Effects of acetic acid on dark cutting beef

by

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A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
May 7, 2017

Keywords: acetic acid, dark cutting, beef

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Abstract

An experiment was conducted to evaluate the effects of an acetic acid product e(Lm)inate ® V distributed by Hawkins, Inc. on meat quality attributes of dark cutting beef strip loins. Three treatment application groups: 0.4%, 1.2%, and 1.6% acetic acid were compared to two USDA Select strip loins (RFN) to evaluate meat quality effects such as pH, color, cook loss, drip loss, rancidity, and sensory characteristics. The e(Lm)inate ®V product was buffered to a pH of 5.0 by Hawkins, Inc. Sixteen dark cutting beef strip loins (DFD) were each cut into four sections; one section was denoted as a control and the remaining sections were classified to a corresponding injection percentage following a replicated Latin square design. On day five after fabrication at the harvest facility, strip loins were sectioned and initial color was evaluated using a Hunter Miniscan XE Plus from the anterior end of each section and initial pH was evaluated using a waterproof pH Spear Double Junction meter (Oakton®, Vernon Hills, IL). The strip loins were then vacuum packaged and stored at 4 ± 2°C for an additional day. On day six, an initial weight was recorded for the sections denoted as one of the three injection percentages and injected using a multi needle pickle injector (Koch Günther Pökelinjektoren) and weighed again to obtain a final weight. After sections were injected, strip loin sections were vacuum packaged and stored at 4 ± 2°C for three days. After completion of the storage period, all sections were cut into three individual steaks. Steaks were recorded for 1) for sensory analysis, 2) TBARS and drip loss, and 3) cook loss and WBSF. Final color and pH readings were obtained from each section during this time. Once all readings were recorded, each of the three steaks was vacuum packaged and stored for future use. Steaks assigned for sensory analysis and TBARS were frozen and steaks assigned for cook loss and WBSF were stored at 4 ± 2°C for 3 days. Data were analyzed using the PROC Mixed procedure of SAS. Initial pH (IpH) values were not different for location

(P>0.54) or treatment (P>0.68). However, a difference was seen (P<0.05) for initial pH comparing the dark cutting (DFD) loins and normal (RFN) loins (6.04 and 5.59 respectively). Final pH (FpH) values did not differ (P>0.39) for the DFD and RFN loins but there was a difference (P<0.04) between treatments. No differences were seen regarding cook loss (CL). With reference to drip loss (DL), there was a difference (P<0.02) between DFD and RFN loins. DFD loins had a lower drip loss percentage exhibiting the increased water holding capacity in DFD meat. Warner-Bratzler shear force (WBSF) values did not differ for treatment (P>0.48) or DFD and RFN loins (P>0.15); however, there was a difference (P<0.01) in location. Initial L* (IL) values were greater for RFN loins compared to DFD loins (P<0.0003). Initial a* (Ia) values had no effect (P>0.13) on DFD or RFN loins regardless of treatment or location. However, there was a difference (P<0.0003) observed in initial b* (Ib) values comparing DFD and RFN loins. Additionally, final L* (FL), final a* (Fa), and final b* (Fb) values were different (P<0.05) for treatment levels in the DFD loins. Moreover, Fb values were greater (P<0.002) for RFN loins compared to DFD loins (15.12 and 12.78, respectively). There was no difference (P>0.05) correlating cooked L* (CookL), cooked a* (Cooka), or cooked b* (Cookb) values. Additionally, cooked internal colorimetric values were recorded and no difference (P>0.05) in L* (CIL) was attributed to DFD and RFN loins, location, or treatment. However, there was a difference (P<0.03) in cooked internal a* (CIa) values comparing DFD and RFN loins. The DFD loins had the greatest a* values at 7.88, whereas the RFN loins had a smaller a* colorimetric value of 6.56. There was no difference for a* values among location (P>0.26) or treatment (P>0.20). Also when cooked internal b* (CIb) values were evaluated, no difference (P>0.05) was seen for DFD and RFN loins as well as location or treatment. The DFD loins had a greater TBARS value (0.2157) compared to RFN (0.1930) loins but there was no difference (P>0.73) recognized.

Sensory attributes were analyzed and there was no difference ($P>0.05$) between initial juiciness (IJ), sustained juiciness (SJ), initial tenderness (IT), sustained tenderness (ST), and beef flavor intensity (BFI). Nonetheless, there was a difference ($P<0.03$) for off flavor intensity (OFI) with reference to treatment.

Acknowledgments

First and foremost I would like to thank God for leading me to where I am today. Without His love and mercy I would not be here. He has blessed me beyond measure and I am thankful that this is the road He has chosen for me. Whenever times were tough and I thought I couldn't make it through, I leaned on my favorite Bible verse Philippians 4:13 which says, "I can do all things through Christ who strengthens me". I look forward to how He uses me for His Kingdom in the future.

I would like to thank my girlfriend Megan for all of her support and love. Without her, not only would I not have pursued a meat science degree, I wouldn't be the person I am today. Every day I am thankful God joined us together and that when I get off track she is right there to help guide me back. Thank you for standing by me when times were tough and being there to talk to. You have been my rock here on earth and all of my accomplishments are due to you always helping me along the way.

I would also like to thank the Animal Science Faculty and Staff for all their help throughout my years at Auburn. I have learned more than I ever thought I could in such a short period of time. Without their help, this degree would not be possible. I would like to say thank you to all my friends and graduate students who have helped get me where I am: Derek Griffing, Brad Baker, Judson Smith, Whitney Magee, Katie Sowell, Nathan Tapp, Caroline Davenport, and all the Animal Science graduate students. Thank you for all your support and friendship.

To the Meat Lab staff: Barney Wilborn, T. J. Mayfield, Susan Dale, and all the student employees thank you for all each of you have done to make my time at Auburn that much more special. The life lessons Barney has given me the last few years are invaluable and will forever

be remembered. Thank you Barney for always being there to listen even when I made no sense and giving me advice on anything I needed. The skills I have learned just by being around you have enabled me to strengthen my abilities in many aspects of life. Thank you T. J. for your friendship and for always allowing me to get my hands dirty. The skills you have taught me will help further my career and for that I will always be indebted to you.

I would also like to thank Dr. Lisa Kriese-Anderson, Patty Tyler, and Brian Smith for all your help. Without you being there, my project would have been a struggle to complete. Thank you for putting in the time and work to help me accomplish my goals.

I also want to thank Dr. Mulvaney and Dr. Mullenix for your support and agreeing to be on my committee. I am thankful to have had the opportunity to learn from you and being better equipped for my next journey. Thank you for always being there to talk to and helping me become a better person.

Last but certainly not least I would like to thank Dr. Bratcher for the countless things you have done to help me. I can't describe in such little space how thankful I am for you. Every day I am blessed to have met you and the privilege of working under your guidance. Thank you for always being there to help me with anything I needed and having your door open to come to when I didn't know where to turn. You took a chance on me when most others wouldn't and for that I am forever thankful. You have taught me how to be better at anything life throws my way. Thank you for your patience and allowing me to grow as a person. Without you I would not be ready for the next chapter of my life. Thank you again for all you have done.

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Literature Review

Introduction

Consumer appeal of lean color is a driving factor in beef retail acceptance, and thus influences the likelihood of purchase for both consumers and purveyors in the food service industry for retail and wholesale beef products (Carpenter et al., 2001). As it relates to the beef industry, dark, firm, and dry lean otherwise known as "dark cutting" meat is characterized by an apparent dark purplish-red color as a result of a pH greater than 5.7 due to a depletion of muscle glycogen prior to harvest resulting in minimal conversion to lactic acid. Dark cutting beef has little to no acceptance among consumers and food service chefs pertaining to the purchase of premium retail cuts from the rib or loin when compared to a normal beef carcass exhibiting a bright, cherry-red colored lean with a pH ranging from 5.4-5.6 (Lawrie & Ledward, 2006; Bass et al., 2008; Aalhus et al. 2009). Additional literature has stated postmortem competition for oxygen between myoglobin and mitochondria is a primary factor for dark, firm, and dry beef (Manchi et al. 2009).

Despite an increase in animal welfare and handling awareness, as well as correct industry implementation, the percentage of dark cutting beef carcasses has increased since the early 2000's. According to the 2011 National Beef Quality Audit (NBQA), 3.2% of slaughter cattle were dark cutters; an increase from both the 2000 and 2005 NBQA which indicated 2.3% and 1.9% of slaughter cattle were dark cutters, respectively (McKenna et al., 2002; Garcia et al., 2008; Moore et al., 2012). Furthermore, a carcass deemed as a dark cutter will be discounted a minimum of one-third grade up to a full grade. Additionally, some dark cutting carcasses will go un-graded and thus be merchandized as "no-roll" beef. Currently the majority of lean from a beef carcass evaluated as a dark cutter is used for ground beef production due to the visually apparent

dark lean color pigmentation. Therefore, given current prices across all beef cattle industry sectors, even a 1% decrease in the percentage of dark cutting beef carcasses would result in substantial monetary losses totaling into the millions of dollars.

Research on dark cutting beef has primarily focused on pre-harvest management to reduce incidence of dark cutting beef. Boleman et al. (1998) stated that dark cutting cattle resulted in a discount of $6.08 per head harvested within the United States in the 1995 NBQA audit. Studies performed by Scanga et al. (1998) found a combination of improved implant strategies, followed by the use of good handling practices, well designed handling facilities, and proper hauling practices would reduce the incidence of dark cutting beef (Smith et al., 1995). Regardless of these pre-harvest management improvements over time across the beef industry, dark cutting beef continues to be an issue.

A study conducted by Bass et al. (2008) found not all muscles in a dark cutting beef carcass exhibit such a drastic lean color change. Middle-meats express the greatest lean color change in dark cutting beef carcasses; whereas other muscles appear to be the same as those found in “normal” beef carcasses. This is primarily due to individual muscle variation found in wholesale cuts. However, much of the carcass value is obtained from the rib and loin.

In an effort to improve lean color, studies have primarily utilized lactic acid (Sawyer et al. 2008; Apple et al. 2014). However, little research has been conducted using other organic acids to improve the lean color of dark cutting beef. Recently, large meat processors such as Cargill Incorporated in Wayzata, MN have focused their attention on adding value to dark cutting meat through an acidification process that would improve consumer appeal. On November 12, 2013, the company received a patent (Patent NO.: US 8,580,326 B2) for the process. As stated by Sawyer et al. (2008), further research is warranted to investigate the impact

of acidic marination on palatability attributes of fresh and cooked color stability of dark cutting beef. Thus, newly innovative research specifically focusing on adding value in terms of lean color appeal and shelf-life to the rib and loin of dark cutting carcasses using previously under-utilized Generally Recognized as Safe Compounds (GRAS) would be of great value to all facets of the food industry.

Limited research has been performed on the meat quality aspects of dark cutting beef (Apple et al. 2014). Previous studies have recommended the sensory aspects of dark cutting beef be further examined. Savell (2013) stated that there is no real palatability issue with dark cutting beef which is why it is used in the foodservice industry. According to Apple et al. (2014), little is known about the differences in other palatability attributes between high and low pH beef, and there are some contradictions among the limited number of studies. Therefore, comparative research among dark cutting meat and "normal" meat would further enhance the food industry through the improvement of dark, firm, and dry lean color and shelf-life.

Animal Welfare

Animal welfare best practices continues to grow and maintain importance in all sectors of the beef industry for both animal husbandry and improvement in meat quality. These practices are a combination of animal handling, facility design, animal behavior analysis, and overall environmental improvements to reduce the stress on the animal. Initially, the study of animal welfare began in the early 1800s in the United States as a response to appease consumers due to ethical concerns of the livestock industry both on the farm and at harvest facilities (Lyles & Calvo-Lorenzo, 2014). In modern times, welfare concerns pertain to weather conditions, uses of technological advancements in the nutrition and biomedical fields, animal transportation as well as concerns from the past that are still present today such as poor handling and husbandry (Lyles

& Calvo-Lorenzo, 2014). Today, not only consumers, but the industry itself including corporations responsible for food service establishments, are requiring animal welfare monitoring for both ethical and economic benefits. In the past, the McDonald's Corporation and Wendy's International have used their economic power and status to improve animal welfare in the livestock industry (Grandin, 2006). In 1999, the McDonald's Corporation with the aid of Temple Grandin began auditing a total of 50 pork and beef harvest plants with an emphasis placed on handling and stunning at the harvest facility. The audit utilized a numerical objective scoring system to evaluate five basic measurements which ranged from the percentage of animals stunned on the first attempt, vocalization, and the percentage of animals that fell down or were electrically prodded upon movement to a restraining chute prior to immobilization. Based on data collected in 2003 and compared to the data from the original audit, beef cattle harvest facilities showed the greatest improvement overall with a significant decrease in vocalization and a significant increase in the percentage of animals stunned on the first shot (Grandin, 2006). Additionally, some of the most common reasons for failing an audit were: untrained employees, physical distractions such as objects causing animals to hesitate, and equipment issues due to overuse. Just like food safety, standards and requirements in animal welfare in the animal protein industry can only be maintained and improved through the continuation of industry audits by food service corporations and related buyers (Grandin, 2006). Moreover, as cited by Lyles & Calvo-Lorenzo (2014) in reference to a personal communication that took place in Stillwater, OK based on how beef production should be defined for the present and future, "producing safe, quality beef with long-term economic viability, stewardship of natural resources, and responsibilities to communities, family, and animals". Large corporations

have the greatest advantage on improving animal welfare through their buying power which improves conditions (Grandin, 2006).

Animal handling remains a concern for consumers regarding to the improper use of equipment and handling techniques. Most of the beef cattle raised for harvest in the United States are born in the Southeast. Grandin (2006) stated when a problem arises when handling cattle, it is the responsibility of the handler to assess the situation to decipher what is causing a problem instead of resorting to improper use of electric prods. Many cow-calf producers are heavily involved in the production process. At a young age, calves are introduced to human contact, different facility designs, and learn how to eat and drink from troughs. Not only are all these steps helpful for the producer but, the calves benefit from it as well. The more calves are around humans, they tend to be calmer and less prone to stress. According to Becker & Lobato (1997), Zebu-cross calves that were handled gently tried to escape less, exhibited less aggression, and showed more curious behaviors compared to calves that have not been handled. Some of the most common problems that arise from handling are hesitation by the cattle due to shiny reflections, dangling chains, seeing people, or air being blown on the faces of the cattle (Grandin, 1996). Therefore, it is essential to make sure that the working facilities at all phases of the beef industry remain free of these obstacles. When moving cattle through a facility, darker areas tend to deter cattle, so with the aid of light to these darker areas, cattle movement will be facilitated. Grandin (2006), surveyed 24 beef, veal, pork, and sheep slaughter plants and assessed five criteria based upon animal handling techniques. Of these criteria, vocalization was deemed an area of concern and in one beef plant, an 8% vocalization score decreased to 0% after the addition of a light source in a dark area. When distractions are eliminated from a working facility, cattle will move more quietly into the stunning area (Grandin, 2006). In addition to the

areas of concern addressed by Grandin (2006), solid walls lining the entire facility will facilitate cattle movement due to the inability of the cattle to see people or any other distractions outside the working areas.

In order to keep cattle as calm as possible, handlers should be trained to move animals quieter by knowing which position to be in at a particular time (Grandin, 1980). As of 2006, Grandin has reviewed over 30 years of experience from working facilities and states that handlers would always correctly move the animals but when moving became difficult, the handlers resorted back to "old, rough ways" and inadvertently knew that the use of electric prods were being used more. One way to limit these instances is monitoring the percentage of cattle being moved with a prod and the percentage that fall down as a result (Grandin, 2006). Numerical scoring allows for precise monitoring and enables management to determine if what they are doing is working. "What one person may consider proper handling, another person may think is abusive" (Grandin, 2006). Therefore, it is important to maintain sufficient guidelines such as numerical scoring to determine what is or is not working. There have been several harvest facilities to resort to the use of a vibration prod to move cattle. In these plants, the handlers were trained to use these prods as the main source to move the animals and only use an electric prod if the animal refused to move. The problem that arose from such practices caused the handlers to use excessive force without the use of an electric prod on animals that were not moving in order to receive an acceptable electric prod score. Instances such as these caused the American Meat Institute to allow only 25% of the animals to be electrically prodded (Grandin, 2006). In 1999, Temple Grandin conducted surveys in the United States, Canada, and Australia within 48 harvest facilities. Cattle vocalizations were of particular interest due to handling and equipment. When cattle stopped moving through the working facilities, 95% or more were

prodded and therefore had a significant increase in cattle vocalizations. Throughout industry, there are many different types of electric prods. Some plants utilized a severe shock in which caused most of the cattle to vocalize whereas other plants use only a 15 V shock that rarely causes vocalization. However, in many of the harvest plants, the primary driving aid is a flag stick, plastic bag, and/or a plastic paddle stick. The only time an electric prod is used is when the primary driving aid fails to move the cattle (Grandin, 2001). In addition to electric prods, shouting and whistling also caused cattle to have an increased heart rate compared to the sound of a gate slamming (Waynert et al., 1999). Grandin (2001) concludes that with proper handling techniques and the elimination of physical distractions, the use of electric prods will decrease. According to Savell & Smith (2009), cattle that are affected by short-term stress, improper handling, or over-exertion prior to harvest have a condition referred to as "fiery fat" in which the subcutaneous fat contains more blood in the peripheral capillaries. The fat is unable to drain properly and results in an abnormal appearance. Dark colored lean is also a condition closely correlated to "fiery fat". This meat is unable to develop a bright, cherry-red appearance that is desirable upon exposure to oxygen. When the period of time is short prior to harvest, the cattle should be allowed to relax for 24 hours before harvest. Dark cutting beef results from long-term stress such as transportation, comingling new animals, and new environments. Beef Stress Syndrome is an extreme case of improper handling. These cattle exhibit fear in which adrenaline production increases, metabolism of muscle glycogen initiates, and the meat has a dark, firm, and sticky appearance at harvest (Savell & Smith, 2009).

Pre-slaughter Stress Factors

Pre-slaughter stress, both short-and long-term, is one factor leading to dark, firm, and dry beef lean (Miller, n. d.). Ante-mortem stress factors have proven to be detrimental to post-

mortem muscle pH, which results in quality defects (Apple et al., 1995). A multitude of sources are responsible for pre-slaughter stress prior to harvest; including: human handlers, facilities, natural environmental factors, transportation prior to harvest, and genetic predisposition (Grandin, 1980). As a result of these sources, cattle respond to pre-slaughter stress through fear, dehydration, hunger, physical activity and fatigue, and possible physical injury all leading to a decline in meat quality. These factors decrease meat quality, as well as consumer appeal and acceptance (Ferguson & Warner, 2008). Glycogen depletion prior to harvest is a direct result of physical exertion and psychological stress (Immonen & Puolanne, 2000; Nockels et al. 1996). The best way to reduce dark, firm, and dry meat is to properly manage animals during the ante-mortem stages (Hedrick et al. 1959; Grandin, 1992; and Shackelford et al. 1994). Glycogen depletion can be a result of several pre-slaughter stress factors such as; time and handling during transportation (Arthington et al., 2003), lairage (Warriss, 2003), climatic factors (Kreikemeier et al., 1998; Silva et al., 1999), and co-mingling (Apple et al., 1995). In an evaluation performed by Scanga et al. (1998), data from a three year period was collected from nine commercial feedlots. In this evaluation, 2,672,223 total cattle were observed with 18,106 being dark cutters that equated to $4,024,058 in losses. Results showed that mean percentages of dark cutters per pen were different demonstrating that the condition was a result of different management philosophies or to structural components of the feedyards. In addition, a study performed in Spain examined the effects of lairage on meat quality. The study consisted primarily of Holstein cattle penned in various groups with an average lairage time of 12.3 +/- 6.06 hours. Results showed that lairage time affected post-mortem pH. Cattle housed for less than 8.16 hours were 10.85% of 5,456 observations with a 24 hour post-mortem pH greater than or equal to 5.8. As waiting time increased to at least 15.8 hours, meat pH greater than or equal to 5.8 increased to

21.09% (Mach et al., 2008). However, these results contrasted Mounier et al. (2006) in which meat pH 24 hours post-mortem decreased in bulls as lairage time increased. Furthermore, results from Mounier et al. (2006) suggested that bulls be in lairage longer than 17 hours to decrease the occurrence of high muscle pH post-mortem.

In addition, the number of cattle within one pen was evaluated to correlate any interaction between over-stocking and meat quality in the study performed by Mach et al. (2008). Results concluded that stocking density had no effect on meat pH 24 hours post-mortem. Nonetheless, when gender and stocking density were evaluated, steers had an increased post-mortem meat pH greater than or equal to 5.8. In agreement with Fisher et al. (1997), decreasing stocking rates at slaughter facilities could have a major impact on the occurrence of meat pH 24 hours post-mortem greater than or equal to 5.8 in steers than in heifers. This phenomenon could be due to the increased physical activity and physiological stress in males compared to females (Kreikemeier et al., 1998).

Research has indicated differences in the occurrence of dark, firm, and dry meat between *Bos indicus* and *Bos taurus* influenced cattle as well as gender differences between steers and heifers (Tatum et al., 2007). Hoffman et al. (1998) and Shackelford et al. (1994) state that one reason for the differing levels of glycogen may be due to gender. When sex attributes in a study by Scanga et al. (1998) were evaluated, intact heifers produced a higher incidence of dark cutting beef than steers or spayed heifers. The maximum temperatures of intact heifers were recorded from 1 to 2 d before harvest and when the average environmental temperature was above 35°C, the rate of dark cutters increased. When average environmental temperatures were below 0°C and the precipitation was greater than 5 mm, heifers produced more dark cutting carcasses than when the average temperatures were above 0°C. Steers with an average temperature below 0°C

showed no effect in the occurrence of dark cutting beef. Temperatures were recorded on 1, 2, and 3 d prior to harvest when the absolute difference between the daily high and low temperatures exceeded 5.6°C. Results concluded that dark cutting beef from steers increased. Also at 2 and 3 d before harvest when daily temperatures were above 5.6°C, heifers produced a higher mean percentage of dark cutting carcasses, this suggests that extreme temperature fluctuations over 1 to 3 d induces stress and the occurrence of dark cutting carcasses may increase. Sex and aggressive use of implants were two main factors in this evaluation that resulted in dark cutters. Results showed that heifers have a higher risk of producing dark cutters than steers or spayed heifers (Scanga et al., 1998). However, in a study performed by Mach et al. (2008), the meat pH 24 hours post-mortem was higher in steers ($\geq$5.8) than in heifers, which supported previous studies done by Hoffman et al. (1998) and Shackelford et al. (1994).

Further research by Fleming & Luebke (1981), Voisinet et al. (1997a), and Voisinet et al. (1997b) all showed that females had a more excitable personality and fearfulness was greater in females that have not calved. Voisinet et al. (1997a) reported that heifers had an increased "borderline" dark cutting condition which could explain why females in Scanga et al. (1998) were more at risk of producing dark cutting beef especially if given exogenous estrogen. In contrast, according to the 2011 National Beef Quality Audit, 61.24% of the steers had more dark cutting carcasses than the heifers at 38.76%.

Sensory Characteristics

Dark cutting beef not only has an apparent color variation, it also has variation in tenderness as well (Silva et al. 1999). When beef reaches a post-mortem pH of 6.0 or greater 24 hours after harvest, meat quality becomes of major concern. Consumers find the characteristics of dark cutting beef undesirable. Moreover, the economic losses due to dark cutting beef are

substantial as evident by penalties of 30 to 60% in carcass value with a pH greater than 5.8 in the Spanish meat industry (Mach et al., 2008). In addition to color, tenderness, and pH, the accumulation of organisms to unacceptable levels causes the development of off-odors as well as the presence of slime (Gardner et al., 2001).

Tenderness

Tenderness is a major concern for consumers when determining beef satisfaction. Consumers are able to differentiate between tenderness discrepancies and therefore will pay premiums for guaranteed tenderness (Boleman et al., 1997; Shackelford et al., 2001). However, according to Brooks et al. (2000), beef tenderness still remains an issue despite the advancements in understanding how tenderness is affected in the beef carcass. Moreover, Katsaras & Peetz (1990) revealed that dark, firm, and dry meat is typically very tender but not in the same aspects as normal meat. DFD beef has a spongy and mealy texture compared to RFN meat. Furthermore, Dransfield (1981) studied the reactions of consumers to evaluate tenderness and pH and concluded that DFD meat was more tender compared to normal pH beef. This phenomenon was explained by Katsaras & Peetz (1990) who reported that a possible explanation for DFD carcasses being more tender is due to upon heating, the fragmentation of myofibrils was larger in DFD meat compared to normal pH meat. Additionally, DFD meat had much smaller cooking losses as well. Warner-Bratzler shear force values increase as cattle become more excitable compared to calmer pen mates (Falkenberg et al., 2005; Voisinet et al., 1997).

To further understand the interaction between animal behavior and the effects on meat quality, a study by King et al. (2006) evaluated 144 steers sorted into three contemporary groups (A, B, & C). Calves in group A consisted of Bonsmara-Romosinuano crosses (31) and were implanted once with Component E-C upon feedlot arrival. Group B calves were Angus yearling

steers (49) and implanted with Revalor-S upon feedlot arrival. Group C consisted of Angus calf-fed steers (48) that were implanted with Component E-C at the time of arrival at the feedlot and re-implanted again with Component TES after 70 days on feed. Temperament scores were evaluated using infrared technology placed 1 m from the working chute. As cattle pass the first set of sensors, a timer started and continued until the animal passed the second set of sensors 1.82 m beyond the first set. An evaluator (Grandin, 1993), assessed each animal with a chute score of 1-5 with one being calm, no movement and five defined as rearing, struggling violently. These scores were evaluated prior to the animal entering the squeeze chute in a weigh box. In addition to the exit velocities and chute scores, pen scores were assigned to each animal as well. A handler walked through a small pen with 4-5 cattle and assigned a pen score of 1-5 with one being not excited by humans to 5 defined as running into fences.

Once cattle were determined to be harvested, they were transported to the slaughter facility and harvested within 4 hours upon arrival using industry standards. Muscle pH was evaluated at 0.5, 4, 7, 12, 24, and 48 hours post-mortem. Within each group, cattle were identified as calm, intermediate, or excitable. Group C steers identified as excitable (n=9) showed higher exit velocities at nearly 4.0 m/s whereas group C steers classified as calm (n=10) had the lowest exit velocities at approximately 1.0 m/s. The chute scores were not statistically different ($P>0.05$)in contrast to other studies by Wulf et al. (1997), which showed significant differences ($P<0.05$) between temperament and meat quality. King et al. (2006) proposes one reason for the discrepancy is the handling experiences of the animals prior to the study. Cattle in this study were penned in contemporary groups and chute scores were assigned on the farm, in addition to the numerous times the cattle were worked through the facility during the experiment.

Temperament category did not affect meat quality factors such as marbling. The three groups combined had quality grades of high select. These results contradict Voisinet et al. (1997) in which animals with more excitable temperaments assessed in the chute score were more prone to display borderline dark cutting lean. King et al. (2006) showed no incidence of dark cutting beef. Meat pH at 0.5 hours post-mortem in calm cattle was 6.1 which were slightly higher than the intermediate and excitable cattle at 6.0 and 5.9. Although these results are minimal, after harvest, the cattle were electrically stimulated which in turn would cause an accelerated post-mortem metabolism rate. Tenderness was assessed using Warner-Bratzler shear force values. Steers in group C with excitable temperaments had higher shear force values when evaluated 7 and 21 days post-mortem from *M. longissimus lumborum* steaks compared to calm and intermediate cattle with the same group. Group A steers shear force values did not differ in regards to temperament except for days 3, 14, and 21 post-mortem in which the excitable steers showed higher shear force values. Additionally, group B steers shear force values did not differ at any point post-mortem.

pH

There have been many studies stating the effect of various pre-harvest factors on beef pH, but information regarding how the pH is affected by the interaction of these pre-harvest factors remains uncertain (Mach et al., 2008). Research conducted by Mach et al. (2008) demonstrated the impact of various stress factors and how they impact beef muscle pH. Cattle arrived at the harvest facility and were sorted into 1 of 82 pens depending on gender, origin, group size, and age. All cattle were harvested using conventional captive bolt stunning and results such as lairage, number of animals per pen, gender, and the number of animals harvested were recorded for further analysis. The study was composed of primarily Holstein cattle (51%), however there

were 12 different breed types included as well. Meat pH was measured in the longissimus dorsi muscle at 24 hours post-mortem and any carcasses with a pH lower than 5.8 were categorized into normal quality, whereas any carcasses exhibiting a pH reading greater than 5.8 were grouped into devaluated meat quality conforming to Spanish industry standards with aid of research from Viljoen et al. (2002). After meat pH 24 hours post-mortem was evaluated, 13.89% of the cattle exceeded a pH of 5.8. However, studies conducted in the United States by Kreikemeier & Unruh (1993) showed cattle with 24 hour post-mortem meat pH greater than 5.8 at only 1.7% of 8,000 carcasses.

At the time of purchase, consumers are able to see the color of dark cutting beef and select against buying the meat (Viljoen et al., 2002). This has been an ongoing issue for the beef industry for many years dating back to 1965 when Hedrick noted that consumers were selecting against fresh dark cutting beef. Hedrick (1965) hypothesized that differences in palatability between normal and dark cutting beef were unclear. Tenderness, juiciness, and flavor are all associated with palatability. A consumer study conducted in England by Dransfield (1981) assessed flavor attributes of DFD. Normal beef pH was more acceptable than the DFD beef due to the conclusion that people preferred the stronger beef flavor. According to Katsaras & Peetz (1990), DFD meat represents a stale (flat) off-flavor compared to normal meat.

Flavor

Flavor attributes regarding normal and DFD meat were further analyzed by Viljoen et al. (2002). In this study, consumer panels were utilized to compare the acceptability of DFD and normal beef in the raw and cooked form. The flavor of DFD meat was expected to be less acceptable compared to normal pH meat. Respondents (n=64) consisted of beef eating consumers who evaluated DFD and normal pH steaks in the raw and fried form. The consumers

ate beef at least once a week and were present for two evaluation sessions where they visually evaluated raw steaks and sensory attributes of fried steaks. The consumers were asked to evaluate acceptability of the appearance and color as well as overall acceptability of raw steaks. Respondents ranked raw and fried steaks on a 9 point scale with 1 being noted as totally unacceptable and 9 being noted as very acceptable. Additionally, the consumers were asked to specify which steak they preferred. Results showed a difference in acceptability ($P<0.01$) as more consumers preferred the normal pH raw steak with means of 6.1 than the raw DFD steak with means of 5.0.

Color

Continuing with the results of Viljoen et al. (2002), color proved to be an important factor for consumer acceptance. Dumont (1981) reported that the importance of color as an indicator of "freshness" is most likely overestimated in the mind of consumers. In addition, Wulf et al. (1996) studied the effects of lean color as it relates to palatability and showed that lean color was notably related to taste panel tenderness scores. Moreover, darker colored lean was expressed as less tender compared to normal and pale meat. Sensory attributes consisted of odor, appearance, color, taste, texture, juiciness, and overall acceptability. When these results were analyzed, there was no difference between consumer acceptability of the fried DFD and normal pH steaks. In general, there was no difference in preference for the fried normal pH and DFD steaks. Conclusions from Viljoen et al. (2002) showed that consumers accepted the raw, normal pH meat more than the raw DFD samples in relation to general appearance ($P<0.05$), color ($P<0.001$), and acceptability ($P<0.01$). Twice as many respondents preferred the raw normal than raw DFD steaks due to the more appealing red color of the normal meat.

The color of cooked dark cutting beef is characterized by a red-pink color which is undesirable for consumer acceptance. Consumers are more concerned about the cooked color of beef rather than the fresh color (Apple et al., 2014). Fresh dark cutting beef is notably less red and yellow than normal pH beef (Wulf et al., 2002; Apple et al., 2005). With the utilization of rotenone, the color of dark cutting beef can be improved to the color of normal pH beef by impeding mitochondrial respiration (Cornforth & Egbert, 1985). To study the effects of organic acids as it relates to improving meat color, Apple et al. (2014) conducted an experiment using normal pH, low Choice, and dark cutting strip loins. Dark cutting sections were either enhanced with lactic acid at rates of 0.15, 0.35, or 0.50% depending upon the experiment, non-enhanced, or served as a negative control. All sections were fabricated into 1.27 cm thick slices and 2.54 cm thick steaks. Steaks were then vacuum packaged in 80% oxygen and 20% carbon dioxide gas with an oxygen-barrier film seal. The packages were placed in an open-topped, coffin-chest display and stored at 2.6°C. Dark cutting sections enhanced with lactic acid were grouped with the non-enhanced Choice and dark cutting sections and placed under 1,600 lx of continuous deluxe warm-white fluorescent lighting for 5 days. Color panelists were trained from the AMSA (1991) guidelines and the fresh beef color was evaluated twice daily on a seven point scale. On days 1, 3 and 5, a steak was randomly chosen from each loin section and was evaluated for instrumental color readings obtained from a Hunter MiniScan XE.

Apple et al. (2014) showed that there were no effects for lactic acid enhancement and retail display duration. Furthermore, instrumental color from the non-enhanced and enhanced dark cutting steaks were darker and closer to the true red axis compared to the Choice sections. Dark cutting sections enhanced with 0.35% lactic acid were lighter and had lower hue angles compared to the non-enhanced dark cutting sections. Within Apple et al. (2014), two

experiments were analyzed. In experiment 1, Choice steaks were redder and more yellow compared to the non-enhanced and enhanced dark cutting steaks after days 1 and 3 of retail display. Additionally, the Choice steaks were noted for having more total color on day 1 than days 3 and 5. However, total color values of the Choice steaks was still greater than the dark cutting sections notwithstanding of enhancement methods on days 1, 3, and 5. Day 3 of display proved to be beneficial for 0.15 and 0.35% lactic acid enhanced dark cutting steaks through greater redness, yellowness, and total color readings than the non-enhanced dark cutting sections. However, the total color, redness, and yellowness values did not differ between the non-enhanced and enhanced dark cutting steaks after day 5 of display. Moreover, dark cutting steaks enhanced with 0.35% lactic acid had redness readings comparable to the non-enhanced Choice steaks on day 5 of display.

Experiment 2 of Apple et al. (2014) showed no interactive effects of retail display duration on any instrumental color readings. However, the Choice steaks had higher redness and yellowness readings than the dark cutting steaks no matter what the enhancement method. The dark cutting steaks enhanced with lactic acid were lighter in color compared to the non-enhanced dark cutting steaks. Furthermore, dark cutting steaks enhanced at 0.35% lactic acid were redder compared to the 0.50% enhanced steaks. Additionally, the enhanced dark cutting steaks were more yellow than the non-enhanced and .50% enhancement. Steaks enhanced at 0.35% had a higher total color value than the 0.50% enhanced steaks and the un-treated dark cutting steaks had the lowest hue angles. Conversely, steaks enhanced with 0.50% lactic acid had the highest hue angles, but hue angle values were inseparable between Choice and dark cutting steaks enhanced with 0.35% lactic acid.

Visually appraised fresh beef color was evaluated in Apple et al. (2014) from the Japanese color scoring standards. Choice steaks in experiment 1 had drastically less color scores compared to the non-enhanced and enhanced dark cutting steaks. Fresh color scores were similar for enhanced and non-enhanced dark cutting steaks on day 1 of display, but dark cutting steaks were noted as being more desirable in terms of color scores by the Japanese scoring standards compared to the enhanced dark cutting steaks on day 2 of display. Steaks enhanced with 0.35% lactic acid had lower color scores than steaks that were either non-enhanced or 0.15% enhanced dark cutting steaks over the final 3 days of retail display. Non-enhanced dark cutting steaks had the highest color scores and the Choice steaks had the lowest color scores in experiment 2. Even though the color score margin was small (0.7 and 0.4 units), dark cutting steaks enhanced with 0.35% lactic acid had higher color scores compared to steaks enhanced with 0.50% lactic acid. Discoloration scores in experiment 1 remained constant throughout the 5 day display among the non-enhanced and enhanced dark cutting steaks. On the contrary, the discoloration scores were decreased for steaks from Choice sections during the final 3 days of display. Moreover, discoloration scores were similar for Choice and dark cutting steaks during days 1 and 2 of display, steaks from Choice strip loin sections had more discoloration than both the lactic acid enhanced and non-enhanced dark cutting sections on days 3, 4, and 5 of display. Furthermore, non-enhanced steaks had greater discoloration scores compared to Choice steaks on days 2 and 4 of display in experiment 2. The discoloration of dark cutting steaks enhanced with 0.35% lactic acid remained unchanged throughout the display period but they were more discolored than the Choice and non-enhanced dark cutting steaks on all 5 days of display. Dark cutting steaks enhanced with 0.50% lactic acid had the most discoloration during the display period, notably on

day 1. Discoloration continued to increase as the retail display period reached days 4 and 5 for steaks enhanced with 0.50% lactic acid.

Continued research by Apple et al. (2014) expanded into cooked color of dark cutting beef. In experiment 1, Choice steaks maintained the best cooked color and degree of doneness scores. Dark cutting steaks enhanced with 0.15% lactic acid received greater cooked and degree of doneness scores when compared to steaks non-enhanced or enhanced with 0.35% lactic acid. However in experiment 2, cooked color and degree of doneness scores comparing Choice steaks and 0.50% lactic acid enhanced steaks did not differ. Moreover, scores proved that non-enhanced steaks ranked the least for cooked color and degree of doneness. However, in experiment 1, the interior color of dark cutting steaks, regardless of enhancement method, were redder and had lower hue angles compared to the internal color of Choice steaks. In experiment 2, redness scores, along with total color for the internal appearance of the cooked steaks enhanced with 0.35% lactic acid and non-enhanced steaks, were greater than the 0.50% lactic acid enhanced steaks. However, cooked 0.50% lactic acid enhanced dark cutting steaks had the highest hue angles while non-enhanced steaks proved to be the lowest in hue angles.

Cooking causes a pigment shift in normal pH meat from red to gray (Mendenhall, 1989). When high pH meat (>6.0) is cooked to the same endpoint temperature, the internal color remains redder and appears under-cooked due to myoglobin protection from heat denaturation (Trout, 1989). According to Gašperlin et al. (2000), cooked dark cutting beef, upon exposure to air, changes color internally through oxygenation and develops the bright red color similar to fresh, normal pH beef. When dark cutting beef meat was ground and treated with lactic acid solutions, elevated myoglobin denaturation during the cooking process along with cooked beef color, proved to be similar to normal pH beef ground meat (Moiseev & Cornforth, 1999). Past

research performed by Sawyer et al. (2008) showed that dark cutting steaks enhanced with 0.5% lactic acid had similar levels of denatured myoglobin as the normal pH steaks. Furthermore, Sawyer et al. (2008) demonstrated that degree of doneness and instrumental cooked color scores were similar between normal pH and dark cutting steaks enhanced with 1.0% lactic acid.

Even though past research regarding dark cutting beef has been limited to ante-mortem stress factors, the results from Apple et al. (2014) show that post-rigor enhancement of whole muscle dark cutting beef with lactic acid can reduce or eliminate the red-pink cooked color and reduce the intense flavor of dark cutting beef. Through lactic acid enhancement, dark cutting beef fresh color can be improved from the unacceptable dark red color to the consumer appealing bright red color.

Shelf-life

Shelf-life is an important factor not only within the beef industry, but the food industry as a whole. Delmore (2009) defined shelf-life as the period of time between when the product is packaged to the time of consumption. The attributes associated with shelf-life are color, appearance, texture, flavor, and nutritive value (Singh & Singh, 2005). Spoilage organisms are responsible for the acceptance of the product and play a role in how long a product can be kept before it is deemed unacceptable. According to Delmore (2009), there seems to be a misunderstanding between spoilage organisms and pathogens. The difference is defined as whether or not the consumer gets sick. Spoilage organisms only change the appearance of the product but do not cause illnesses. Neither pathogens nor spoilage organisms are acceptable in the beef industry, as evident by the continuous research to prevent, reduce, and eliminate them before the product reaches retail stores or the consumer.

Dark, firm, and dry meat has a few positive qualities such as water-holding capacity but is notably recognized for the undesirable traits. Shelf-life remains a concern for DFD meat due to the depleted levels of glycogen. DFD beef and products made from DFD meat have a shorter shelf-life compared to normal meats (Nicol et al., 1970; Bern et al., 1976; Tarrant, 1976). DFD meat spoils faster due to the decreased levels of glucose in which organisms feed on post-mortem. If glucose levels are low, organisms begin feeding on amino acids and causes spoilage (Miller, n. d.) Past studies concluded that the reason for the rapid decline of DFD meat was due to the rate at which spoilage organisms matured at a higher ultimate pH (Tarrant, 1976). This hypothesis occurred when some isolated mesophilic organisms from a spoiled DFD ham were inhibited in normal pH hams (Ingram, 1948). According to Newton & Gill (1981), not all spoilage bacteria will be reduced in the low pH of normal meat. The growth rates of the major aerobic bacteria of fresh meat are unaffected by the normal pH range. According to (Fromm & Monroe, 1965; Rey et al., 1976; Newton & Gill, 1978), the increased pH range only affects the lag phase of these bacteria; however, this has no effect on the rate of spoilage. Nonetheless, certain types of psychotropic bacteria that are repressed by normal pH meat, can multiply on high pH meats (Newton & Gill, 1981). These bacteria species are known as *Acinetobacter* and *Altermonas putrefaciens*. Growth in vacuum packaged meat is affected by bacteria such as *Enterobacter liquefaciens* and *Yersinia enterocolitica*, and occurs more rapidly on DFD meat than normal meat (McMeekin, 1977; Gill & Newton, 1978). Species of *Acinetobacter* are inhibited anaerobically but *Enterobacter liquefaciens* and *Altermonas putrefaciens* can cause odors, thus even small representative numbers can hasten spoilage (Newton & Gill, 1981). Three facultative anaerobes *Yersinia enterocolitica*, *Enterobacter liquefaciens*, and *Altermonas putrefaciens* are prevalent on DFD meat but typically do no contribute to the anaerobic spoilage

flora (Gill & Newton, 1979; Seelye & Yearbury, 1979). *Yersinia enterocolitica* is typically found in normal meat vacuum packaged products. Its growth is increased on vacuum packaged DFD meat, but does not knowingly cause spoilage issues. *E. liquefaciens* growth rate is reduced at a pH of 6.0 or below and causes odors at low cell numbers on DFD meat. *A. putrefaciens* causes the green coloration as a result of the decreased levels of hydrogen sulphide and is responsible for the limited shelf-life of vacuum packaged DFD meat (Newton & Gill, 1981). Therefore, the amount of glucose in the muscle post-mortem can have an effect on spoilage organisms and shelf-life. In order to improve the consumer acceptance of DFD meat, perhaps the utilization of organic acids can have an effect on these spoilage organisms and improve shelf-life.

Organic acids

Research utilizing organic acids on the effects of dark cutting beef remain limited. Much of the research conducted has been on the focus of using organic acids such as lactic acid to improve the lean color of dark cutting beef. According to Jamilah et al. (2008), the antimicrobials most commonly used in research are citric, acetic, and lactic acids. These acids also play a role in flavor development in acidified products as well. The use of antimicrobials depends largely on the product characteristics and legality situations. Beth et al. (2004) stated that the effectiveness of organic acids used as antimicrobials will differ based upon factors such as concentration, pH, molarity, and the concentration of the non-dissociated form. According to Oreskovich et al. (1992), Seuss & Martin (1993), and Aktaş et al. (2003), normal pH beef treated with organic acids can help diminish post-enhancement muscle pH values. Furthermore, Medyński et al. (2000) observed that pH values of normal beef and pork decreased to values less than or equal to 4.3 as the concentration of lactic acid within the marinade increased to greater than or equal to 1%.

The legal limits for organic acids to be used as a spray on pre-chilled carcasses is less than 2.5% (Beth et al., 2004; U.S. Food and Drug Administration, 2014). Using lactic, acetic, and citric acids as a spray have been shown to decrease the growth of spoilage and pathogenic organisms (Dorsa et al., 1997). Spoilage organisms largely accumulate on the beef product surface. Research has been conducted in order to hasten the development of spoilage organisms through organic acid dips and sprays (Siragusa & Dickson, 1992). Bacteria thrive in the pH range of neutrality and begin to spoil meat in the pH range of 6.0-6.5 which is also considered to be dark cutting meat in beef. Preventative measures to control spoilage organisms are to increase the acidity of beef which in turn provides unfavorable conditions for microbial growth (Jamilah et al., 2008).

Citric acid

One of the organic acids used to improve the quality of beef is citric acid. Citric acid is extracted from acidic fruits such as lemons, pineapples, limes, and is also a product of the fermentation of glucose. Citric acid can be further classified as soluble in water and insoluble in fat (Jamilah et al., 2008). Studies have also shown that citric acid is inhibitory to bacteria, yeasts, and molds and inhibits better than lactic and acetic acids (Sorrells, 1989).

Acetic acid

Acetic acid is another commonly used organic acid and is characterized by a pungent odor and taste. Acetic acid has the ability to lower pH, cause disruption within the cell membrane, and is sometimes referred to as vinegar (Jay, 1992). Acetic acid is generally safe to use and has been notably recognized for its effectiveness against *E. coli* O157:H7 and *Salmonella* typhimurium. Acetic acid at a concentration of 3% has also been shown to reduce

cell numbers of *Enterobacteriaceae* in vacuum packaged beef stored at 2.4°C for 6 weeks (Jamilah et al., 2008).

Lactic acid

Lactic acid can be classified into un-dissociated and dissociated forms depending on the pH. In the un-dissociated form, lactic acid is inhibitory to bacteria (Jamilah et al., 2008). Anderson & Marshall (1990) reported that at a combined 100% concentration, lactic acid and acetic acid had a significant effect on lowering the spoilage organism level. Sodium lactate, which is a salt derived from lactic acid has been used primarily within the meats industry as a flavor enhancer, shelf-life extender, and microbiological safety of the product. Moreover, lactic acid has been notably utilized for extending shelf-life in various meat products (Jamilah et al., 2008).

According to Sawyer et al. (2008), studies have shown that lactic acid at 0.25-0.50% can improve post-rigor dark cutting beef by lowering the muscle pH and consequently eradicating the undesirable red-pink color of cooked dark cutting meat. Furthermore, Sawyer et al. (2009) stated that lactic acid can enhance the fresh color of dark cutting beef as well.

In a study conducted by Apple et al. (2014), lactic acid was used to determine the effects on dark cutting beef regarding fresh and cooked color. Beef strip loins used in the experiment were normal pH, low Choice, and dark cutting meat. In experiment 1, the average pH of the Choice and dark cutting beef was 5.37 and 6.70 and in experiment 2 the Choice steaks had an average pH of 5.58 and the dark cutting steaks had an average pH of 6.78. All loins were vacuum packaged and allowed to age an additional 7 days at 2°C. Following aging, all strip loins were removed from the packaging and cut into 2 equal lengths with a 1.27 cm slice taken from each section to measure pre-enhancement longissimus muscle pH. The dark cutting beef in

experiment 1was either randomly categorized into non-enhanced, negative control, or enhanced. All Choice loins served the purpose as a positive control in this experiment. Each strip loin was weighed and the dark cutting loins were enhanced to either 105% (Exp. 1) or 112% (Exp. 2) of the specific section weight with one of the randomly assigned lactic acid solutions by a multi-needle injector. Dark cutting loins were injected with lactic acid at either 0.15 or 0.35% in experiment 1 and the average pH of the solution was 2.83 and 2.70. In experiment 2, the 0.35 or 0.50% solution had an average pH of 2.75 and 1.97. After being placed in a vacuum tumbler, the dark cutting beef loins were allowed to drip for 1 hour before re-weighing**.**

Results from Apple et al. (2014) showed that post-treatment yields from the Choice and dark cutting strip loins were similar. Furthermore, dark cutting loin sections enhanced with 0.35 (Exp. 1) and 0.50% (Exp. 2) lactic acid had 0.8 and 1.9% higher mean post-enhancement yields compared to sections enhanced with 0.15 (Exp. 1) and 0.35% (Exp. 2) lactic acid. Choice sections in experiment 1 had lower pre and post-enhancement pH values compared to the non-enhanced dark cutting strip loin sections. When dark cutting sections were enhanced with 0.15 and 0.35% lactic acid, post-enhancement pH values were not affected at the enhancement target of 105% in experiment 1. Pre-enhancement pH values were lower in Choice sections compared to dark cutting sections in experiment 2, yet post-enhancement pH values of the dark cutting beef samples was decreased by lactic acid enhancement at 115% of the raw product weight. When enhanced with 0.50% lactic acid, post-enhancement pH values were comparable among Choice and dark cutting sections. Additionally, post-enhancement of dark cutting strip loins enhanced at 110% of the raw product weight decreased pH values from 6.37 to 4.10 when the concentration of lactic acid increased from 0 to 2% (Sawyer et al., 2008). Moreover, research performed by Sawyer et al. (2009) showed that the pH of dark cutting strip loins was abbreviated by 0.37 and

1.77 pH units with 0.25 and 0.50% lactic acid enhancement at 110% of the raw weight. Additional pH values post-enhancement were reduced by 1.82 and 2.14 units when enhancement levels were 0.75 and 1% lactic acid.

When moisture analysis was evaluated in Apple et al. (2014), dark cutting sections enhanced with 0.35% lactic acid had greater total moisture than the dark cutting sections enhanced with 0.15% lactic acid in experiment 1. Additionally, regardless of lactic acid enhancement, dark cutting sections had higher percentages of total and bound moisture and lower percentages of free moisture compared to the Choice sections. Total moisture percentages in experiment 2 were greater for dark cutting sections enhanced with 0.5% lactic acid than the non-enhanced dark cutting and Choice sections. However, the non-enhanced dark cutting sections had greater moisture levels compared to the Choice sections. Dark cutting sections left untreated had the most bound moisture and the lowest free moisture percentages in experiment 2 despite the lactic acid enhancement caused a reduction in the amount of bound moisture and increased free moisture proportions compared to the non-enhanced dark cutting sections. Furthermore, the bound and free water percentages from the dark cutting sections enhanced with 0.5% lactic acid did not differ from the Choice sections. According to Wismer Pedersen (1971) and Hamm (1986), normal pH of meat is somewhat greater than the isoelectric point of myofibrillar proteins, and the number of reactive groups that are able to bind water will increase when the muscle pH values are higher than 6.0 or lower than 4.0 (Gault, 1985). Dark cutting beef has more reactive protein side chains and consequently the non-enhanced dark cutting sections in Apple et al. (2014), with mean pH values greater than 6.5, had the highest amount of bound moisture and the least amount of free moisture. Post-enhancement pH values combined with water-holding capacity of dark cutting beef were not affected by lactic acid solutions in

experiment 1. However, reduction of post-enhancement pH of the longissimus muscle enhanced with 0.5% lactic acid culminated the percent of bound and free moisture to be comparable to Choice sections. Results from Sawyer et al. (2008, 2009) were similar to Apple et al. (2014) noting that post-enhancement pH of dark cutting beef was lowered to values closer to the isoelectric point with lactic acid solution.

Organic acids used in the food industry are labeled as Generally Recognized as Safe (GRAS) and are listed in the FSIS regulations for use as an acidifier in meat products at a safe level of up to 2.5% of solution without labeling (FSIS-USDA, 1996). The FSIS has allowed concentrations of acetic, citric, and lactic acids to be used at concentrations of 1.5-2.5% (FSIS-USDA, 1996). Overall, using organic acids in the food industry has been effective in decreasing the bacterial and pathogenic concentrations on carcasses (Dickson & Anderson, 1992).

Conclusion

Meat quality is of upmost importance in regards to consumer acceptability. There are many aspects and production systems that affect meat quality that can have a lasting effect on the overall acceptability of the product. Pre-harvest management could play an important role in reducing the negative beef quality attributes such as bruising and dark cutting beef, costing the beef industry approximately $22 million dollars annually (Virginia BQA, 2010). Through cohesive efforts from producers and industry leaders, new and improved production systems can help ease the stress of livestock species and improve efficiency. Furthermore, even though the majority of research pertaining to dark cutting beef has been attributed to ante-mortem factors, dark cutting beef still remains an issue. Perhaps providing economic incentives to producers who have continued high quality beef would help reduce the amount of dark cutting beef through genetic selection. The next course of action could be to find innovative ways to improve dark

cutting beef once established through the utilization of organic acids. Lactic acid has been the most predominately used organic acid but further research is warranted to study the lasting effects on color stability as well as antimicrobial regulation in dark cutting beef. By using the GRAS list established by the FDA, many more organic acids can be used to help improve dark cutting beef to a state in which the beef can be used for purposes other than ground meat or ready-to-eat products. Through industry leaders and continued research studying the ways in which dark cutting beef can be improved post-mortem, the beef industry as a whole can benefit and continue to produce safe, wholesome, and nutritious products.

Preliminary Research

Dark cutting beef remains an issue as it relates to consumer acceptability. Consumers associate dark cutting beef to be old, short shelf life, and poor flavor (Holmgren and Zobell, n.d.). Perhaps through by using acetic acid, the meat quality attributes associated with dark cutters can be positively affected to a level that closely compares to USDA Select loins. Prior to this study, preliminary research was conducted to facilitate the materials and methods of this study. One cull cow strip loin was fabricated from a beef carcass at the Auburn University Meats Lab. The strip loin was cut into 8 steaks for the analysis of color and drip loss. After fabrication, all steaks were randomly assigned a treatment. Treatments were assigned by obtaining an initial steak weight. After steaks were weighed, treatments were applied as a percentage of the initial steak weight. The steak was weighed in grams and the amount of each treatment was calculated by taking a percentage (0.5%, 1%, 1.5%, 2%, 3%, 4%, and 5%) of the initial weight of the steak. One steak served as the control. The treatment solution e(Lm)inate® V, was provided by a food ingredient company Hawkins Inc.

Color

An initial color reading (L*, a*, and b*) was taken on d 1 of fabrication. The colorimeter used illuminant D_{65}, with a 10° observer angle, and a 2.54 cm aperture. After all initial colorimetric values were recorded utilizing a Hunter Miniscan XE Plus, each steak was injected at the predetermined vinegar percentage with a 10 mL syringe in equal portions throughout the steak. The steaks were then vacuum packaged in 8x13 oxygen impermeable bags and stored at 4± 2°C for 24 hours. When the storage time was complete, a final color value was taken from each steak. Additionally, a subjective color assessment was conducted by the researcher to evaluate any discoloration from slight to extreme color change.

Drip Loss

Drip loss was determined on d 2 after fabrication. Samples were weighed, then suspended by a fish hook (Model 31 number:121 – 2/0, Eagle Claw®) in a 800 mL plastic screw cap container (Nalgene®) and stored for 48 h at a temperature of 4°C. Following the 48 h time period, samples were removed from hooks and blotted to remove excess surface fluid. Samples were then weighed to the nearest 0.1g. Percent drip was calculated by the NPPC (2000) recommended equation.

Results

No statistical analysis was conducted during the preliminary stages. When colorimetric values were evaluated, it was observed that regardless of treatment, L* values increased, a* values drastically decreased, and b* values increased as vinegar concentration increased. Drip loss results reported that the control had the least drip loss of 0.76% compared to the greatest drip loss value of 1.87% for the 5% treatment. Treatment 0.5% had a drip loss value of 1.26%, treatment 1% had a value of 1.13%, and treatment 1.5% had a value of 1.81%.

Subjective color evaluations were taken during drip loss calculations. The samples were removed from the container and given a score of slight to extreme color change. Treatments containing 0.5% vinegar had a slight discoloration, 1% vinegar had a slight discoloration, 1.5% vinegar had a moderate discoloration, and treatments 4% and 5% had extreme discoloration. A conclusion was made from this data that it was unnecessary to include any treatment over 2% vinegar in the official research trial.

A separate analysis was conducted with another cull cow strip loin for cook loss. The loins (n=13) were fabricated at the Auburn University Meats Lab and cut into 13 steaks. In this

analysis, steaks were marinated in e(Lm)inate® V for 24 h and stored at 4°C. One steak served as a control and treatments consisted of 0.5, 1, 2, 3, 5, 10, 15, 20, 50, 70, and 100%.

Cook loss

All streaks were weighed prior to cooking and were cooked to an internal temperature of 71°C with the aid of copper constantan thermocouple wire inserted into the geometric center of the steak and attached to a hand-held Omega data logger HH309A temperature recorder (Omega, Stamford, CT). Steaks were allowed to cool before being weighed to obtain a final cooked weight to calculate cook loss. Results showed that 50% vinegar solution had the greatest cook loss of 23.37% and 10.95% cook loss from 0.5% vinegar. The control steak had a cook loss value of 15.39%. Also during cook loss calculations, the researcher sampled each steak in order to determine a flavor profile for each vinegar percentage. The subjective measurement showed that vinegar concentrations above 3% were very strong in acidic taste. Upon completion of the preliminary research, a conclusion was made that the official study would include treatments 0.5, 1.0, and 1.5% e(Lm)inate ® V solution due to the higher concentrations being non-beneficial.

Materials and Methods

Strip Loins

Research strip loins were provided by Cargill, Inc. in Dodge City, Kansas. Strip loins were fabricated at the harvest facility and vacuum packaged and shipped in a Styrofoam color with ice packets to the Auburn University Meats Lab the following day. Upon arrival, the strip loins remained in the package and placed in a holding cooler at 4 ± 2°C. Samples remained in storage for five days until further use. On day five after fabrication, all loins were randomly assigned a number 1-16 and each loin was sectioned into four pieces. A Latin square design (repeated four times) was used to assign a quadrant either W, X, Y, or Z from cranial to caudal end. Each of the four sections from each loin was assigned a treatment of a control, low, medium, or high concentration of acetic acid. Initial color and pH readings were recorded by calibrating the color and pH meter (Oakton® Vernon Hills, IL) and reading the sections at the most anterior end. The pH readings were taken in duplicate to obtain an accurate representation and values were averaged. Color readings were also obtained by taking two measurements and then averaged through the colorimeter. The colorimeter used illuminant D_{65}, with a 10° observer angle, and a 2.54 cm aperture. All steaks were vacuum packaged and stored at 4 ± 2°C. After a one day storage period, all steaks assigned to a low, medium, or high treatment were injected to obtain a pickup percentage. Sections from the low level treatment group were weighed and injected at the lowest setting on the injector and a final weight was obtained to give an average pickup percent for that treatment group. Similarly, the medium level treatment sections were weighed and injected at a sprayer speed slightly faster than the low level treatment group to obtain an average for that treatment group. Lastly, the high level treatment sections were passed through the injector at a faster sprayer speed and averaged to obtain a high level treatment

percent. After all steaks were weighed, injected, and averaged, each treatment group was assigned a pickup percent of a low level treatment of 0.4%, a medium level treatment of 1.2%, and a high level treatment of 1.6%. All control steaks remained in the cooler until needed. Once the sections were injected, each section was vacuum packaged in 18 X 20 oxygen impermeable bags and stored at 4 ± 2°C for three days. Upon completion of the storage period, all strip loin sections were analyzed for a final color and pH reading at the anterior end and then cut into three individual steaks for further laboratory analysis. Steaks were allocated for sensory analysis, WBSF and cook loss, and one TBARS and drip loss, respectively. Steaks determined for sensory analysis were vacuum packaged and frozen until needed. Steaks for WBSF and cook loss were vacuum packaged and placed in a holding cooler at 4 ± 2°C for an additional three days. Finally, steaks for TBARS and drip loss were taken to the laboratory at Upchurch Hall at Auburn University and a sample was taken from each steak for determination of drip loss. Once the sample was taken from each steak, the steaks were vacuum packaged and frozen.

Drip Loss

A 40 to 50 g sample was obtained from each steak and trimmed of any fat and connective tissue. Samples were weighed, then suspended by a fish hook (Model 31 number:121 – 2/0, Eagle Claw®) in a 800 mL plastic screw cap container (Nalgene®) and stored for 48 h at a temperature of 4°C. Following the 48 h time period samples were removed from hooks and blotted to remove excess surface fluid. Samples were then weighed to the nearest 0.1g. Percent drip was calculated by the following NPPC (2000) recommended equation.

Percent Drip Loss = (Loss in Weight / Initial Weight) x 100

Cook Loss and WBSF

Steaks to be used for cook loss and WBSF were stored at 4 ± 2°C for 48 hours. Steaks were cooked on clam-shell-style grills (Calphalon Removable Plate Grill, Caphalon, Perrysburg, OH), preheated to ~177°C and cooked to an internal temperature of 71°C. Temperatures were monitored with copper constantan thermocouple wire inserted into the geometric center of the steak and attached to a hand-held Omega data logger HH309A temperature recorder (Omega, Stamford, CT). Cook loss values were determined by weighing steaks prior to cooking. After cooking, steaks were allowed to cool and were then reweighed to determine percent cook loss. Also after steaks were cooled, external color readings were obtained by utilizing a Hunter Miniscan XE Plus. The colorimeter used illuminant D_{65}, with a 10° observer angle, and a 2.54 cm aperture. Cooked steaks were then covered in aluminum foil, labeled, and chilled at 4°C for 24 h. Six cores (1.27 cm in diameter) were removed from each steak with a brass cork borer (Model 1601A Series Brass Cork Borer, Boekel Scientific, Feasterville, PA), parallel to the muscle fiber orientation. Each core was sheared once at its center, perpendicular to the muscle fiber orientation, using a TA-XT2i Texture Analyzer shear machine (Texture Technologies Corp., Scarsdale, NY). The peak force measurements were then averaged from the 6 cores from each steak to be used for statistical analysis. The probe was programmed to be lowered 30 mm after detection of resistance. The penetration speed was 3.3 mm/s with a post-test speed of 10 mm/s and a pre-test speed of 2.0 mm/s.

Sensory Evaluation

Randomly selected frozen strip loin steaks were thawed at 4°C for 24 h and cooked as described for WBSF. The steaks were trimmed of external fat and connective tissue. The samples were then cut into 1.27 cm × 1.27 cm × steak thickness portions using a plastic grid, placed in

sample cups and labeled. Sample cups were then placed in pans and kept in a warming oven until served to a trained sensory panel, consisting of 8-14 members. Each panelist was given a sample cup containing 2 samples from each steak for evaluation of initial and sustained juiciness, initial and sustained tenderness, beef flavor intensity, and off flavor intensity on a scale of 1 to 8, where 1 = extremely dry, tough, bland, and uncharacteristic of beef, and 8 = extremely juicy, tender, intense, and characteristic of beef. Panelists evaluated samples in secluded partitioned booths with red incandescent light. Panelists were also instructed to cleanse their palate with a salt-free saltine cracker and a sip of apple juice before each evaluating each sample. A total of 8 sessions (November 14, 17, 18, 28, 29, 30, 2016 and December 1-2, 2016) were utilized to complete sensory evaluation of 80 total samples, 8-12 samples per session.

Thiobarbituric Acid Reactive Substances (TBARS)

Steaks allotted for TBARS analysis were thawed at 4°C for 24 h and followed a modified procedure described by Tarladgis et al. (1960) and performed by Fernando et al. (2003). A 5 g sample was cut from the steak free of fat and connective tissue and blended in a Waring® commercial laboratory blender with 30 mL of deionized water for 1 min and transferred to a 250 mL distillation tube. The blender cup was washed with an additional 20 mL of deionized water and poured into the same distillation tube. A volume of 2.5 mL of 4 N HCl was added to the mixture, stirred, and distilled at a maximum rate until 25 mL of distillate was collected in a 25 mL volumetric flask. After distillation was complete, 5 mL of distillate was pipetted into a 50 mL presterilized centrifuge tube from VWR® in duplicate and 5 mL of .02 M 2-thiobarbituric acid in 90% acetic acid was added and vortexed. The caps were tightly capped and heated in a reciprocal shaking boiling water bath (Thermo Scientific Laboratory Services Equipment) for 30 min and cooled to room temperature. The absorbance was read at 532 nm using a Beckman

Coulter® Du® 730 Life Science UV/Vis spectrophotometer. A K-value was calculated using 1,1,3,3-tetraethoxypropane as the standard and the TBARS readings were recorded by multiplying the absorbance by the K-value of 7.8 (Tarladgis et al. 1960).

Statistical analysis

Statistical analysis was performed with the mixed procedure of SAS version 9.4 (SAS Inst. Inc., Cary, NC). Type-3 tests of fixed effects were analyzed for all variables. Fixed effects were meat (DFD vs RFN), Location, and Treatment. Least means squares were separated by using the DIFF procedure. Statistical significance was reported as P values being ≤ 0.05.

Results and Discussion

pH

When evaluating initial pH (IpH) among DFD and RFN strip loins, a difference ($P<0.05$) was seen comparing the two variables. Table 1 and Table 2 lists the means of the IpH and final pH (FpH) values. The RFN loins showed an IpH of 5.59 compared to the DFD loins with an IpH of 6.04. Previous research states that when beef pH exceeds 6.0 within 24 h after harvest, meat quality can deteriorate and the eating experience is undesirable for the consumer. Furthermore, as the pH increases above 6.0, economic losses begin to increase (Corstiaensen et al., 1981, Pipek et al., 2003, Viljoen et al., 2002 and Wulf et al., 2002). According to Mach et al. (2008), when the pH is greater than 5.8, the carcass is discounted between 30-60% in the Spanish meat industry. Furthermore, a consumer survey in England conducted by Dransfield (1981), reported that consumers preferred normal pH beef over DFD beef due to the stronger beef flavor of the normal steaks. In a study conducted by Viljoen et al. (2002), consumers preferred ($P=0.02$) the color of the normal pH (pH=5.51-5.64) raw steaks compared to the raw DFD (pH=6.15-6.37) steaks. There was no difference among treatments ($P>0.68$) or location ($P>0.54$). Moreover, there was no difference ($P>0.39$) for FpH when comparing RFN (5.67) and DFD (5.87) loins as well as location. However; there was a variation ($P<0.04$) detected within treatment as shown in Table 2. It would be expected that the DFD control (DFDC) sectioned loins would have a higher FpH value compared to the other treatments (0.4%, 1.2%, and 1.6%). As is evident in Table 2, DFDC FpH values were the highest at 5.87 in comparison to 0.4%, 1.2%, and 1.6% with respective FpH values of 5.76, 5.75, and 5.70, which followed the hypothesis trend of increasing e(Lm)inate® V concentrations related to a lower final pH.

Cook Loss

Cook loss was evaluated in conjunction with steaks assigned for WBSF measurements. There was no difference ($P>0.05$) among DFD or RFN strip loins, location or treatment. The cook loss values when comparing DFD and RFN steaks was 19.25% for DFD steaks and 20.20% for RFN steaks. It was hypothesized that the DFD steaks would have a higher cook loss value due to the loss of water binding ability during cooking. In a dissertation conducted by Grayson (2014) at Texas A&M University, cook loss decreased as pH increased and severe dark cutting beef had the lowest cook loss ($P<0.05$) whereas normal beef had the highest cook loss values ($P<0.05$). Results from the present study were not different comparing DFD and RFN steaks which are in agreement with Purchas and Aungsupakorn (1993), who reported no difference in cook loss values comparing bulls and steers with a different pH. Although the findings of the present study report that DFD steaks had numerically lower cook loss values compared to RFN steaks, no statistical difference was observed. However, the results of the present study begin to follow the trend which are in agreement with Bouton et al. (1971, 1972a) who report that as pH increases, cook loss decreases. Dransfield (1981) and Purchas (1990) convey that meat with a higher pH result in more open protein structure which allows more water to be incurred within the myofibril. When relating treatments to cook loss values, there was also no difference observed. The highest cook loss values within treatments was 1.2% (Y) with a 20.19% cook loss compared to the lowest cook loss percent at 19.10% for the DFDC steaks. These results are shown in Table 2.

Drip Loss

Drip loss was conducted on d 3 after injection with e(Lm)inate® V. As is shown in Table 2, there was no difference ($P>0.09$) among treatments. The greatest drip loss effect recorded for

1.6% (Z) e(Lm)inate® V with a value of 1.27%. The least drip loss was identified for the DFDC streaks at 0.91%. Due to the high water holding capacity of the DFD steaks, it was expected that drip loss values would be the lowest for the DFDC steaks compared to the RFN steaks. Analyzing DFD and RFN steaks proved the hypothesis that DFD steaks had lower drip loss values. In a Canadian study evaluated by Holdstock et al. (2014), dark cutting carcasses were categorized into two different dark cutting classifications based upon pH of the *Longissimus thoracis*. Classic dark cutters had a pH greater than 6.0 and atypical dark cutters had a pH of less than 6.0. Holdstock et al. (2014) reported that classic dark cutters resulted in lower drip loss values compared to atypical dark cutters and the control had the greatest drip loss value ($P<0.05$). Results from the present study are in agreement with the findings of Holdstock et al. (2014). There was a difference ($P<0.02$) comparing DFD and RFN steaks as DFD steaks drip loss values were 0.82% compared to RFN steaks with a value of 1.23%.

Warner Bratzler Shear Force (WBSF)

Warner Bratzler Shear Force values were obtained on d 6 after injection. The assumption prior to this study was that there would be a difference of WBSF values within strip loins. As muscle location moved from anterior to posterior toward the sirloin, the more connective tissue would be present, and therefore lead to increased toughness. Katsaras and Peetz (1990) reported that meat from DFD carcasses are typically very tender but in a different manner than normal meat. Katsaras and Peetz (1990) also state that a possible explanation for the increased tenderness of DFD beef relates to the increased fragmentation of the myofibrils during heating compared to normal beef. As reported by Wulf et al. (2002), cooked longissimus muscles from DFD carcasses had 46% higher shear force values compared to normal carcasses. Furthermore,

Wulf et al. (2002) described there was considerable variation among tenderness among DFD carcasses compared to normal carcasses within the longissimus (P<0.0002).

Results of the present study show that regardless of DFD or RFN meat, there was no difference. Moreover, when Holdstock et al. (2014) evaluated shear force values among normal and DFD meat, the atypical (pH<6.0) steaks from the *longissimus thoracis* had the greatest (P<0.05) shear force values of 8.06 kg. Shear force values for the classic (pH>6.0) steaks were 5.47 kg and the control steaks had a shear force value of 6.18 kg. Although there was no difference within the present study, WBSF values were numerically higher for the DFD steaks compared to the RFN steaks which contradicts results from Holdstock et al. (2014) even though there was variation in muscle location. Dransfield (1981) and Purchas (1990) report that as pH increases, the structure within proteins are more open, which allows more water to be in the myofibril and less structural components within a cross-section. Therefore, a lesser shear force would be required to shear the meat and would positively impact tenderness. However, these conclusions do not explain the discrepancy in the results of the present study comparing DFD and RFN steaks.

When examining location, there was a difference (P<0.01) identified. Location W, which was the most anterior, was evaluated by WBSF to be the most tender at a force value of 2.89 kg. WBSF means are reported in Table 3. Location X had a shear force value of 3.01 kg and Location Z, which was most posterior, had a shear force value of 3.12 kg. Furthermore, Location Y had the greatest shear force value at 3.48 kg. Previous research has evaluated the effect of WBS on location within the same muscle. Ramsbottom et al. (1945) reports that the caudal end of the longissimus was the most tender whereas Martin et al. (1971) report that the cranial end of the longissimus was the most tender. Furthermore, Jeremiah and Murray (1984) state that there is

no effect within the longissimus as it relates to location. Thus, it is evident that within research study, WBS values are contradicting. In contrast to the present study in which the *longissimus lumborum* muscle was evaluated, Wheeler et al. (1996) report that there is no effect on longissimus muscle location in relation to shear force. In addition, the location within the *longissimus thoracis* and *lumborum* were no different ($P>0.05$) among shear force. The discrepancy between the present study and the study conducted by Wheeler et al. (1996) evaluating location effect within a muscle on WBS could be a result of coring. Both studies cored the muscle parallel to the muscle fiber orientation; however, there could have been more connective tissue within Location Y that could not be seen which would explain the increased toughness within that location. There were no differences within treatments ($P>0.48$) or between DFD and RFN steaks ($P>0.15$).

Color

In this study, all initial color measurements were taken in the same anterior location within strip loin sections. Initial color scores are reported in Tables 4 and 5. Initial colorimetric scores are compared for DFD and RFN loins in Table 5 which lists the means of the colorimetric values L*, a*, and b*. There was a difference ($P<0.0003$) observed for initial L* (IL) values comparing DFD and RFN loins which are in agreement with Wulf et al. (2002) who reported that normal carcasses (pH=5.53) had a L* value (recorded from the longissimus) of 40.6 compared to the DFD carcasses (pH=6.00) with a L* value of 34.0 ($P<0.0001$). The greatest L* values were RFN (41.42) strip loin sections compared to DFD L* values at 32.89 which were in agreement with the hypothesis that DFD meat would have a lower L* value due to the darker appearance. The initial color readings were done prior to any treatments so there was no difference in initial L*, a*, and b* values when comparing treatments. Initial a* (Ia) values showed no difference

(*P*>0.13) between DFD and RFN loins which contradict the results from Wulf et al. (2002) that report the a* value for normal carcasses was 27.5 compared to the DFD carcasses with an a* of 20.6 (*P*<0.0001). The RFN loins had the greatest a* value of 16.17 compared to the DFD loins with an a* value of 14.30. In addition, there was not a difference (*P*>0.39) among locations. When comparing initial b* (Ib) values to DFD and RFN loins, there was a difference (*P*<0.0003). RFN sections had the greatest b* value at 15.30 compared to DFD sections with a b* value of 10.94. These results agree with Wulf et al. (2002) who report that the b* value for the normal carcasses was 12.4 and the b* value for the DFD carcasses were 7.4 (*P*<0.0001). Also, regardless of treatment, there was no difference (*P*>0.61) when comparing location.

Final colorimetric values were taken on d 3 after injection and read from the anterior location. Table 4 and 5 report the means for the final colorimetric values. There was no difference (*P*>0.07) in L* values comparing DFD and RFN loins. DFD loins had the lowest L* values of 37.72 compared to an L* value of 40.99 for the RFN loins. There was also no difference (P>0.05) for L* values comparing location. However, a difference (*P*<0.0001) was identified when comparing treatments. The greatest final L* (FL) value among treatments was 42.55 for 1.6% (D). Treatment A (0.4%) had a final L* value of 38.60. Treatment B (1.2%) obtained a final L* value of 40.13 and treatment C (control) had the smallest final L* value of 36.14. The results followed the hypothesis trend that final L* would increase as treatment percentage increased. Final a* (Fa) values did not differ for DFD and RFN loins (*P*>0.59) or location (*P*>0.79). Yet, there was a difference (P<0.001) within treatments. The greatest final a* value was 15.22 for treatment A (0.4%). Treatment B (1.2%) acquired a final a* value of 12.27 and treatment C (control) had a final a* value of 14.99. Treatment D (1.6%) had a final a* value of 12.07. The hypothesis was that as treatment increased, the a* value would decrease. As the

results indicate, there was a slight increase in final a* value between treatment C to treatment A, however; the hypothesis was validated as the other treatments were evaluated. Furthermore, final b* (Fb) colorimetric values were not different ($P>0.16$) within location. There was a difference ($P<0.002$) between DFD and RFN loins. RFN loins had the greatest final b* value of 15.12 compared to the DFD loins with a final b* value of 12.78. When treatment was evaluated there was a difference ($P<0.02$) detected. Treatment A had the greatest final b* value of 14.49, treatment B had a value of 13.77, treatment C had the lowest value of 13.13, and treatment D had a value of 14.42.

Cooked external colorimetric values were recorded on d 6 after injection. The means for cooked color are reported in Tables 6 and 7. There was no difference ($P>0.05$) in cooked external color values between DFD and RFN loins, location, or treatment for L*, a*, and b*. Cooked internal values were also recorded on d 6 after injection. Cooked internal L* values did not differ ($P>0.05$) for DFD and RFN loins, location, or treatment. However, it was hypothesized that there would be a difference for a* values between DFD and RFN steaks. According to Mendenhall (1989), when normal beef is cooked, the color changes from a red appearance to a gray appearance whereas Trout (1989) report that when dark cutting beef (pH>6.0) is cooked to an identical end-point internal temperature, the color appears to be redder and undercooked due to myoglobin being protected from heat denaturation. Furthermore, Gašperlin et al. (2000) state that when cooked dark cutting beef is exposed to oxygen, the internal color may become oxygenated, therefore, developing a bright red color that imitates fresh normal beef. Previous research suggests that DFD meat has a persistent pink cooked color which results in a greater a* value (Sawyer et al. 2008, 2009). Viljoen et al. (2002) reported that there was no difference ($P>0.75$) when consumers rated the cooked DFD and normal steaks for color. The DFD steaks

were scored by the consumers to be 6.0 and the normal steaks were scored at 5.9. The scale utilized by Viljoen et al. (2002) was where 1=totally unacceptable and 9=very acceptable. Cooked internal a* values comparing DFD and RFN steaks are reported in Table 9. In this study, cooked internal a* values were different ($P<0.03$) between DFD and RFN steaks. DFD steaks had a greater a* value of 7.88 compared to the RFN a* value of 6.56 which supported the hypothesis. These results are in agreement with Apple et al. (2014) who report that regardless of lactic acid treatment (0.15, 0.35%) there was a difference ($P<0.05$) between the dark cutters and normal pH strip steaks in regards to internal cooked a* value. Results from Apple et al. (2014) report that the dark cutting control strip steaks had a cooked internal a* value of 18.08 compared to the cooked internal a* value of the normal strip steaks (15.96). There were no differences for a* values among location ($P>0.26$) or treatments ($P>0.20$) within the present study. When evaluating internal b* values, there was no difference ($P>0.05$) on DFD and RFN steaks, location, or treatment which agree with findings from Apple et al. (2014) that report b* values were not different ($P \geq 0.35$) for DFD steaks regardless of treatment with lactic acid compared to normal beef.

Thiobarbituric Acid Reactive Substances (TBARS)

TBARS values were frozen on d 3 after injection and results were recorded after samples were thawed starting on d 14-21. Results for TBARS means are reported in Tables 1 and 2. It was conjectured that as the concentration of vinegar increased, the TBARS value would decrease. However, there was no difference ($P>0.73$) among DFD and RFN steaks. DFD steaks had a greater TBARS value of 0.2157 mg MDA/kg compared to the RFN steaks with a value of 0.1930 mg MDA/kg. There was no difference between location ($P>0.86$) and treatment ($P>0.24$) which rejected the hypothesis that as the percent vinegar increased, the TBARS value would

decrease. Treatment A had a value of 0.1991 mg MDA/kg, treatment B had a value of 0.2096 mg MDA/kg, treatment C had the lowest value of 0.1569 mg MDA/kg, and treatment D had the greatest value of 0.2519 mg MDA/kg. Based upon the results of this study, it could be beneficial to add an antioxidant in conjunction with the vinegar to help retard rancidity.

Sensory Evaluation

Sensory evaluation steaks were frozen on d 3 after injection and were performed between d 6 and 24. Sensory scores are reported in Table 3. There were no differences ($P>0.05$) between DFD and RFN samples, treatment, or location for initial juiciness, sustained juiciness, initial tenderness, sustained tenderness, and beef flavor intensity. According to Wulf et al. (2002), cooked beef palatability was lower for DFD carcasses compared to normal carcasses. Wulf et al. (2002) also reported that sensory panel data revealed less tender longissimus, gluteus medius, and semimembranosus for DFD carcasses than normal carcasses.

When tenderness scores within the present study were reported, the sensory panelists responses were compared to WBSF measurements and the results had a strong correlation among location. As stated before, Location W, which was the most anterior location, had the lowest shear force value at 2.89 kg. Location W was also reported by the panelists to have the greatest initial tenderness score of 5.89. Furthermore, Wulf et al. (2002) reported that the sensory panel rated DFD longissimus steaks much tougher as well as a much lower percentage of very tender steaks compared to normal beef. Also Grayson (2014) reported that there were differences ($P<0.05$) among tenderness between DFD and normal steaks which disagree with the results of the present study.

As mentioned previously, sensory panel juiciness and beef flavor intensity scores were not different in the present study. These results are in agreement with Wulf et al. (2002) who

report that dark cutting carcasses had no effect (P>0.05) on sensory panel juiciness and flavor intensity. Dransfield (1981) reported that DC steaks had less beef flavor than normal pH steaks. On the other hand, Grayson (2014) reported that severe (pH=6.89) and moderate (pH=6.59) dark cutters were juicier than normal (pH=5.66) steaks as rated by a trained sensory panel.

Additionally, it was hypothesized that trained sensory panelist would be able to detect increasing levels of vinegar. The means for off flavor intensity (OFI) among treatments are reported in Table 8. There was a difference (P<0.03) among treatments as treatment A had an OFI score of 7.71. Treatment B had an OFI of 7.53. Treatment C had the greatest OFI score of 7.76 and treatment D had the lowest OFI score of 7.47. When DFD and RFN steaks were compared for OFI, there was no difference (P>0.78). RFN steaks had an OFI of 7.65 and the DFD steaks had an OFI of 7.59, which reject the hypothesis that panelists would be able to detect an off flavor between the DFD and RFN steaks. However, Wulf et al. (2002) report that longissimus steaks from DFD carcasses resulted in more off flavors than the normal steaks yet, there was no difference (P>0.05) reported for off flavors between DFD and normal gluteus medius steaks or between DFD and normal semimembranosus steaks. Grayson (2014) also reported that severe and moderate dark cutters had greater (P<0.05) scores for “rancid” compared to normal steaks.

Implications

There are some aspects of shelf life that warrant further research regarding the effect of e(Lm)inate ®V on dark cutting beef. According to Hawkins Inc. e(Lm)inate® V has the ability to extend shelf life and is shown to reduce *Listeria* in formulated sausages. There were no shelf life or microbial research conducted within this study therefore; the effects of shelf life and microbial eradication of dark cutting beef require investigation. Due to the importance of color

appearance and stability among consumers, it may be warranted to investigate the effects of vinegar at an array of different levels to observe the cooked internal color of dark cutters as well as the addition of a phosphate to help facilitate internal color in the cooked product. After evaluation of the results, e(Lm)inate® V was only sufficient at altering the final raw color and pH to a level that closely represents a USDA Select strip loin. e(Lm)inate® V did not have a large effect on cook loss, WBSF, TBARS, and cooked internal color. Therefore, e(Lm)inate® V used alone would most likely not be a viable option in industry. However, results do suggest that it would be valuable to investigate the use of e(Lm)inate® V in conjunction with an antioxidant and/or functional ingredient used for binding water. The synergistic effects could improve raw and cooked color and increase water holding capacity in the raw product while reducing cook loss. Furthermore, the effects that acetic acid has on microbial survival could be beneficial to use e(Lm)inate®V as a antimicrobial carcass spray in combination with citric or lactic acid in future studies.

Table 1. LSMEANS and SEM of pH, cook loss, drip loss, and TBARS for DFD and RFN loins.

Meats	Initial pH	Final pH	Cook Loss (%)	Drip Loss (%)	TBARS(MDA mg/kg)
RFN	5.59±0.21[a]	5.67±0.22	20.20±1.74	1.23±0.17[a]	0.19±0.06
DFD	6.04±0.08[b]	5.87±0.08	19.25±0.61	0.82±0.06[b]	0.22±0.02

[abc] Means with common superscripts in the same column are not different (P>0.05).

Table 2. LSMEANS and SEM of pH, cook loss, drip loss, and TBARS for loins within treatments.

Treatments (%)	Initial pH	Final pH	Cook Loss (%)	Drip Loss (%)	TBARS(MDA mg/kg)
0	5.81±0.11	5.87±0.12^{b}	19.10±1.20	0.91±0.13	0.16±0.04
0.4	5.82±0.11	5.76±0.12^{bc}	19.59±1.20	0.99±0.13	0.20±0.04
1.2	5.81±0.11	5.75±0.12^{cd}	20.19±1.20	0.92±0.13	0.21±0.04
1.6	5.81±0.11	5.70±0.12^{cd}	20.03±1.20	1.27±0.13	0.25±0.04

^{abc} Means with common superscripts in the same column are not different (P>0.05).

Table 3. LSMEANS and SEM of WBSF and sensory evaluation for Location (Loc).

Loc	WBSF (kg)	Initial Juiciness	Sustained Juiciness	Initial Tenderness	Sustained Tenderness	Beef Flavor	Off Flavor
W	2.89±0.29[b]	5.41±0.20	5.35±0.19	5.89±0.26	5.82±0.24	6.06±0.08	7.65±0.20
X	3.01±0.29[b]	5.58±0.20	5.43±0.19	5.86±0.26	5.81±0.24	5.88±0.08	7.66±0.20
Y	3.48±0.29[a]	5.53±0.20	5.40±0.19	5.80±0.26	5.69±0.24	5.92±0.08	7.52±0.20
Z	3.12±0.29[b]	5.51±0.20	5.33±0.19	5.86±0.26	5.80±0.24	5.85±0.08	7.66±0.20

[abc] Means with common superscripts in the same column are not different (P>0.05).

An eight-point scale was used for the evaluations of initial and sustained juiciness, initial and sustained tenderness, beef flavor, and off flavor (1= extremely dry, extremely tough, extremely bland, extreme off flavor to 8= extremely juicy, extremely tender, extremely intense, and no off flavor).

Table 4. LSMEANS and SEM of raw colorimetric values for DFD and RFN loins within treatments.

Treatments (%)	Initial L*	Initial a*	Initial b*	Final L*	Final a*	Final b*
0	37.31±1.11	15.07±0.63	13.13±0.59	36.14±1.04^{b}	14.99±0.56^{a}	13.13±0.46^{bc}
0.4	37.19±1.11	15.30±0.63	13.22±0.59	38.60±1.04^{c}	15.22±0.56^{a}	14.49±0.46^{a}
1.2	36.93±1.11	15.24±0.63	13.12±0.59	40.13±1.04^{c}	12.27±0.56^{b}	13.77±0.46^{ab}
1.6	37.19±1.11	15.33±0.63	13.02±0.59	42.55±1.04^{d}	12.07±0.56^{b}	14.42±0.46^{a}

^{abc} Means with common superscripts in the same column are not different (P>0.05).

Table 5. LSMEANS and SEM of raw colorimetric values for DFD and RFN loins.

Meats	Initial L*	Initial a*	Initial b*	Final L*	Final a*	Final b*
RFN	41.42±2.04[a]	16.17±1.14	15.30±1.06[a]	41.00±1.65	13.85±0.71	15.12±0.69[a]
DFD	32.89±0.72[b]	14.30±0.40	10.94±0.38[b]	37.72±0.58	13.43±0.25	12.78±0.24[b]

[abc] Means with common superscripts in the same column are not different (P>0.05).

Table 6. LSMEANS and SEM for external and internal cooked colorimetric values for DFD and RFN loins.

Meats	Cooked External L*	Cooked External a*	Cooked External b*	Cooked Internal L*	Cooked Internal a*	Cooked Internal b*
RFN	48.77±4.13	6.82±1.10	12.74±2.00	54.28±1.49	6.56±0.54[a]	13.50±1.08
DFD	41.37±1.46	8.01±0.39	13.66±0.71	54.18±0.53	7.88±0.19[b]	15.47±0.38

[abc] Means with common superscripts in the same column are not different (P>0.05).

Table 7. LSMEANS and SEM for external and internal cooked colorimetric values within treatments.

Treatments (%)	Cooked External L*	Cooked External a*	Cooked External b*	Cooked Internal L*	Cooked Internal a*	Cooked Internal b*
0	44.79±2.38	7.52±0.64	13.03±1.19	53.80±0.92	7.39±0.38	14.01±0.66
0.4	42.97±2.38	7.52±0.64	12.44±1.19	54.23±0.92	7.37±0.38	14.94±0.66
1.2	46.41±2.38	7.31±0.64	13.50±1.19	54.72±0.92	7.44±0.38	14.72±0.66
1.6	46.11±2.38	7.29±0.64	13.82±1.19	54.18±0.92	6.69±0.38	14.27±0.66

[abc] Means with common superscripts in the same column are not different (P>0.05).

Table 8. LSMEANS and SEM for off flavor intensity within treatments.

Treatments (%)	Off Flavor Intensity
0	7.76 ± 0.12^{a}
0.4	7.71 ± 0.12^{ac}
1.2	7.53 ± 0.12^{bc}
1.6	7.47 ± 0.12^{b}

^{abc} Means with common superscripts in the same column are not different (P>0.05).

Table 9. LSMEANS and SEM for cooked internal colorimetric values.

Meats	L*	a*	b*
RFN	54.28±1.49	6.56±0.54[a]	13.50±1.08
DFD	54.18±0.53	7.88±0.19[b]	15.47±0.38

[abc] Means with common superscripts in the same column are not different (P>0.05).

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Appendix

Supplemental table 10. LSMEANS, SEM, and P-values of initial pH for meat and location.

	Initial pH	p-value
Meat		
RFN	5.57±0.21	0.05
DFD	6.04±0.08	0.05
Location		
W	5.83±0.11	0.54
X	5.81±0.11	0.54
Y	5.81±0.11	0.54
Z	5.81±0.11	0.54

Treatments (0, 0.4, 1.2, 1.6%) acetic acid.

Supplemental table 11. LSMEANS, SEM, and P-value of final pH for meat, location, and treatment.

	Final pH	p-value
Meat		
RFN	5.67±0.22	0.39
DFD	5.87±0.08	0.39
Location		
W	5.72±0.12	0.36
X	5.76±0.12	0.36
Y	5.77±0.12	0.36
Z	5.83±0.12	0.36
Treatment (%)		
0	5.87±0.12	0.04
0.4	5.76±0.12	0.04
1.2	5.75±0.12	0.04
1.6	5.70±0.12	0.04

Supplemental table 12. LSMEANS, SEM, and P-value of cook loss for meat, location, and treatment.

	Cook Loss (%)	p-value
Meat		
RFN	20.20±1.74	0.61
DFD	19.25±0.61	0.61
Location		
W	19.25±1.20	0.80
X	19.34±1.20	0.80
Y	20.33±1.20	0.80
Z	19.98±1.20	0.80
Treatment (%)		
0	19.10±1.20	0.83
0.4	19.59±1.20	0.83
1.2	20.19±1.20	0.83
1.6	20.03±1.20	0.83

Supplemental table 13. LSMEANS, SEM, and P-value of drip loss for meat, location, and treatment.

	Drip loss (%)	p-value
Meat		
RFN	1.23±0.17	0.02
DFD	0.82±0.06	0.02
Location		
W	0.89±0.13	0.14
X	0.93±0.13	0.14
Y	1.03±0.13	0.14
Z	1.24±0.13	0.14
Treatment (%)		
0	0.91±0.13	0.09
0.4	0.99±0.13	0.09
1.2	0.92±0.13	0.09
1.6	1.27±0.13	0.09

Supplemental table 14. LSMEANS, SEM, and P-value of WBS for meat, location, and treatment.

	WBSF (kg)	p-value
Meat		
RFN	2.73±0.51	0.15
DFD	3.52±0.18	0.15
Location		
W	2.89±0.29	0.01
X	3.01±0.29	0.01
Y	3.48±0.29	0.01
Z	3.12±0.29	0.01
Treatment (%)		
0	3.08±0.29	0.48
0.4	3.11±0.29	0.48
1.2	3.01±0.29	0.48
1.6	3.29±0.29	0.48

Supplemental table 15. LSMEANS, SEM, and P-value of initial L* for meat, location, and treatment.

	Initial L*	p-value
Meat		
RFN	41.42±2.04	0.0003
DFD	32.89±0.72	0.0003
Location		
W	37.14±1.12	0.63
X	37.47±1.12	0.63
Y	37.09±1.12	0.63
Z	36.90±1.12	0.63
Treatment (%)		
0	37.31±1.12	0.85
0.4	37.19±1.12	0.85
1.2	36.93±1.12	0.85
1.6	37.19±1.12	0.85

Supplemental table 16. LSMEANS, SEM, and P-value of initial a* for meat, location, and treatment.

	Initial a*	p-value
Meat		
RFN	16.17±1.14	0.13
DFD	14.30±0.40	0.13
Location		
W	15.42±0.63	0.39
X	15.04±0.63	0.39
Y	15.02±0.63	0.39
Z	15.45±0.63	0.39
Treatment (%)		
0	15.07±0.63	0.87
0.4	15.30±0.63	0.87
1.2	15.24±0.63	0.87
1.6	15.33±0.63	0.87

Supplemental table 17. LSMEANS, SEM, and P-value of initial b* for meat, location, and treatment.

	Initial b*	p-value
Meat		
RFN	15.30±1.06	0.0003
DFD	10.94±0.38	0.0003
Location		
W	13.35±0.59	0.61
X	13.08±0.59	0.61
Y	12.94±0.59	0.61
Z	13.11±0.59	0.61
Treatment (%)		
0	13.13±0.59	0.93
0.4	13.22±0.59	0.93
1.2	13.12±0.59	0.93
1.6	13.02±0.59	0.93

Supplemental table 18. LSMEANS, SEM, and P-value of final L* for meat, location, and treatment.

	Final L*	p-value
Meat		
RFN	40.99±1.65	0.07
DFD	37.72±0.58	0.07
Location		
W	40.36±1.04	0.0526
X	38.54±1.04	0.0526
Y	40.18±1.04	0.0526
Z	38.34±1.04	0.0526
Treatment (%)		
0	36.14±1.04	<0.0001
0.4	38.60±1.04	<0.0001
1.2	40.13±1.04	<0.0001
1.6	42.55±1.04	<0.0001

Supplemental table 19. LSMEANS, SEM, and P-value of external a* for meat, location, and treatment.

	Final a*	p-value
Meat		
RFN	13.85±0.71	0.59
DFD	13.43±0.25	0.59
Location		
W	13.21±0.56	0.79
X	13.76±0.56	0.79
Y	13.79±0.56	0.79
Z	13.80±0.56	0.79
Treatment (%)		
0	14.99±0.56	<0.0001
0.4	15.22±0.56	<0.0001
1.2	12.27±0.56	<0.0001
1.6	12.07±0.56	<0.0001

Supplemental table 20. LSMEANS, SEM, and P-value of final b* for meat, location, and treatment.

	Final b*	p-value
Meat		
RFN	15.12±0.69	0.002
DFD	12.78±0.24	0.002
Location		
W	14.27±0.46	0.16
X	14.14±0.46	0.16
Y	14.09±0.46	0.16
Z	13.31±0.46	0.16
Treatment (%)		
0	13.13±0.46	0.02
0.4	14.49±0.46	0.02
1.2	13.77±0.46	0.02
1.6	14.42±0.46	0.02

Supplemental table 21. LSMEANS, SEM, and P-value of cooked external L* for meat, location, and treatment.

	L*	p-value
Meat		
RFN	48.77±4.13	0.10
DFD	41.37±1.46	0.10
Location		
W	46.57±2.38	0.33
X	44.91±2.38	0.33
Y	45.10±2.38	0.33
Z	43.70±2.38	0.33
Treatment (%)		
0	44.79±2.38	0.11
0.4	42.97±2.38	0.11
1.2	46.41±2.38	0.11
1.6	46.11±2.38	0.11

Supplemental table 22. LSMEANS, SEM, and P-value of cooked external a* for meat, location, and treatment.

	a*	p-value
Meat		
RFN	6.82±1.10	0.31
DFD	8.01±0.39	0.31
Location		
W	7.23±0.64	0.23
X	7.85±0.64	0.23
Y	7.02±0.64	0.23
Z	7.55±0.64	0.23
Treatment (%)		
0	7.52±0.64	0.91
0.4	7.52±0.64	0.91
1.2	7.31±0.64	0.91
1.6	7.29±0.64	0.91

Supplemental table 23. LSMEANS, SEM, and P-value of cooked external b* for meat, location, and treatment.

	b*	p-value
Meat		
RFN	12.74±2.00	0.67
DFD	13.66±0.71	0.67
Location		
W	12.79±1.19	0.73
X	13.64±1.19	0.73
Y	12.90±1.19	0.73
Z	13.46±1.19	0.73
Treatment (%)		
0	13.03±1.19	0.44
0.4	12.44±1.19	0.44
1.2	13.50±1.19	0.44
1.6	13.82±1.19	0.44

Supplemental table 24. LSMEANS, SEM, and P-value of TBARS for meat, location, and treatment.

	TBARS (mg MDA/kg)	p-value
Meat		
RFN	0.1930±0.06	0.73
DFD	0.2157±0.02	0.73
Location		
W	0.2243±0.04	0.86
X	0.1901±0.04	0.86
Y	0.2110±0.04	0.86
Z	0.1922±0.04	0.86
Treatment (%)		
0	0.1569±0.04	0.24
0.4	0.1991±0.04	0.24
1.2	0.2096±0.04	0.24
1.6	0.2519±0.04	0.24

Supplemental table 25. LSMEANS, SEM, and P-value of initial juiciness for meat, location, and treatment.

	Initial juiciness	p-value
Meat		
RFN	5.42±0.33	0.62
DFD	5.60±0.12	0.62
Location		
W	5.41±0.20	0.79
X	5.58±0.20	0.79
Y	5.53±0.20	0.79
Z	5.51±0.20	0.79
Treatment (%)		
0	5.39±0.20	0.56
0.4	5.61±0.20	0.56
1.2	5.46±0.20	0.56
1.6	5.57±0.20	0.56

Supplemental table 26. LSMEANS, SEM, and P-value of sustained juiciness for meat, location, and treatment.

	Sustained juiciness	p-value
Meat		
RFN	5.28±0.30	0.54
DFD	5.48±0.11	0.54
Location		
W	5.35±0.19	0.91
X	5.43±0.19	0.91
Y	5.40±0.19	0.91
Z	5.33±0.19	0.91
Treatment (%)		
0	5.26±0.19	0.47
0.4	5.49±0.19	0.47
1.2	5.34±0.19	0.47
1.6	5.42±0.19	0.47

Supplemental table 27. LSMEANS, SEM, and P-value of initial tenderness for meat, location, and treatment.

	Initial tenderness	p-value
Meat		
RFN	5.95±0.45	0.70
DFD	5.76±0.16	0.70
Location		
W	5.89±0.26	0.94
X	5.86±0.26	0.94
Y	5.80±0.26	0.94
Z	5.86±0.26	0.94
Treatment (%)		
0	5.81±0.26	0.49
0.4	5.99±0.26	0.49
1.2	5.79±0.26	0.49
1.6	5.83±0.26	0.49

Supplemental table 28. LSMEANS, SEM, and P-value of sustained tenderness for meat, location, and treatment.

	Sustained tenderness	p-value
Meat		
RFN	5.88±0.42	0.64
DFD	5.68±0.15	0.64
Location		
W	5.82±0.24	0.76
X	5.81±0.24	0.76
Y	5.69±0.24	0.76
Z	5.80±0.24	0.76
Treatment (%)		
0	5.82±0.24	0.25
0.4	5.92±0.24	0.25
1.2	5.65±0.24	0.25
1.6	5.74±0.24	0.25

Supplemental table 29. LSMEANS, SEM, and P-value of beef flavor intensity for meat, location, and treatment.

	Beef Flavor Intensity	p-value
Meat		
RFN	5.96±0.10	0.47
DFD	5.89±0.03	0.47
Location		
W	6.06±0.08	0.14
X	5.88±0.08	0.14
Y	5.92±0.08	0.14
Z	5.85±0.08	0.14
Treatment (%)		
0	5.84±0.08	0.24
0.4	6.03±0.08	0.24
1.2	5.89±0.08	0.24
1.6	5.94±0.08	0.24

Supplemental table 30. LSMEANS, SEM, and P-value of off flavor intensity for meat, location, and treatment.

	Off Flavor Intensity	p-value
Meat		
RFN	7.65±0.19	0.78
DFD	7.59±0.07	0.78
Location		
W	7.63±0.12	0.52
X	7.66±0.12	0.52
Y	7.52±0.12	0.52
Z	7.66±0.12	0.52
Treatment (%)		
0	7.76±0.12	0.03
0.4	7.71±0.12	0.03
1.2	7.53±0.12	0.03
1.6	7.47±0.12	0.03

Supplemental table 31. LSMEANS, SEM, and P-value of cooked internal L* for meat, location, and treatment.

	L*	p-value
Meat		
RFN	54.28±1.49	0.95
DFD	54.18±0.53	0.95
Location		
W	54.30±0.92	0.96
X	54.43±0.92	0.96
Y	54.03±0.92	0.96
Z	54.17±0.92	0.96
Treatment (%)		
0	53.80±0.92	0.69
0.4	54.23±0.92	0.69
1.2	54.72±0.92	0.69
1.6	54.18±0.92	0.69

Supplemental table 32. LSMEANS, SEM, and P-value of cooked internal a* for meat, location, and treatment.

	a*	p-value
Meat		
RFN	6.56±0.54	0.03
DFD	7.88±0.19	0.03
Location		
W	7.00±0.38	0.26
X	7.03±0.38	0.26
Y	7.71±0.38	0.26
Z	7.15±0.38	0.26
Treatment (%)		
0	7.39±0.38	0.20
0.4	7.37±0.38	0.20
1.2	7.44±0.38	0.20
1.6	6.69±0.38	0.20

Supplemental table 33. LSMEANS, SEM, and P-value of cooked internal b* for meat, location, and treatment.

	b*	p-value
Meat		
RFN	13.50±1.08	0.09
DFD	15.47±0.38	0.09
Location		
W	14.60±0.66	0.85
X	14.69±0.66	0.85
Y	14.40±0.66	0.85
Z	14.25±0.66	0.85
Treatment (%)		
0	14.01±0.66	0.31
0.4	14.94±0.66	0.31
1.2	14.72±0.66	0.31
1.6	14.27±0.66	0.31