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Impact of Carcass Chilling System on Carcass Characteristics and Biochemical Changes in Beef Carcasses

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IMPACT OF CARCASS CHILLING SYSTEM ON CARCASS CHARACTERISTICS AND BIOCHEMICAL CHANGES IN BEEF CARCASSES

BY

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This thesis is approved as a creditable and independent investigation by a candidate for the master's degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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ABSTRACT

IMPACT OF CARCASS CHILLING SYSTEM ON CARCASS CHARACTERISTICS AND BIOCHEMICAL CHANGES IN BEEF CARCASSES

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Effective and efficient chilling of beef carcasses will directly influence meat quality and consumer acceptance. Over the past century the United States' meat industry has evolved, and spray chilling system have become common practice utilized by the beef industry. However, many of the chilling systems presently in use were designed to chill carcasses averaging lighter than the average beef hot carcass weight of today. Beef hot carcass weight has increased 68 kilograms from 1991 to 2019. The increase in beef hot carcass weight could result in longer chilling periods and present possible challenges for chilling systems used by the beef industry and impact meat quality. The impact of carcass chilling system on protein degradation postmortem has not been heavily investigated, however; the degradation of desmin early postmortem has been found to play a vital role in tenderness development.

The objective of this thesis was to determine the impact of air and spray chilling systems on carcass characteristics, instrumental color, and biochemical changes (temperature and pH decline of round, loin, and chuck primal, and protein degradation) in beef carcasses. Temperature decline was recorded by data loggers that were inserted into the round, loin, and chuck at varying positions and remained during the 24-hour chilling period. At 24 hours postmortem, a single 2.54 cm steak was removed from the

longissimus lumborum to be utilized for protein degradation analysis. There was no impact of chilling treatment on carcass characteristics, instrumental color, pH decline, or protein degradation. Spray chilling beef sides enhanced temperature decline in the round, loin, and chuck. Internal temperature decline differed at data logger position in the round and chuck. Tissues closer to the surface reached lower temperatures and chilled faster in both treatments. Additional research is needed to fully understand the impact of spray chilling systems on protein degradation postmortem in beef carcasses.

CHAPTER 1: REVIEW OF LITERATURE

LYDIA MARGARET HITE

Introduction

Efficaciously chilling beef carcasses following slaughter will directly impact ultimate meat quality and consumer appeal. Carcass chilling removes heat generated by metabolism following slaughter to allow carcass temperature to decline prior to fabrication. Prior to the use of refrigeration units, slaughter would occur in cooler seasons and product would be stored in caves (Savell, S. L. Mueller, & B. E. Baird, 2005; Zhang, Mao, Li, Luo, & Hopkins, 2019). The use of caves to provide a cooler environment eventually adapted into cellars being built for food preservation. When caves were being utilized, ice was gathered from frozen lakes to ensure cool temperatures for products. The use of caves and food preservation cellars was replaced in the early 1900s with the first refrigeration system being built. Over the past century the United States' meat industry has evolved, and spray chilling system have become common practice utilized by the beef industry. However, many of the chilling systems presently in use were designed to chill carcasses averaging 500 pounds compared to the average beef hot carcass weight of today which is averaging close to 850 pounds.

Spray Chilling

Spray chilling of beef carcasses is a widely accepted system in the United States. Spray chilling involves repeatedly spraying cold water onto carcasses. Initially, the suggested duration for spray chilling was the first 3-8 h postmortem (Hippe, Field, Ray,

& Russell, 1991). The process of spray chilling was invented and patented by Swift & Company in 1968 (Heitter, 1975; Savell, 2012). The system, called Clor-Chil, was offered to other companies willing to pay royalties. By 1975, all slaughter facilities owned by Swift Fresh Meats Company were utilizing this system. A major economic concern in the beef industry is carcass shrinkage during chilling. Clor-Chil was designed to accomplish three main objectives: 1) effectively reduce carcass shrinkage loss due to moisture evaporation during chilling, 2) reduce spoilage bacteria, and 3) sustain an acceptable product (Heitter, 1975; Savell, 2012). Clor-Chil sprayed a water and chlorine solution intermittently during chilling to reduce moisture lost during chilling and to destroy any bacteria present. Clor-Chil sprayed beef carcasses showed a 94.5% - 98.5% reduction in bacterial count (Heitter, 1975).

The patented process expired in the late 1980s allowing for wider adoption of spray chilling systems by beef processing facilities. Carcass chilling systems often utilize different combinations of chilling methods including chilled air, a combination of chilled air and sprayed water, varying wind speed and relative humidity. The use of increased air velocity and decreased temperature decreases chilling time for carcasses.

While the Clor-chil system involved spraying a water and chlorine solution, other systems involve spraying chilled, potable water alone. The liquid solution is typically delivered through polyvinyl chloride (PVC) pipes with staggered sprinkler nozzles positioned parallel to the rails of the chilling cooler. The PVC pipes are suspended above the rails to spray the liquid on the posterior end of the carcass allowing water to flow downward to the anterior end closest to the ground (Zhang et al., 2019).

As mentioned, the purpose of spray chilling carcasses is to reduce weight loss during postmortem chilling (Allen, Hunt, Filho, Danler, & Goll, 1987). Designing and implementing a spray chilling system to chill beef carcasses involves optimizing several factors including carcass type, interval between each spray, the duration of the spray, number of sprays per spray cycle, the temperature of the chilling cooler, fan speeds, number of fans, and the final spray time prior to carcass weighing (Zhang et al., 2019). Clor-chil, the most common system utilized during the 1980s, sprayed a cold water and chlorine solution during the first fourteen hours of chilling. The application time of spray chilling systems often varies among plants and the amount of liquid that is applied to carcasses during this time frame is often unknown (Greer & Jones, 1997). One of the more common spray chilling systems utilized for beef carcasses today involves spraying at intervals for the first 14 h of chilling with the remaining time allocated to allow the surface of the carcass to dry for application of grading stamps (Savell, 2012). Greer and Jones (1997) estimated between 8 and 12 h of spray chilling using four 60 s cycles per hour was needed to reduce beef carcass shrinkage (Greer & Jones, 1997). This was suggested as the optimal spray chilling period to reduce shrink but not for reduction of carcass temperature. The application time necessary to optimize heat removal is unknown because the efficiency and implementation of spray chilling has been primarily focused on reducing carcass shrinkage, not chilling.

Carcass chilling systems face the challenge of removing heat from the deeper tissue (Zhou, Xu, & Liu, 2010). Other challenges include minimizing carcass shrinkage, accommodating increasing carcass weights, and managing the rate of chilling. A chilling rate that is too slow or too rapid can result in inferior meat quality (Van Moeseke, De

Smet, Claeys, & Demeyer, 2001). The rate of chilling is influenced by many factors such as carcass size, carcass shape, adipose tissue content, subcutaneous fat thickness covering the carcass, cooler temperature, humidity, and air flow pattern (Savell et al., 2005).

Shrinkage

Shrinkage is caused by free water loss or evaporation of water through drip loss from the carcass (Savell et al., 2005). The amount of shrinkage a beef carcass will experience during the first 24 h post-mortem has been studied extensively and reported differently throughout research studies in the late 1970 and 1980s. Heitter (1975) reported application of Clor-Chil to beef carcasses resulted in a reduction in shrinkage between 0.5% and 1.25%. Allen et al. (1987) compared spray chilled beef sides to conventionally air chilled sides. The spray chilled sides were sprayed with 3 °C chilled water during the first 8 hours of chilling and were reported to have 1.14% less shrink than the conventionally chilled sides. Hippe et al. (1991) compared conventionally air chilled beef sides to spray chilled beef sides with shrinkage measured at 0-, 2-, 4-, 6-, and 24-hours. Spray chilling decreased shrink at 2-, 4-, 6-, and 24-hours postmortem compared to air chilled beef sides. Greer and Jones (1997) concluded a linear relationship existed between spray-chill duration and carcass weight loss that for every hour of spray chilling, carcass shrinkage was reduced 0.08g / 100g.

Another method investigated to reduce carcass shrinkage is shrouding carcasses. Shrouding is the use of a chlorinated soaked cloth that is wrapped around the carcass to smooth the fat and reduce cooler shrink. (Kastner, 1981) reported that shrouded beef carcasses chilled by conventional air overnight experienced a 0.75% to 2% reduction in

shrink compared to non-shrouded carcasses. Jones and Robertson (1988) evaluated shrouded and unshrouded beef sides that were spray chilled for 0 h or 4 h. The unshrouded and shrouded beef sides had decreased temperature in the *longissimus dorsi* after 4 hours of spray chilling compared to 0 hours of spray chilling. Lee et al. (2006) evaluated shrouded and unshrouded beef sides that were spray chilled or conventionally chilled and showed the unshrouded spray chilled beef sides had the least amount of shrink after 24 hours.

Temperature decline

Carcasses experience many biochemical changes, including pH and temperature decline during the first 24 hours post-mortem. There is an emerging interest in research focused on the rate of temperature decline of beef, lamb, and pork carcasses. The rate of heat loss from a carcass is impacted by several factors including heat transfer between the surface of the carcass and specific muscles, heat movement within a specific muscle at the surface of the carcass, and the environment of the chilling cooler (Levy, 1986).

Some research has evaluated heat movement and temperature decline in specific muscles of a beef carcass. Lee et. al (2006) evaluated beef sides that were shrouded or unshrouded and chilled postmortem either by conventional (air only) or spray chilling. The *longissimus dorsi* (LD) and *semimembranosus* (SM) muscles in both shrouded and unshrouded spray chilled sides had lower muscle temperatures than muscles in the conventionally chilled sides at 8 hours (Lee, Hawrysh, Jeremiah, & Hardin, 2006).

Hippe et al. (1991) reported conflicting results to Lee et. al (2006) where there were no differences in temperatures of SM, LD, or *M. serratus ventralis* muscles between

spray chilled and conventionally chilled beef sides. (Wiklund, Kemp, leRoux, Li, & Wu, 2010) evaluated spray chilling of red deer carcasses and the impact on deep leg temperature and surface temperature. Spray chilling was applied for 6 hours of the chilling cycle as intermittent sprays. The deep leg temperature was measured in the *semimembranosus* close to the pelvic bone and the surface temperature was measured in the rib cage area (Wiklund et al., 2010). The final surface temperature of spray chilled carcasses, collected at 24 hours, was lower than air chilled. There were no differences in final deep leg temperatures between air and spray chilled carcasses (Wiklund et al., 2010). Strydom and Buys (1995) similarly concluded no differences in chilling rates between spray chilled and conventionally chilled beef sides, except for the *M. longissimus thoracis,* which chilled more quickly in spray chilled sides.

There is limited research on temperature decline and temperature decline in specific muscles of beef carcasses during chilling. This limitation could present possible challenges, with the potential for beef hot carcass weights to continue increasing for the near future (USDA, NASS 2019). The first National Beef Quality Audit (NBQA) was conducted in 1991 to provide baseline carcass data for the beef industry and has been conducted approximately every five years since. The United States Department of Agriculture (USDA) publishes the Annual Livestock Slaughter Summary for total livestock slaughter and red meat production in the United States. The data gathered from the NBQA and the annual USDA summary indicate hot carcass weights have been increasing over the past thirty years (USDA-ERS, 2020; NBQA, 2016). Since 1991, weight of the average A maturity beef carcass has increased in hot carcass weight by 68 kilograms (USDA, NASS 2019). Specifically, the average beef hot carcass weight has

increased 68 kilograms from 1991 to 2019, with the average hot carcass weight for steers and heifers increasing 323 kilograms to 391 kilograms respectively (USDA, NASS 2019). This increase in hot carcass weight could present challenges to some chilling systems as heavier beef carcasses can require a longer chilling period postmortem due to elevated temperature postmortem (Egolf et al., 2020). A longer chilling period is due to larger primals and carcasses reaching increased temperatures postmortem. This could be due to biochemical heat generation in the carcass. Heavier carcasses could generate more ATP. More ATP results in increased energy being released during hydrolysis of ATP (Reis, Farage, de Souza, & de Meis, 2001).

There is little research on the impact of spray chilling on the rate of temperature, however; temperature decline in the carcass is crucial to meat quality and rigor formation in carcasses. When a beef carcass is chilled too quickly, muscles may experience a phenomenon referred to as cold shortening. Cold shortening or cold induced toughening, has been heavily investigated since the 1960s (Locker, 1985). Cold shortening was defined by Locker and Hagyard (1963) as shortening occurring at high temperatures coupled with the onset of rigor mortis, however; at low temperatures shortening typically begins rapidly and immediately. Locker and Hagyard (1963) stated minimum shortening occurs between 14 – 19 °C. Locker and Hagyard (1963) concluded that isolated beef *psoas* muscle shortens more at 2 °C than at 37 °C. At 2 °C the average shortening percentage was 39 % and at 37 °C shortening was 33%. Contraction is caused by a failure of function by the calcium pump in the sarcoplasmic reticulum due to the low temperature. The improper function of the calcium pump prevents calcium from being bound in the sarcoplasmic reticulum resulting in a release of calcium into the sarcoplasm.

The release of calcium and remaining ATP left in the muscle causes contraction. The muscles that are excised pre-rigor and are impacted by cold shortening can decrease in length by about 60% (Aberle, Forrest, Gerrard, & Mills, 2012). Cold shortening can impact a carcass through two main aspects: temperature and pH at the time point for onset of rigor mortis (Hannula & Puolanne, 2004).

The rate of carcass temperature decline has also been reported to influence eating experiences for consumers. Chilling rates that are too slow or too rapid can result in inferior meat quality (Van Moeseke et al., 2001). Carcasses can experience cold shortening due to rapid temperature decline prior to the onset phase of rigor. Different muscles within a carcass have been investigated to determine their impact on chilling and potential to cold shorten.

Two conditions, which are a result of extreme change in temperature in carcasses prior to the completion of rigor, are thaw rigor and heat rigor. Thaw rigor occurs when muscles are frozen prior to rigor mortis and is a more severe form of cold shortening (Aberle et al., 2012; Dransfield, 1996; Kim et al., 2012; H. W. Kim et al., 2012). When these muscles are thawed, calcium is released suddenly into the sarcoplasmic reticulum and allows for muscle contraction to occur and results in tougher meat (Aberle et al., 2012; Dransfield, 1996; Kim et al., 2012; H. W. Kim et al., 2012). Thaw rigor causes a physical shortening by 80% of the original length of the muscles (Aberle et al., 2012). Heat rigor is when muscles undergo rapid pH decline coupled with high temperatures, typically up to 50 °C. Heat rigor causes shortening in muscles due to an early onset of rigor and depletion in ATP from the muscles (Aberle et al., 2012; Savell et al., 2005).

The majority of spray chilling research in the beef industry has been focused on shrinkage of beef carcasses. There is little research on temperature decline in beef carcasses and the impact on meat quality. The potential continuation for increase in beef hot carcass weights presents the challenge of evaluating technology utilized in the beef industry to decrease carcass temperature while maintaining meat quality.

Quality traits

Most research to date has focused on the impact of spray chilling on improving carcass yield with minimal focus on the benefit of spray chilling to carcass quality. Jones and Robertson (1988) concluded that spray chilling beef carcasses did not influence loin muscle color at 24 h post-slaughter compared with air chilled (control) sides. However, the spray chilled sides tended to have decreased luminosity (*Y)* compared to the control sides. Fat color from the spray chilled sides that were sprayed for 8 h had higher *Y* values than the control sides (Jones & Robertson, 1988). Similarly, Greer and Jones observed spray chilling of beef sides did not affect lean color but there were differences in fat color. The L* value of the fat over the chuck, rib, and hip was consistently higher after 4, 8, 12, and 16 h of spray chilling compared to air chilled sides. While a* and b* values of these locations were generally decreased for spray chilled sides after 12 and 16 h of spray chilling. Following the 16 h of spray chilling, the fat surfaces of each location developed a washed out, grey appearance (Greer & Jones, 1997).

pH decline

One significant biochemical change experienced postmortem is pH decline. Several factors can impact the rate and extent of pH decline following exsanguination. Living muscle has a pH of 7.4 and meat has a pH of 5.3 to 5.6 (Aberle et al., 2012; Smulders, 1992). Postmortem pH decline can be influenced by environmental stressors that can impact meat quality following slaughter. One negative meat quality condition caused by stress is dark, firm, and dry (DFD) meat (Aberle et al., 2012; Lister, 1988). DFD is typically observed in beef carcasses compared to other species and is a condition resulting from chronic stress prior to slaughter, including handling, transportation, environmental temperatures, feed restrictions, and mixing of animals. Chronic stress prior to slaughter results in negative meat quality attributes and limits pH decline, leading to DFD conditions (Hedrick, 1959; Voisinet, Grandin, O'Connor, Tatum, & Deesing, 1997).

Following slaughter under normal conditions, glycogen in the muscles is converted to lactic acid until a final pH of about 5.5 is reached (Newton & Gill, 1978). Chronic stress causes glycogen to be depleted from the muscles prior to slaughter, thus limiting the accumulation of lactic acid and resulting in a final pH greater than 5.8 (Lawrie, 1988; Miller, 2007; Newton & Gill, 1978; Scanga, Belk, Tatum, Grandin, & Smith, 1998; Wulf, Emnett, Leheska, & Moeller, 2002). Dark, firm and dry meat is visually unpleasing to consumers, however research concerning the eating quality is limited (Wulf et al., 2002). Wulf et al. (2002) reported that cooked *longissimus* from DFD carcasses had higher shear force values and greater shear force variation than those from normal carcasses. Viljoen et al. (2002) investigated consumer sensory evaluations of raw normal pH steaks and raw DFD steaks. General appearance, color, and overall

acceptability were preferred for the raw normal pH steaks compared to the raw DFD steaks. Research on the impact of spray chilling on pH decline of beef carcasses is limited. However, Jones and Robertson (1988) evaluated the effect of spray chilling on pH decline of beef carcasses and concluded spray chilling had no influence on the rate of pH decline.

Tenderness

Smith and Carpenter (1974) defined palatability as the collective response to flavor, juiciness, and tenderness of cooked meat. Tenderness has been identified as the most important trait that impacts the overall eating experience for consumers (Savell et al., 1987). Beef research in the meat industry over the past twenty-five years has focused heavily on meat tenderness. The emphasis on beef tenderness is expected to continue with further improvements in tenderness (O'Quinn, Legako, Brooks, & Miller, 2018). National Beef Tenderness Surveys have collected data since 1990 and every five years since 2005, to verify the improvements made across the industry. The most recent survey published in 2015 reported that over 95% of top blade, top loin, and porterhouse steaks are classified as very tender at the retail level. Consumer panelists evaluated samples for overall liking, tenderness liking, tenderness level, flavor liking, and juiciness liking. For the panelist ratings, the top blade steak was given among the highest for all attributes (Martinez et al., 2017). O'Quinn et al. (2018) evaluated the risk of palatability failure due to the unacceptable level of one or more of the following traits: tenderness, juiciness, and flavor. They concluded the failure of a single palatability trait increases the likelihood of overall palatability failure (O'Quinn et al., 2018).

Tenderness is impacted by three mechanisms, including proteolysis of myofibrillar proteins, sarcomere length, and connective tissue content (Koohmaraie, Kent, Shackelford, Veiseth, & Wheeler, 2002). Tenderness can be influenced by numerous factors such as pH (Huff-Lonergan et al., 2002), muscle temperature (Mohrhauser, Lonergan, Huff-Lonergan, Underwood, & Weaver, 2014), and chilling system (Prado & de Felício, 2010) that work through the three primary mechanisms.

Taylor et al. (1995) found that postmortem tenderization involves three interacting events: 1) an increased rigidity or strengthening followed by a weakening of the actin/myosin interaction, 2) disruption and weakening of the connections between thin filaments in the I-band and the Z-disk, and 3) degradation of the costameres and intermyofibril linkages. These three events during postmortem storage have a large impact on postmortem tenderness (Taylor, Geesink, Thompson, Koohmaraie, & Goll, 1995).

Taylor et al. (1995) concluded during postmortem tenderization approximately 65 to 80% will occur during the first 3 to 4 days postmortem. Degradation to desmin, nebulin, titin and vinculin were contributed within 3 days postmortem in the *semimebranosus*, with little or no degradation to the Z-disk during these days. Investigating degradation of desmin, or other proteins within the costamere, could indicate tenderization development specifically in samples collected during early postmortem.

The effects of postmortem conditions and meat quality aspects has been investigated to determine the impact on desmin degradation postmortem. Huff-Lonergan et al. (1996) concluded desmin was degraded more rapidly postmortem in low shear force

samples than high shear force samples. Carlson et al. (2017) concluded samples from fresh pork loins with low star probe values exhibited more degradation of troponin-T, desmin, filamin and titin compared to samples with high star probe values. To fully understand how tenderness development occurs, it is important to understand how muscles are organized and the structure of muscle cells.

Muscle structure

Muscle is comprised of cells that are multinucleated, striated, and highly organized (Feher, 2012; Huff Lonergan, Zhang, & Lonergan, 2010). Whole muscles are made up of groups of muscle bundles and muscle bundles are composed of groups of muscle fibers. A muscle fiber is comprised of multiple myofibrils, which are responsible for muscle contraction (Huff Lonergan et al., 2010). Filamentous structures were found to link myofibrils to the sarcolemma (Craig & Pardo, 1983) with the structure being named costamere. Myofibrils are composed of structures called sarcomeres, which are organized within the myofibril in a boxcar like fashion (Aberle et al., 2012). Sarcomeres are the functional unit of the muscle (Aberle et al., 2012; Fraterman, Zeiger, Khurana, Wilm, & Rubinstein, 2007). Each sarcomere is formed between two Z - lines / disks (Hopkins, 2006).

Proteins are located in and around the sarcomere and it is estimated that there are over 65 proteins have within the structure (Fraterman et al., 2007) that interact to perform different functions (Huff Lonergan et al., 2010). The costamere contains several proteins including vinculin and desmin, found at each level of the I-band in skeletal muscle (Taylor et al., 1995). Vinculin and desmin are found to be two important proteins in the

costamere and they extend from the costamere to encircle the Z-disk within muscle cells (Richardson, Stromer, Huiatt, & Robson, 1981; Robson et al., 1984; Taylor et al., 1995).

Sarcomeres are measured in length from one end, known as the Z-disk, to the other and on average is 2.5 μm in resting skeletal muscle (Aberle et al., 2012; Cross, West, & Dutson, 1981). The length of the sarcomere, which is established at the completion of rigor, can influence tenderness (Huff Lonergan et al., 2010). Shorter sarcomeres commonly result in tougher product (Locker & Hagyard, 1963). Sarcomeres consist of protein dense A-band and a less protein dense I-bands (Huff Lonergan et al., 2010). The striated appearance of muscle cells is the result of the highly organized of the sarcomeres. Myofibrils contain striations due to the alternating light and dark bands (Aberle et al., 2012). The light bands found in the myofibrils are the I-band which contain the thin filaments (Hanson & Huxley). The dark bands found in the myofibrils are the A-band which contain the thick filaments (Hanson & Huxley). The A-band can contain portions of the thin filaments depending on the state of contraction (Hanson & Huxley)

There are three main categories of proteins in the sarcomere: regulatory, cytoskeletal, and contractile, each with different associated functions (Aberle et al., 2012). Actin and myosin are contractile proteins, proteins responsible for the contraction mechanism and very abundant in the myofibrils (Aberle et al., 2012). The sarcomere is comprised of overlapping thin and thick filaments that interact to cause muscle contraction (Huff Lonergan et al., 2010). The thick filament is predominantly composed of the protein myosin, which consists of a rod region and a head region that interacts with thin filament. Actin is the protein that interacts with myosin and comprises the majority of the thin filament. Regulatory proteins regulate the contractile process in the sarcomere

(Aberle et al., 2012). Two regulatory proteins include troponin and tropomyosin. Cytoskeletal proteins provide support and structure for the sarcomere, including desmin, titin, and nebulin (Aberle et al., 2012). These three proteins are involved in the alignment of the sarcomeres.

The costameres are found near the surface of the muscle cell, which would allow for direct exposure to calcium leaking into the muscle cell, causing damage by weakening the sarcolemma during postmortem storage (Jeacocke, 1993; Ouali, 2007; Taylor et al., 1995). Taylor et al. (1995) investigated if z-disk degradation is responsible for postmortem tenderization. Taylor et al. (1995) concluded within the first 24 hours postmortem the costameres were heavily degraded and after 72 hours postmortem the sarcolemma is separated from the myofibril in all fibers. As mentioned, desmin is found in the costamere and extends from the costamere to encircle the Z-disk within muscle cells (Richardson et al., 1981; Robson et al., 1984; Taylor et al., 1995)

Desmin is an intermediate filament protein (Bär, Strelkov, Sjöberg, Aebi, & Herrmann, 2004; Granger & Lazarides, 1979; Huff Lonergan et al., 2010). The structure of desmin consists of an alpha-helical rod, a non-alpha-helical head, and a carboxy-terminal tail (Bär et al., 2004; Geisler & Weber, 1982). The head and tail region of desmin assist in proper filament assembly around the myofibrils (Bär et al., 2004). Desmin is known to play a role in the development of tenderness. During postmortem storage desmin is degraded and the structure of the myofibrils is weakened (Huff Lonergan et al., 2010; Huff-Lonergan et al., 1996; Melody et al., 2004; Taylor et al., 1995). In postmortem muscle, intact desmin (55 kDa) is degraded by calpain-1 at the head and tail region (Baron, Jacobsen, & Purslow, 2004).

The intermediate filaments are located near the Z-lines of myofibrils to connect adjacent myofibrils to the costameres found on the sarcolemma (Aberle et al., 2012; Huff Lonergan et al., 2010; Taylor et al., 1995) and are responsible for integrity of the muscle cells (Huff Lonergan et al., 2010). Desmin degradation has a significant impact on meat quality due to the role in maintaining integrity of the muscle cells (Huff-Lonergan et al., 1996; Taylor et al., 1995).

The calpain system

The calpain system, which can be found within a muscle cells and other tissues, consists of calcium dependent proteases known as calpains and their specific inhibitor calpastatin. The calpains are considered key regulators of postmortem proteolysis (Koohmaraie et al., 2002). Calpain can exist in different isoforms with the most well studied being calpain-1 and calpain-2 (Koohmaraie et al., 2002). Calpain-1, previously known as μ-calpain, has been reported to be primarily responsible for protein degradation postmortem (Chen et al., 2011; Huff-Lonergan et al., 1996). Calpain-2, previously known as m-calpain, has very limited activity postmortem due to its elevated calcium requirements (Chen et al., 2011). Calpain-1 requires micro-molar (μM) concentrations of calcium and calpain-2 requires milli-molar (mM) concentrations of calcium to be activated (Chen et al., 2011).

Calpastatin is the endogenous inhibitor of calpain and is found in tissues that contain calpain (Chen et al., 2011; Huff Lonergan et al., 2010). During beef aging, calpain-1 is responsible for degradation of myofibrillar proteins, including desmin

(Geesink, Kuchay, Chishti, & Koohmaraie, 2006; Huff-Lonergan et al., 1996; Mohrhauser, Underwood, & Weaver, 2011; Taylor et al., 1995).

Calpain-1 consist ofs an 80 kDa and a 28 kDa subunit (Geesink et al., 2006; Goll, Thompson, Li, Wei, & Cong, 2003; Huff Lonergan et al., 2010; Suzuki et al., 1990). The 80 kDa subunit is comprised of four domains, I, II, III, and IV, each having different sequences. Domain I is the N-terminal domain and domain II is the primary catalytic domain (Huff Lonergan et al., 2010). Domain III and IV both have sequences that are responsible for predicting E-F hand calcium binding sites (Huff Lonergan et al., 2010).

Calpain-1 requires calcium to be activated, when incubated with calcium, calpain-1 autolyzes (self-degrades) (Huff Lonergan et al., 2010). During autolysis, degradation of the 80 kDa and 28 kDa subunits occurs. The 80 kDa subunit of calpain-1 is not active and reduces to 78 kDa. The 78 kDa subunit of calpain-1 is active and this is reduced to the 76 kDa subunit of calpain-1 that was previously active. (Goll et al., 2003; Huff Lonergan et al., 2010). Autolysis also lowers the amount of calcium required to activate calpain-1. The calcium concentration to activate calpain-1 is reduced from 3-50 μM to 0.5-2.0 μM for half maximal activity (Goll et al., 2003).

The costameres are found near the surface of the muscle cell, which would allow for direct exposure to calcium leaking into the muscle cell. Calcium leaking into the muscle cell activates calpain and can cause damage to the sarcolemma during postmortem storage (Jeacocke, 1993). Damage to the costamere can cause some damage to the sarcolemma and allow for more calcium to be leaked into the muscle cell (Ouali, 2007; Taylor et al., 1995). The calpain system in muscles heavily impacts proteolysis. The impact of spray chilling treatments on early postmortem proteolysis is unknown.

Summary

A beef carcass will undergo many biochemical changes following slaughter and during the chilling period prior to fabrication, including temperature and pH decline, as well as tenderness development. Carcass chilling systems have been shown to impact temperature decline, with some improving carcass yields and replacing moisture loss. Many slaughter facilities have implemented spray chilling systems to replace moisture loss and improve carcass yields. Majority of spray chilling research to date has been focused on preventing shrink in beef carcasses.

Tenderness development has been found to occur early postmortem. A structure within the sarcomere, known as the costamere, includes the protein desmin. The degradation of these structures during the first 4 days postmortem have been concluded to have an important role on tenderness development. The potential continuation for increase in beef hot carcass weights presents the challenge of evaluating technology utilized in the beef industry. There is little research investigating chilling systems, specifically spray chilling, and the impact on temperature decline, pH decline and protein degradation in different primals of beef carcasses.To further understand the impact of carcass chilling systems, the objective of this thesis is to determine the effect of air and spray chilling systems on carcass characteristics and biochemical changes in beef carcasses.

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CHAPTER 2: EFFECTS OF AIR AND SPRAY CHILLING SYSTEMS ON CARCASS CHARACTERISTICS AND BIOCHEMICAL CHANGES IN BEEF CARCASSES

LYDIA MARGARET HITE

ABSTRACT

Spray chilling systems have been utilized in the beef industry to replace moisture loss and improve yields during carcass chilling. The impact of spray chilling beef carcasses on protein degradation, temperature decline, and pH decline is largely unknown. The objective of this study was to determine the effect of air only and spray chilling systems on beef carcass characteristics, protein degradation, and pH and temperature decline. Ten beef carcasses were used, the left side of each carcass was chilled by air movement only, (3.8 °C and air movement of 3500 m^3 /min) (AIR) and the right side was spray chilled with cycles of 5.5 °C water and air movement of 3500 m^3 /min) (SPRAY). Temperature data loggers were placed in different positions in the round, loin, and chuck of each side. Data loggers were placed in the 5 cm, 10 cm, 15 cm, and 20 cm positions for the round. Data loggers were placed in the 10 cm, 15, cm, and 20 cm positions for the chuck. Due to the loin being a smaller primal, a single data logger was placed in the 10 cm position. pH was measured at various timepoints postmortem and carcass data were collected. A steak was removed from the *longissimus lumborum* and sliced into smaller samples and aged for 1-, 3-, 5-, 7-, 14-, or 21-days for proteolysis. Data were analyzed using the MIXED procedure of SAS with fixed effect of time,

treatment, logger position, aging d, and their interaction where appropriate. No differences in carcass characteristics, instrumental color, desmin degradation, or pH decline ($P > 0.05$) were observed between treatments. A treatment by time interaction was observed for temperature decline in the loin ($P = < 0.0001$). A treatment by position interaction was observed for temperature decline in the chuck ($P = 0.0045$). Sides from the SPRAY treatment had lower temperatures than AIR sides at the 10 cm position (20.84 ± 0.38 °C vs 21.74 ± 0.34 °C; $P = 0.02$) in the chuck. Sides from the SPRAY treatment did not differ from the AIR sides at the 15 cm or 20 cm position ($P > 0.05$) in the chuck. A treatment by time interaction was observed for temperature decline in the chuck ($P = 0.0003$). A position by time interaction was observed for temperature decline in the chuck ($P = 0.0039$). At 720 minutes, halfway through the chilling treatments, the 10 cm position had a lower temperature (18.88 ± 0.49 °C) compared to the 15 cm position (21.28 ± 0.24 °C; $P < 0.0001$) and the 20 cm position (21.05 ± 0.38 °C; $P < 0.0001$) in the chuck. There was no difference in temperature between the 15 cm and 20 cm position at 720 minutes in the chuck. A day effect was observed for intact desmin ($P < 0.0001$). A day effect was observed for desmin ($P < 0.0001$). Day 14 and day 21 had decreased ratio for intact desmin compared to day 1, 3, 5, and 7. A treatment by position by time interaction was observed for temperature decline in the round ($P = < 0.0001$; Figure 2.9). The positions closer to the surface of the round reached decreased temperatures compared to the deeper positions. As time increase, temperature decreased for all positions in the round. The SPRAY sides had decreased temperatures at all positions compared to the AIR sides. Treatment did not impact pH decline for the round, loin, or chuck ($P > 0.05$). However, a time effect was observed in each primal ($P < 0.01$),

overall as time increased, pH decreased. Spray chilling of beef carcasses resulted in lower internal temperatures in the round, loin and chuck compared to the air chilled sides. These data suggest that spray chilling helps chill carcasses more rapidly, however; additional research is needed to optimize spray chilling systems.

INTRODUCTION

Carcasses undergo chilling after slaughter to ensure food safety, reduce shrinkage, maximize shelf life, and develop tenderness and color of the final product (Savell et al., 2005). Wind speed and relative humidity can be altered within a chilling environment to improve chilling efficiency and effectiveness of heat transfer from a carcass. The addition of intermittent spray chilling is also common in many beef processing facilities. Spray chilling systems have been utilized in the meat industry since the 1960s, with the primary goal of replacing moisture lost during chilling thus improving carcass yield. Spray chilling is the process of repeatedly spraying cold water onto carcasses Initially, the suggested duration for spray chilling was the first 3-8 h postmortem (Hippe et al., 1991). The first patented spray chilling system was introduced in 1968 by Swift Fresh Meats Company and was known as Clor-Chil (Heitter, 1975). Clor-Chil was developed to reduce carcass shrinkage and involved spraying a water and chlorine solution intermittently during chilling to reduce moisture lost during chilling and improve product safety. Clor-Chil was reported to reduce bacterial count by 94.5% - 98.5% (Heitter, 1975). Spray chill systems that only apply water have also been utilized by the beef industry to chill carcasses.

Research has been heavily conducted to compare the impact of different chilling systems on shrinkage of beef carcasses postmortem. The majority of spray chilling research in the beef industry has been focused on shrinkage of beef carcasses. There is little research the impact of spray chilling on temperature decline, pH decline, or protein degradation in beef carcasses and the impact on meat quality. The potential continuation for increase in beef hot carcass weights presents the challenge of evaluating technology utilized in the beef industry to decrease carcass temperature while maintaining meat quality.

Aside from the chilling systems utilized and the environment of the chilling cooler, other factors can impact the heat transfer and reduction of temperature in a carcass including the heat transfer between specific muscles and the surface of the carcass and heat movement within a specific muscle (Levy, 1986). Investigation into temperature decline of individual muscles during chilling is lacking with few published studies.

The objective of this study was to determine the effects of spray chilling beef carcasses on temperature decline, carcass characteristics, instrumental color, protein degradation, pH decline, and ultimate pH. It was hypothesized that spray chilling beef carcasses during chilling will result in lower temperatures within muscles compared to air chilling without impacting carcass characteristics, pH decline or protein degradation.

MATERIALS AND METHODS

Spray chilling and temperature decline

Ten commercial beef animals were harvested at the South Dakota State University Meat Laboratory across two harvest days (n = 5/d) twenty-one days apart. Hot carcass weight (HCW) for each side was recorded prior to entering the chilling cooler. The left side of each carcass was assigned to be chilled by air movement only (AIR) and the right side was assigned to the spray chilling treatment (SPRAY). The AIR treatment had an average cooler temperature of 3.8 °C (ranging from 2.5 - 6.0 °C) and air movement at 3500 m^3 /min that was controlled by two portable fans (MaxxAir 24" Multi-Purpose Tilt Fan – Yellow; Airxcel, Wichita, KS) placed on opposite ends of the chilling cooler to allow for circulation of air throughout the cooler. The SPRAY treatment was exposed to the same temperature and air movement conditions as the AIR treatment with the addition of an intermittent spray of chilled water.

To chill water for the spray chill system, potable tap water was piped through 36.58 m copper tubing (Streamline copper plumbing tube 1.27 cm 36.6 m Soft Coil; Mueller Industries, Collierville, TN) surrounded by an ice/water slurry to lower the temperature of the water to an average of 5.5 °C. Ice was added to the water periodically to ensure consistent cooling. The source water flowing into the chilling system was 16.6°C. After circulating through the chilling system, the water was cooled to 5.5 °C.

To apply the chilled water to carcasses, sprinkler heads (Rain Bird 2.43 m by 4.58 m Shrub Head Sprinkler; Rain Bird, Azusa, CA) were attached to polyvinyl chloride (PVC) pipe, which was connected to hoses that were attached to the copper tubing located in the ice/water slurry. The design of the PVC pipe was to include two sections for sprinkler heads to be hung above the rails in the chilling cooler. The sprinkler heads were in an inverted orientation on the PVC pipe section. Each section consisted of 90°

and 45° angled sprinkler heads to allow water to be sprayed directly on the carcass (Figure 2.1) including eight sprinkler heads total per section. A programmed timer, Model K2000 Timer (Scott's Sales; McCallsburg, Iowa) was attached to the system to allow for scheduled spraying of water through the sprinkler heads to the SPRAY treatment sides. The timer was programmed to alternate between the two sections of the carcasses spraying the left section, then switching to the right section. The timer would spray for one hundred and sixty seconds then would be off for thirty-two minutes and repeat for 24 h for 45 cycles during the chilling period. The spray duration and off period was the same for each section.

Temperature data loggers (ThermaData stainless steel USB temp data logger; ThermoWorks, American Fork, UT) were placed in the round, loin, and chuck of each side at approximately 60 minutes postmortem, prior to entering the chill cooler, and temperature was recorded at multiple positions every 30 minutes. Loggers were placed in the center of the round at the 20, 15, 10, and 5 cm position (Figure 2.2). Loggers were placed in the chuck immediately posterior to the foreleg in the pocket between the brisket and the chuck at the 20, 15, and 10 cm position (Figure 2.3). A single data logger was placed in the 10 cm position at the third lumbar vertebra (Figure 2.4).

Carcass data and instrumental color collection

At 24 h postmortem, carcasses were ribbed between the 12th and 13th ribs and temperature data loggers were removed. Hot carcass weight (HCW) for each side was recorded at 1- and 2-d postmortem to calculate weight loss between the two days during air chilling after spray chilling had stopped. Ribeye area (REA), backfat thickness (BFT),

kidney, pelvic, and heart fat (KPH), and marbling scores were measured by trained personnel at 1 day postmortem. USDA yield grades were calculated using HCW, REA, BFT, and KPH. Instrumental color (L^*, a^*, b^*) of the *longissimus dorsi* muscle of each side was collected at 1 day postmortem using a colorimeter (Chroma Meter CR 410; Konica Minolta, Inc., Tokyo, Japan) following a 30-minute bloom period. The colorimeter was calibrated using a standard white plate with a 2° observer, 50 mm aperture, and C illuminance. Following the bloom period, a single 2.54 cm steak was removed from the *longissimus lumborum* from each side and sliced into 25 – 50 gram samples to be utilized for proteolysis. Samples were vacuum packaged (3 mm; Koch Supplies, Riverside, MO) and aged for 1-, 3-, 5-, 7-, 14-, or 21-days postmortem at 4°C and then frozen at -20°C.

Protein extraction

The aged samples were prepared for protein extraction by slicing into smaller sections and powdered in liquid nitrogen for thirty seconds in stainless steel blender cups (Model SS 110, Waring Products Division, New Hartford, CT) until samples were uniform in consistency. Powdered samples were stored in 7.62 x 12.7 cm sample bags (Fisher, Hanover Park, IL) at -20°C until further analysis. Powdered samples were used to create protein samples for gel electrophoresis and Western Blots using methods by Melody et al. (2004) with several modifications. Following powdering, 0.60 g of each sample were weighed out Powdered samples and homogenized using an overhead stirrer model RZR1; Heidolph, Schwabach, Germany) in 10 ml of whole muscle buffer (2% Sodium Dodecyl Sulfate [SDS], 10 mM Sodium Phosphate, pH 7.0) to extract

myofibrillar proteins. Homogenized samples were centrifuged for 15 minutes at 1500 x g at 25°C. Protein concentrations of the supernatant were determined in duplicate by diluting samples with a 1:20 dilution in double distilled deionized water. A Lowry protein assay (RC/DC Protein Assay Kit; Bio-Rad Laboratories, Hercules, CA) was used to determine protein concentrations. The protein assay was analyzed using a spectrophotometer at 750 nm wavelength (SpectraMax 190; Molecular Devices, Sunnyvale, CA). The associated software (SoftMax Pro 6, version 6.2.1; Molecular Devices) to the spectrophotometer evaluated protein concentrations compared to a standard curve. Protein gel samples were prepared with a final sample concentration of 4 mg/ml and stored at -20°C. Prior to running western blots, load checks were conducted on 15% sodium dodecyl sulfate polyacrylamide separating gels (SDS-PAGE; acrylamide: N-N'-bis-methylene acrylamide = 100:1, 0.1% SDS, 0.05% TEMED, 0.5% Ammonium Persulfate [APS], and 0.375 M Tris HCl, pH 8.8) with 5% stacking gels (acrylamide: N-N'-bis-methylene acrylamide = 100:1, 0.1% SDS, 0.125% TEMED, 0.075% APS, and 0.125 M Tris HCl, pH 6.8) to ensure proper dilution of each sample. Gels were run using a mini gel electrophoresis unit (model SE-260; Hoefer Scientific, Holliston, MA) at 120 v for 390 v h. Gels were stained using Coomassie blue (40% Methanol, 7% Glacial Acetic Acid, 53% ddH_2O, 0.1% Coomassie brilliant blue R-250) for 24 h. Gels were destained using 40% methanol and 7% glacial acetic acid.

Western blot analysis

A 10% SDS-PAGE gel was used for quantification of intact desmin using 40 μg of protein sample run at 120 v for 240 v h. After the completion of electrophoresis,

gels were transferred to a polyvinylidene difluoride (PVDF; Immobilon – P; Merck Millipore, Darmstadt, Germany) membrane with a pore size of 0.45 μm using a TE-22 transfer unit (Hoefer Scientific, Holliston, Massachusetts) at 135 v h. The membrane was emersed in transfer buffer (24 mM Tris, 186 mM Glycine, and 15% Methanol) at 4°C using a refrigerated water bath (IsoTemp, model 6200 R28; Thermo Fisher Scientific, Asheville, NC). After the transfer was complete membranes were blocked in a 0.5% non-fat dry milk solution for 1 h. Membranes were incubated overnight in the primary antibody (1:80,000 Rabbit anti-desmin; Iowa State University, courtesy of the Lonergan Lab) at 4°C. The next day, membranes were warmed to room temperature for one h and then washed with PBS-Tween (66 mM Sodium Phosphate, 0.1 M NaCl, and 0.1% Tween-20) three times,10 minutes per wash. Following the final wash, a secondary antibody was applied to each membrane (1:20,000 goat anti-rabbit horseradish peroxidase; Product #31460 Thermo Fischer Scientific, Asheville, NC) and incubated for 1 h. Blots were again washed three times (10 min per wash) with PBS-Tween. Membranes were developed using an ECL Prime detection kit (GE Healthcare, Lafayette, CO). Images were gathered using the similar imaging system mentioned previously using chemiluminescence. AlphaView SA software (Protein Simple; San Jose, CA) was used to allow for visualization and quantification for the disappearance of 55 kDa bands for intact desmin. Intact Desmin was analyzed as a ratio to an internal standard across all western blots. The internal standard consisted of a composite sample equally represented by both treatments and time points across all western blots to control for inter gel variation.

pH decline

A portable Orion Star A221 pH meter (Thermo Scientific, Beverly, MA) and KNIpHE probe (model 9121 APWP; Orion, Thermo Scientific) were used to measure pH at 0 h (prior to each side entering the chilling cooler), then at 1-, 2-, 4-, 6-, 12-, and 24-h after entering the chilling cooler. The pH meter was calibrated before each measurement with standard pH 4.0 and pH 7.0 buffers. The pH probe was inserted on the posterior side of the carcass just beneath the fat surface in the *semimembranosus* of the round and on the dorsal side of the *longissimus lumborum* of the loin. Due to the location of the *serratus ventralis* of the chuck, pH was measured on the ventral side of the carcass just beneath the fat surface in chuck immediately posterior to the leg in the pocket between the brisket and the chuck. At each time point a different insertion point was used.

Ultimate pH

The powdered samples from protein extraction were used to collect ultimate pH. Five grams of day 3 powdered samples were weighed out and homogenized with 45 mL of distilled deionized water. A benchtop Orion 370 PerpHecT LogR pH meter (Thermo Scientific, Beverly, MA) and KNIpHE probe were used to ultimate pH. The pH meter was calibrated before each measurement with standard pH 4.0 and pH 7.0 buffers.

Statistical analysis

Data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC v 9.4). Temperature decline from the round and chuck were analyzed separately as repeated measures with time, treatment, and logger position and their interactions as fixed

effects. Temperature decline from the loin was analyzed as a repeated measure with time and treatment and their interactions as fixed effects. Carcass characteristics and instrumental color data were analyzed using fixed effects of treatment. pH data were analyzed separately by primal using fixed effects of treatment, time, and their interactions. Ultimate pH data were analyzed using fixed effect of treatment. Western blot analysis data were analyzed as repeated measures with aging day, treatment, and their interactions as fixed effects. Kill day was tested as a covariate and was not significant. Hot carcass weight was used as a covariate. Heterogeneous compound symmetry was used for a covariate structure for all data. Statistical significance was considered at an alpha of $P \leq 0.05$.

RESULTS AND DISCUSSION

Carcass characteristics

As expected, carcass characteristics did not differ between treatments ($P > 0.05$; Table 2.1), as treatment sides were from the same animal. Mean HCW was 400.6 ± 14.1 kg for AIR sides and 395 ± 14.1 kg for SPRAY sides prior to sides entering the chilling cooler. Mean REA was 14.4 ± 0.71 cm^2 for AIR sides and 14.9 ± 0.71 cm^2 for SPRAY sides and mean BFT was 0.395 ± 0.07 cm for AIR sides and 0.435 ± 0.07 for SPRAY sides. Mean marbling score was 505.0 ± 39.31 for AIR sides and 508.0 ± 39.32 for SPRAY sides. Mean KPH was 2.5% for AIR and SPRAY sides. Mean yield grades was 3.051 ± 0.093 for AIR and 3.038 ± 0.093 for SPRAY sides.

Longissimus dorsi color analysis

No differences in instrumental L*, a*, or b* ($P > 0.05$; Table 2.2) were observed for the *longissimus dorsi* between treatments. Greer and Jones (1997) evaluated lean color collected at three locations in the *longissimus thoracis* (LT) and also reported no influence of spray chilling on L*, a*, and b* of beef sides. Greer and Jones (1997) also evaluated fat color at two locations on the chuck, rib, and hip 24 h postmortem. In contrast to lean color, fat color was impacted by spray chilling, with L* values being consistently higher for spray chilled sides compared to conventionally chilled carcasses (Greer & Jones, 1997). The a* and b* values of fat were generally lower for spray chilled carcasses compared to conventionally chilled sides with the most significant difference occurring after 16 h of spray chill. After 16 h of spray chilling it was noted that the fat surfaces developed a washed out, grey appearance (Greer & Jones, 1997). Fat color was not evaluated in the present study.

Temperature decline

Loin

Due to the smaller size of the loin primal, only a single data logger was used to collect temperature at the 10 cm position. A treatment by time interaction was observed for temperature decline in the loin ($P = < 0.0001$; Figure 2.5). At 0 minutes, the initial time the data loggers were inserted, there was no difference in temperature between AIR and SPRAY sides ($P > 0.05$). At 60 minutes, SPRAY sides had a decreased temperature compared to AIR sides (34.5 ± 0.71 °C vs 36.7 ± 0.71 °C; $P = 0.0239$). At 60 minutes,

SPRAY sides had decreased temperature compared to AIR sides (34.46 ± 0.71°C vs 36.75 ± 0.71°C; $P = 0.0239$). At 720 minutes, halfway through the chilling treatments, SPRAY sides had a decreased temperature compared to AIR sides (8.25 ± 0.75°C vs 12.93 ± 0.75°C; $P < 0.0001$). At 1440 minutes, the final chilling treatment time point, SPRAY sides continued to have decreased temperature compared to AIR sides (3.49 ± 1.17 °C vs 7.22 ± 1.17 °C; $P = < .0245$). For both treatments, as time increased, temperature decreased ($P < 0.0001$). Similar evidence was observed by (Strydom & Buys, 1995) when comparing chilling rates between spray chilled and conventionally chilled beef sides. Strydrom and Buys (1995) observed the *longissimus thoracis* chilled quicker in spray chilled beef sides compared to conventionally chilled beef sides.

Chuck

A treatment by position interaction was observed for temperature decline in the chuck ($P = 0.0045$; Figure 2.6). Sides from the SPRAY treatment had lower temperatures than AIR sides at the 10 cm position (20.84 ± 0.38 °C vs 21.74 ± 0.34 °C; $P = 0.02$). Sides from the SPRAY treatment did not differ from the AIR sides at the 15 cm or 20 cm position ($P > 0.05$). In both AIR and SPRAY treatment sides, the 10 cm position had lower temperatures compared to the 15 cm and 20 cm positions.

A treatment by time interaction was observed for temperature decline in the chuck ($P = 0.0003$; Figure 2.7). At 0 minutes, the initial time the data loggers were inserted, there was no difference in temperature between AIR and SPRAY sides ($P > 0.05$). At 720 minutes, halfway through the chilling treatments, SPRAY sides had decreased temperatures compared to AIR sides (20.00 ± 0.37 °C vs 20.79 ± 0.33 °C; $P =$

0.54). At 60 minutes, AIR and SPRAY sides did not differ in temperature ($P > 0.05$). At 720 minutes, halfway through the chilling treatments, SPRAY sides had decreased temperature compared to AIR sides (20.00 ± 0.38 °C vs 20.80 ± 0.33 °C; $P = 0.05$). At 1440 minutes, the end of the chilling treatments, AIR and SPRAY sides did not differ in temperature ($P > 0.05$). For both treatments, as time increased, temperature decreased ($P < 0.0001$).

A position by time interaction was observed for temperature decline in the chuck ($P = 0.0039$; Figure 2.8). At 0 minutes, the initial time the data loggers were inserted, there was no difference in the 10 cm, 15 cm, or 20 cm positions ($P > 0.05$). The temperature at 10 cm position was lower than temperatures at 15 cm and 20 cm positions for most time points. At 60 minutes, the 10 cm position had a lower temperature (39.26 ± 0.19 °C) compared to the 15 cm position (39.62 ± 0.28 °C; $P = 0.001$) and the 20 cm position (39.48 ± 0.14 °C; $P = 0.005$). There was no difference in temperature between the 15 cm and 20 cm position ($P > 0.05$) at 60 minutes. At 720 minutes, halfway through the chilling treatments, the 10 cm position had a lower temperature (18.88 ± 0.49 °C) compared to the 15 cm position (21.28 ± 0.24 °C; $P < 0.0001$) and the 20 cm position (21.05 ± 0.38 °C; $P < 0.0001$). There was no difference in temperature between the 15 cm and 20 cm position at 720 minutes. At 1440 minutes, the end of the chilling treatments, there was no difference in the 10 cm, 15 cm, or 20 cm positions ($P > 0.05$).

Round

A treatment by position by time interaction was observed for temperature decline in the round ($P < 0.0001$; Figure 2.9). At the initial time of data logger's placement, there

was no difference between AIR 15 cm, SPRAY 20 cm, SPRAY 15 cm, SPRAY 10 cm, or AIR 20 cm positions ($P > 0.05$). The AIR 15 cm position had the most increased temperature (40.58 ± 0.62 °C) compared to AIR 5 cm position (30.51 ± 2.2 °C; $P < 0.0001$). There was no difference in the SPRAY 5 cm and AIR 5 cm position at 0 minutes ($P > 0.05$).

At 60 minutes, the SPRAY 20 cm position had increased temperature (40.71 ± 0.18 °C) compared to SPRAY 15 cm position (40.26 ± 0.13 °C; $P = 0.03$) and the AIR 20 cm position (40.08 ± 0.27 °C; $P = 0.05$). The SPRAY 5 cm and AIR 5 cm positions had the most decreased temperatures and did not differ (25.19 ± 0.52 °C and 24.44 ± 1.48 °C, respectively) at 60 minutes. The SPRAY 5 cm and AIR 5 cm positions had the most decreased temperatures at all time points during the chilling treatments (Figure 2.9).

At 720 minutes, halfway in the chilling treatments, the AIR 20 cm and SPRAY 20 cm positions had similar temperatures (24.38 ± 0.39 °C; 24.30 ± 0.28 °C; $P > 0.05$) and were elevated compared to all other treatments. From 60 minutes to 720 minutes, temperature decreased for all data logger positions. ($P > 0.05$). The SPRAY 15 cm (22.68 ± 0.28 °C) and AIR 15 cm (22.67 ± 0.38 °C) were similar in temperature, both positions both had increased temperatures compared to SPRAY 10 cm (19.29 ± 0.30 °C) and AIR 10 cm (19.22 ± 0.41 °C) positions. At 720 minutes, there was no difference in the SPRAY 10 cm and AIR 10 cm positions. There was no difference in the SPRAY 5 cm and AIR 5 cm position ($P > 0.05$). The SPRAY 5 cm (9.55 ± 0.37 °C and AIR 5 cm (9.34 ± 0.55 °C) positions had the most decreased temperatures compared to all positions at 720 minutes.

At 1440 minutes, the end of the chilling treatments the SPRAY 20 cm position had the most increased temperature (14.27 ± 0.44 °C) but did not differ from the AIR 20 cm position (13.56 ± 0.64 °C; $P > 0.05$). The SPRAY 10 cm (11.05 ± 0.51 °C; $P < 0.0001$) and AIR 10 cm (10.32 ± 0.71 °C; $P < 0.0001$) positions were similar in temperature and had decreased temperatures compared to the SPRAY 20 (14.27 ± 0.44 °C) and AIR 20 cm (13.56 ± 0.64 °C) positions.

In the round, the SPRAY 5 cm and AIR 5 cm positions had the most decreased temperatures at all time points during the chilling treatments (Figure 2.9). Allen et al. (1987) compared conventional (air only) chilled beef sides to spray chilled sides. The spray chilled sides were sprayed with 3 °C chilled water during the first 8 h of chilling. Jones and Robertson (1988) evaluated the impact of spray chilling beef carcasses on shrinkage and meat quality. Temperature was collected between the 12th and 13th ribs in the *longissimus dorsi* (LD) and at a depth of 8 cm into the *semimembranosus* (SM). Similar to the current study, spray chilling significantly impacted the rate of chilling in the LD and the SM with a greater effect in the SM. The increase in the SM was suggested due to the position of the muscle in the round being closest to the water sprayed from the chilling system (Jones & Robertson, 1988).

pH decline

Treatment did not impact pH decline for the round, loin, or chuck ($P > 0.05$; Table 2.3). However, a time effect was observed in each primal ($P < 0.01$; Table 2.3), overall, as time increased, pH decreased. In the loin, pH at 0, 1, and 2 hrs (6.56 ± 0.08, 6.49 ± 0.09, and 6.37 ± 0.11, respectively) was similar ($P > 0.05$). At 4 hr postmortem, pH began to decline with 4 hr pH (6.20 ± 0.10) being decreased compared to 0 ($P =$

0.0014) and 1 (P = 0.01) hr. Though the 12 hrs postmortem pH in the loin numerically decreased (6.11 ± 0.14) it was not different at 2, 4, 6, and 12 hrs (P > 0.05).

A similar effect was observed in the chuck, pH at 0, 1, and 2 hrs (6.58 ± 0.07, 6.51 ± 0.07, 6.46 ± 0.08, respectively) was similar (P > 0.05). At 4 hr postmortem, pH began to decline with 4 hr pH (6.32 ± 0.08) being decreased compared to 0 (P = 0.0003) and 1 (P = 0.0097) hr. The 12 hrs postmortem in the chuck pH was decreased (6.05 ± 0.09) compared to 0, 1, 2, 4, and 6 hrs (P < 0.05).

In the round, pH at 0 (6.66 ± 0.10), had increased pH compared to 1 (6.32 ± 0.14; P < 0.0007), 2 (6.15 ± 0.14), 4 (6.32 ± 0.08), 6 (6.23 ± 0.08), and 12 (6.05 ± 0.09) hrs postmortem. There was no difference in pH at 1 and 2 hrs postmortem in the round (P < 0.05). Though the 12 hrs postmortem pH in the round numerically decreased (5.75 ± 0.11) it was not different at 4, 6, and 12 hrs (P > 0.05).

No treatment effect was observed for ultimate pH (AIR = 5.66 ± 0.02, SPRAY = 5.70 ± 0.02; P = 0.3363). The ultimate pH observed for AIR and SPRAY treatments were in the normal range of pH for meat which is 5.3-5.8. Temperature and pH decline early postmortem to play a vital role in tenderization of meat (Huff Lonergan et al., 2010; Locker & Hagyard, 1963).

Desmin degradation

No treatment effects were observed for disappearance of intact desmin (P > 0.05; Table 2.4). A day effect was observed for intact desmin (P < 0.0001; Figure 2.10). Day 1 and day 3 were statistically similar (P = 0.806). Day 5 and day 7 were statistically similar (P = 0.175). Day 14 and day 21 were statistically similar (P = 0.538). Day 5 and day 7 had decreased ration for intact desmin compared to day 1 and day 3. Day 14 and

day 21 had decreased ratio for intact desmin compared to day 1, 3, 5, and 7. This decrease was expected as myofibrillar proteins degrade during postmortem storage (Huff-Lonergan et al., 1996; Koohmaraie, Kennick, Elgasim, & Anglemier, 1984). The first 24 h postmortem are crucial for impacts on meat quality, specifically with the rate of pH and temperature decline impacting tenderness (Huff Lonergan et al., 2010; H. W. Kim et al., 2012; Kim, Warner, & Rosenvold, 2014; Marsh, Lochner, Takahashi, & Kragness, 1981; Savell et al., 2005). Mao et al. (2012) concluded rapid chilling of beef sides decreased the degradation rate of desmin and troponin-T with the effects weakening gradually as aging postmortem increased.

CONCLUSION

The present study demonstrates spray chilling of beef carcasses has the potential to positively impact carcass chilling and decrease internal temperatures in the round, loin, and chuck when compared to air chilling. Internal temperature decline differed at data logger position in the round and chuck, tissues closer to the surface were lower and carcasses chilled faster in both AIR and SPRAY treatments. There was no impact of chilling treatment on carcass characteristics, instrumental color, pH decline, or protein degradation. Additional research is needed to fully understand the impact of spray chilling systems on protein degradation postmortem in beef carcasses. These data suggest spray chilling helps chill carcasses more rapidly, without impacting *longissimus dorsi* color or proteolysis of desmin.

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Table 2.1. Least square means for carcass data of sides assigned air (AIR) or spray (SPRAY) chilling treatments

Variable	AIR	SPRAY	SEM	*P* – value
HCW, kg	400.6	395.0	14.13	0.697
Ribeye area, cm^2	14.4	14.9	0.708	0.448
Backfat, cm	0.395	0.435	0.068	0.565
Marbling score[1]	505.0	508.0	39.31	0.940
Yield grade	3.051	3.038	0.093	0.922

[1] Marbling Score: 300 = $Slight^0$, 400 = $Small^0$, 500 = $Modest^0$, 600 = $Moderate^0$

Table 2.2. Least square means for effects of carcass chilling treatment air (AIR) or spray (SPRAY) on instrumental color[1] of *longissimus dorsi* muscle of beef sides

Variable	AIR	SPRAY	SEM	*P* – value
L^*	39.42	39.66	0.901	0.800
a^*	24.29	23.29	0.544	0.083
b^*	8.94	8.47	0.415	0.274

[1] L^* = lightness (0 = black, 100 = white) , a^* = redness (- value = red, + value = green), and b^* = yellowness (- value = blue, + value = yellow)

Table 2.3. Least square means for primal (loin, round, and chuck) pH decline of beef sides. pH[1] was recorded at time of carcasses entering the cooler (0), 1-, 2-, 4-, 6-, and 12-h postmortem.

Time (h)	0	1	2	4	6	12	*P* – value
Loin pH	6.56 ± 0.08^{a}	6.49 ± 0.09^{a}	6.37 ± 0.11^{abcd}	6.20 ± 0.10^{bcd}	6.22 ± 0.13^{bcd}	6.11 ± 0.14^{d}	0.0022
Chuck pH	6.58 ± 0.07^{a}	6.51 ± 0.07^{a}	6.46 ± 0.08^{ab}	6.32 ± 0.08^{bc}	6.23 ± 0.08^{c}	6.05 ± 0.09^{d}	<.0001
Round pH	6.66 ± 0.10^{a}	6.32 ± 0.10^{b}	6.15 ± 0.14^{bcd}	5.91 ± 0.12^{de}	5.93 ± 0.10^{cde}	5.75 ± 0.11^{e}	0.0022

[1] pH was recorded just beneath the fat surface of the longissimus lumborum of the loin and the semimembranosus of the round, and in the chuck immediately posterior to the leg just beneath the fat surface in the pocket between the brisket and the chuck.

Figure 2.1. Two section spray chilling system suspended above beef sides spraying water during chilling period.

Figure 2.2. Temperature data loggers were placed in the center of the round in the 20-, 15-, 10-, and 5-cm position.

Figure 2.3. Temperature data loggers were placed in the chuck immediately posterior to the foreleg in the pocket between the brisket and the chuck in the 20-, 15-, and 10-cm position.

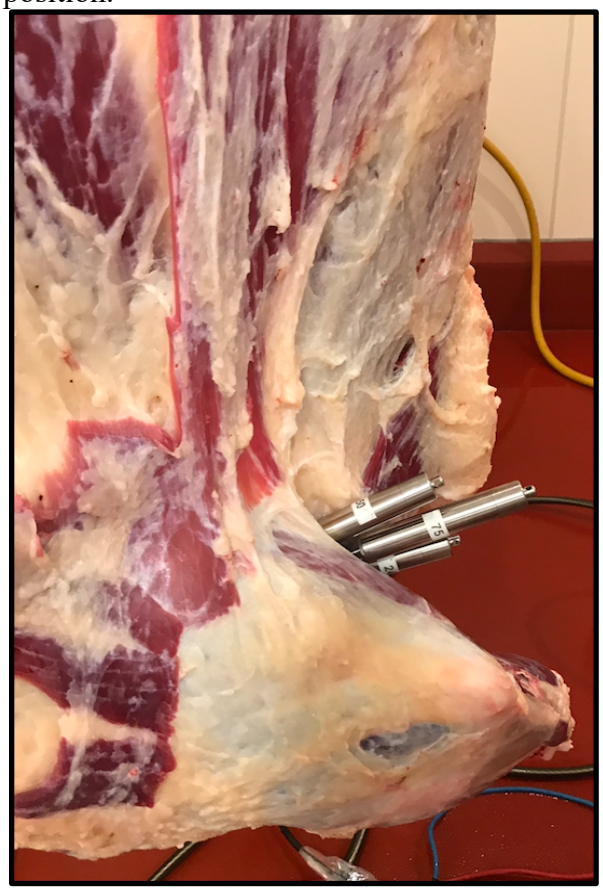


Figure 2.4. A temperature data logger was placed in the 10 cm position at the third lumbar vertebra in the loin.

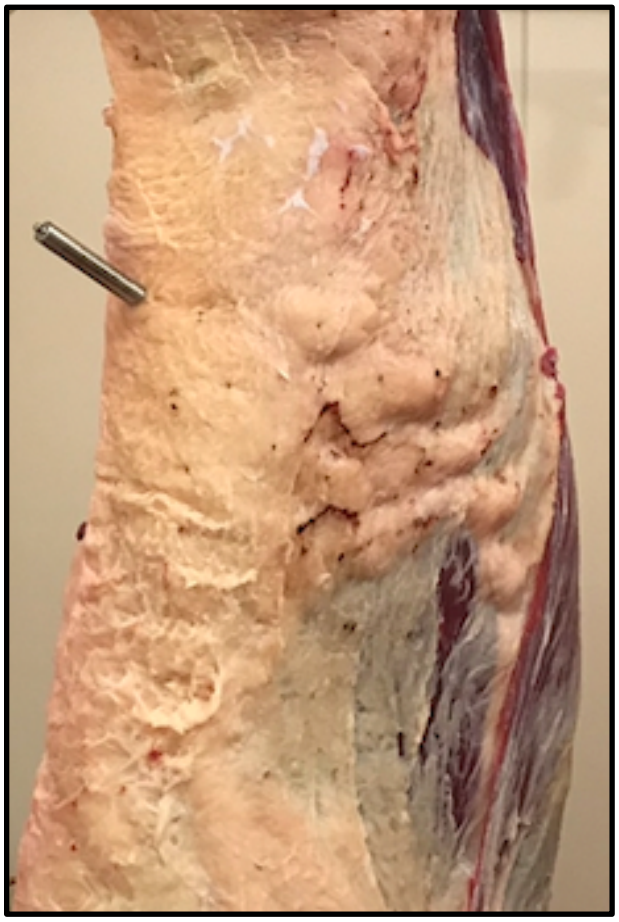


Figure 2.5. Least square means for beef loin primal internal temperature decline of air (AIR) and spray (SPRAY) treatment sides. Temperature recorded at the 10 cm position every 30 minutes at the third lumbar vertebra in the loin.

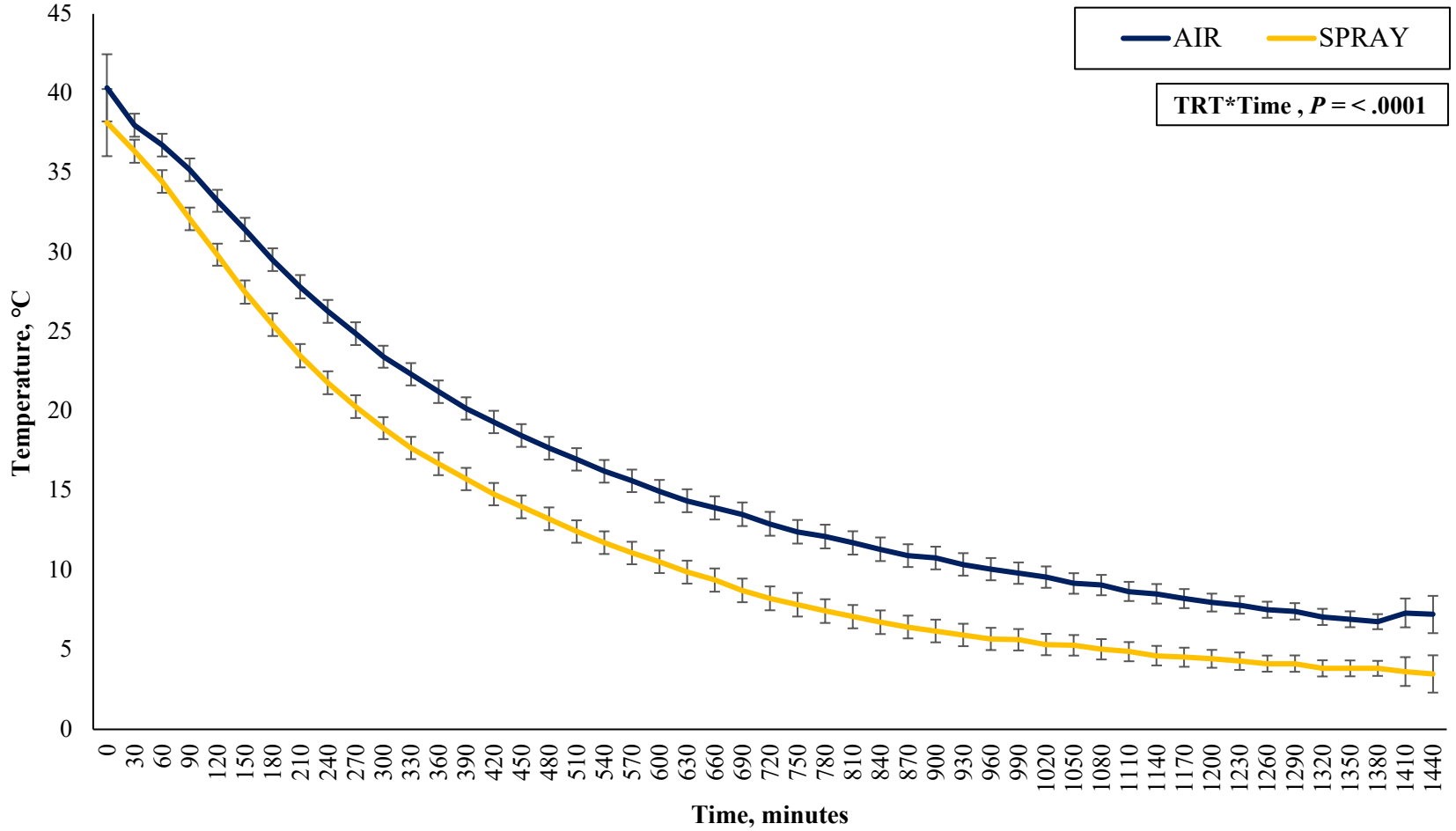
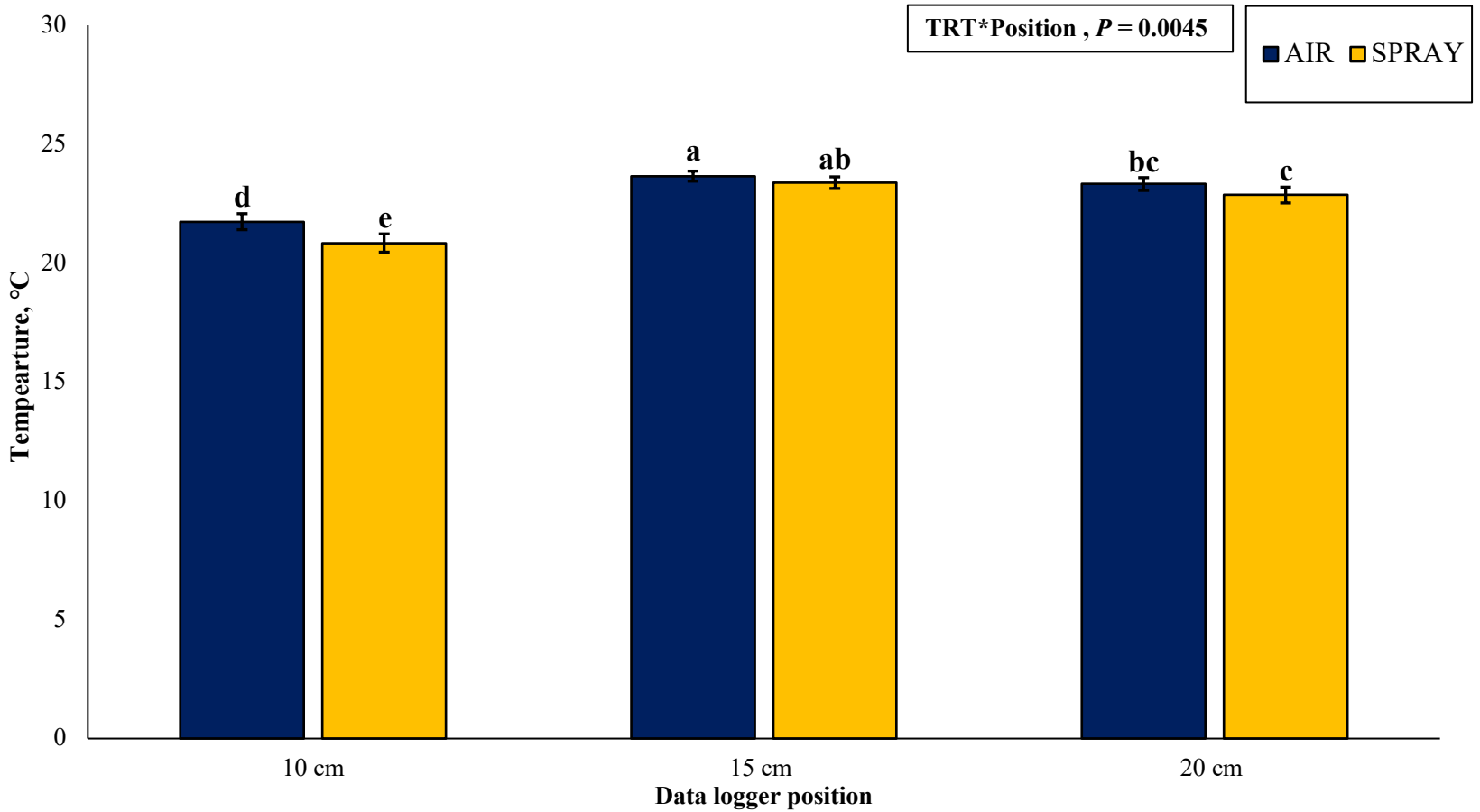


Figure 2.6. Least square means for beef chuck primal means for treatment and position of data loggers. Temperature recorded at the 10-, 15-, and 20-cm positions every 30 minutes in air (AIR) and spray (SPRAY) treatment sides in the chuck immediately posterior to foreleg in the pocket between the brisket and the chuck.



[abcde] Means lacking common superscripts differ $P < 0.05$

Figure 2.7. Least square means for beef chuck primal internal temperature decline of air (AIR) and spray (SPRAY) treatment sides. Temperature recorded every 30 minutes in the chuck immediately posterior to the foreleg in the pocket between the brisket and the chuck.

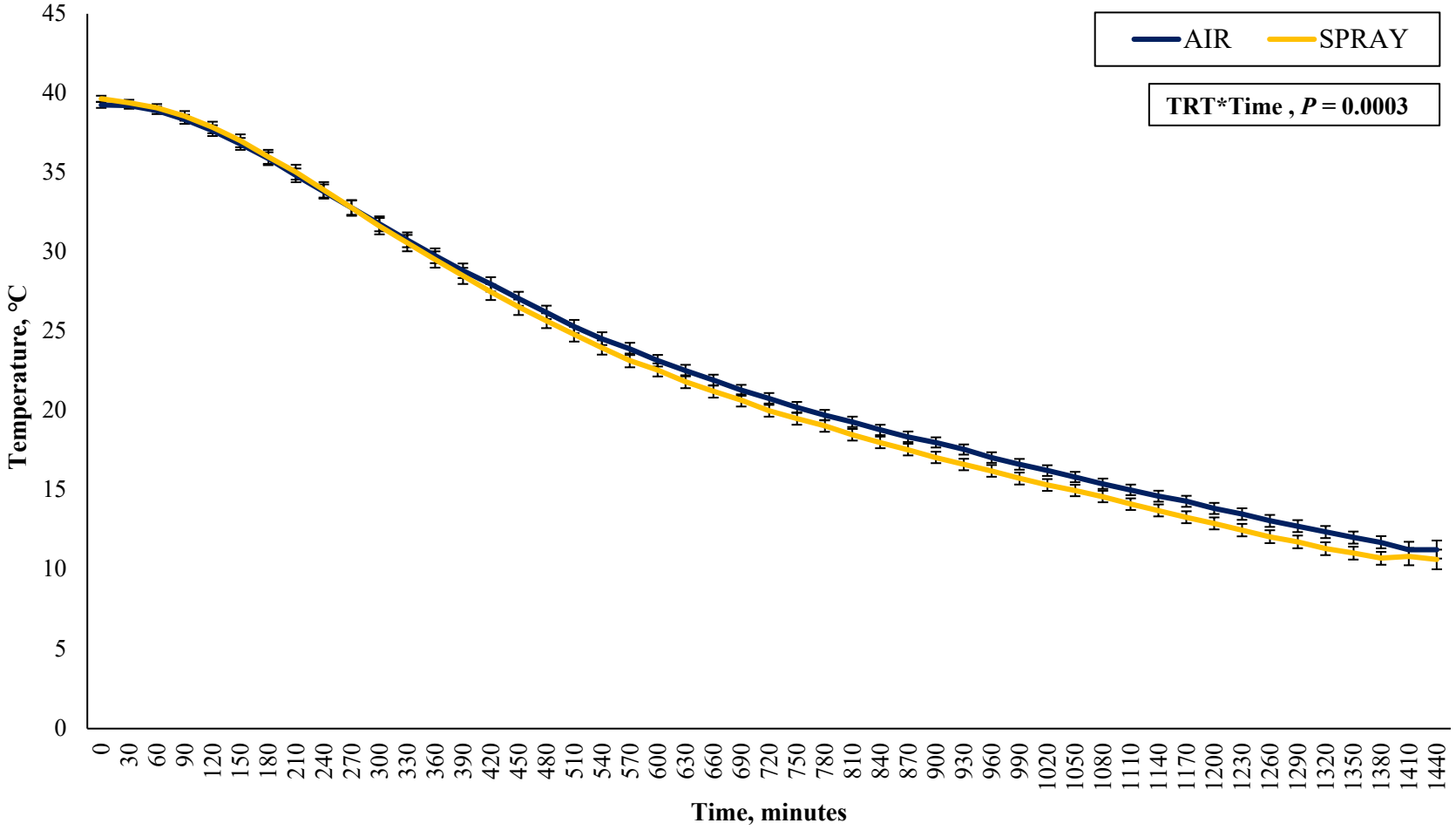


Figure 2.8. Least square means for beef chuck primal internal temperature decline at the 10-, 15-, and 20-cm positions. Temperature recorded every 30 minutes in the chuck immediately posterior to the foreleg in the pocket between the brisket and the chuck.

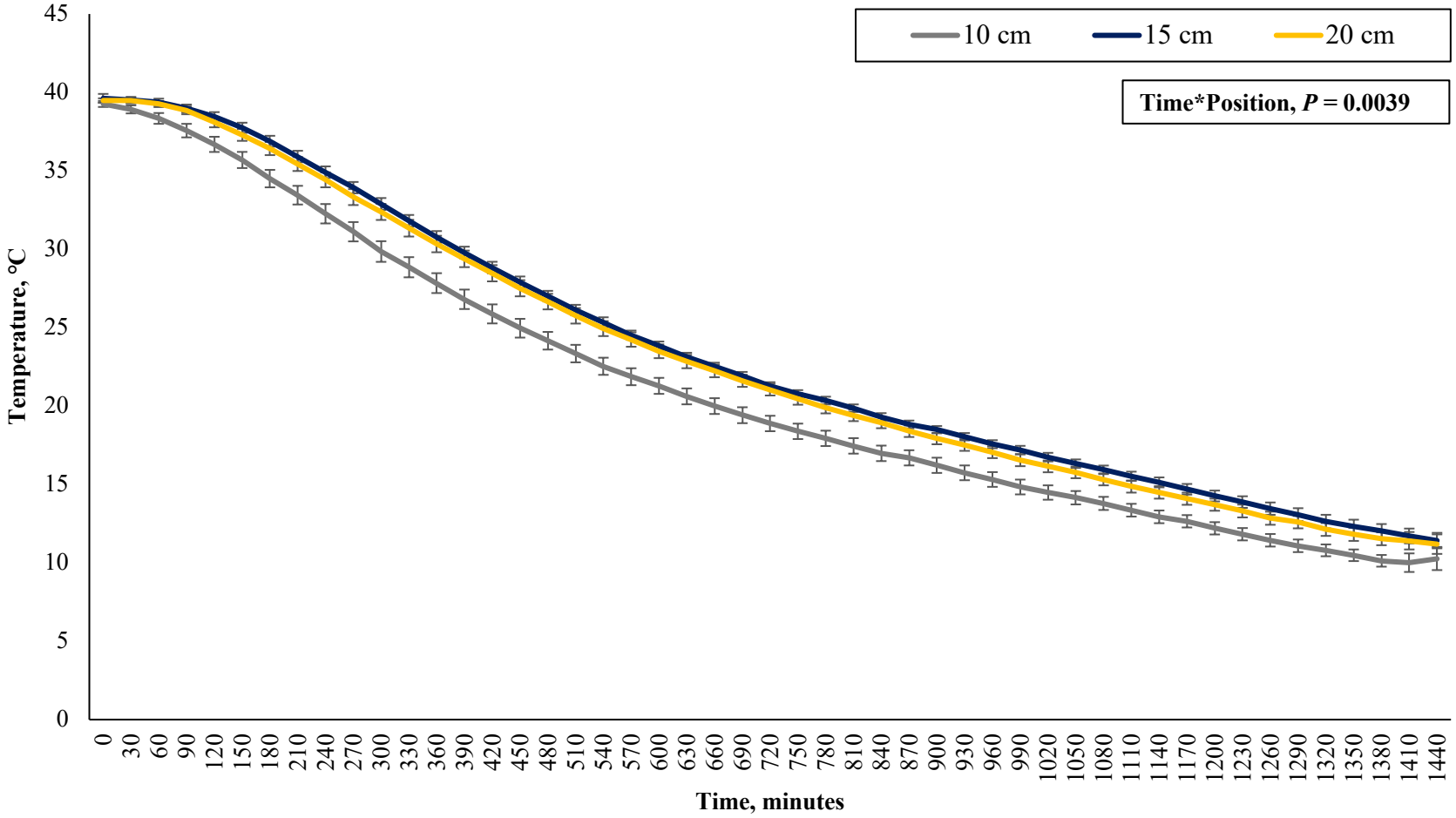


Figure 2.9. Least square means for beef round primal internal temperature of air (AIR) and spray (SPRAY) treatment sides. Temperature recorded at the 5-, 10-, 15-, and 20-cm positions 30 minutes in the center of the round primal.

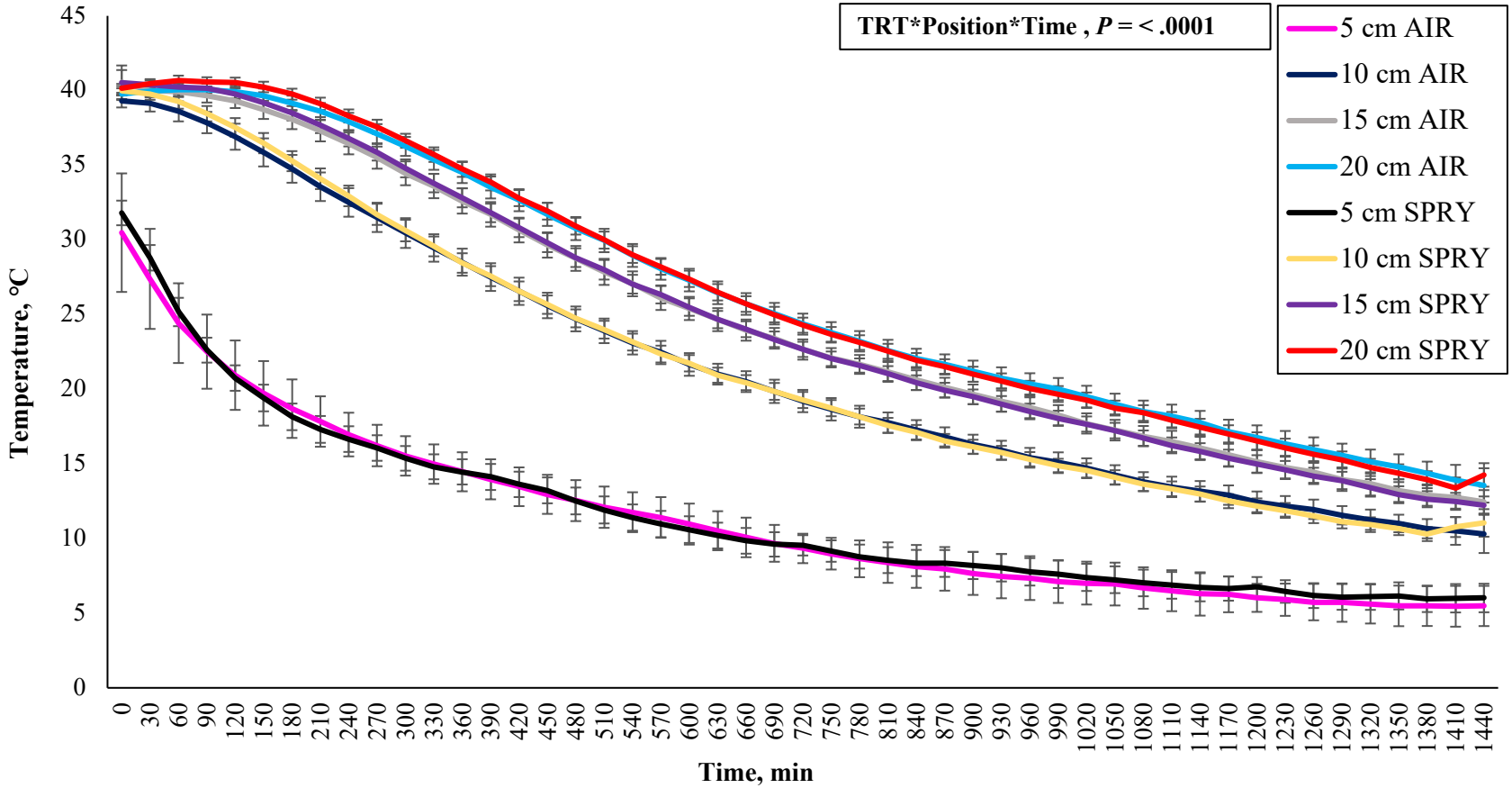
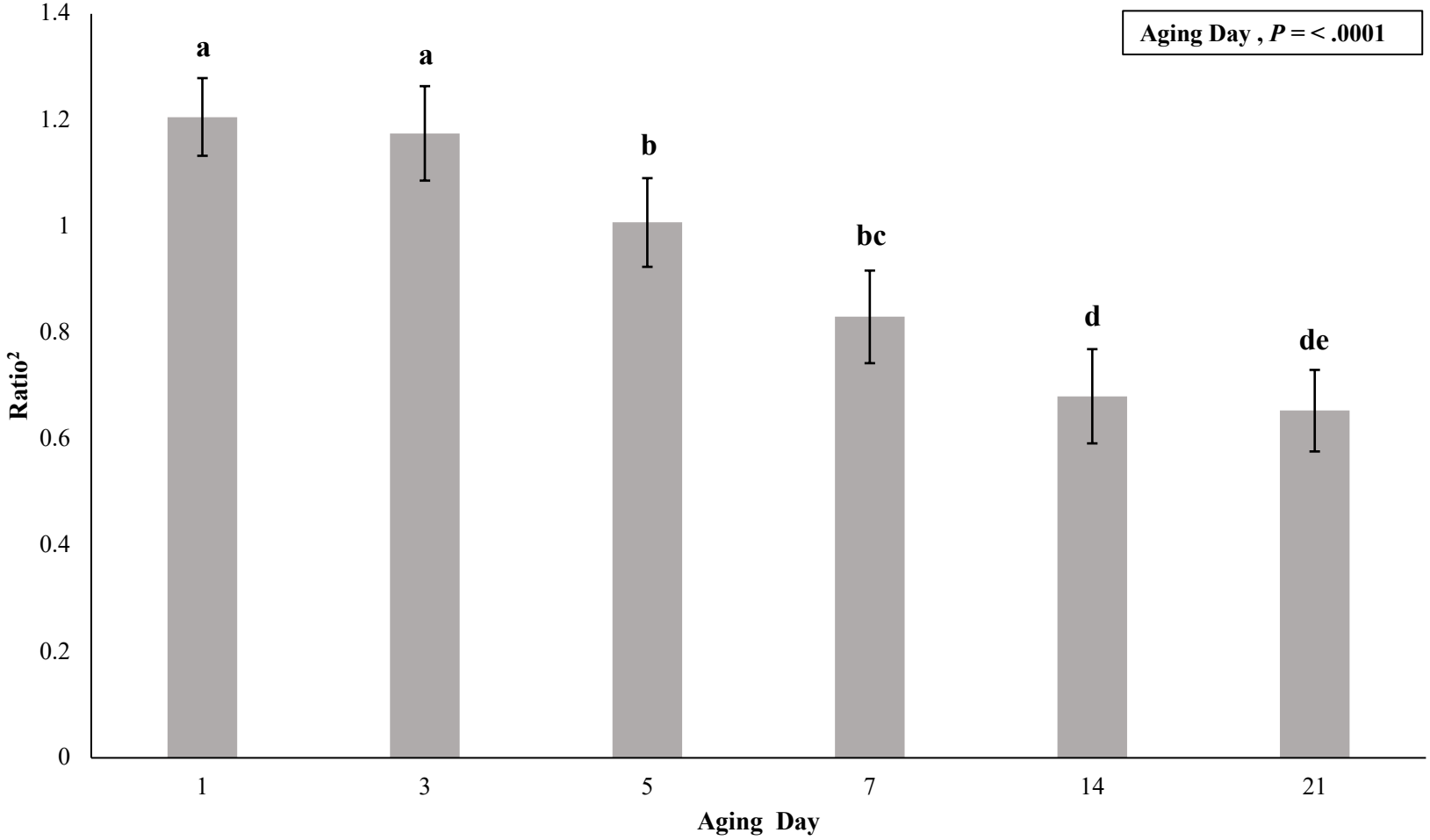


Table 2.4. Least square means for impact of chilling system treatment on proteolysis of intact desmin (55 kDa)[1]

Treatment	AIR	SPRAY	SEM	*P* – value
Intact desmin	0.916	0.946	0.091	0.743

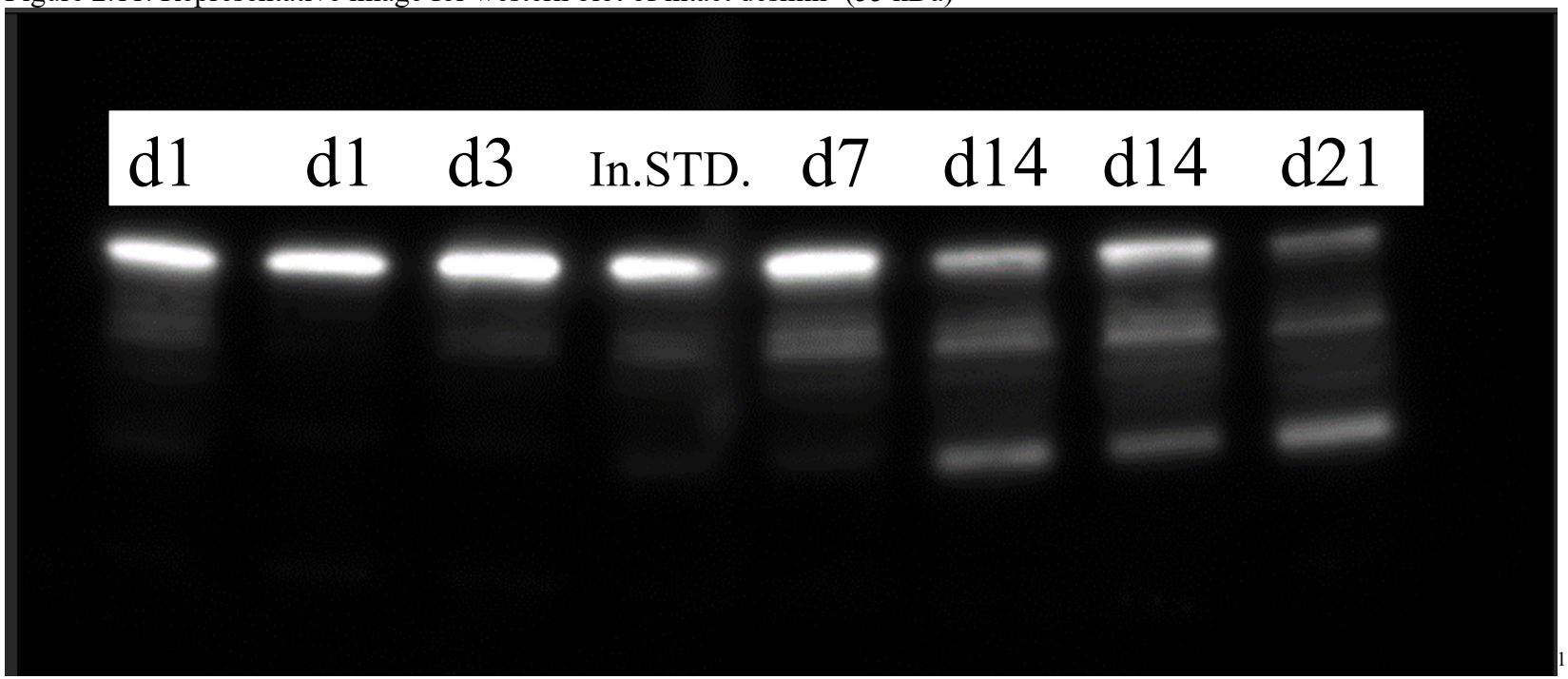
[1] Expressed as a ratio of intact desmin to an internal standard

Figure 2.10. Least square means for aging day proteolysis of intact desmin (55 kDa)[1]



[1] Expressed as a ratio of intact desmin to an internal standard
[abcde] Means lacking common superscripts differ $P < 0.05$

Figure 2.11. Representative image for western blot of intact desmin[1] (55 kDa)



[1]AlphaView SA software was used to allow for visualization and quantification for the disappearance of 55 kDa bands for intact desmin. Intact Desmin was analyzed as a ratio to an internal standard (In.STD.) across all western blots. The internal standard consisted of a composite sample equally represented by both treatments and time points across all western blots to control for inter gel variation. d1 = sample aged for 1 day postmortem, d3 = sample aged for 3 days postmortem, d7 = sample aged for 7 days postmortem, d14 = sample aged for 14 days postmortem, d21 = sample aged for 21 days postmortem.