whipple et al., 1990; Shackelford et al., 1991,1994), muscle (Koochmaraie et al., 1988c) and with the use of beta-adrenergic agonists (β -agonists; Wheeler and Koochmaraie, 1992; Pringle et al., 1993). The callipyge genetic condition in lambs also caused differences in postmortem proteolysis. Lambs that exhibit the callipyge condition have much higher calpastatin levels compared to normal lambs (Koochmaraie et al., 1995; Geesink and Koochmaraie, 1999) resulting in decreased tenderness throughout postmortem aging. Many different factors are involved in the amount of postmortem proteolysis that occurs. Maximization of the calpain system is one step towards improving overall tenderness.

2.1.4 Marbling

Intramuscular fat, known as marbling, is another factor that could influence tenderness. Intramuscular fat is normally the last depot of fat to be deposited and can vary across muscles, breeds, genders and planes of nutrition. In addition, the use of growth promotants could influence deposition of intramuscular fat. Historically, emphasis has been put upon the amount of marbling in the longissimus at the 12th rib cross-section of beef carcasses in the assignment of USDA quality grades. However, higher quality grades may not always translate into a desirable rating for tenderness or shear force (Smith et al., 1987). Smith and Carpenter (1974) developed four theories to aid in the explanation of how intramuscular fat could aid in tenderness. The theories are commonly named the bite theory, the strain theory, the lubrication theory and the insurance theory. The bite theory indicated that with deposition of more intramuscular

fat, the lower the bulk density of the sample would be. Lowering the bulk density in addition to fat being less resistant to shearing could result in a perceived or possibly real increase in tenderness. The strain theory utilized the knowledge that intramuscular fat is deposited in the perivascular cells inside the walls of the perimysium or endomysium. Deposition of fat in these areas was thought to put strain on connective tissue, decreasing the strength of connective tissue and thus decreasing the resistance to shearing or chewing by connective tissue. The lubrication theory indicated that with an increase in intramuscular fat surrounding the muscle fibers, there was a lubricating effect. They hypothesized that with an increase in the juiciness, there also could be a perceived increase in tenderness. The insurance theory hypothesized that increased intramuscular fat would allow for cooking to a higher endpoint temperature without causing adverse effects on palatability including tenderness. The theories presented by Smith and Carpenter (1974) indicated that with increased intramuscular fat, decreases in tenderness could be mitigated. However, research has been mixed on the effect that intramuscular fat has upon tenderness. Some researchers have found low to moderate correlations between intramuscular fat and tenderness, while other have found no correlation between the two.

Cover et al. (1956) conducted research to determine the relationship of fatness in yearling steers to tenderness using braising and broiling cookery methods. They used loin and bottom round steaks in the evaluations. They found a low, but significant correlation between amount of estimated marbling and tenderness scores of panelists for the bottom round steaks, however there was not a significant correlation for the loin

steaks. Shear force measurements also were not correlated to marbling except for braised bottom round steaks. Tuma et al. (1962) also conducted research to determine the influence that marbling and animal age had on tenderness and flavor. Animals were classified in three age categories: 18 months, 42 months and 90 months of age. They also were divided into two marbling categories: slight marbling and slightly abundant marbling. Tenderness was measured using shear force and trained sensory panel evaluation. They determined that amount of marbling did not affect trained sensory panel scores for tenderness, however, shear force values were lower ($P < 0.005$) for animals that were classified having slightly abundant marbling. There was an interaction between animal age and marbling level. Animals that were 18 months old did not differ in tenderness between marbling scores, whereas the 42 and 90 month old animals with slightly abundant marbling were more tender compared to the slight marbling animals of the same age. Results from this study indicated that in animals of typical slaughter age (18-24 months of age), marbling does not have an influence on tenderness rating of panelists or shear force, but may play a larger role in tenderness of older animals. Parrish et al. (1973) conducted an experiment to determine if the amount of marbling affected palatability scores or shear force measurements. In addition, they also conducted research to determine if there was an interaction between amount of marbling and endpoint cooking temperature on palatability scores. Results from their experiment indicated that there was no difference in tenderness scores or shear force values when comparing slight, modest and moderately abundant marbling scores. They also determined that higher amounts of marbling could not offset the decrease in palatability

that was caused by increased endpoint internal temperature. Other researchers have found that marbling does not have a significant role in the tenderness of meat or there are no differences in tenderness between marbling groups (Berry et al., 1974; Carpenter et al., 1972; Dryden and Maechello, 1970; Garcia-de-Siles et al., 1977; Jeremiah, 1996; Parrish et al., 1973, 1979; Romans et al., 1965; Walter et al., 1965;), with many attributing variation in tenderness due to other factors.

Wheeler et al. (1994) examined the effect of marbling on tenderness in *Bos taurus* and *Bos indicus* cattle. Within *Bos taurus* cattle, there was an improvement in tenderness measured by shear force as marbling increased from Traces to Slight and Slight to Small. However, there was no difference in tenderness found between Small, Modest and Moderate amounts of marbling within *Bos taurus*. In *Bos indicus* influenced cattle there also was improvement as marbling increased from Trace to Slight and Slight to Small amounts of marbling. Tenderness measured by trained sensory panelists found similar trends in both *Bos taurus* and *Bos indicus*. Steaks with Traces and Slight marbling were less tender than steaks with to Small, Modest, or Moderate levels of marbling, *Bos taurus* cattle. In *Bos indicus* cattle, as marbling increased from Traces to Slight or Small, there was also an increase in tenderness. They determined that overall, there was a small, positive association of marbling score with tenderness, although marbling accounted for a maximum of 5% of the variation in tenderness. They also concluded that there would need to be another method used to estimate tenderness in conjunction with marbling to better segregate carcasses based upon palatability.

Smith et al. (1984) compared differences in USDA marbling groups within differing carcass maturity groups. Within the A and A+B maturity groups, longissimus tenderness scores from trained sensory panelists increased linearly as marbling increased with differences found when comparing practically devoid, traces, slight and small to modest, moderate, slightly abundant and abundant marbling. In comparison, marbling score was not different among any levels in the semimembranosus muscle for the same carcasses. Shear force values followed the same trend as trained sensory tenderness evaluation for both muscles. Shear force was also measured on biceps femoris and semitendinosus steaks. There were no differences in shear force between marbling groups for the biceps femoris. Shear force values of the semitendinosus were mostly the same among marbling level comparisons, however steaks with slightly abundant and modest marbling had lower shear force compared to traces marbling. For loin steaks in the A and A+B maturity groups, differences in marbling explained 34.3% and 32.7% of the variability of overall palatability scores. As age increased, this percentage decreased, indicating there were other factors affecting tenderness. The amount of variation in overall palatability for the other muscles tested was much lower and marbling was determined to be of limited value when determining the tenderness of the semimembranosus, semitendinosus and biceps femoris.

Neeley et al. (1998) conducted the Beef Customer Satisfaction survey. The survey examined moderate-to-heavy beef users preferences for overall-like, tenderness, juiciness, flavor desirability and flavor intensity across USDA quality grades, cut and city. Tenderness responses had two main effect interactions: USDA quality grade X city

and cut X city. Consumers in Chicago and Philadelphia were determined to be “US Choice” cities, while Houston and San Francisco were determined to be “US Select” cities. Consumers from Chicago and Philadelphia found Top Choice steaks to be more tender ($P < 0.05$) compared to the other quality grades. Houston and San Francisco consumers did not perceive a difference between any of the USDA quality grades. The authors were unable to determine whether the lack of difference perceived by Houston and San Francisco consumers were based on preference or learned behavior. Behrens et al. (2005) also conducted a Beef Customer Satisfaction survey on top round steaks. They found that quality grade significantly affected the tenderness evaluation of samples. Top Choice samples were rated more tender ($P < 0.05$) compared to High Select by consumers. Both of these surveys indicated that with in certain consumers, amount of marbling or quality grade does affect the perception of tenderness, with improved tenderness scores related to higher quality grades.

Nishimura et al. (1999) hypothesized that during the deposition of intramuscular fat, structural weakening of the endomysium and perimysium occurred and resulted in an increase in tenderness in Japanese black cattle. Through scanning electron micrographs, differences in the connective tissue organization were examined. Samples from Japanese cattle that were highly marbled revealed that when intramuscular fat was deposited between the endomysium and the muscle fiber that the endomysium was being damaged. Shear force was not measured on cooked meat samples, but rather was measured on the connective tissue alone in longissimus and semitendinosus muscles with a method developed by Nishimura et al. (1998). In the longissimus, the connective

tissue shear force measurement increased until 24 months of age and then decreased. The semitendinosus continued to increase in connective tissue shear force until 32 months of age. These findings support the differences found in the longissimus muscle electron micrographs and the lack of differences in the semitendinosus electron micrographs. Finally, they determined that animals with the propensity to deposit at least 8% intramuscular fat (corresponded to moderate level of marbling in the United States), could cause the damages to the intramuscular connective tissue found in this research and could ultimately have a role in tenderness. Other researchers have found that marbling can have an influence on tenderness, whether it was sensory tenderness or shear force measurements (Campion et al., 1975; Dolezal et al., 1982; Jennings et al., 1978; Jones and Dolezal 1994; Tatum et al., 1980). Those that found a relationship between tenderness and marbling often had low relationships even though they were statistically significant, with few having moderate relationships.

The results of how intramuscular fat affect tenderness are mixed. Although marbling may influence tenderness, these differences could also be attributed to other factors such as connective tissue (amount or heat stability), amount of myofibrillar degradation or contractile state of the sarcomeres in the muscle. Differences in sensory tenderness scores between marbling groups could be the result of halo effects from increases in juiciness as marbling level increased. Although addition of intramuscular fat may not cause an increase in tenderness, it could have aided in the prevention of toughening from cold-shortening. Overall, there is a weak, positive relationship between

marbling and tenderness with large differences in marbling needed to see meaningful differences in tenderness.

There are several factors that affect tenderness in beef. Improvements in a single factor that affected tenderness might not have translated into improvements in tenderness due to another factor. Rhee et al. (2004) concluded that it was not any single factor (sarcomere length, connective tissue or postmortem proteolysis) that affected tenderness, but the combination of factors that affected tenderness was the most important.

2.2 Exogenous factors that affect tenderness

2.2.1 Growth promotants

The use of growth promotants is a common practice in the cattle industry. Growth promotants can be defined as any product that is used to promote growth or growth efficiency in the animal that would otherwise not occur in the absence of the product. Common growth promotants are estrogenic or anabolic steroid-based implants and the feeding of beta-adrenergic agonists (β -agonists). Growth promotants are utilized during the finishing phase of the cattle industry to increase growth and profitability.

There are several different types of implants available to promote growth in cattle. In addition to these different types, there are many different implant strategies that have been implemented based upon the ultimate goal of the producer. Implants commonly are either estrogenic or anabolic steroid-based or a combination of both. Implants that contained trenbolone acetate were of specific concern and hypothesized to have detrimental effects on tenderness (Schneider et al., 2007). Mixed results have been found concerning the effect that growth implants have on tenderness. Some researchers

have determined that there are no differences in shear force for implanted cattle (Barham et al., 2003; Reiling and Johnson, 2003). Others have found that use of implants in various combinations or strategies have a negative impact upon tenderness (Samber et al., 1996; Roeber et al., 2000; Boles et al., 2009). The majority of these studies where implants were detrimental to tenderness involved various amounts of trenbolone acetate, indicating that it is a major component in causing the decrease in tenderness.

Nichols et al. (2002) reviewed the effects that steroid implants had upon tenderness. Nineteen studies were included in the review to determine differences in Warner-Bratzler shear force between implant treatments. Of the 19, three studies found detrimental effects on Warner-Bratzler shear force, two found positive effects on Warner-Bratzler shear force and the remainder had no difference in Warner-Bratzler shear force for implanted versus non-implanted cattle. They further examined differences in taste panel tenderness scores. Thirteen studies were included in the review. Three out of thirteen studies found detrimental effects on tenderness related to implanted cattle. In contrast, two studies found positive effects on tenderness when cattle were implanted with steroid implants. The remaining 8 studies found no difference between implanted and non-implanted cattle for taste panel tenderness.

Increased growth has also occurred when animals are fed β -agonists. β -agonists are agents that when added to feed of animals, results in an increase in protein mass through hypertrophy along with a decrease in fat mass (Mersmann, 1998). In addition to an increase in muscle mass, feed efficiency is also increased (Wheeler and Koohmaraie, 1992; Pringle et al., 1993; Rathmann et al., 2012). However, tenderness has been shown

to be negatively affected by the feeding of β -agonists (Koohmaraie et al., 1996; Gruber et al., 2008; Brooks et al., 2009; Kellermeier et al., 2009; Leheska et al., 2009; Garmyn et al., 2010; Boler et al., 2012; Rathmann et al., 2012; Arp et al., 2013). The increase in muscle mass is most likely linked to higher calpastatin levels and reduced protein turnover. Higher calpastatin levels also most likely cause the decrease in tenderness that is found when β -agonists are used (Kretchmar et al., 1990; Koohmaraie and Shackelford, 1991a; Koohmaraie et al., 1991b; Wheeler and Koohmaraie, 1992; Pringle et al., 1993). Use of β -agonists results in more saleable yield for each animal that consumes it, however, the detrimental effect on tenderness is concerning.

2.2.2 Suspension method/carcass manipulation

Several strategies for stretching muscles more than they would normally be stretched when carcasses are hung by the Achilles tendon have been investigated in order to improve tenderness. Improvement of tenderness by prerigor manipulation such as aitch-bone hanging or cutting certain skeletal restraints would result in muscles with longer sarcomeres when compared to muscles from normally hung carcasses. Consideration of other factors that influence tenderness, such as connective tissue amount would also be imperative to determining if manipulation of the carcass would improve tenderness of specific muscles.

Herring et al. (1965a) found that when a carcass was restrained in place with the limbs perpendicular to the body (normal walking position) prior to rigor mortis, there were differences in sarcomere length. The psoas major, latissimus dorsi, infraspinatus, rectus femoris, and triceps brachii were all shortened, while the semimembranosus,

longissimus dorsi, semitendinosus, adductor, biceps femoris and gluteus medius were stretched. For muscles that were shortened, fiber diameter was also larger whereas those that were lengthened were smaller. The psoas major, latissimus dorsi and rectus femoris had the largest increases in shear force due to the shortening of the sarcomeres. Other muscles that were shortened did not have as large of differences in shear force values. The longissimus dorsi, semitendinosus, adductor, biceps femoris and gluteus medius had large reductions in shear force values when muscles were stretched by changing carcass position prerigor.

Smith et al. (1971) investigated different prerigor treatments to determine their effects on tenderness. These treatments included suspension via the obturator foramen, severing of the spinal vertebrae in various places and a combination of both. Severance of the vertebral column resulted in steaks with lower shear force values and higher sensory tenderness ratings for steaks from the neck, chuck and loin. Suspension of the carcass from the obturator foramen in combination with severance of the vertebral column resulted in the largest increase in tenderness for the neck, chuck and loin.

Carcass suspension via the obturator foramen was named the Tenderstretch. The Tenderstretch, although increased tenderness, was not widely adopted by the meat industry due to the need for increased cooler space and the change in shaped of some cuts, which made it not efficient for the meat industry. One company has implemented the technique of severing the vertebral column in their facilities with a cut being made at the 7th lumbar vertebrae prior to rigor mortis. The cut in the vertebrae occurs where the 12th rib surface will eventually be exposed for carcass grading. The cut at the 7th lumbar

vertebrae was a cut that was going to occur at/or before grading anyway, therefore it was easily implemented.

Carcass manipulation of any kind would currently require the retraining of employees that fabricate carcasses. In order for carcass manipulation to improve tenderness to be a viable option for the meat industry, the increase in tenderness would have to be worth the cost of teaching employees new fabrication techniques and any other expenses that would be incurred due to the changes and make a profit. In addition, carcass manipulation has been shown to stretch sarcomere lengths, however, muscles of high connective tissue may not improve in sensory tenderness even with significant sarcomere stretching.

2.2.3 Electrical stimulation

Electrical stimulation was developed as a method to mitigate the effects of cold-shortening or thaw shortening (Chrstall and Hagyard, 1976). The theory behind electrical stimulation was to find a way to greatly diminish the amount of ATP present in the muscle prior to the onset of rigor mortis through acceleration of glycolysis. Two types of electrical stimulation were developed: low-voltage and high-voltage. Low voltage stimulation is widely used in most harvesting facilities with few using high-voltage. Low-voltage electrical stimulation does minimal if any structural damage to the carcass. In comparison, high-voltage electrical stimulation causes tearing in the muscle and the possibility of bones breaking in the carcass (Takahashi et al., 1984).

Carse (1973) determined that the use of electrical stimulation could accelerate the decline in pH and therefore the rate of glycolysis postmortem. This was believed to aid

in the prevention of cold-shortening and thaw shortening and therefore increase tenderness. He found that as the voltage of electrical stimulation increased, the amount of time it took lamb carcasses to decline to a pH of 6.0 decreased. Others continued research to determine how much electrical stimulation affected tenderness. Chrystall and Hagyard (1975) examined how high-voltage electrical stimulation affected tenderness in lambs. Carcasses were stimulated using 3600 volts for a total of 55 seconds using pulses of electricity. They measured the rate of postmortem glycolysis using pH decline in the loin of the carcasses. They found that stimulation of carcasses caused a marked acceleration of glycolysis. Carcass were then frozen 1 hr postmortem after measurement of stiffness in the leg. Steaks were removed from frozen carcasses and cooked from the frozen state. They found that muscles from the loin and leg of electrically stimulated lambs were markedly more tender in comparison to unstimulated lambs. They determined that the increase in glycolysis also prevented cold-shortening or thaw shortening as both of these conditions were absent from the steaks of stimulated carcasses that were cooked from the frozen state. They found no other quality attributes were affected by electrical stimulation and suggested that research into electrical stimulation on beef carcasses should be examined.

Davey et al. (1976) studied the effect of high-voltage electrical stimulation of beef carcasses on tenderness. At 30 min postmortem, right sides of carcasses were stimulated from 30 sec to 10 min at 3600 volts, with left sides being unstimulated controls. Beef carcasses were chilled for 24 hrs at 10°C prior to boning and cutting steaks. Half of the steaks were frozen while the remainder were left to age for 2 days.

Much like Chrystall and Hagyard (1976), postmortem glycolysis was accelerated as measured by pH decline. Carcasses that only received 30 seconds of stimulation had faster rates compared to unstimulated carcasses, but those stimulated for longer periods of time had a much faster decline in pH, indicating the need for at least 1 minute of stimulation to have a significant impact on increasing the rate of glycolysis postmortem. Ultimate pH was not affected by electrical stimulation. Shear force measurements in the longissimus dorsi were lower when comparing the frozen or 2 d aged steaks. In addition, they noted that variation in tenderness was much lower in samples from electrically stimulated carcasses compared to the unstimulated carcasses. They also found that electrically stimulated carcasses received higher sensory tenderness rating for either frozen or 2 d aged samples.

Savell et al. (1977) examined the effect that a commercially available 100-volt electrical stimulator would have on tenderness in beef, lamb and goat carcasses. They determined that electrical stimulation at this intensity decreased shear force and increased sensory flavor ratings for the loin in beef carcasses. Steaks from the shoulder, loin and leg were tested for lamb and goat carcasses. Electrical stimulation decreased shear force in the loins, but was less effective in the leg and shoulder steaks. They concluded that low-voltage electrical stimulation could increase tenderness in certain muscles of all three species and also improve flavor scores in beef. Savell et al. (1978b) further researched the effect on beef tenderness by electrical stimulation including aging time. They found that at 7 d of aging, there was no difference between control and electrically stimulated samples for shear force or sensory tenderness, flavor or overall

palatability. However at 21 days of aging, electrically stimulated samples had lower shear force values, but no differences in sensory tenderness, flavor or overall palatability. In comparing 21 d aged control steaks with 7 day aged electrically stimulated steaks, electrically stimulated samples had lower shear force values. This result indicates that with the addition of electrical stimulation, aging time could be reduced, making movement of product quicker without risking tenderness. Others have also found positive relationships between electrical stimulation and tenderness (Savell et al., 1978a; Savell et al., 1979; Riley et al., 1980; McKeith et al., 1981; Savell et al., 1981; Uytterhaegen et al., 1992; Geesink et al., 2001; Hwang and Thompson, 2001; King et al., 2004), depending upon how it was applied.

2.2.4 Blade tenderization

Blade tenderization is a method in which wholesale cuts or steaks are tenderized using small sharp blades to cause physical disruption to muscle fibers and connective tissue. Cuts that are inherently tough due to various factors can be blade tenderized to improve tenderness. Blade tenderization has also been used on rib, loin and sirloin cuts to minimize variation in tenderness and provide a more uniform, tender cut to restaurants. Seideman et al. (1977) used blade tenderization in an attempt to make a tougher cut (semitendinosus) comparable in tenderness to a tender cut (psoas major). They found that blade tenderizing the semitendinosus two or three times resulted in shear force values that did not differ from untenderized psoas major. However, this did not translate to sensory tenderness scores, indicating that cuts with higher connective tissue or inherently tough meat that has been blade tenderized may not be

interchangeable with normally tender cuts. Tatum et al. (1978) examined the effects of blade tenderization on steer, cow and bull carcasses to determine if toughness related to increased age can be mitigated for the biceps femoris, semimembranosus and longissimus muscles. They determined that blade tenderizing steaks from cows and bulls would not result in steaks that were as tender as steaks from steers. Others have shown the positive effect between blade tenderization and tenderness of steaks from young animals (Bowling et al., 1976; Davis et al., 1977; Hayward et al., 1980; Benito-Delgado et al., 1994; Jeremiah et al., 1999; King et al., 2009).

Although improvement in tenderness occurred when meat was blade tenderized, blade tenderization introduces the opportunity for bacteria that are present on the blades or surface of the subprimal to be introduced into the middle of the muscle. Muscle that has not been penetrated is sterile and can be cooked to a lesser degree of doneness without food safety risks. Outside cut surfaces where bacterial contamination is likely heated to high enough temperatures to kill surface bacteria. Steaks cooked to a lesser degree of doneness that have also been blade tenderized could pose a food safety risk if bacteria are present on the inside of the muscles. Methods to mitigate bacteria on the outside of meat that is to be blade tenderized and proper sanitation of blade tenderizing equipment is essential to prevent food-borne illness.

2.3 Factors that influence dark-cutting beef

Dark-cutting beef is a condition that is caused by long-term physiological stress prior to slaughter. During antemortem stress, storages of glycogen in the muscle are greatly reduced. Postmortem anaerobic glycolysis relies upon storages of glycogen in

the muscles in an attempt to maintain homeostasis in the cells. The by-product of anaerobic glycolysis in skeletal muscle is lactic acid. Build up of lactic acid is the cause of normal postmortem pH decline in muscle. In muscles where there is reduced glycogen, less lactic acid is produced, which results in a smaller decline in pH postmortem, resulting in high ultimate pH and dark-cutting in beef. The degree or severity of dark-cutting is proportional to the reduction in glycogen in the muscle and can vary in color from a slightly darker red color to almost black.

Lawrie (1958) wrote a review paper concerning physiological stress in relation to dark-cutting beef. Considering previous research, he determined that dark-cutting in cattle was due to higher ultimate pH versus having higher concentrations of myoglobin. Lawrie (1958) furthered the research to examine factors that could affect the ultimate pH of an animal. Methods that would cause reduction of muscle glycogen were said to be fasting, exhausting exercise and training, all of which could be considered stress. Stress in animals can be induced by other factors than those listed by Lawrie (1958). The other factors that have been researched are: animal handling, management practices (including the use of growth promotants and construction of facilities), climate (in relation to seasonal spikes in dark-cutting) and genetics. Steps to minimize some of the above stresses have aided in the reduction of dark-cutting.

2.3.1 Animal influence on dark-cutting

The response to stress is different between animals, with little knowledge of how a specific animal will respond to stress. Wulf et al. (2002) conducted research to determine the relationship between glycolytic potential and dark-cutting as evaluated by

ultimate pH. When comparing the ultimate pH of the longissimus muscle with glycolytic potential, a curvilinear relationship was found. The results showed that animals with a glycolytic potential of below 100 $\mu\text{mol/g}$ were associated with higher ultimate pH, while those animals with higher than 100 $\mu\text{mol/g}$ had no effect on ultimate pH. This relationship was most likely due to animals having differing amount of initial glycogen stored in the muscle. For animals with higher amounts of glycogen present naturally, stress does not reduce glycogen levels to the point of causing dark-cutting. However, animals with lower amounts of glycogen when exposed to the same stress will result in dark-cutting due to the glycolytic potential falling below the threshold. This animal-to-animal variation in glycolytic potential can assist in explaining why animals from the same pen, exposed to the same stress are dark-cutters while others are not.

2.3.2 Animal handling and management

The minimization of stress as a result of good animal handling practices has been shown to reduce the incidence of dark-cutting in cattle. Animal handling includes how the animals are handled at the farm/feedlot, transportation to the packing plant and handling of cattle from the time they are unloaded to slaughter. During all of these times, implementation of good animal handling practices can aid in the reduction of the incidence of dark-cutters. Proper training of employees in handling practices is an ongoing process. Warriss (1990) reviewed proper movement of animals by humans in regards to prevention of stress. He emphasized that moving cattle in larger groups were easier than moving an individual animal due to herd instinct. In addition, the employee should place themselves at approximately a 45-60° angle from a line from the shoulder

of the animal or the shoulder of the lead animal in a group, making it easier to move the animals.

Another handling practice that resulted in increased dark-cutters is mixing of pens. Cattle are known to have a hierarchical system of dominant animals. During the social regrouping of cattle pens, the rearrangement of the hierarchy results in cattle that display different behaviors (mounting, head butting and chin resting.) while trying to establish the new hierarchy. During these behaviors, large amounts of energy (glycogen in the muscle) are expended. When given enough time to rest after mixing, the opportunity to restore muscle glycogen may prevent dark-cutting. Kenny and Tarrant (1987) demonstrated that the amount of mounting and other behaviors that occurred during mixing of animals was minimized when an electric grid (grid made up of electrically-charged wires) was placed over the pens and effectively reduced the amount of dark cutting. Work by McVeigh et al. (1982) supports the findings that when new animals are mixed that behavioral stresses cause decreases in muscle glycogen.

Management practices have been identified as one of the sources where increases in the occurrence of dark-cutting beef have occurred. Management practices can include the use of implants or the addition of beta-adrenergic agonists. Scanga et al. (1998) examined the type of implant used and implant strategies on the incidence of dark-cutting in beef. They found that steers implanted and reimplanted with combination types (contained androgen and estrogen) of implants were higher in percentage of dark-cutters and had a greater number of pens with 6% or higher incidence of dark-cutting. Results were mixed for heifers with the highest numerical dark-cutters occurring when

first implant was androgen-based and reimplant was estrogen-based. In regards to implant strategy, they found that cattle re-implanted less than 100 d prior to harvest had a higher percentage of dark-cutting per pen with the exception of steers re-implanted with androgen-based implants and heifers re-implanted with estrogen-based implants. Control of type and strategy in regards to implants can be a method to minimize dark-cutting in cattle.

Tarrant (1989) suggested that the addition of β -agonists also have the potential to increase incidence of dark-cutting. Using the knowledge that β -agonists cause breakdown of glycogen during protein synthesis, he hypothesized that eventually, aggressive use of β -agonists in addition to the normal stresses from handling would result in an increase in dark-cutting cattle, although this has not been confirmed by research.

2.3.3 Weather

Dramatic changes in weather conditions have been shown to cause an increase in the incidence of dark cutting. During the year, season or time of year affects the amount of dark cutting that occurs. Times that included warmer days and colder nights (spring and fall) have been shown to have higher instances of dark-cutting cattle (Tarrant and Sherington, 1980). The cause of this is shivering (Lawrie, 1958; Tarrant, 1989). Shivering requires a great amount of energy from the muscles, reducing the amount of glycogen present prior to slaughter. In addition to shivering, other weather patterns such as thunderstorms or extreme heat, where animals are stressed for extended amount of time could also increase the incidence of dark-cutting in cattle.

2.4 Effects of dark-cutting on tenderness and flavor

2.4.1 Tenderness

Tenderness has been shown to vary across pH levels. In a review by Hedrick (1965) increased ultimate pH was related with an increase in tenderness, although the mechanism was not discussed. In contrast, many studies show a curvilinear relationship, where peak toughness occurred when the ultimate pH of the longissimus muscle was 6.0 (Purchas, 1990). Dransfield (1981) indicated that differences in tenderness depended mainly upon variations in myofibrillar contractile state, connective tissue proteins and water and their interactions with cooking conditions. They discussed that higher pH increased water holding capacity and therefore was partially responsible for increasing tenderness. The mechanism they believed to be responsible for the increase in tenderness was a more open muscle structure. Bouton et al. (1972a) also demonstrated that when ultimate pH was increased, there was an increase in water holding capacity, which is likely related to differences in tenderness. Dutson (1983) indicated that at higher pH, there were increases in degradation of Z-lines, possibly leading to increases in tenderness. He also determined that troponin-t degradation was greater in higher pH samples. He hypothesized that differences in degradation could be attributed to increased μ -calpain activity.

2.4.2 Flavor

Little research has been conducted on the flavor of dark-cutting beef due to the lack of consumer demand. The dark colored lean is undesirable to consumers and would most likely not sell in the form of steaks or roasts. Wulf et al. (2002) determined that

steaks from dark-cutting cattle had more “off-flavors” as determined by trained sensory panel analysis. They determined that flavors such as peanutty, sour and bitter were higher in dark-cutters in comparison to controls. Further research on flavors present in dark-cutting cattle is much needed. Yancey et al. (2005) conducted trained sensory analysis of flavor as affected by pH. They found that carcasses with high pH were lower in brown/roasted and sour flavors, higher in rancid flavor and no difference in bloody/serumy or metallic flavors. Differences in flavor were apparent in both studies with higher incidence of “off-flavors” and could result in negative consumer acceptability, but would need further research.

3. MATERIALS AND METHODS

3.1 Carcass selection

Carcass selection occurred in a commercial harvesting facility. The harvesting facility applied low-voltage electrical stimulation to carcasses in order to promote the onset of rigor mortis. Carcasses exhibiting dark colored lean in the ribeye at the 12th rib were selected as they were presented for carcass grading. A complimentary carcass with similar amounts of intramuscular fat and normal lean color was selected from the same commercial lot to serve as a control (n=160, mean pH=5.7). Dark colored lean carcasses were classified in one of the following categories: severe (SEDC, n=40, mean pH=6.9), moderate (MODC, n=40, mean pH=6.6), mild (MIDC, n=40, mean pH=6.4), or shady (SHDC, n=40, mean pH=6.1) dark-cutter (DC) using online pH measurements. Least square means were calculated to determine differences in pH (Table 2). One measurement of online pH for each carcass was conducted using an Omega PHE-2385 (Omega Corporation, Stamford, CT) pH probe, in addition to an Omega PHAT-222 (Omega Corporation, Stamford, CT) temperature probe inserted approximately 1.5 cm in the medial portion of the longissimus lumborum muscle. A two-point (4.0 and 7.0) calibration occurred in buffers that were at the temperature of the cooler prior to selection of carcasses. Probes were calibrated at least every hour if not more often until selection was complete. When probes were not in use, they were stored in 4.0 pH buffer and monitored. During this monitoring, if probes deviated from 4.0, they were recalibrated. Carcass grades for DC and normal cohorts were Certified Angus Beef, U.S. Choice, U.S. Select or U.S. Standard. Distribution of carcasses into Certified

Angus Beef, U.S. Choice, U.S. Select or U.S. Standard grades were 0, 1, 3 and 36 for SEDC; 0, 2, 3, and 35 for MODC; 0, 6, 8 and 26 for MIDC; 0, 6, 16 and 18 for SHDC; and 8, 83, 67 and 2 for NC. Marbling scores were collected using the VGB 2000 camera grading system. Marbling scores ranged from traces to slightly abundant. Distribution of marbling scores for traces, slight, small, modest, moderate and slightly abundant were 0, 12, 14, 7, 5, and 2 for SEDC; 1, 2, 14, 7, 2, and 2 for MODC; 0, 8, 18, 9, 3 and 2 for MIDC; 0, 16, 13, 7, 3, and 1 for SHDC; and 2, 69, 54, 29, 5 and 1 for normal cohorts.

3.2 Sample preparation

Strip loins (IMPS #180) were collected from each left carcass side, vacuum packaged and returned to USMARC. Strip loins were checked to ensure package integrity upon return, and “leakers” were repackaged. Strip loins were placed in a cooler at 2°C, wrapped with black plastic to exclude light and allowed to age to 14 d postmortem. At the conclusion of the aging period, strip loins were cut into sections or 2.54 cm steaks for various analyses including 1 steak for slice shear force (SSF) and 2-6.35 cm sections for trained sensory panel training and evaluation. Sections utilized for trained sensory evaluation and training were vacuumed-packed and frozen at -30°C until the start of sensory evaluation.

3.3 Cooking loss and slice shear force

Fresh, 2.54cm thick longissimus lumborum steaks were equilibrated at 5°C until the internal temperature reached 5°C (approximately 24 hrs). Raw and cooked weights were collected to determine cook loss percentage with the equation of [(raw weight-

cooked weight)/raw weight]*100. Steaks were cooked on a belt grill using a program designed to achieve a final internal temperature of 71°C (Wheeler et al., 1998). After steaks exited the belt grill, a needle thermocouple probe (Type J, (Omega Engineering, Stamford, CT)) was inserted into the geometrical center of the steak and temperature was monitored using a Cole-Parmer handheld thermometer (Cole-Parmer, Vernon Hills, IL) until maximum temperature was reached.

Slice shear force (SSF) values were determined using the USMARC protocol for the longissimus muscle (Shackelford et al., 1999). Immediately after peak temperature and cooked weight were collected, the lateral end of the steak was trimmed off to square up the steak. A second cut was made to obtain a 5 cm section from the lateral end. Using a slice shear force box and a double-bladed knife, a 1 cm thick slice was taken from the 5 cm section parallel to the muscle fibers. This slice was sheared using an Instron machine (Instron Model 4411, Canton, MA) equipped with a slice shear force blade. Maximum force was recorded in kilograms.

3.4 Sarcomere length

Sarcomere length determination was made using the helium-neon laser diffraction method as described by Cross et al. (1981) using the USMARC sarcomere length protocol (Wheeler et al., 2002). Cooked LL half slices after SSF determination were frozen at -30°C until sarcomere length could be measured. In the preparation of samples, the half slices were thawed at 5°C and the outside cooked surfaces were removed from the slices. Six cubes (devoid of connective tissue and fat, with fibers running longitudinally) were cut from each sample (3 from each half slice). The six

cores were placed in 0.2 M sucrose in 0.1 M NaHPO₄ buffer prior to determining sarcomere length (Koolmees, 1986). Fibers were teased apart and placed on a clear glass microscope slide. Six laser diffraction patterns were recorded per cube, on one paper, for a total of 36 sarcomere lengths per sample. Diffraction patterns were scanned into JPEG images and ImagePro (Media Cybernetics, Rockville, MD) software was used to measure the distance between diffraction bands. Sarcomere length was determined

using the equation by Cross et al. (1981) which is $\mu = \frac{0.6328 * D * \sqrt{\frac{T^2}{D} + 1}}{T}$, where D is the distance from specimen to diffraction pattern screen in millimeters (set to 100 mm), T was the spacing between diffraction bands in millimeters.

3.5 Immunoblotting

Analysis of extent of postmortem proteolysis was determined by western blotting to determine the percentage of desmin degradation in each sample (Wheeler, et al., 2002). Cooked longissimus lumborum half slices remaining after SSF (trimmed of exterior cooked surface) were frozen in liquid nitrogen, powdered and stored at -80°C until analysis occurred. Each dark-cutting classification and the matching control were represented on one gel. Order was rotated to block for potential gel location effects. At-death standards from longissimus muscle were used to normalize the data within blots and determine differences in desmin degradation.

3.6 Trained sensory panel

Six highly trained and experienced sensory panelists from USMARC were utilized for descriptive flavor analysis. Panelists were trained on the Beef Flavor

Lexicon developed by Adhikari et al. (2011) and descriptive attributes (tenderness and juiciness) according to the methods described by Cross et al. (1978). The trained panelists completed a two week refresher training and validation at the conclusion of the refresher training to ensure accuracy and precision.

Frozen longissimus sections designated for sensory panel evaluation or training were cut frozen using a bandsaw. After facing each section, 2-2.54cm steaks were collected from each of the sections. Frozen weights were collected in order to determine thaw loss. After weighing, frozen steaks were rinsed with water to remove any particles that could have been deposited from the bandsaw, vacuum packaged and placed back in the freezer at -30°C.

Sensory steaks were cooked in the same manner as for SSF to an endpoint temperature of 71°C. After the outside edges of each steak was trimmed away, the steaks were placed in a plexiglass guide to be cut into 1 cm X 1 cm X steak thickness cubes. For each panelist, cubes were randomly selected and placed in a white paper soufflé cup with a three-digit random code.

Sensory panelists were seated in booths with red lights (with an average candlefoot of 14.5) over head to prevent bias based upon color. Sensory panelists evaluated tenderness and juiciness on an 8-point scale as described by AMSA (1995) (1=extremely tough/extremely dry, 2= very tough/very dry, 3=moderately tough/moderately dry, 4=slightly tough/slightly dry, 5=slightly tender/slightly juicy, 6=moderately tender/moderately juicy, 7=very tender/very juicy, 8=extremely tender/extremely juicy). In addition, panelists were evaluated major and minor flavor

attributes as described by Adhikari et al. (2011) (Major: beef flavor identity, brown/roasted, bloody/serummy, fat-like, metallic, liver-like, green-haylike, umami, overall sweet, sweet, sour, salt, bitter, sour aromatics; Minor: animal hair, barnyard, burnt, rancid, heated oil, chemical, apricot, green-grasslike, asparagus, musty/earthy/hummus, cumin, floral, beet, chocolate/cocoa, spoiled-putrid, dairy, buttery, cooked milk, sour milk/sour dairy, refrigerator stale, soapy and warmed-over) on a point scale (0=none-15=extremely intense) as described in Table 1. In order to evaluate overall sweet and sour aromatics, panelists were required to sniff the samples prior to flavor evaluation.

3.7 Statistical analysis

Slice shear force, sarcomere length, percentage of desmin degradation and trained sensory panel analysis for tenderness, juiciness and descriptive flavor attributes were analyzed using PROC GLIMMIX in SAS (SAS Corp, Cary, NC). Analysis of variance was used with the fixed effect of DC class and random effect of kill date for thaw loss, cook loss, SSF, sarcomere length and percentage of desmin degradation. For trained sensory panel analysis, analysis of variance was used with the fixed effects for the model of DC class and panelist. When panelist was significant ($P < 0.05$), the interaction of dark-cutter classification and panelist was tested. When panelist or panelist by dark-cutter classification was significant, reviews of each trait was conducted to determine if the interaction was meaningful. It was determined that neither the panelist nor panelist by dark-cutter classification interaction was meaningful and it was removed from the model resulting in data being averaged across panelists. Random

effects for the sensory panel analysis were serving order, kill date and sensory day. Least squares means were in SAS using the PDIFF function of SAS with a P-value of $P < 0.05$. Correlation analyses were conducted using PROC CORR function in SAS (SAS Corp, Cary, NC). Relationships between SSF, desmin degradation, sarcomere length, pH and cook loss were examined.

4. RESULTS AND DISCUSSION

4.1 Slice shear force

Means for SSF are present in Table 3. Results for SSF were curvilinear relative to the pH of the dark-cutting groups, with mild DC and shady DC having higher ($P<0.05$) SSF values compared to severe DC, moderate DC and normal cohorts. Many researchers have shown that as ultimate pH increased, differences in tenderness occurred (Miles and Lawrie, 1970; Bouton et al., 1971, 1972a; Jeremiah et al., 1991). Carcasses with intermediate pH (5.9-6.2) were toughest compared to carcasses with either normal pH or pH greater than 6.2 (Bouton et al., 1972b; Freeden et al., 1974; Fjelknerr-Modig and Ruiderus, 1983; Watanabe and Devine, 1996a; Watanabe et al., 1996b; Wulf et al., 2002), although mechanisms to cause differences were not well understood. A possible cause of SSF differences could include increased water-holding capacity. Correlation analysis determined that slice shear force was correlated ($P<0.05$; $R^2=0.12$) although the relationship was weak. Dransfield (1981) and Purchas (1990) indicated that as pH increased, the structure of protein was more open, allowing for more water to be present in the myofibril and less structural components in a cross-section. Less structural components present in a cross-section would require less force to shear through and therefore increase tenderness. However, this theory does not explain the toughening that occurs at intermediate ultimate pH. It is likely that more than one factor contributed to the higher SSF values at intermediate ultimate pH, such as pH and temperature combination needed for sufficient calcium release into the sarcoplasm to optimally activate μ -calpain.

Current variation in tenderness of beef could be partially explained by carcasses that have an intermediate ultimate pH. These carcasses (shady DC and mild DC) often possessed color that was slightly darker, but would likely still be purchased by consumers or utilized by the restaurant/hotel industry. These carcasses are also routinely included in the U.S. Choice or U.S. Select product lines. Determination of the cause of toughening in carcasses with intermediate pH or solutions for it would be a large step towards minimizing the variation in tenderness that occurs in the beef industry.

4.2 Sarcomere length

Means for sarcomere length are presented in Table 4. Sarcomere lengths for all dark cutter classifications were different ($P < 0.05$) when compared to normal cohorts. In this study, as pH increased, sarcomere length decreased ($P < 0.05$). Mean sarcomere length for moderate DC and severe DC were shorter compared to shady DC and normal cohorts. Also, mild DC and shady DC were shorter ($P < 0.05$) compared to normal cohorts. These results agreed with Purchas (1990) in that as pH increased, sarcomere length decreased; however he found that above a pH of 6.3, there was a slight increase in sarcomere length, which was not found in the current study.

Generally, sarcomere length is related to tenderness (Locker, 1960; Herring et al., 1965a, b and 1967; Marsh and Leet, 1966; Hoestetler et al., 1970), where longer sarcomeres tend to result in increased tenderness. However, the differences found among DC classes in sarcomere length were not consistent with the differences in SSF, indicating that another factor other than sarcomere length was most likely the cause of

the differences found in tenderness. Sarcomere length was moderately correlated with slice shear force ($P < 0.01$; $R^2 = -0.31$), where as sarcomere lengths were longer, SSF was lower.

4.3 Postmortem proteolysis

All dark-cutting classes had lower ($P < 0.01$) percentages of desmin degradation compared to normal cohorts (Table 3). Additionally, there were no differences ($P > 0.05$) between dark-cutting classifications in desmin degradation (Table 4). Desmin degradation was also moderately correlated with SSF ($P < 0.01$; $R^2 = -0.46$) and pH ($P < 0.01$; $R^2 = -0.27$), where desmin degradation decreased as SSF and pH increased. Differences in desmin degradation are usually the most important factor to explain differences found in longissimus tenderness. However, the results of this experiment indicated that other factors also were impacting tenderness as measured by SSF. Normal cohorts had a greater ($P < 0.05$) amount of desmin degradation compared to severe DC and moderate DC although there were no differences in SSF. Differences in SSF that occurred between shady DC, mild DC and normal cohorts were due somewhat to differences in desmin degradation. However, differences in SSF between severe DC and moderate DC compared to mild DC and shady DC samples cannot be explained by desmin degradation.

When considering differences in SSF among dark-cutter classes, it is also possible that structural proteins not measured were responsible for the differences in tenderness. Watanabe and Devine (1996a) found a curvilinear relationship in the amount of titin and nebulin that was degraded. Titin and nebulin both had the slowest rate of

degradation at intermediate pH levels and could contribute to the toughness of meat with an intermediate ultimate pH when no difference in desmin degradation was found.

4.4 Trained sensory panel

Trained sensory panel analysis detected differences ($P < 0.05$) for tenderness and juiciness between dark-cutter classes and normal cohorts (Table 3). Severe DC were rated highest ($P < 0.05$) and “moderately tender”, followed by moderate DC, mild DC, normal cohorts and shady DC were rated lowest ($P < 0.05$) and “slightly tough”. Sensory panel tenderness results were very similar to the results of SSF, with SEDC being the most tender and SHDC being the least tender for both. Wulf et al. (2002) found similar results with intermediate pH longissimus samples rated tougher compared to normal pH longissimus samples. Lower tenderness for intermediate pH samples that are regularly included in U.S. Choice and U.S. Select product lines could result in a negative eating experiences. After identifying carcasses with intermediate pH, implementation of a mitigating strategy would be needed to prevent negative tenderness experience for consumers. Possible solutions to mitigate toughness could be the use of extended aging, freezing or blade tenderization. These solutions would cause physical damage to the myofibrils, improving tenderness (Hiner et al., 1945; Seideman et al., 1977; Koohmaraie, 1992; Wantanabe and Devine, 1996).

As severity of DC increased, sensory panel juiciness scores increased. Severe, moderate and mild DC were all juicier ($P < 0.05$) compared to shady DC. Severe and moderate DC were juicier than normal cohorts. These results supported evidence that in meat with higher ultimate pH, water-holding capacity was increased (Dransfield, 1981;

Purchas, 1990). Although differences were found between DC and normal cohorts, means for all DC classes and normal cohorts were “slightly juicy”. In contrast, Wulf et al. (2002) found no difference in sensory panel juiciness scores between normal pH carcasses and intermediate pH carcasses.

Trained descriptive flavor analysis also detected differences ($P < 0.05$) in the following flavor attributes from the 36 flavor attributes evaluated: brown/roasted, fat-like, metallic, overall sweet, sour, salty, rancid, musty/earthy/hummus, heated oil and chemical (Table 5). Samples from severe DC were lower ($P < 0.05$) in brown/roasted flavor compared to mild DC and normal cohorts, which agreed with Yancey et al. (2005). Fat-like flavor increased as ultimate pH increased. Severe DC had the highest fat-like flavor ($P < 0.05$) compared to other DC classes and normal cohorts. Normal cohorts had the lowest fat-like flavor ($P < 0.05$). Normal cohorts had higher ($P < 0.05$) metallic scores compared to moderate DC and severe DC. Also, there were no differences in metallic flavor between DC classes. Wulf et al. (2002) found no differences between intermediate ultimate pH and normal ultimate pH for metallic flavor, which agrees with this work.

Moderate dark-cutters had the highest overall sweet scores (a combination of sweet flavor and aroma), but were not ($P > 0.05$) different from severe DC or shady DC. Severe, moderate and shady dark-cutters were higher ($P < 0.05$) in overall sweet when compared to normal cohorts. Differences in overall sweet could be related to the amount of browning on the outside of the samples. Caramelized sugar products from Maillard reactions likely give off a sweeter aroma and flavor. Visual observation of samples from

dark-cutters indicated they had a greater amount of browning on the surface, although brown/roasted flavor was lower. Sour flavor was lower ($P<0.05$) in dark-cutters compared to normal cohorts as would be expected with higher pHs. Yancey et al. (2005) found that normal pH beef had higher sour flavor scores compared to dark-cutting beef and attributed it to differences in postmortem lactic acid levels or lactic acid producing bacteria in vacuum packaging over longer aging times. Salt flavor was highest ($P<0.05$) in the normal cohorts, mild DC and shady DC compared to moderate DC and severe DC. Lower salt flavor in moderate DC and severe DC compared to cohorts could be a result of dilution of flavor due to higher water-holding capacity (Lawrie and Ledward, 2006).

Wulf et al. (2002) determined that there were more “off-flavors” associated with DC, which was also found in this experiment. Severe and moderate dark-cutters had higher ($P<0.05$) scores for rancid flavor compared to mild DC, shady DC and normal cohorts. Yancey et al (2005) also found higher pH beef were more rancid compared to normal pH beef and determined that higher pH beef was more susceptible to fat oxidation. Heated oil flavor was higher ($P<0.05$) in severe DC compared to moderate DC, shady DC and normal cohorts. The level of detectable heated oil notes was small and likely not of practical significance. Chemical flavor was higher ($P<0.05$) in severe dark-cutters in comparison to normal cohorts. Chemical flavor means were also very small and likely not very meaningful. Musty/earthy/hummus flavor was higher ($P<0.05$) in severe DC and moderate DC compared to mild DC, shady DC and normal cohorts.

Overall, differences in flavor were related to differences in the severity of DC. As severity of DC increased, rancidity and musty/earth/hummus flavors increased as

well as fat-like, metallic and overall sweet. However, as severity of DC increased, brown/roasted, sour and salty flavor intensity decreased. Lawrie and Ledward (2006) indicated that swelling of proteins that occurs with increased ultimate pH could be responsible for the dilution of overall flavor intensity and possibly other specific flavors. Severe and moderate dark-cutters tended to be on the extreme end of differences in flavor. These extreme differences coupled with visually unappealing lean color would likely be undesirable to the consumer.

4.5 Thaw loss and cook loss

Thaw loss for sensory evaluation samples were lower ($P<0.05$) for severe DC and moderate DC compared to shady DC and normal cohorts (Table 6). There were no differences for thaw loss for sensory samples between moderate DC and mild DC or between shady DC and normal cohorts. Cook loss was lower ($P<0.05$) for all DC classes when compared to normal cohorts for slice shear force and sensory evaluation samples. Mean cook loss decreased linearly as pH increased (Table 6), with severe DC having the lowest ($P<0.05$) cook loss. Normal cohorts had the highest ($P<0.05$) cook loss. Cook loss for SSF and sensory evaluation samples were highly negatively correlated ($P<0.01$; $R^2=-0.85$ and $R^2=-0.82$, respectively), indicating that as pH increased, cook loss decreased. These results are in agreement with McClain and Mullins (1969) and Bouton et al. (1971, 1972a), where higher pH resulted in lower cook loss; however they disagree with those of Purchas and Aungsupakorn (1993), which found no difference in cook loss between bulls and steers with differing pH. In meat with higher pH, it has been determined that the protein structure is more open (Dransfield, 1981 and Purchas, 1990),

resulting in a greater amounts water to be present in the myofibril and less structural component present. Lower SSF values from severe and moderate DC are partially explained by this; however, higher SSF values from intermediate pH are not explained by thaw or cook loss. In addition, juiciness scores also were reflective of differences found in thaw loss and cook loss. Severe DC had the lowest thaw loss and cook loss and highest ($P<0.05$) juiciness scores.

5. CONCLUSION

In summary, differences found in tenderness, juiciness and flavor were dependent upon the severity of dark-cutting. Tenderness was lowest at intermediate pH as determined by SSF and trained sensory panel tenderness scores. Segregation of these carcasses would add the ability to implement other techniques, like extended aging, freezing or blade tenderization, to resolve problems with tenderness. Although significant differences were detected in juiciness, means for DC classes and normal cohorts were rated as “slightly juicy” and would most likely not impact palatability as much as tenderness and flavor would. Differences in undesirable flavor notes found across dark-cutting classes were mostly in severe and moderate DC, which likely do not reach retail outlets. The decreased tenderness of shady DC that end up in U. S. Choice and Select products could be causing negative consumer satisfaction.

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APPENDIX A

Table 1. Definition and reference standards for beef descriptive flavor and aromatic attributes¹

Sensory	Attribute Definition	Reference, standard flavor scale value unless otherwise defined
Animal Hair	Aromatic perceived when raw wool is saturated with water	Caproic acid (Hexanoic acid)=12.0
Apricot	Fruity aromatics that can be described as specifically apricot	Sunsweet dried apricot=7.5
Asparagus	Slightly brown, slightly earthy green aromatics associated with cooked asparagus	Asparagus water=6.5
Barnyard	Combination of pungent, slightly sour, hay-like aromatics associated with farm animals and the inside of a horn	White pepper in water=4.0
Beef identity	Amount of beef flavor identity in the sample	Beef brisket=4.0 Swanson' beef broth=5.0 80% lean ground beef=7.0
Beet	Dark, damp-musty-earthly note associated with canned red beets	Beet water=4.0
Bitter	Fundamental taste factor associated with a caffeine solution	0.05% Caffeine solution=2.0 0.08% Caffeine solution=5.0 0.15% Caffeine solution=10 0.20% Caffeine solution=15
Bloody/serumy	Aromatic associated with blood on cooked meat products. Closely related to metallic aromatic	USDA Choice strip steak=5.5 Beef brisket=6.0
Brown/Roasted	Round, full aromatic generally associated with beef suet that has been broiled	Beef suet=8.0 80% lean ground beef=10.0
Burnt	Sharp/acrid flavor note associated with over roasted beef muscle, something over baked or excessively browned in oil	Alf's red wheat puffs=5.0
Buttery	Sweet, dairy-like aromatic associated with natural butter	Land O'Lakes unsalted butter=7.0
Chemical	Aromatic associated with garden hose, hot Teflon pan, plastic packaging and petroleum-based products such as charcoal lighter fluid	Chlorox in water=6.5

Table 1. Definition and reference standards for beef descriptive flavor and aromatic attributes, continued¹

Sensory	Attribute Definition	Reference, standard flavor scale value unless otherwise defined
Chocolate/Cocoa	Aromatics associated with cocoa beans and powdered cocoa and chocolate bars. Brown, sweet, dusty, often bitter aromatics	Hershey cocoa power in water=3.0 Hershey chocolate kiss=8.5
Cooked Milk	Combination of sweet, brown flavor notes and aromatics associated with heated milk	Mini Babybel Original Swiss Cheese=2.5 Roberts whole milk=4.5
Cumin	Aromatics commonly associated with cumin and characterized as dry, pungent, woody and slightly floral	McCormick ground cumin=7.0
Dairy	Aromatics associated with products made from cow's milk, such as cream, milk, sour cream or buttermilk	Roberts 2% milk=8.0
Fat-like	Aromatics associated with cooked animal fat	Hillshire Farms Lit'l Beef Smokies=7.0 Beef suet=12.0
Floral	Sweet, light, slightly perfume impression associated with flowers	Welch's White grape juice=5.0
Green-Grasslike	Sharp, slightly pungent aromatics associated with green/plant/vegetable matter such as parsley, spinach, pea pod fresh cut grass, etc.	Fresh parsley water=9.0
Green-Haylike	Brown/green dusty aromatics associated with dry grasses, hay, dry parsley and tea leaves	Dry parsley=6.0
Heated Oil	Aromatics associated with oil heated to a high temperature	Wesson vegetable oil=7.0
Liver-like	Aromatics associated with cooked organ meat/liver	Beef liver=7.5 Brauschweiger liver sausage=10.0
Metallic	Impression of slightly oxidized metal, such as iron, copper and silver spoons	0.10% potassium chloride solution=1.5 USDA Choice strip steak=4.0 Dole canned pineapple juice=6.0

Table 1. Definition and reference standards for beef descriptive flavor and aromatic attributes, continued¹

Sensory	Attribute Definition	Reference, standard flavor scale value unless otherwise defined
Musty/Earthy/ Hummus	Musty, sweet, decaying vegetation	1000 ppm of 2,6,- Dimethylcyclohexanol in propylene glycol=9.0
Overall Sweet	Combination of sweet taste and sweet aromatics	Post Shredded Wheat Spoon Size=1.5 Hillshire Farms Lit'l Beef Smokies=3.0
Rancid	Aromatic commonly associated with oxidized fat and oils. These may include cardboard, painty, varnishy and fishy	Wesson Vegetable Oil (heat 3 minutes)=7.0 Wesson Vegetable Oil (heat 5 minutes)=9.0
Refrigerator Stale	Aromatics associated with produce left in refrigerator for an extended period of time and absorbing a combination of odors (lack of freshness/flat)	80% lean ground beef (1 day old)=4.5
Salty	Fundamental taste factor of which sodium chloride is typical	0.20% NaCl solution=2.5 0.35% NaCl solution=5 0.50% NaCl solution=8.5 0.70% NaCl solution=15
Soapy	Aromatic commonly found in unscented hand soap	Ivory bar soap=6.5

Table 1. Definition and reference standards for beef descriptive flavor and aromatic attributes, continued¹

Sensory	Attribute Definition	Reference, standard flavor scale value unless otherwise defined
Sour	Fundamental taste factor associated with a citric acid solution	0.05% Citric acid solution=2 0.08% Citric acid solution=5 0.15% Citric acid solution=10 0.20% Citric acid solution=15
Sour Aromatics	Aromatics associated with sour substances	Roberts buttermilk=5.0
Sour Milk/Sour Dairy	Sour, fermented aromatics associated with dairy products such as buttermilk and sour cream	Laughing Cow Light Swiss cheese=7.0 Roberts buttermilk=9.0
Spoiled-Putrid	Presence of inappropriate aromatics and flavors that is commonly associated with the products. It is a foul taste and/or smell that indicates the products is starting to decay and putrefy	Dimethyl disulfide in propylene glycol (10,000 ppm)=12.0
Sweet	Fundamental taste factor associated with a sucrose solution	2% Sucrose solution=2 5% Sucrose solution=5 10% Sucrose solution=10 15% Sucrose solution=15
Umami	Flat, salty, somewhat brothy. The taste of glutamate, salts of amino acids and other molecules called nucleotides	0.035% Accent Flavor Enhancer Solution=7.5
Warmed-over	Perception of a product that has been previously cooked and reheated	80% lean ground beef (reheated)=6.0

¹Intensities of flavor attributes are on a 16 point scales where 0=none and 15=extremely intense from Adhikari et al. (2011)

Table 2. Least square means and standard error of the mean for pH

Degree	Mean	SEM
Severe DC ¹	6.89 ^a	0.02
Moderate DC ¹	6.59 ^b	0.02
Mild DC ¹	6.36 ^c	0.02
Shady DC ¹	6.10 ^d	0.02
Normal	5.66 ^e	0.01

¹DC=dark-cutter

^{a,b,c,d,e}Means lacking a common superscript within a column are different (P<0.05)

Table 3. Least square means and standard error of the mean for slice shear force, sensory tenderness and juiciness

Degree	Slice Shear Force ¹		Tenderness ²		Juiciness ²	
	Mean	SEM	Mean	SEM	Mean	SEM
Severe DC ³	16.6 ^a	1.12	6.5 ^a	0.11	5.9 ^a	0.10
Moderate DC ³	19.2 ^a	1.12	6.1 ^b	0.11	5.7 ^b	0.09
Mild DC ³	22.8 ^b	1.11	5.2 ^c	0.11	5.4 ^c	0.09
Shady DC ³	25.2 ^b	1.12	4.7 ^e	0.11	5.2 ^d	0.10
Normal	17.6 ^a	0.76	5.0 ^d	0.07	5.3 ^d	0.08

¹Expressed in kg

²1=extremely tough/dry, 2=very tough/dry, 3=moderately tough/dry, 4=slightly tough/dry, 5=slightly tender/juicy, 6=moderately tender/juicy, 7=very tender/juicy, 8=extremely tender/juicy

³DC=dark cutter

^{a,b,c,d,e}Means lacking a common superscript within a column are different (P<0.05)

Table 4. Least square means and standard error of the mean for sarcomere length and desmin degradation

Degree	Sarcomere Length ¹		Desmin Degradation ²	
	Mean	SEM	Mean	SEM
Severe DC ³	1.66 ^a	0.02	49.89 ^a	4.13
Moderate DC ³	1.67 ^{ab}	0.02	40.80 ^a	4.11
Mild DC ³	1.71 ^{bc}	0.02	42.16 ^a	4.10
Shady DC ³	1.74 ^c	0.02	43.30 ^a	4.13
Normal	1.86 ^d	0.01	60.27 ^b	2.38

¹Expressed in μm

²Expressed as percentage of desmin degraded

³DC=dark-cutter

^{a,b,c,d}Means lacking a common superscript within a column are different ($P < 0.05$)

Table 5. Least square means, standard error of the mean and root mean square error for trained sensory panel descriptive flavor attributes¹

Attribute	SEDC ²		MODC ²		MIDC ²		SHDC ²		Normal		P-Value	RMSE ⁴
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM		
Beef Identity	4.1	0.10	4.2	0.10	4.3	0.10	4.2	0.10	4.3	0.09	0.06	0.32
B/R ³	2.0 ^a	0.10	2.2 ^a	0.10	2.3 ^a	0.10	2.2 ^a	0.10	2.3 ^b	0.09	<0.01	0.39
B/S ³	2.7	0.08	2.8	0.08	2.8	0.08	2.8	0.08	2.9	0.06	0.07	0.34
Fat-like	2.2 ^a	0.09	2.0 ^b	0.09	1.9 ^b	0.09	1.5 ^c	0.09	1.4 ^d	0.07	<0.01	0.38
Metallic	1.7 ^c	0.07	1.8 ^{bc}	0.07	1.9 ^{ab}	0.07	1.9 ^{bc}	0.07	2.0 ^a	0.06	<0.01	0.31
Liver-like	0.1	0.02	0.1	0.02	0.1	0.02	0.1	0.02	0.1	0.02	0.74	0.12
Umami	2.2 ^c	0.07	2.2 ^{bc}	0.07	2.4 ^{ab}	0.07	2.3 ^{ab}	0.07	2.4 ^a	0.05	<0.01	0.49
OSweet ³	0.8 ^{ab}	0.04	0.8 ^a	0.04	0.7 ^{bc}	0.04	0.7 ^{ab}	0.04	0.6 ^c	0.03	<0.01	0.23
Sweet	1.0	0.04	1.0	0.04	1.0	0.04	1.0	0.04	1.0	0.03	0.19	0.23
Sour	1.6 ^c	0.09	1.7 ^{bc}	0.09	1.8 ^{bc}	0.09	1.8 ^b	0.09	2.2 ^a	0.07	<0.01	0.42
Salty	0.9 ^b	0.04	0.9 ^b	0.04	1.1 ^a	0.04	1.0 ^{ab}	0.04	1.1 ^a	0.03	<0.01	0.23
Bitter	0.6	0.06	0.6	0.06	0.6	0.06	0.6	0.06	0.7	0.04	0.38	0.35
Rancid	0.3 ^a	0.03	0.3 ^a	0.03	0.2 ^b	0.03	0.1 ^c	0.03	0.1 ^c	0.02	<0.01	0.18
M/E/H ³	0.3 ^a	0.03	0.3 ^a	0.02	0.1 ^b	0.02	0.1 ^{bc}	0.02	0.0 ^c	0.02	<0.01	0.14

¹Scores based on 16 point scale where 0=none and 15=extremely high; attributes with means of <0.1 were not reported

²SEDC=severe dark-cutter; MODC=moderate dark-cutter; MIDC=mild dark-cutter; SHDC=shady dark-cutter

³B/R=brown/roasted, B/S=Bloody/Serumy, OSweet=overall sweet, M/E/H=musty/earthy/hummus

⁴Root mean square error

^{a,b,c,d}Means lacking a common superscript within a row are different (P<0.05)

Table 6. Least square means and standard error of the mean for thaw loss and cook loss for sensory analysis samples and cook loss for slice shear force

Degree	Sensory				Slice Shear Force	
	Thaw Loss ¹		Cook Loss		Cook Loss ²	
	Mean	SEM	Mean	SEM	Mean	SEM
Severe DC ³	1.7 ^a	0.33	11.4 ^a	0.26	10.0 ^a	0.28
Moderate DC ³	2.0 ^a	0.33	12.8 ^b	0.26	10.8 ^b	0.27
Mild DC ³	2.6 ^b	0.33	14.0 ^c	0.26	11.9 ^c	0.27
Shady DC ³	3.2 ^c	0.33	15.6 ^d	0.26	12.8 ^d	0.27
Normal	3.3 ^c	0.30	17.0 ^e	0.17	14.9 ^e	0.22

¹Values expressed as a percentage of thaw loss calculated using the equation $[(\text{frozen weight} - \text{thawed weight}) / \text{frozen weight}] * 100$

²Values expressed as a percentage of cook loss calculated using the equation $[(\text{raw weight} - \text{cooked weight}) / \text{raw weight}] * 100$

³DC=Dark cutter

^{a,b,c,d,e}Means lacking a common superscript within a column are different (P<0.05)