2015

The effect of blast chilling on fresh pork quality in cuts from the Longissimus dorsi, Psoas major, Semimembranosus, and Triceps brachii

Aaron M. Blakely
Iowa State University

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The effect of blast chilling on fresh pork quality in cuts from the Longissimus dorsi, Psoas major, Semimembranosus, and Triceps brachii

by

Aaron M. Blakely

A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Meat Science

Program of Study Committee:
Steven M. Lonergan, Major Professor
Kenneth J. Prusa
Joseph G. Sebranek

Iowa State University
Ames, Iowa
2015

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ABSTRACT

Pork carcasses (n=40) were selected on four nonconsecutive slaughter dates. All carcasses were selected within the range of 54-57% fat-free lean and weighed between 86 and 91 kg. Carcasses were split approximately 45 minutes postmortem and alternating sides were assigned either blast chill (BC) or conventional chill (CC) treatment. Sides subjected to BC treatment were chilled at -32°C for 90 minutes and held at 2°C for 22 h. Sides in CC treatment group were spray-chilled and held at 2°C overnight. Temperature was continuously recorded at the tenth rib in the Longissimus dorsi (LM) in two sides from each treatment on each slaughter date. Additional temperature and pH measurements were taken in the LM upon entering chilling treatment, exiting chilling treatment (approximately 2 h postmortem in CC sides), 4, 22 and 30 h postmortem. Cuts from the LM, Psoas major (PM), Semimembranosus (SM), and Triceps brachii (TB) were collected approximately 24 h postmortem and pH was recorded approximately 30 h postmortem. The SM was further divided into a superficial (SMS) and deep (SMD) portion. A trained sensory panel (n=4) evaluated aged (PM 7 d, SM 8 d, LM 10 d, TB 13 d) fresh pork for juiciness, tenderness, chewiness, flavor, and off-flavor. Warner-Bratzler shear (WBS) and star probe measurements were collected on sensory sampling day. Calpastatin activity, \( \mu \)-calpain autolysis, desmin, and troponin-T (TnT) degradation were evaluated in the LM on samples aged 2 d postmortem. TnT degradation was further evaluated in the LM at 10 d postmortem. Desmin degradation was analyzed in cuts from the TB, SMS, SMD, and PM at two and ten days postmortem. The LM from BC sides had lower (\( P <0.05 \)) temperature exiting chilling treatment (21.8°C CC, 9.7°C BC), 4 h (13.3°C CC, 3.8°C BC), 22 h (4.2°C CC, 1.4°C BC).
BC), and 30 h (0.4°C CC, -0.2°C BC) postmortem. The lower temperature was associated with a higher ($P<0.05$) pH measured at 4 h (6.09 CC, 6.34 BC), 22 h (5.81 CC, 5.89 BC), and 30 h (5.68 CC, 5.74 BC) postmortem. Blast chilling resulted in higher ($P<0.05$) 30 h postmortem pH (5.68 CC, 5.73 BC) in the SM from BC sides. Roasts from the PM in BC sides had increased ($P<0.05$) purge loss (0.48% CC, 0.74% BC). Chops from the LM of BC sides had increased ($P<0.05$) cook loss (22.37% CC, 24.24% BC). Roasts from the PM of BC sides were more ($P<0.05$) juicy (7.50 CC, 8.30 BC), less chewy (2.80 CC, 2.10 BC), and more tender (7.90 CC, 8.60 BC). Chops from the LM in BC sides had greater ($P<0.05$) WBS (2.00 CC, 2.30 BC). Chilling was found to significantly ($P<0.05$) impact color in the SM with BC sides showing darker color score (3.00 CC, 3.20 BC) and lower Hunter a (16.35 CC, 16.02 BC). Chilling did not have an effect on postmortem proteolysis across muscle groups. This study confirmed that chilling has different impacts across muscles. This could be due to differences in carcass location, muscle composition, and fiber type.
CHAPTER 1

GENERAL INTRODUCTION

The influence of temperature decline in postmortem muscle has been shown to have an impact of fresh pork quality (Rosenvold et al., 2010; Rybarczyk et al., 2015; Shackelford et al., 2012). Blast chilling systems subject carcasses to -32°C temperatures for up to three hours postmortem (Huff-Lonergan and Page, 2001) and have been utilized to reduce the occurrence of pale, soft, and exudative pork. By rapidly decreasing muscle temperature, denaturation of proteins caused by low pH and high temperature environments can be avoided (Offer, 1991). However, with rapid temperature reduction, pork may experience cold shortening that will have a negative impact on tenderness and water holding capacity. Shackelford et al. (2012) found increased slice shear force in the Longissimus (LM) produced from a processing facility that utilized CO₂ stunning and a blast chilling regime. Other studies have found improved water holding capacity and color stability in the loin and ham that were produced using a blast chilling system (McFarlane and Unruh, 1996; Ohene-Adjei et al., 2002; Springer et al., 2003). These studies demonstrate that fresh pork quality may be improved with the use of a blast chilling regime. The current study evaluated the influence of blast chilling on muscles from various locations throughout the carcass. Sides were split and alternating sides assigned to a blast chill or conventional chilling treatment allowing for within carcass comparisons of the effect of chilling on fresh pork quality. It was hypothesized that due to the influence of muscle fiber type and location within carcass, the effect of blast chilling on fresh pork quality was not uniform throughout muscles.
Thesis Formatting

This thesis was organized into four chapters. Chapter one provides a general outline of the topic of pork chilling and development of fresh pork quality. Chapter two is a review of literature on topics relative to the topic of fresh pork quality. Chapter three is titled “The Effect of Blast Chilling on Fresh Pork Quality in Cuts from the Longissimus dorsi, Psoas major, Semimembranosus, and Triceps brachii” and is prepared for submission to the *Journal of Animal Science*. Chapter four contains a general conclusion for the entire thesis.
CHAPTER 2
REVIEW OF LITERATURE

INTRODUCTION

Consumer perception of fresh pork quality is affected by many ante-mortem and post-mortem variables. Selective breeding has increased fresh pork quality and reduced the occurrence of extreme cases of pale, soft, and exudative (PSE) pork (Barbut et al., 2008). Consumer demand for fresh pork products has changed the composition of the typical market pig (National Pork Board, 2013) and with increased world population, we are efficiently producing more pork per animal than ever before. Pork production in the United States reached 10,578,636,363 kg of pork with 113,163,000 animals in 2012 (National Pork Board, 2013). This is an increase when compared to 1980 when the United States produced 7,553,181,818 kg with 96,074,000 animals (National Pork Board, 2013). Pork carcass weights have increased 15 kg since 1980 (93 kg/carcass 2012 vs 78 kg/carcass 1980) (National Pork Board, 2013). This increase in production has the potential to impact fresh pork quality (Lonergan et al., 2001).

Consumers will give preference and pay a higher premium to pork that they deem to be of better quality. Fresh pork that is less marbled with darker color has been found to be given greater visual preference by consumers (Brewer and McKeith, 1999; Brewer et al., 2001; Font i Furnols et al., 2012). However, chops that were more highly marbled were rated more tender and juicy in sensory analysis, and more likely to be purchased (Brewer and McKeith, 1999; Brewer et al., 2001; Font i Furnols et al., 2012). Color, marbling, and package appearance are driving factors that influence the probability of initial purchase of fresh pork products. However, sensory qualities of juiciness,
tenderness, chewiness, and flavor are what drive the repeated purchase of fresh pork by consumers (Devol et al., 1988; Huff-Lonergan et al., 2010).

**Measurements of Meat Quality**

Definition of fresh pork quality can vary from palatability, texture, and color to processing functionality and yield (Bray, 1966). Regardless of which definition may be accepted, the events of the conversion of muscle to meat and postmortem carcass handling have the potential to greatly influence fresh pork quality attributes. The definition and quantification of these attributes allows comparison of a multitude of factors that contribute to fresh pork quality.

Measurements may be taken instrumentally or subjectively. Many meat quality studies use human sensory panels as measures of pork quality. Depending on the study, there are a variety of ways in which a sensory panel can be conducted (Miller, 1998). Emphasis should be placed on reducing variation in human sampling error, sample preparation, and sampling procedure. For the purpose of this literature review, emphasis will be placed on trained sensory analysis. Sensory panels may be conducted to measure several traits at once. In the instance of meat quality tenderness, chewiness, juiciness, and flavor intensity are common measurements (Miller, 1998). In trained analysis, panelists are selected and trained on attributes in sessions prior to data collection. This is an important step to establish a baseline for methods of analysis and provide a reference of each attribute for future comparisons (Miller, 1998). Ultimately, sensory evaluation provides descriptive evidence of treatment effect as the product is consumed.

Instrumental evaluations of quality will also allow evaluation of treatment effect on resulting quality. These evaluations provide measurable quality differences between
samples. Warner-Bratzler shear (WBS) force measures the force required to shear a core (1.27cm diameter) of a sample in half (Wheeler et al., 1997). WBS measurements have been found to be a good means of evaluating tenderness in fresh pork products (Van Oeckel et al., 1999). Correlations between predicted tenderness using WBS and sensory evaluation may range from 0.72 (Destefanis et al., 2008) to 0.98 (Peachey et al., 2002) in beef sensory analysis. Variations in correlations may be caused by the amount of training sensory panelists receive (Peachey et al., 2002). Yet this method of analysis provides repeatable objective measurements that may not be obtained by other methods (Hovenier et al., 1993; Wheeler et al., 1997). Star probe analysis is another objective measure of texture. Star probe analysis measures the force required to puncture and compress a sample to 80% of its original height (Huff-Lonergan et al., 2002). Much like WBS, star probe analysis is a moderate, yet repeatable predictor of tenderness (Huff-Lonergan et al., 2002).

Measurements of moisture retention can be measured as purge loss and cooking loss. The measurement of purge can be conducted following aging and is represented as weight loss of raw product. Cook loss is measured as weight loss due to cooking. Both of these quality traits help explain the juiciness of fresh pork products. Drip loss in particular has been negatively correlated with firmness of pork (Huff-Lonergan et al., 2002). Furthermore, drip loss can be an indicator of rapid pH decline or low ultimate pH resulting in denaturation of proteins, increased drip loss, and decreased technological value of the product (Monin and Sellier, 1985; Offer, 1991; Kapper et al., 2014).

Color is an important characteristic in the evaluation of fresh pork quality. Consumers want to buy a product that is visually appealing (Brewer and McKeith, 1999;
Brewer et al., 2001; Font i Furnols et al., 2012). Color measurement may be conducted objectively using colorimeters, or visually using a standardized scale. Colorimeters, such as the Hunter color system measure color values on three scales (AMSA, 2012). The first scale is known as L value that is a measure of a sample's lightness or darkness. L values are measured on a scale of 0-100 with higher values representing lighter color and lower values representing darker color. Hunter a-value represents red to green and is represented by positive to negative values. A positive a-value indicates a greater intensity of red value whereas a negative a-value represents a greater intensity of green value. The b-value of the Hunter color system is representative of intensity of yellow and blue. Again, a positive to negative b-value is assigned with a positive b-value indicating a greater intensity of yellow and a negative b-value indicating a greater intensity of blue (Hunter Laboratory Associates, 2012).

A second system for color measurement is known as the CIE L* a* b*. This system evaluates colors on the same planes as the Hunter Lab system with L* representing white to black, a* representing red to green and b* representing yellow to blue. However, this system is calculated as the cube root function of the original CIE X, Y, Z system where Hunter L a b values are calculated as the square root function (Hunter Laboratory Associates, 2012).

An additional evaluation of color may be done with hue and saturation index. Hue may be used to describe difference in color between samples, as well as discoloration. Hue is calculated using the values obtained from CIE L* a* b* values and is represented as hue angle=[arctangent(b*/a*)] (AMSA, 2012). Saturation index describes the intensity or “depth” of the color, and is calculated as saturation index=
\[(a^2 + b^2)^{1/2}\]. Measurements of hue and saturation may help describe pigments of myoglobin present in a sample based on intensity of red (a*) or yellow (b*) observed. These calculations may be more descriptive in correlations of perceived color and might be more descriptive than simple L* a* b* values (AMSA, 2012).

Differences in observer, aperture, and light source may play a role in observed color score. Observation angle accounts for observed color based on a typical field of vision. It is recommended a 10° observer be used in meat color analysis (Hunter Laboratory Associates, 2012) although a 2° observer may be used as well and was used before the development of the 1976 CIE color score system (AMSA, 2012). In a survey of research articles Tapp et al. (2011) found very little research conducted on the influence of observer and color measurements. But it is thought that the 10° observer is a better representation of visual observations made by the human eye (AMSA, 2012).

Aperture size is an important consideration when evaluating color scores across studies. As aperture size decreases, color scores may also decrease with most noticeable reductions in a* values (AMSA, 2012). It is important to select aperture size adequate to collect multiple measurements across the entire sample. Light sources vary in color spectra evaluated. Light source A is recommended for meat evaluation due to its reflectance of red color in particular. However the use of C or D light sources is common because they simulate incandescent retail light display (AMSA, 2012).

Processed meat quality may be measured in similar methods to fresh pork quality. Important factors of processed meat are yield, measured as sliceability, color, and water holding capacity (Van de Perre et al., 2010). Measurements of fresh meat quality, such as color and drip loss serve as predictors of ultimate processed meat quality showing the
irreversible effect of fresh pork defects such as pale, soft, and exudative (PSE) meat (Van de Perre et al., 2010).

It is important to understand the underlying mechanisms that play a role in the development of fresh pork quality. The objective of this literature review is to build this understanding from the level of a single muscle fiber to the postmortem processes that affect fresh and processed meat.

**Muscle Structure and Function**

The interaction between proteins that comprise muscle tissue has a profound effect on the resulting fresh meat quality. To understand how these proteins may affect product quality, it is important to understand how they are structurally arranged and the function they serve in living tissue as well as in postmortem conversion of muscle to meat.

Skeletal muscle fibers are long thread like structures that range from 10 to 100 microns in diameter and may run the entire length of a muscle (Huxley, 1958). Skeletal muscle fibers consist of a sarcolemma, the outer cell membrane of the muscle fiber. A fluid sarcoplasm is the muscle fiber cytoplasm, and surrounds myofibrils. The sarcoplasm is composed mostly of water, glycogen, and among other proteins, the oxygen transport protein myoglobin (Giles, 1962; Garry and Mammen, 2007). The sarcoplasmic reticulum and transverse tubule (t-tubule) system work to transmit stimulus for skeletal muscle contraction. The sarcoplasmic reticulum is the calcium reservoir for muscle and is responsible for the release and uptake of calcium to the muscle sarcoplasm throughout the contraction and relaxation cycle (Huxley, 1964).
Skeletal muscle structure begins at the attachment to bone by tendons that are comprised of the connective tissue protein, collagen (Diament et al., 1972). Collagen is also the protein that divides muscles into bundles, sheaths, and fibrils (Jarvinen et al., 2002). Many muscle bundles, also known as fasciculi, are surrounded by a perimysium layer and comprise an entire muscle unit (Jarvinen et al., 2002). These fasciculi may be divided again into muscle fibers, and surrounded by another connective tissue layer known as the endomysium (Rowe, 1978). Ultimately fasciculi are divided into individual muscle cells known as muscle fibers, which are enclosed by yet another layer of connective tissue known as the endomysium (Rowe, 1978). Muscle fibers contain the muscle fiber mitochondria and nucleus, as well as myofibrils. Myofibrils are the muscle structures that are responsible for muscle relaxation and contraction. The interactions between myofibril proteins are responsible for production of the power stroke in living tissue and the increased toughness in postmortem conversion of muscle to meat (Huxley, 1958; Wheeler and Koohmaraie, 1994).

When skeletal muscle fibers are viewed under a light microscope they appear to have striations (Huxley, 1958). These striations are due to alternating A-bands (dark bands) and I-bands (light bands) (Locker and Leet, 1975), and are the site of the two filament proteins whose interaction with one another are responsible for contraction and relaxation (Huxley and Niedergerke, 1954). The darker bands are known as anisotropic or A-bands. The major motor protein that comprises 55% of the A-band is myosin. Myosin measures 110 Å in diameter and when viewed with polarized light causes a birefringent refraction (Huxley and Hanson, 1954). The light band is singly refractive when viewed with polarized light and is referred to as the isotropic or I-band (Huxley and...
Hanson, 1957). This band is much thinner, measuring 40 Å in diameter and is comprised of the motor protein actin (Huxley and Hanson, 1954; Huxley, 1973). The I-band is interrupted by a structure known as the Z-disk (Huxley and Hanson, 1954), this portion of the myofibril is comprised of several cytoskeletal proteins that will be detailed later in this literature review.

A visibly less dense area bisects the A-band, and is referred to as the H-zone. The H-zone contains primarily the tail portion of myosin (Pepe, 1967). Collectively these structures comprise sarcomeres. Each sarcomere is contained by Z-disks that bisect an I-band, and an A-band that is bisected by an H-zone that is divided by an M-line (Huxley and Niedergerke, 1954). Sarcomere length may be determined by measuring from Z-disk to Z-disk and is commonly 2.0-2.5 µm in length in relaxed muscle and 1.7-2.0 µm in contracted muscle (Huxley and Niedergerke, 1954; Gordon et al., 1966).

**Muscle Proteins**

Myosin is the major motor protein located in the thick filament. Myosin is a long rod shaped protein with two globular heads. The head region of myosin is actin activated, and has attachment sites for actin as well as an adenine binding site (Moore et al., 1970; Walker et al., 1982). The presence of inorganic molecules such as adenosine triphosphate (ATP) and adenosine diphosphate (ADP) at this site assist in regulating the weak and strong force binding of myosin to actin (Hill et al., 1983).

Actin is the major myofibril protein located in the thin filament. Actin filaments are comprised of many smaller globular actin molecules that are bound together forming a double helix filamentous structure known as F-actin (Murray and Weber, 1974; Erickson, 1989). Actin possesses attachment sites that allow myosin heads to bind and
ultimately produce the force of contraction. These binding sites are covered by the protein tropomyosin when there is no stimulus for contraction in the form of increased calcium concentrations in muscle sarcoplasm (Murray and Weber, 1974). Upon binding of calcium to the troponin complex, binding sites are uncovered and myosin is able to attach to actin. This stimulates the hydrolysis of ATP and produces force to shorten sarcomeres and contract muscle (Murray and Weber, 1974).

**Cytoskeletal proteins**

In addition to the motor proteins listed above, there are several other proteins that are essentials to maintaining muscle structure throughout contraction and relaxation. The protein titin is the largest known protein with a molecular weight of 3,700 kDa (Huff-Lonergan et al., 1995). This protein originates in the Z-line and spans to the M-line (Fürst et al., 1988). Under stress, titin provides elasticity to the sarcomere allowing the muscle to return to its original length. Titin provides the framework necessary for other aforementioned proteins to assemble upon (Wang et al., 1984).

With a molecular weight of 600-900 kDa (Huff-Lonergan et al., 1996b), nebulin is the second largest known protein. Nebulin runs parallel to the thin filament and much like titin serves as an anchor for other motor proteins (Labeit et al., 1991). Because nebulin runs parallel to the thin filament it is believed that it may serve as a “ruler” for sarcomere assembly, as well an anchor point for the thin filament (Labeit et al., 1991).

Intermediate filament proteins comprise a small portion of the muscle fiber proteins. The intermediate filament protein desmin is an integral protein in the organization of the Z-lines and serves to anchor adjacent myofibrils at the level of the Z-line. Desmin also is an attachment point for the sarcolemma (Paulin and Li, 2004).
Desmin runs approximately 55 kDa on SDS-PAGE gels, and is 10 nm thick (Ohashi and Maruyama, 1979; O’Shea et al., 1981).

Vinculin is an intermediate protein with a molecular weight of 116 kDa (Tidball, 1991), and along with the proteins talin and integrin, are responsible for linking actin molecules with the extracellular matrix (Monti et al., 1997). Degradation of these proteins postmortem has been shown to have an impact on WHC and tenderness (Morrison et al., 1998; Melody et al., 2004) because of their importance in structural integrity in muscle fibers.

**Muscle Contraction**

The contraction of muscle requires several steps. Stimulus for contractions is transmitted from the brain to the neuromuscular junction at the sarcolemma. The sarcolemma possesses tubules that extend into muscles cells to assist in the transmission of signal. These tubules are known as transverse or T-tubules (Peachey, 1965). The primary function of the T-tubule systems is the transmission of signal from the sarcolemma to the sarcoplasmic reticulum (Peachey, 1965). Once stimulus reaches the T-tubule, a calcium voltage activated channel is opened releasing calcium into the sarcoplasmic reticulum via the ryanodine receptor. The function of the sarcoplasmic reticulum is to regulate the sarcoplasmic calcium concentration of myofibrils by sarcoplasmic reticulum calcium ATPase (SERCA) pumps (Bendall, 1975; Higgins et al., 2006). Once calcium is released into the sarcoplasm, it binds to the myofibrillar protein troponin-C (TnC) (Greaser and Gergely, 1971). TnC is a portion of the troponin myofibrillar protein complex. The troponin proteins are located on the protein tropomyosin, and are incorporated on top of the actin filament (Blumenschein et al.,
The troponin proteins work in concert to regulate the contraction of muscle. The binding of calcium to TnC causes a conformational change to troponin-I (TnI), which then moves troponin-T (TnT) (Blumenschein et al., 2005). Troponin-T then acts on tropomyosin causing the protein to shift its location on actin. The displacement of tropomyosin allows binding sites to be opened on actin allowing myosin heads to interact with actin (Flicker et al., 1982).

The attachment of myosin heads to actin requires the presence of adenosine triphosphate (ATP) (Huxley, 1973). The presence and binding of the ATP causes a conformational change in the myosin nucleotide binding sites, allowing myosin heads to bind to actin in a weak force confirmation (Prochniewicz et al., 2004). With ATP attached and myosin bound to actin, the myosin head has ATPase activity (Lowey et al., 1969). The hydrolysis of ATP to ADP, the release of phosphate, and actin are regulated by magnesium (Potter and Gergely, 1974), and cause another conformational change in the myosin head. This conformational change straightens the myosin molecule giving the power stroke step required for generation of force (Rayment et al., 1993; Prochniewicz et al., 2004). Once phosphate is released, conformation of myosin once again changes and myosin binding affinity for actin is reduced and affinity for ATP is increased (Rayment et al., 1993). Therefore with ATP available, myosin will preferentially bind with ATP, releasing actin and allowing for the cycle to repeat. In the absence of ATP, myosin will remain bound to actin forming rigor bonds.

**Muscle Metabolism**

As previously mentioned, ATP plays a vital role in the contraction of muscle. It serves as an energy source to regulate the uptake of calcium into the muscle sarcolemma.
from the sarcoplasmic reticulum during relaxation via SERCA pumps (Bendall, 1975; Higgins et al., 2006). ATP also provides energy for the association of actin and myosin which ultimately results in contraction. Upon the hydrolysis of ATP by myosin (Lynn and Taylor, 1970), ATP must be replenished to continue to drive muscle contraction and relaxation. A readily available, but quickly depleted supply of creatine phosphate is used to produce ATP when muscle is under high stress conditions. Upon depletion, regeneration of creatine phosphate relies on creatine kinase and oxidative phosphorylation to replenish supply (Giannesini et al., 2003). Skeletal muscle produces ATP via the TCA cycle under aerobic conditions (Ohlendieck, 2010). The TCA cycle utilizes glycogen as an energy input for a net result of 36 ATP.

In glycolysis, glucose is converted to pyruvate. Under anaerobic conditions, pyruvate is oxidized to lactate by the enzyme lactate dehydrogenase (Ohlendieck, 2010). The buildup of lactate in postmortem muscle results in a drop in pH in tissue and is an important factor in conversion of muscle to meat and ultimate fresh pork quality (Offer, 1991). The complete degradation of glycogen to glucose-6-phosphate for use in glycolysis is reliant on the activity of the enzymes glycogen phosphorylase and glycogen debranching enzyme (GDE) (Brown et al., 1966). The activity of GDE postmortem will ultimately have an effect of the rate of glycolysis postmortem (Kylä-Puhju et al., 2005). The study by Kylä-Puhju et al. (2005) found that GDE activity in postmortem muscle is temperature sensitive. When muscle temperature is decreased below 39°C GDE activity decreased and activity was limited below 15°C. The study by Kylä-Puhju et al. (2005) found that in muscles with more rapid temperature decline, ultimate pH was higher. The
higher ultimate pH was attributed to loss in GDE activity early postmortem and therefore slowing glycolysis and pH decline.

**Muscle Fiber Type**

The metabolic pathway utilized to produce ATP in living tissue is also dependent on the muscle fiber type (Dubowitz and Pearse, 1960; Brooke and Kaiser, 1970). Muscle fibers may be classified by their myosin ATPase activity. Myosin ATPase activity is dependent on myosin isoforms present (Schiaffino and Reggiani, 1994). Through the classification of fibers based on myosin ATPase activity, up to eight different muscle fiber classes exist (Yellin and Guth, 1970). Generally speaking, fiber types are typically classified as three main types. Type I, type IIa, and type IIb (Christensen et al., 2004). Type I fibers are known as red or slow twitch muscle fibers (Taylor, 1972). Type I fibers have a high proportion of myoglobin (4-12 mg/g tissue) (Beecher et al., 1965), myoglobin adds pigment to muscle tissue and is also a carrier of oxygen (Wittenberg et al., 1975). Slow twitch fibers are found more in tissues that require continuous, low contraction force activity (Dubowitz and Pearse, 1960). Type I fibers are smaller in diameter (2,000 µm²) (Quiroz-Rothe and Rivero, 2004) than other muscle fiber types and upon contraction generate less force (Dubowitz and Pearse, 1960). Red (type I) muscle fibers typically have less muscle glycogen and a greater proportion of mitochondria making them consistently able to utilize oxidative metabolism for ATP production (Schiaffino and Reggiani, 2011).

The second major muscle fiber type is known as type IIb or white muscle fibers. Type IIb fibers are larger (4,000 µm²) (Quiroz-Rothe and Rivero, 2004) than type I and are used for short burst, high energy contractions (Edgerton and Roy, 1991). Type IIb
muscle fibers have a greater proportion of muscle glycogen stores for use in glycolysis (Schiaffino and Reggiani, 2011). With increased reliance on glycolysis, the need for oxygen in muscle is lower than in type I fibers. Therefore, white muscle fibers have a lower concentration of myoglobin (2 mg/g tissue) (Beecher et al., 1965; Wittenberg et al., 1975). Myoglobin also adds the characteristic red or pink pigment to muscle, so with decreased amounts of myoglobin, type IIb fibers can be expected to be paler products giving type IIb muscle fibers their characteristic “white” name. Generally, the pH in type IIb fibers has been found to decline at a faster rate than type I fibers due to their reliance on anaerobic metabolism and increase in lactate production (Klont et al., 1998). Rate of postmortem pH decline has been shown to greatly impact fresh pork quality (Offer, 1991).

The third muscle type is known as an intermediate type IIa fiber. Type IIa fibers are both glycolytic and oxidative, are intermediate size (2,215 µm²) (Quiroz-Rothe and Rivero, 2004), and have contraction rates similar to fast twitch (type IIb) fibers (Christensen et al., 2004; Schiaffino and Reggiani, 2011). The composition of all three of these fibers will make up an entire muscle each providing a different ratio based on muscle use and skeletal location.

Conversion of muscle to meat

Following stunning the animal is exsanguinated. Blood is removed that constitutes up to 3.55% of the live weight of the animal (Gardner et al., 2004). Blood serves to deliver oxygen to tissue, regulate body temperature, deliver nutrients, and remove waste. Upon exsanguination, muscle is no longer able to utilize oxygen to produce ATP via the TCA cycle and must rely on the creatine phosphate and glycolysis
to produce ATP. In addition, the utilization of glycolysis results in a buildup of lactate that cannot be removed without a blood supply. An effect of acidic pH on muscle fibers has been found to lower calcium activated myosin ATPase activity (Bowker et al., 2004) and has been suggested to have an effect on calcium binding sensitivity in the actin filament (Blanchard et al., 1984). This twofold effect of reduced ATPase activity in myosin and decreased calcium regulation in the thin filament will reduce the hydrolysis of ATP by myosin. With reduced ATP production and lowered pH resulting in reduced ATPase activity, permanent actin and myosin cross bridge formation resulting in rigor. The initial toughening immediately following slaughter (first 24 hours postmortem) is known as rigor shortening (Koohmaraie et al., 1996; Weaver et al., 2009).

Pale, soft and exudative pork may be produced from animals that experience extreme stress immediately prior to slaughter from shipping, handling, stunning, or are carriers of the halothane gene (Bendall and Wismer-Pedersen, 1962; Wismer-Pedersen, 1959). Severe cases of PSE are attributed to a condition known as porcine stress syndrome (PSS) that is caused by a mutation known as the Halothane gene (Barbut et al., 2008). The Halothane gene impacts the ryanodine receptor, causing excess calcium release into muscle fibers from the sarcolemma. Cheah and Cheah (1976) found that twice the amount of calcium was released in animals that were carriers of the Halothane gene when compared non mutant pigs. The release of calcium stimulates the activity of the enzyme pyruvate dehydrogenase (PDH). With increased activity of PDH, pyruvate is converted to acetyl-CoA. Increased levels of acetyl-CoA have a negative feedback on PDH leaving an excess of pyruvate available for use in glycolysis. Pyruvate has a negative feedback effect on glycolysis and needs to be further processed to continue ATP
production (Felipe, 2013). Increased calcium levels stimulate lactate dehydrogenase causing an increase of production of lactate from pyruvate (Cheah and Cheah, 1976). Because this reaction occurs immediately following exsanguination, carcass temperatures will be in the range of living tissue or slightly above. The combination of high postmortem temperature and rapid lactate production causing rapid pH decline leads to denaturation of myofibrillar and sarcoplasmic proteins. These factors will have an irreversible and detrimental effect in water holding capacity, color, and texture of fresh pork products (Briskey and Wismer-Pedersen, 1961). Color and texture are largely influenced by the denaturation of myosin. Myosin contributes to the formation of rigor bonds in postmortem muscle. Upon denaturation of myosin during the conversion of muscle to meat, there will be a reduction in extracellular space in the myofibril (Barbut et al., 2008). This will cause an increase in purge from meat containing the sarcoplasmic protein myoglobin. Increased purge will also cause increased light scattering and pale color. Ultimately these products will have pale soft and exudative qualities.

Briskey and Wismer-Pedersen (1961) reported muscles that were more exterior in carcasses chilled more rapidly and showed fewer PSE like attributes when compared to more interior muscles that chilled slower. The rate of postmortem pH decline and ultimate pH of muscle are reliant on species and fiber type. The typical living pH of pork is 7.4 and fresh pork will reach and ultimate pH of 5.5 six to twelve hours postmortem (Lawrie et al., 1960). Stressed pigs or pigs that exhibit PSS due to mutations in the ryanodine receptor caused by the Halothane gene will reach ultimate pH early postmortem (Bendall and Swatland, 1988) causing PSE defects in fresh pork products.
Short term stress prior to slaughter caused by handling, transportation, or stunning (Martínez-Rodríguez et al., 2011) will have an effect on fresh pork quality similar to PSE characteristics. An increase in stress immediately prior to slaughter will result in increased lactate production, increased muscle temperature, and decreased muscle glycogen content (Støier et al., 2001; Rosenvold and Andersen, 2003; Lindahl et al., 2006). The combination of these factors will lead to increased protein denaturation and drip loss in pork. Young et al. (2009) found that pigs that were stressed but allowed to rest prior to slaughter produced pork with improved drip loss but was tougher than pigs that were handled normally. The study by Young et al. (2009) suggested that the effects of pre-slaughter stress resulted in increased muscle oxidative stress and therefore has an effect on postmortem tenderization.

An alternate defect that carcasses may experience postmortem is known as cold shortening. Cold shortening was defined in beef as the rapid decline in muscle temperature to 14-19°C before the onset of rigor mortis (Locker and Hagyard, 1963). Cold shortening occurs when carcasses are quickly chilled before the completion of rigor, causing tougher than typical fresh pork and may have additional moisture loss (James et al., 1983). This toughening is due to the loss of functionality of the sarcoplasmic reticulum making it no longer able to bind calcium and releasing calcium to the muscle fiber. In pork, Bendall (1975) found the additional release of calcium is coupled with increased myosin ATPase activity suggesting increased muscle contractions. With increased contraction, sarcomere length is decreased and has a negative impact on tenderness in all red meat species (Herring et al., 1965; Bouton et al., 1973; Bendall, 1975). However, cold shortening is dependent upon muscle fiber type. There is a great
difference in cold shortening susceptibility between muscles that can be correlated to muscle myoglobin content (Bendall, 1975). Muscles that have a greater proportion of type I fibers are more susceptible to cold shortening because they undergo rigor mortis at a slower rate than those with greater proportions of type IIb fibers. Bendall (1975) found that due to the wide variation of muscle fiber types between muscles, pork carcasses make an excellent model for the formation of cold shortening and variation of shortening among muscle fiber type.

**Postmortem Muscle**

Postmortem handling of carcasses has the potential to greatly impact fresh pork quality. One way in that fresh pork quality may be improved is through the initial chilling of the carcasses following slaughter. Conventional methods of chilling include spray chilling, delay chilling, and blast chilling (Savell et al., 2005).

Blast chilling is commonly accomplished through the use of chilling tunnels and high velocity forced air onto carcasses resulting in an environment that may reach -40°C. Carcasses are maintained at this temperature for up to three hours (Huff-Lonergan and Page, 2001). By rapidly chilling muscle, GDE activity is decreased therefore slowing glycolysis and lactate production (Kylä-Puhju et al., 2005). The effect of rapid chilling has been demonstrated to reduce the effect of high postmortem temperature and rapid pH decline leading to pale, soft, and exudative (PSE) products. Borchert and Briskey (1964) demonstrated that by submerging sections of pork loins in liquid nitrogen immediately postmortem, color was improved and with equilibration at 4°C purge loss was reduced. Due to cost and worker safety, this is an improbable method of carcass chilling industry wide. More practical applications of blast chilling have been found to have an improved
effect on water holding capacity (WHC) and color. Results from previous studies on the improvement of these traits have been inconsistent (Kerth et al., 2001; Rees et al., 2003).

Tenderness is a major contributor to consumer perception of fresh pork quality (Devol et al., 1988; Moeller et al., 2010). The initial toughening immediately following exsanguination is due to the interaction between the muscle proteins actin and myosin (Koohmaraie et al., 1996). Upon exsanguination, muscle no longer has a means to maintain homeostasis. Muscle mitochondria are deprived of oxygen resulting in the inability to synthesize ATP. Additionally, the sarcoplasmic reticulum is no longer able to regulate calcium transport to the sarcolemma. With calcium available, troponin is not able to regulate myosin binding to actin results in irreversible actomyosin cross bridging causing shortened sarcomeres and permanent muscle contraction. The degree of sarcomere shortening has been found to be one of several indicators of fresh meat tenderness. Muscle that is contracted to a greater extent will have shorter sarcomeres and will likely be tougher (Dransfield and Lockyer, 1985; Wheeler et al., 2000).

Postmortem proteolysis may also be affected by extreme sarcomere shortening. Weaver et al. (2009) found that shortened muscle exhibits less postmortem proteolysis and decreased \( \mu \)-calpain activity. Thus, a two-fold effect of toughness due to shortened sarcomeres and limited improvements in tenderness through postmortem proteolysis could lead to tougher meat products. The calpain system works to degrade protein in living muscle and meat (Goll et al., 1992). With limited \( \mu \)-calpain activity in cold shortened muscle, protein degradation is limited in beef (Weaver et al., 2009) and may have a similar effect in fresh pork. Additionally, rapid temperature decline has been shown to affect calpain activity in beef, slowing initial degradation and limiting the
extent of proteolysis (Mohrhauser et al., 2014). Studies have also shown that there is a correlation between calpain activity, protein degradation, and WHC (Huff-Lonergan and Lonergan, 2005). If the effects of blast chilling are negatively impacting proteolysis and causing cold shortening, sensory characteristics of fresh pork may be compromised resulting in tougher product as well as decreased juiciness resulting in poorer quality fresh pork.

μ-Calpain

Calpains were first described as calcium-activated sarcoplasmic factors in 1963 (Guroff, 1964), and are composed of several different isoforms of calpain. The calpain system acts as a cysteine protease in both living tissue and meat. This literature review will focus on the effects and effectors of calpain in postmortem tissue. The two calpains of most interest are known as μ-calpain and m-calpain. Each is named for the amount of calcium required by each for activity with μ-calpain requiring 5-70 µM calcium and m-calpain requiring 100-2000 µM calcium for activity (Goll et al., 1992). A study conducted by Geesink et al. (2006) found that mice that exhibited a μ-calpain knockout gene had less postmortem protein degradation than their wild-type counter parts. Postmortem degradation of desmin, troponin-T (TnT), as well as other cytoskeletal proteins (Goll, 2003) is a good indication of overall postmortem tenderization and resulting fresh beef tenderness (Young et al., 1980; Huff-Lonergan et al., 1996a).

Calpain is comprised of an 80 kDa subunit and a 28 kDa subunit. Suzuki and Ohno (1990) reported the 80 kDa subunit contributes the protease activity of the proteinase and is unique to each isoform of calpain while the 28 kDa subunit is similar in each. The 80 kDa subunit is comprised of four domains, simply named I, II, III, and IV, while the 28
kDa domain is comprised of two domains (V and IV’) (Suzuki and Ohno, 1990).

Domain II of the 80 kDa subunit works as the main protease of the enzyme (Ono et al., 1998). Domain II would be constantly active, however the interactions of domains IV and IV’ inhibit domain II’s proteolytic activity (Emori et al., 1988). Domain IV and IV’ contain EF hand domains which change conformation upon binding of calcium allowing for activation (Suzuki and Ohno, 1990). Upon activation, each subunit’s molecular mass will decrease. Hathaway et al. (1982) reported polypeptide fragments at 76 kDa upon activation of the 80 kDa subunit, while the 28 kDa subunit was reduced to 18 kDa.

The changing conditions of postmortem muscle have been shown to have a substantial effect on µ-calpain activity (Mohrhauser et al., 2014; Yin et al., 2014). Koohmaraie (1992) found that as pH declined, but temperature remained constant (25°C), the rate of autolysis of µ-calpain’s 80 and 30 kDa band increased. However, as temperature decreased, the rate of autolysis slowed. This was further investigated by Mohrhauser et al. (2014) who found that protein degradation, specifically TnT, was increased in beef that were incubated at 22°C than those incubated at 4°C. The study by Mohrhauer et al. (2014) further shows that µ-calpain activity is temperature as well as pH dependent.

**Calpastatin**

Another component of the calpain system known to have an effect on calpain activity is known as calpastatin. This endogenous protease inhibitor is a regulator of calpain in addition to calcium (Goll et al., 1992). Calpastatin is described as a competitive inhibitor of m-calpain as well as µ-calpain (Maki et al., 1988). Much like m- and µ-calpain, calpastatin’s inhibitory ability is related to calcium concentrations (Ma et
Calpastatin works to inhibit calpain by directly binding to calpains regulatory site (Kawasaki et al., 1993). Upon binding of calpastatin to calpain, Kawasaki et al. (1993) found autolysis to be completely halted when specific regions of each inhibitory domain were removed, despite calcium incubation levels or temperature. These results indicate that each inhibitory domain of calpastatin are regulated by interactions with several subdomains identified by Ma et al. (1993) as subdomains A, B, and C, with subdomain B being essential for the inhibition of calpain activity, and subdomains A and B having no inhibitory capabilities.

Calpastatin consists of four inhibitory domains (I, II, III, and IV) (Maki et al., 1988). Each inhibitory domain has the capacity to inhibit activity of one μ- or m-calpain molecule (Emori et al., 1988). Therefore, it is possible for one calpastatin molecule to effectively inhibit multiple calpain molecules (Maki et al., 1988). Within each inhibitory domain, there are three homologous subdomains (A, B, and C). The binding of calpain to calpastatin is dependent on calcium levels in the cell. The calcium required for inhibitory action is less than the requirements for μ-calpain activation. Kapprell and Goll (1989) found that the inhibitory activity of calpain requires less calcium than that required by μ-calpain. These results indicate that calpastatin has an opportunity to inhibit μ-calpain activity before μ-calpain is able to be activated. Once calcium is removed from calpastatin, calpastatin becomes unbound, making the inhibition of calpain by calpastatin a reversible process (Cottin et al., 1981; Ma et al., 1993). The inhibition of calpain by calpastatin has been shown to be increased in cold-shortened beef (Zamora et al., 1998). Thus a two-fold effect of shortened sarcomeres and limited postmortem proteolysis in cold-shortened pork may lead to tougher fresh pork (Drainsfeld and Lockyer, 1985).
The reported molecular weight of calpastatin is highly variable and has been reported to range from 34 to 300 kDa (Goll, 2003). The nature of calpastatin contributes to the wide range of molecular weight estimates. First calpastatin exists in random coil form, a contributing factor in the range of estimated weight in size exclusion chromatography. Because of the high number of charged amino acids that compose calpastatin, SDS-PAGE molecular weights may over estimate calpastatin’s true molecular weight (Takano and Murachi, 1982). Additionally, because calpastatin is readily degraded by calpain in postmortem muscle, the isolation of the molecule is complicated and again adds to the variation in reported molecular weights (Otsuka and Goll, 1987). Another explanation of the variation in molecular weights of calpastatin is due to the presence of several different isoforms that are formed because of alternative splicing and the presence of different promoters, which may also play a role in the regulation of calpains by calpastatin (Cong et al., 1998; Huff-Lonergan and Lonergan, 1999; De Tullio et al., 2007). Ciobanu et al. (2004) demonstrated that variations of calpastatin haplotypes present in different breeds of pigs can account for variations in fresh pork tenderness and juiciness due to calpastatin’s regulation of calpains role in postmortem proteolysis.

While it is known that calpastatin is an inhibitor of the calpains, the exact mechanism of inhibition in postmortem muscle is still up for debate. Doumit and Koohmaraie (1999) found calpastatin degraded upon incubation with μ- and m-calpain among other proteases. However, immunoreactive fragments of the degradation product of calpastatin closely resembled those that were found in aged lamb longissimus. This study indicates that calpastatin not only inhibits calpain activity, but serves as a substrate
for µ- and m- calpain proteolysis. The postmortem regulation of proteolysis by calpain and regulation of the calpain system by calpastatin is essential in the development of fresh meat quality. With increased ionic strength of postmortem pork, calpastatin has been found to have an increased inhibitory effect on µ-calpain (Maddock et al., 2005). The influence of calcium in cold-shortened pork may have an impact on calpastatin inhibition as well as sarcomere length.

**Meat Quality**

Meat quality may be defined as carcass composition and conformation. However, the consumer will perceive the products eating quality with the traits of tenderness, chewiness, juiciness and flavor. These traits will ultimately have an impact on the likelihood of repeat business by a consumer. It is important to understand the relationship of postmortem conditions on the effect of the sensory attributes of fresh meat products. This literature review briefly introduced how quality may be measured. The following section will outline how quality will develop postmortem.

**Color**

Myoglobin is the sarcoplasmic protein that is responsible for the characteristic red color of meat. Muscle myoglobin concentration will vary with muscle type, age, and species. Myoglobin contains a single globin peptide and an iron containing heme ring with six binding sites (Suman and Joseph, 2013). Four of these sites are bound by nitrogen, one site is bound to the globin and the final site is a free binding site. The oxidation state of the heme ring, and the molecule bound to the open site determine the color observed in fresh meat (Suman and Joseph, 2013). Oxidized iron is known as ferric iron and will give a brownish color to meat products. This pigment is known as
metmyoglobin. Reduced iron without bound oxygen will result in purplish meat color
and is known as deoxymyoglobin. With the addition of oxygen to the reduced myoglobin
molecule, meat will develop the generic bright cherry red color (Suman and Joseph,
2013). In PSE conditions, the high postmortem temperature and low pH causes an
increase in myofibrillar protein denaturation and precipitation of sarcoplasmic proteins
cauing increased light scatter and the characteristic light color (MacDougall, 1982).

In living tissue, iron is maintained in a reduced state by the enzyme
metmyoglobin reductase (Hagler et al., 1979). The activity of metmyoglobin reductase
has also been attributed to improvements in color shelf life of fresh pork (Zhu and
Brewer, 1998). This enzyme is heat sensitive under low pH conditions and contributes to
the decreased color stability in PSE products (Zhu and Brewer, 1998). In high pH
conditions (5.8-6.2) (Zhu and Brewer, 1998) of postmortem tissue, metmyoglobin
reductase activity is maintained and improvements in color stability may be made. Thus
if pH decline may be mediated in postmortem muscle, gains in fresh pork color could be
made.

**Tenderness**

Tenderness is a major attribute that will determine a consumer’s preference for a
particular product. Improved tenderness is more likely to be correlated with improved
overall acceptanc for the product when compared to other sensory characteristics (Enfält
et al., 1997). Much work has been done in the past to predict and ensure a product is
consistently tender. However, tenderness is a complex trait that may be affected by many
different intrinsic qualities of muscle fiber type, muscle location, and animal variations.
Muscle fiber type will not only influence the development of meat quality, but the inherent qualities of muscle that will influence the consumers eating experience. Slow twitch (type I) or intermediate fibers (type IIa) fibers have a smaller cross sectional area (Jeong et al., 2010; Quiroz-Rothe and Rivero, 2004) and can be expected to be more tender than type IIb fibers. Additionally, with increased fiber number per cell, there may be improved sensory quality due to the presence of intramuscular fat or marbling. Marbling is the contribution of the endomysium surrounding muscle fibers and may serve to break up muscle tissue adding flavor and potentially improving tenderness (Devol et al., 1988; Huff-Lonergan et al., 2002). However, the true contribution of marbling to improved tenderness in pork is still up for debate with previous work indicating weak (Huff-Lonergan et al., 2002) or no correlation (Jeong et al., 2010) between improved marbling and tenderness rating.

As previously discussed, blast chilling of pork carcasses can lead to cold shortening and has been shown to have an impact on tenderness of fresh pork (Dransfield and Lockyer, 1985). In addition to shortened sarcomere lengths, cold shortening may also have a negative effect on postmortem degradation of myofibrillar proteins that play a role in the tenderization of pork during aging (Iversen et al., 1995). The postmortem proteolysis of proteins results in ultrastructure modification that may be seen as increased separation between myofibrils and increased I-band fracturing resulting in improved tenderness (Wheeler and Koohmaraie, 1994; Taylor et al., 1995). Degradation of key proteins titin, nebulin, filamin, desmin and troponin are all good indicators of total postmortem degradation and degree of tenderness in beef (Huff-Lonergan et al., 1996a; Morrison et al., 1998; Zhang et al., 2006). The location of each of these proteins explains
the changes in structure of myofibrils during postmortem aging. Titin and nebulin are anchored in the Z-disk and extend through the I-band. Upon degradation thin filament rigidity is decreased resulting in disruption of lateral muscle fiber structure increasing space between muscle fibers (Greaser and Gergely, 1971; Huff-Lonergan et al., 1996b; Melody et al., 2004). Degradation of filamin, a protein located at the junction of the cell membrane and the Z-disk, has been reported to be responsible for the lateral conformation of muscle fibers up postmortem degradation (Huff-Lonergan et al., 1996a).

The degradation of desmin, an intermediate filament protein, is also responsible for the lateral degradation of muscle fibers (Huff Lonergan et al., 2010) that assist in tying the myofibril together and may have an impact on water holding capacity of pork (Melody et al., 2004). The degradation of desmin in beef and pork has been shown to be correlated with improved tenderness of aged samples (Huff-Lonergan et al., 1996a; Wheeler et al., 2000; Wheeler et al., 2002). It is known that postmortem proteolysis is more rapid in pork than in beef (Koohmarae et al., 1991). The use of western blotting to determine the desmin degradation is therefore a good indicator of tenderness of the sample and may be used as a predictor of fresh meat quality (Huff-Lonergan et al., 1996a; Melody et al., 2004; Wheeler et al., 2000; Wheeler et al., 2002).

Another protein that may be used as a predictor of tenderness is troponin-T. Troponin-T plays an integral part in the regulation of actin and myosin cross bridging. Throughout postmortem aging, this protein is degraded and the presence of a 28 kDa and a 30 kDa degradation product may be detected using monoclonal antibodies and SDS-PAGE (Huff-Lonergan et al., 1996a). Rate of troponin-T degradation as measured by intensity of 30 kDa band has been shown to be a good indicator of tenderness of resulting
fresh pork (Lonergan et al., 2001). There is still some discussion as to whether the degradation of troponin-T is simply an indicator of overall proteolysis (Huff-Lonergan et al., 1996a), or if the degradation troponin-T does in fact have a significant effect on muscle structure and tenderness (Melody et al., 2004; Mohrhauer et al., 2014). It has been suggested that because of its integral part in the regulation of contraction, upon degradation the improved tenderness experienced may be due to changes in interactions of thick and thin filaments (Huff-Lonergan et al., 2010).

The presence of connective tissue will play a role in the development of meat tenderness. Connective tissue is made of primarily of the proteins collagen and elastin (Nishimura et al., 2008). Collagen content will vary from muscle to muscle based on use and development (Diamant et al., 1972; Nishimura et al., 2008; 2009). With increased collagen content from such muscles as the biceps femoris when compared to the psoas major, toughness of the muscle has been found to increase (Nishimura et al., 2009). It has also been demonstrated that as animals age, collagen will crosslink and become less heat-soluble once again resulting in tougher product (Nishimura et al., 2008). There are a multitude of other factors which have already been discussed that contribute to fresh pork tenderness. The contribution of collagen concentration between muscles can also account for variations in tenderness between fresh pork cuts (Wheeler et al., 2000). Furthermore, the presence of collagen will have a negative effect on the processing functionality of fresh pork in processed products.

**Water Holding Capacity**

The ability of muscle to retain water in fresh pork products is important not only from the standpoint of fresh muscle quality, but also as a source of potential lost revenue
for a producer. Muscle which may experience poor water holding capacity may be losing weight in the form of purge (drip) loss or cook loss. The idea of purge loss is the inability of a product to hold water, without any exterior factors, such as cooking or processing, affecting the product. The actual amount of loss is typically measured as percentage of fresh pork weight lost and can be as high as 10% in carcasses exhibiting pale, soft and exudative quality defects (Melody et al., 2004). Typically, in PSE carcasses, the combination of high carcasses temperature and low pH will increase protein denaturation and will have an effect on WHC (Offer, 1991, Deng et al., 2002).

Muscle is composed mostly of water. Depending on muscle composition, water constitutes up to 70-75% of muscle tissue (Offer and Trinick, 1983; Huff-Lonergan and Lonergan, 2005). A majority of water is held within the muscle fiber, occupying the area between muscle filaments (Offer and Trinick, 1983; Offer and Cousins, 1992). This portion of water within the muscle is known as entrapped or immobilized water, and is held within the muscle by the fibers themselves and is not bound to proteins. Immobilized water is most susceptible to loss in normal postmortem conditions due to the spatial changes that occur during the onset of rigor with increased interaction between myosin and actin causing shortening of sarcomeres (Honikel et al., 1986). The decrease of sarcomere length leaves less room between myofibrils in which water can be held, and is lost as purge or drip through openings in the sarcomeres that have become known as “drip channels” (Honikel et al., 1986; Offer and Cousins, 1992; Huff-Lonergan and Lonergan, 2005). It should be apparent that in events which may cause extreme shortening of myofibrils, more water may be lost as purge or drip. These instances of
cold-shortening have been previously discussed as having a negative impact on
tenderness of the product and reducing consumer appeal.

Another portion of water in muscle is known as bound water. Bound water is
tightly bound to muscle proteins and is not easily removed from muscle fibers from external factors such as cooking due to positive and negative charges on proteins. The portion of bound water is extremely small (<10%) (Huff-Lonergan and Lonergan, 2005). Bound water is however susceptible to net charge changes, such that occurs in postmortem muscle with the buildup of lactate. As lactate increases in postmortem muscle, pH declines. As pH falls, it will approach the isoelectric point (5.2-5.4) of the functional protein myosin (Huff-Lonergan and Lonergan, 2005). At the isoelectric point of myosin, the net charge will be zero. As myosin reaches its isoelectric point, protein will have a lower affinity for water. The lowered affinity for water will result in water lost as purge or drip (Huff-Lonergan and Lonergan, 2005). Another effect will be seen in muscle structure. Again, as muscle reaches a net charge of zero, electrostatic repulsion between muscle fibers will decrease. Will less repulsion between fibers, space in fibers will decrease leaving less room for water storage (Offer, 1991). Product that has ultimate pH of greater 6.4, (van der Wal et al., 1988) results in a product defect referred to as dark firm and dry. These products will have high water binding and reduced purge loss. The higher pH in these instances causes increased net negative charge of myofibrillar proteins and increased affinity for water molecules. This product appears dry, has improved water holding capacity, (van der Wal et al., 1988) and has been shown to be more tender (Lonergan et al., 2007).
Intermediate filament proteins such as desmin are responsible for linking adjacent myofibrils together to assist in ensuring uniform contraction of muscle as a whole (Huff-Lonergan et al., 2010). Upon the process of postmortem tenderization, intermediate proteins like desmin are degraded. This allows muscles to create more space for water storage between myofibrils (Kristensen and Purslow, 2001; Melody et al., 2004). Early degradation in pork may account for some improvements in purge loss after the onset of rigor mortis by preventing drip channel formation and keeping water within muscle structure.

**Protein Functionality in Processed Products**

The quality of processed meats is reliant on the quality of the fresh meat that goes into the product. Water holding capacity, slice yield, and color are all reliant on the quality of myofibrillar and sarcoplasmic proteins that go into the making of the product. The extractability of myosin in particular will have an effect on the technological quality of the product as it is the protein responsible for bind in processed products (Yasui et al., 1982). The sensitivity of myosin to degradation at high temperatures and, in particular, low pH has already been discussed. The rapid chilling of these proteins has been shown to improve WHC, color, and texture of processed products (Tomović et al., 2008; 2013). The effect of muscle location in particular will have a role in the development of processed meat quality particularly in section and formed meats. The slower chilling of deeper ham muscles may be having an effect on processed ham quality as deeper portions chill slower while pH decline is normal (Ohene-Adjei et al., 2002; Van de Perre et al., 2010). In PSE carcasses the rapid pH decline leads to partial denaturation of myosin
heads and decreased ATPase activity (Torley et al., 2000) making myosin unable to
dissociate from actin and decreasing myosin solubility and protein functionality.

**Conclusion**

There are a multitude of factors that contribute to fresh pork quality. Inherent
features of pork contribute to the variation of color, texture and sensory which a
consumer will experience when purchasing fresh pork. Current chilling of pork carcasses
has been shown to have an effect on fresh loin quality. However, the effect of chilling
and location of various muscles is not well documented. The present study was designed
to test the hypothesis that the effect of blast chilling on fresh pork quality is dependent on
muscle composition, metabolism, and location.
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CHAPTER 3

THE EFFECT OF BLAST CHILLING ON FRESH PORK QUALITY IN CUTS FROM THE LONGISSIMUS DORSI, PSOAS MAJOR, SEMIMEMBRANOSUS, AND TRICEPS BRACHII

A manuscript prepared for submission to the Journal of Animal Science


*Iowa State University, Department of Animal Science, Ames, IA 50011
†Iowa State University, Department of Food Science and Human Nutrition, Ames, IA 50011
‡JBS, Greely, CO 80631
Abstract

The objective of this study was to investigate the effects of blast chilling on sensory and proteolysis in cuts from the *Longissimus dorsi* (LM), *Psoas major* (PM), *Semimembranosus* (SM), and the *Triceps brachii* (TB). The SM was further divided into a superficial (SMS) and the deep (SMD) portions. Carcasses were selected for fat-free lean (FFL) and HCW approximately 45 minutes postmortem. Carcasses were split and sides were assigned to either blast chill (BC, -32°C for 90 minutes) or conventional chill (CC, spray chilled at 2°C for 24 h) regimens. BC sides had lower ($P<0.05$) temperature exiting chilling treatment (CC 21.8°C approximately 2 h postmortem, BC 9.7°C BC), 4 h (CC 13.3°C, 3.8°C BC), 22 h (CC 4.2°C, BC 1.4°C), and 30 h (CC 0.4°C, BC -0.2°C) as measured at the tenth rib in the LM. This was associated with a higher ($P<0.05$) pH in BC sides 4 h (CC 6.09, BC 6.34), 22 h (CC 5.81, BC 5.89) and 30 h (CC 5.68, BC 5.74) postmortem. Chilling regime resulted in higher ($P<0.05$) 30 h postmortem pH in the SM from BC sides (CC 5.68, BC 5.74). Cuts from BC sides had increased ($P<0.05$) purge loss in the PM (CC 0.48%, BC 0.74%) and increased ($P<0.05$) cook loss in chops from the LM (CC 22.37%, BC 24.24%). Trained sensory analysis (n=4) found that the PM from BC sides was more juicy (CC 7.50, BC 8.30), less chewy (CC 2.80, BC 2.10), and more tender (CC 7.90, BC 8.60). Chops from the LM of BC sides had greater Warner-Bratzler shear force (CC 2.00, BC 2.30). Color was affected in the SM with BC sides showing darker color score (CC 3.00, BC 3.20) and reduced Hunter a value (CC 16.35, BC 16.02). Chilling treatment did not have an effect on postmortem proteolysis across muscle groups. This study confirmed that chilling has different impacts across muscle groups which may be caused by location, rate of chilling, and fiber type.
**Introduction**

Leaders in the meat industry now realize that importance of meeting consumer demands for tender, juicy and safe fresh pork products as emphasis shifts from a commodity to a product focus in the global marketplace. It is clear that improved swine genetics and chilling rate have reduced the incidence of pork with poor water holding capacity and color. Once the slaughter process begins, one of the first impacts upon pork quality is the reduction of carcass temperature. This step in the process is vital to reduce or inhibit microbial growth. The rate and extent of carcass chilling has been shown to negatively impact fresh pork quality by influencing sarcomere length and postmortem proteolysis (Bendall, 1975; Shackelford et al., 2012). Other parameters of color and water holding capacity have been shown to be improved with the implementation of blast chilling systems (Jones et al., 1993). McFarlane and Unruh (1996) found that loins from blast chilled sides had reduced purge loss without a negative impact on tenderness. Rybarczyk et al. (2015) found similar improvements loins of BC carcasses with improved drip loss but with a negative effect on sensory tenderness. These varied results in tenderness and water holding capacity have also been found in the ham (Springer et al., 2003; Ohene-Adjei et al., 2002). Due to location and fiber type differences across muscles, the effect of chilling will not have a uniform impact on fresh pork quality. The effect of temperature decline across different muscles is not yet well defined. Therefore the objectives of the current study were to 1.) Define the effect of blast chilling on fresh pork quality in cuts from the *Longissimus dorsi, Psoas Major, Semimembranosus,* and the *Triceps brachii.* 2.) Characterize the effect of chilling on muscle location in the
*Semimembranosus* superficial and deep portions. 3.) Evaluate the effect of blast chilling on cook ham yield.

**Materials and Methods**

All animals were slaughtered at JBS Marshalltown using carbon dioxide stunning. Pork carcasses (n=40) were selected in groups of ten at four nonconsecutive slaughter dates (11/2/2012, 2/8/2013, 5/29/2013, and 8/30/2013). Carcasses were selected on the processing floor, immediately following evisceration. Criteria for selection included HCW (86-91 kg) and fat-free lean (FFL) (54-57%). After selection, carcasses were split and alternating sides assigned to blast chill (BC) or conventional chill (CC) treatment approximately 45 minutes post slaughter. Sides that received BC treatment were subjected to -32°C chilling cycle for 90 minutes while CC sides were spray chilled and held at 2°C. Loin temperatures were continuously recorded in two sides from each treatment at each slaughter date using LogTag data loggers (LogTag Recorders Limited, Auckland New Zealand). Tenth rib LM pH was recorded in each carcass entering chilling treatment, approximately 4, 22, and 30 h postmortem. Tenth rib loin temperature measurements were recorded entering chilling treatment, exiting chilling treatment (approximately 2 h postmortem in CC sides), and approximately 4, 22, and 30 h postmortem. All carcasses were held at 2°C overnight and processed the following day.

**Sample Collection**

Sides were processed at JBS approximately 26 h postmortem. The Longissimus dorsi (LM), Triceps brachii (TB), Semimembranosus (SM) and Psoas major (PM) were collected from each carcass, vacuum packed and transported on ice to the Iowa State University sensory evaluation lab. Fresh pork was aged (PM 7 d, SM 8 d, LM 10 d and
TB 13 d) under refrigeration (4°C) and used to determine treatment effect on ultimate pH, purge loss, cook loss, star probe, Warner-Bratzler shear force (WBS) and sensory quality. Purge measurements were collected following aging. Samples were removed from their vacuum bag and weighed. The weight of the bag was then subtracted from the weight of purge remaining in the bag and purge was determined as a percentage of the original muscle weight \([(\text{initial weight-final weight})/\text{initial weight}] \times 100\). Ultimate pH was recorded on whole cuts and 2.54 cm thick chops (LM and SM) were prepared. Hunter L a b values were collected using a Minolta Chroma Meter fitted with a 50mm aperture and a D65 light source with a 0° observer. Color scores (National Pork Board (1999), standard six point scale, 1=pale pinkish gray to white; 6= dark purplish red) marbling scores (National Pork Board (1999), standard ten point scale, 1= 1.0% intramuscular fat; 10= 10.0% intramuscular fat) were assigned to chops (SM and LM).

**Sensory Analysis**

Raw weights were collected on chops (LM and SM) and roasts (PM and TB) for cook loss determinations. Chops were cooked in clamshell grills to an internal temperature of 68°C. Roasts (PM and TB) were cooked in a rotary hearth oven at 176°C to an internal temperature of 68°C. Cooked weight was then collected and samples were divided into 2.54 cm square cubes for trained sensory analysis (n=4). Two additional chops were cooked using the same procedure as listed above. These chops were allowed to cool to room temperature and an Instron Universal Testing Machine (Instron Industrial Products, Grove City, PA) fitted with Warner-Bratzler shear force head was used to determine Warner-Bratzler shear force values (Gruber et al., 2008). A five point probe
was fitted to the Instron and compressed the sample to 80% of its height to determine star
probe value (Lonergan et al., 2007).

Sensory analysis was conducted to determine tenderness, chewiness, juiciness,
flavor, and off-flavor. Trained panelists (n=4) evaluated samples on a ten point anchored
scale. A lesser value represents a lesser degree of a trait and a greater values represents a
greater degree of the trait (1=tough, 10=tender; 1=not chewy, 10=chewy; 1 = dry, 10=
juicy; 1=no pork flavor, 10=intense pork flavor; 1=no off-flavor, 10=intense off-flavor).
Panelists were required to complete a 6-mo training period. During this period, they were
provided with samples that exhibited extremes of the sensory attributes measured.
Following training, panelist served a 6-mo probationary period. Data collected during
this probationary period were compared to data collected from existing highly trained
panelist. Data from individual panelist were not used until the panelist consistently
performed with existing highly trained panelist performance. Panelists were served 1.27
cm cubes of each sample immediately following cooking. Unsalted crackers and ice
water were provided between samples (Huff-Lonergan et al., 2002).

**Whole Muscle Protein Extracts**

Samples aged two and ten days postmortem were frozen in liquid nitrogen and
then powdered using a Waring® blender (Waring Commercial, New Hartford CT).
Frozen powdered samples were extracted using modified methods of (Huff-Lonergan et
al., 1996). Samples (0.5g) were individually homogenized in 10ml of solubilizing buffer
(2% wt/vol SDS, 10mM sodium phosphate, pH 7.0). Protein concentration was
determined using a Lowry protein assay with premixed reagents (DC protein assay, Bio-
Rad Laboratories, Hercules, CA) samples were diluted to 6.4 mg/ml and whole muscle
sample extracts were prepared following methods of (Huff-Lonergan et al., 1996), final protein concentration of extracted samples was 4 mg/ml.

**SDS-PAGE and Western Blot Analysis**

Desmin degradation on all samples (PM, TB, SMS, SMD and LM) aged 2 d and 10 d postmortem was determined using 10% polyacrylamide separating gels (acrylamide/bisacrylamide=100:1 [wt/wt], 0.1% [wt/vol] SDS, 0.05% [vol/vol] tetramethylenediamine (TEMED), 0.05% [wt/vol] ammonium persulfate (AMPER), and 0.5 M Tris HCl pH 8.8). To determine troponin-T degradation, samples (LM aged 2 d and 10 d) were run on 15% polyacrylamide gels. 8% polyacrylamide gels were used for determination of μ-calpain (LM aged 2 d) autolysis. All gels used a 5% polyacrylamide stacking gel (acrylamide/bisacrylamide=100:1 [wt/wt], 0.1% [wt/vol] SDS, 0.125% [vol/vol] TEMED, 0.075% [wt/vol] AMPER, and 0.125 M Tris HCl pH 6.8). For desmin degradation and μ-calpain autolysis analysis, 40 µg of sample was loaded in each lane, while 20 µg of sample was used in troponin-T degradation determination. Gels (10 cm wide x 8 cm tall) were run in SE 260 Hoefer Mighty Small II (Hoefer, Inc., Holliston, MA) electrophoresis units. The running buffer was a solution composed of 25 mM Tris, 192 mM glycine, 2 mM EDTA, and 0.1% [wt/vol] SDS. Desmin gels were run for approximately 375 volt-hours, troponin-T gels were run for approximately 360 volt-hours and μ-calpain gels were run for approximately 350 volt-hours. Proteins from gels were transferred to polyvinylidene difluoride (PVDF) membrane as described by Melody et al. (2004). Blots were blocked for one hour at room temperature using 5% non-fat dry milk [wt/vol] in PBS-Tween (80 mM Na₂HPO₄, anhydrous, 20 mM NaH₂PO₄, 100 mM NaCl, 0.1% [vol/vol] polyoxyethylene sorbitan monolaurate [Tween-20]. Blocking solution
was then discarded and primary antibodies were added to PBS-Tween in the following dilutions, desmin- 1:40,000 using rabbit anti-desmin polyclonal antibody produced in house, troponin-T- 1:40,000 using monoclonal mouse anti-troponin-T (T6277, JLT-12, Sigma, Saint Louis, MO) and µ-calpain- 1:5,000 using monoclonal mouse anti-µ-calpain (MA3-940, Thermo Scientific, Rockford, IL). Blots were incubated in 10 ml of diluted antibody solution overnight at 4°C or for an hour and a half at room temperature. Blots were then washed in three, ten minute intervals using 10 ml of PBS-Tween before one hour incubation in secondary antibody at room temperature. Dilutions for secondary antibodies in PBS-Tween are as follows: desmin- 1:20,000 using goat anti-rabbit antibody (31460, Thermo Scientific, Rockford, IL), troponin-T- 1:30,000 using goat anti-mouse antibody (A2554, Sigma, Saint Louis, MO), and µ-calpain- 1:10,000 using goat anti-mouse antibody (A2554, Sigma, Saint Louis, MO). Following secondary antibody incubation, blots were again washed three times in 10 minute intervals using 10ml of PBS-Tween before detection using a chemiluminescent detection kit (ECL Prime, GE Healthcare, Piscataway, NJ). Densities of labeled proteins were quantified using ChemiImager 5500 (Alpha Innotech, San Leandro, CA) and Alpha EaseFC (v. 2.03; Alpha Innotech, San Leandro, CA). Desmin degradation was indicated by a decrease in the intensity of the approximate 55 kDa band and compared against a reference collected from a pork LM aged seven days postmortem (Figure 1), troponin-T degradation was indicated by an increase in intensity of the approximate 30 kDa band and compared against a reference collected from a pork LM aged seven days postmortem (Figure 2), and 2 d µ-calpain autolysis was indicated by an increase in the approximate 76 kDa band and compared to LM reference aged 12 h postmortem (Figure 3).
Calpastatin Assay

Calpastatin activity was determined on LM samples at 2 d postmortem following procedures of Lonergan et al. (2001). Fresh muscle was finely minced and 10g was homogenized in 30ml extraction buffer (100 mM Tris-HCl pH 8.3, 10 mM mercaptoethanol, 10mM EDTA, 100mg/L ovomucoid) using a Polytron PT 3100 (Lucerne, Switzerland). The solution was then centrifuged at 25,000 x g for 20 min and filtered through cheesecloth. Samples were then dialyzed for at least six hours against 40ml of 40mM Tris-HCl pH 7.4, 1mM EDTA. Following dialysis, samples were heated for 20 min in a 95°C water bath and immediately chilled in ice cold water for 15 minutes. Samples were transferred to 30 ml centrifuge tubes, centrifuged at 25,000 x g for 20 min, filtered through cheesecloth and volumes recorded for calpastatin activity calculation. Calpastatin activity was then determined using a casein substrate according to (Koohmaraie, 1990) with modifications. Samples were added to test tubes in 500, 750 and two 1000 µl volumes. Each tube was then brought to 1 ml using TE (40 mM Tris-HCl, 1 mM EDTA, pH 7.4). Blank standard tubes were filled with 1 ml TE and 1000 µl sample tubes were brought to 2 ml using TE and served as negative controls. 50 µl of purified m-calpain was then added to all tubes except the blank standards. Casein buffer (100 mM Tris-acetate, 7 mg/ml casein, and 1 mM sodium azide, 0.2% MCE, pH 7.5) was added in 1 ml volumes to each tube. Negative control tubes received 100 µl of EDTA, the remaining tubes (positive controls, samples and blanks) each received 100 µl CaCl₂. All tubes were then briefly vortexed and incubated in a 25°C water bath for one hour. 2 ml of 0.5% trichloroacetic acid was then added to tubes and centrifuged at 1,500 x g for 20 minutes. Assays were conducted and absorbance measured at 278 nm. As calpain
activity increased absorbance will also increase, therefore calpastatin activity may be calculated as the amount of calpastatin required to inhibit 1 unit of lung m-calpain (Koohmaraie, 1990).

**Sarcomere Length Determination**

One LM chop was collected 30 h postmortem, frozen, and shipped to Dr. Carol Lorenzen’s laboratory at the University of Missouri for sarcomere length determination using light diffraction (Dolazza and Lorenzen, 2014)

**Ham Processing**

The bottom ham containing the biceps femoris and semitendinosus were collected at the same time as previously mentioned muscles following slaughters one (11/2/12), three (5/29/13) and four (8/30/13). These muscles were divided into deep and conventional chilled sides and processed into hams. At two days postmortem muscles were injected to 25% of raw weight using a brine containing sodium chloride (11%), sugar (6.6%), sodium tripolyphosphate (2.2%), sodium erythorbate (0.295%) and sodium nitrite (0.08%). The injected muscles were then tumbled for two hours at 4°C, stuffed into 10 cm cellulose casings, raw weights were recorded and hams were cooked in an ALKAR smokehouse (ALKAR Lodi, WI) following the cook schedule detailed in Table 1. Both treatments were cooked at the same time to ensure uniform cooking cycle was achieved. Cooking yield was recorded and measured as (stuffed weight-cooked weight/stuffed weight)*100.

**Statistical Analysis**

Data were analyzed using mixed linear model (PROC MIXED, SAS Enterprise Guide 5.1, SAS Inst. Inc., Cary NC). The model included fixed effects of Treatment
(TRT), Harvest, and TRT*Harvest. Carcasses (n=40) served as random effects. To
determine the effect of carcass composition, covariates of HCW, FFL, and the quadratic
of each were added to the model. Least square means and standard errors are reported for
each trait. Ham processing yields were analyzed using a mixed linear model, fixed
effects of TRT, Harvest and TRT*Harvest was assigned. Ham was used as a random
effect. Locational effect of blast chilling (SMS and SMD) used fixed effect of TRT,
Harvest, TRT*Harvest, Location and Location*TRT and carcass as a random effect.
Significance level was determined at \( P \leq 0.05 \).

**Results and Discussion**

Average HCW was 89.0 ± 1.3 kg, average fat free lean (FFL) was 56.4 ± 0.8%
across carcasses (n=40). Continuous measurements of temperature decline in the LM at
the tenth rib are represented in Figure 4. Additional LM temperature measurements were
taken upon entering chilling treatment, exiting chilling treatment, approximately 4, 22
and 30 h postmortem and are represented in Table 2. Temperature decline of the LM in
BC sides was more rapid than CC sides. Sides had similar temperature at approximately
one hour postmortem. However, as expected BC sides had lower temperature exiting
chilling treatment (approximately 2 h postmortem in CC sides) 4, 22, and 30 h
postmortem temperature.

The LM of BC sides had higher pH at 4, 22, and 30 h postmortem (Table 2). No
treatment effect was observed in ultimate (10 day) pH in chops from the LM. Ultimate
pH was not affected by chilling rate in previous studies (Rybarczyk et al., 2015;
Shackelford et al., 2012; Springer et al., 2003). The results from the current experiment
confirm those results and suggest that rate of pH decline is influenced by blast chilling, a hypothesis supported by Kylä-Puhju et al. (2005).

Blast chilling resulted in higher pH in the SM at 30 h postmortem (Table 3). Chilling had no effect on ultimate (8 d) pH in the SM. Previous work by Jones et al. (1993); Tomović et al. (2008); and Rosenvold et al. (2003) also found no difference in one day postmortem pH of the SM after exposure to similar blast chilling regimens. The SM was divided into a superficial (SMS) and a deep (SMD) portion to determine the effect of chilling on different locations throughout the muscle. Chilling regimen did not have an effect in the SMS or SMD on 30 h postmortem pH. Analysis of 30 h pH in SMS and SMD did however find a significant difference between the SMS and SMD of CC sides while this difference was not found in BC sides. It is likely that the superficial portion chilled at a more rapid rate than the deep portion due to more direct exposure to air temperature resulting in decreased pH decline. This result may also demonstrate the insulating effect of the outer portion of the muscle.

It is interesting to note the similarities in chilling effect on the SM and the LM 30 h postmortem pH. It has been shown that the SM and the LM have a large proportion of Type IIb fibers (Christensen et al., 2004; Melody et al., 2004), the current study shows the influence of fiber type on postmortem pH may be similar despite chilling method. Differences in pH decline and its relation to fiber type was discussed by Kylä-Puhju et al. (2005). Kylä-Puhju et al. (2005) demonstrated that activity of glucose debranching enzyme (GDE) was lower for darker muscle fibers when compared to lighter muscle fibers. Kylä-Puhju et al. (2005) also showed that low temperature slowed the rate
postmortem pH decline, and is potentially confirmed by the present study with LM pH declining at slower rate in BC sides.

**Proteolysis**

Chilling regime had no effect on intact desmin intensity as measured by the 55 kDa band in the LM (Table 4), PM, SM, or TB following aging for 2 or 10 d (Table 5). Furthermore, there was no difference in troponin-T degradation product as measured by the 30 kDa band in the LM aged 2 and 10 d postmortem (Table 4). Calpastatin activity at 2 d postmortem in the LM was not influenced by chilling treatment (Table 4). Pork is known to age more rapidly than beef or lamb (Koohmaraie et al., 1991). This may be a reason why differences in degradation were not detected between chilling regimens at our measured aging times. Chilling did not have an effect on μ-calpain activity in the LM 2 d postmortem (Table 4).

Cruzen et al. (2013) measured troponin-T degradation at 0, 1, 3, and 7 d postmortem between two lines of pigs selected for feed intake efficiency. Between days three and seven there was a significant decrease in the 30 kDa degradation product of troponin-T in a line of gilts selected for increased residual feed intake. As meat ages, there should be a continued increase in the 30 kDa product. This may not necessarily be the case because the 30 kDa band will continue to degrade to lower molecular weights with aging and may not be picked up on standard SDS-PAGE gels. It is possible that this occurred in our 10 d samples from the LM and there was actually a difference in rate of tenderization earlier than 10 d postmortem but after 2 d postmortem. Results of intermediate time points in this study could provide evidence of differences in tenderization before sensory analysis was conducted.
Water Holding Capacity

Least squares means of treatment on purge loss and cook loss are summarized in Table 6. Chilling regime did not have an effect on purge loss in cuts from the LM, SM or TB. Roasts from the PM of BC sides had greater drip loss than CC sides. Melody et al. (2004) found that the PM in pork has a greater proportion of type I and type IIa myosin heavy chain (MHC) isoforms when compared to the LM. Bowker et al. (2004) suggested that differences in muscle fiber type may play a role in fiber sensitivity to calcium activated ATPase activity in postmortem pH conditions with fast twitch (RST) fibers having greater calcium sensitivity resulting in increased myosin ATPase activity and increased pH decline. Differences in muscle fiber type in relation to postmortem metabolism and contraction have been found. Previous studies have reported that in slow twitch (type I) fibers, the sarcoplasmic reticulum is less developed and is less able to regulate the release of calcium to the sarcoplasm in times of high demand or at low temperatures (Cassens and Newbold, 1967; Damiani and Margreth, 1994). This makes red fibers more susceptible to cold shortening because of increased contraction and pH decline.

Because of the PM’s exterior location in a carcass, it is less insulated and it is likely that temperature decline will be most rapid in this muscle, and will experience a cold shortening effect that will decrease myofibrillar space for water storage (Honikel et al., 1986; Offer and Cousins, 1992). Offer and Cousins (1992) found that the formation of drip in event of sarcomere shortening could be explained by the formation of “drip channels” allowing water to escape from muscle fiber to the endomysium and ultimately lost from muscle tissue. This could potentially explain the increased purge loss in roasts
from the PM of BC sides. If the PM did in fact chill more rapidly in BC sides there may have been a cold shortening effect, creating drip channels and increasing purge lost.

Increased purge in the PM could also be explained by formation of ice crystals on the surface of muscle tissue. Rapid chilling of pork, specifically by air chilling, has been found to lead to ice crystal formation throughout muscle structure (Hansen et al., 2003; Ngapo et al., 1999). When muscle thaws, there is a significant increase in purge loss due to ice formation and melting.

Chops from the LM of BC sides had increased cook loss while effect of chilling on cook loss was not found in the PM, SM, or TB (Table 6). One possible explanation could be in the proteolysis that occurred in the first 24 h postmortem. O’Halloran et al. (1997) found that in beef LM with accelerated pH decline there was evidence of increased proteolysis. Similarly, Melody et al. (2004) hypothesized that specifically in the PM an increase in pH decline early postmortem may have had an effect on not only rate of μ-calpain autolysis but also on association of μ-calpain to Z-line structures. The current study found no chilling effect on degradation of desmin, troponin-T, or μ-calpain autolysis in the LM at 2 days postmortem (Table 4). A possible explanation of differences detected in cook loss in the LM may be from lack of early proteolysis of intermediate filaments like desmin in BC sides having a negative effect on cook loss post aging. Similar to the previous effect on drip channel formation in cold shortened muscle, linkage of myofibrils in the LM may have an effect on water loss during cooking due to sarcomere shortening. This greater contraction during cooking could be a result of lack of early postmortem degradation of intermediate filament proteins which link muscle fibers to the sarcolemma and other muscle fibers at the Z-line causing increased purge.
loss (Bee et al., 2007; Kristensen and Purslow, 2001; Melody et al., 2004; Morrison et al. 1998). Likewise, the increase in purge in the PM of BC sides could be a result of early proteolysis contributing to improved purge in the PM from CC sides. Pomponio et al. (2010) found that early postmortem pH decline influenced μ-calpain activity with muscle with faster postmortem pH decline having increased μ-calpain activity postmortem. However after aging, myofibril degradation was not found to be different between muscles. Melody et al. (2004) found that degradation of desmin in the PM began as early as 45 minutes postmortem in conventionally chilled pork carcass. If temperature decline was rapid enough in the PM of BC sides, sarcomeres may have shortened resulting in greater purge loss.

**Sensory Analysis**

Chops from the LM of BC sides had increased WBS values (Table 7). Pomponio et al. (2010) found that early pH decline caused an increased activation of μ-calpain and decreased activity as early as 1 h postmortem resulting in faster myofibril fragmentation. However the early effect of increased fragmentation was lost after 3 days of storage. While results from the current study show no difference in desmin or troponin-T degradation. Degradation of proteins like vinculin, titin, and nebulin were not measured and may explain improved WBS values (Taylor et al., 1995) of CC carcasses due to increased degradation by μ-calpain prior to two days postmortem. Results from the current study mimic previous results (Shackelford et al., 2012) that also found an increased toughness in the LM as determined by slice shear force value, but found no difference in proteolysis after 15 days aging. Increased WBS values were not reflected in sensory analysis scores. While significant, the small difference (300g) was likely not
detected by panelist between treatments. In beef, a difference of 1 kg in WBS was required for panelist to rank a sample considerably tougher (Huffman et al., 1996; Destefanis et al., 2008).

Roasts from the PM of BC sides were juicier, more tender and less chewy than roasts from CC sides (Table 8). Blast chilling had an effect in the SM with decreased Hunter a value indicating a lower intensity of red pigment, and a darker observed color score (Table 9). No treatment effect was found in cuts from the TB (Table 10). Improved tenderness and chewiness were not reflected in WBS or Star Probe measurements in the PM.

Koohmaraie et al. (1998) found that the freezing of restrained lamb longissimus prevented toughening by slowing glycolysis and decreasing intensity of rigor formation. Lamb which was normally considered tough due to the inhibition of μ-calpain degradation by calpastatin had improved tenderness because of an increase in sarcomere length when restrained. Because of the PM’s location in pork carcasses during processing, the muscle is stretched (Herring et al., 1965). The combined effect of limited rigor shortening due to rapid chilling and restrained muscle may help explain improved sensory scores.

**Influence of Carcass Composition**

The experimental approach in this study was designed to evaluate the effect of chilling on fresh pork quality while accounting for variations which may arise in individual animal, between muscles, and carcass composition. Analysis of dependent variables with HCW and FFL included in the model as covariates confirmed that HCW and FFL had no effect on treatment (Table 11, Table 12).
Cooked Ham Yields

Cooked whole muscle hams were produced from the *Biceps femoris* and *Semitendinosus*. A majority of previous work done on carcass chilling and processed hams focused on improving ham quality from products prone to PSE quality defects (Hambrecht et al., 2004; Tomović et al., 2013; Rosenvold et al., 2010). With BC, there would be better expected water holding capacity, color and texture compared to the CC counterparts. As in other muscles BC would increase rate of temperature decline, increase 30 h pH, and prevent denaturation of proteins that may occur from slow chilling and low pH (Milligan et al., 1998; Rosenvold et al., 2010). In the current study, chilling treatment was not found ($P$< 0.38) to have an effect on cook yields in hams (92.41 ± 0.40 CC, 92.78 ± 0.40 BC) from three harvest dates.

Implications

Postmortem pH decline, protein degradation, and changing postmortem muscle structure impacts fresh pork quality. The current study confirms the influence of chilling on fresh pork quality varies with muscle metabolism and carcass location. Postmortem proteolysis and changes in muscle biochemistry are responsible for the development of fresh pork quality. Slowing the rate of postmortem pH decline in the LM has a direct effect on the rate and extent of normal changes in the conversion of muscle to meat. These changes include protein degradation and denaturation. However, no difference in proteolysis was found in the current study. The influence of chilling on early postmortem muscle modification may warrant further investigation.
LITERATURE CITED


Figure 1. Representative western blot, ratio of 55 kDa desmin band was compared to a reference *Longissimus dorsi* sample aged 7 d postmortem.
Figure 2. Representative western blot, ratio of degraded troponin-T was compared to a reference Longissimus dorsi sample aged 7 d postmortem.
Figure 3. Representative western blot, ratio of autolyzed μ-calpain was compared to a reference Longissimus dorsi sample aged 12 h postmortem.
Table 1. Cook schedule for boneless hams.

<table>
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<th>Step</th>
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<th>Wet bulb temperature</th>
<th>Relative humidity</th>
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<td>40 m</td>
</tr>
<tr>
<td>Cook</td>
<td>77°C</td>
<td>-</td>
<td>-</td>
<td>30 m</td>
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<td>85°C</td>
<td>100%</td>
<td>Cook to 71°C internal</td>
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<tr>
<td>Cold shower</td>
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<td>-</td>
<td>30 m</td>
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</table>
Figure 4. Temperature decline of *Longissimus dorsi* (LM) as measure at the tenth rib with continuous measurements.

* Means represent 8 sides from each treatment group. Mean temperatures collected on four non-consecutive slaughter dates with two sides from each treatment per slaughter.
<table>
<thead>
<tr>
<th>Time postmortem</th>
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<th>SEM</th>
<th>P-value</th>
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<td>0.22</td>
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<tr>
<td></td>
<td><strong>Blast Chilled Sides</strong></td>
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<tr>
<td>4 h</td>
<td>6.09</td>
<td>0.07</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>22 h</td>
<td>5.81</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>30 h</td>
<td>5.68</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Ultimate¹</td>
<td>5.68</td>
<td>0.03</td>
<td>0.86</td>
</tr>
<tr>
<td><strong>Temperature</strong></td>
<td><strong>Conventional Chilled Sides</strong></td>
<td>37.9°C</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td><strong>Blast Chilled Sides</strong></td>
<td>38.0°C</td>
<td></td>
</tr>
<tr>
<td>Entering chilling treatment</td>
<td><strong>Conventional Chilled Sides</strong></td>
<td>21.8°C</td>
<td>2.45</td>
</tr>
<tr>
<td></td>
<td><strong>Blast Chilled Sides</strong></td>
<td>9.7°C</td>
<td></td>
</tr>
<tr>
<td>4 h</td>
<td>13.3°C</td>
<td>1.48</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>22 h</td>
<td>4.2°C</td>
<td>1.15</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>30 h</td>
<td>0.4°C</td>
<td>0.07</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

*Indicates significant effect of treatment, measurements taken at tenth rib.

**Means reported from LM of BC (n=40) or CC (n=40) sides

¹Ultimate pH represent pH measurement taken 10 days postmortem

²Approximately 2 h postmortem in CC sides
Table 3. Effect of chilling on pH in cuts from the *Psoas major, Triceps brachii, and Semimembranosus*

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Conventional Chilled Sides</th>
<th>Blast Chilled Sides</th>
<th>SEM</th>
<th><em>P</em>-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>30 h postmortem pH</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Psoas major</em></td>
<td>6.00</td>
<td>6.05</td>
<td>0.05</td>
<td>0.29</td>
</tr>
<tr>
<td><em>Triceps brachii</em></td>
<td>5.99</td>
<td>5.96</td>
<td>0.05</td>
<td>0.56</td>
</tr>
<tr>
<td><em>Semimembranosus</em></td>
<td>5.68</td>
<td>5.73</td>
<td>0.02</td>
<td>0.002</td>
</tr>
<tr>
<td>superficial</td>
<td>5.70</td>
<td>5.74</td>
<td>0.02</td>
<td>0.11</td>
</tr>
<tr>
<td><em>Semimembranosus</em></td>
<td>5.66</td>
<td>5.72</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>deep</td>
<td>(0.05)²</td>
<td>(0.43)²</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Ultimate pH</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Psoas major</em></td>
<td>6.01</td>
<td>6.02</td>
<td>0.05</td>
<td>0.90</td>
</tr>
<tr>
<td><em>Triceps brachii</em></td>
<td>6.01</td>
<td>6.00</td>
<td>0.05</td>
<td>0.86</td>
</tr>
<tr>
<td><em>Semimembranosus</em></td>
<td>5.77</td>
<td>5.80</td>
<td>0.04</td>
<td>0.47</td>
</tr>
</tbody>
</table>

*Indicates significant effect of treatment.  
**Means reported of pH from BC (n=40) or CC (n=40) sides.  
¹Ultimate pH represents pH on day of sensory analysis (PM 7 d, SM 8 d, TB 13 d).  
²*P*-value representative between muscle locations. Fixed effects of TRT, harvest, harvest*TRT, muscle, and muscle*TRT with carcass serving as random effect used for analysis.
Table 4. Effect of chilling on proteolysis in the *Longissimus dorsi*

<table>
<thead>
<tr>
<th></th>
<th>Conventional Chilled Sides</th>
<th>Blast Chilled Sides</th>
<th>SE</th>
<th><em>P</em>-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sarcomere length</td>
<td>1.41µm</td>
<td>1.40µm</td>
<td>0.01</td>
<td>0.27</td>
</tr>
<tr>
<td>Ratio of intact desmin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 d postmortem&lt;sup&gt;1&lt;/sup&gt;</td>
<td>2.27</td>
<td>2.31</td>
<td>0.23</td>
<td>0.87</td>
</tr>
<tr>
<td>Ratio of intact desmin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 d postmortem&lt;sup&gt;1&lt;/sup&gt;</td>
<td>1.97</td>
<td>1.75</td>
<td>0.19</td>
<td>0.25</td>
</tr>
<tr>
<td>Ratio of degraded</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>troponin-T 2 d</td>
<td>0.13</td>
<td>0.11</td>
<td>0.01</td>
<td>0.15</td>
</tr>
<tr>
<td>postmortem&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ratio of degraded</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>troponin-T 10 d</td>
<td>1.02</td>
<td>1.06</td>
<td>0.06</td>
<td>0.52</td>
</tr>
<tr>
<td>postmortem&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calpastatin activity&lt;sup&gt;3&lt;/sup&gt;</td>
<td>1.22</td>
<td>1.23</td>
<td>0.05</td>
<td>0.82</td>
</tr>
<tr>
<td>Ratio of autolyzed µ-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>calpain 2 d</td>
<td>133.10</td>
<td>136.25</td>
<td>4.04</td>
<td>0.44</td>
</tr>
<tr>
<td>postmortem&lt;sup&gt;4&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>*</sup>Indicates significant effect of treatment.

<sup>**</sup>Least square means reported for each trait.

<sup>1</sup>55 kDa Band Intact Band (Figure 1).

<sup>2</sup>30 kDa Band Degradation Product (Figure 2).

<sup>3</sup>Units of calpastatin activity per gram of tissue 2d postmortem.

<sup>4</sup>Measured as 76 kDa degradation product (Figure 3).
Table 5. Effect of chilling on desmin degradation in the *Psoas major, Semimembranosus, and Triceps brachii*

<table>
<thead>
<tr>
<th></th>
<th>Conventional Chilled Sides</th>
<th>Blast Chilled Sides</th>
<th>SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ratio of Intact Desmin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Psoas major</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 d postmortem</td>
<td>3.90</td>
<td>3.68</td>
<td>0.19</td>
<td>0.24</td>
</tr>
<tr>
<td>10 d postmortem</td>
<td>3.64</td>
<td>3.74</td>
<td>0.24</td>
<td>0.68</td>
</tr>
<tr>
<td><em>Semimembranosus superficial</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 d postmortem</td>
<td>2.97</td>
<td>2.95</td>
<td>0.23</td>
<td>0.92</td>
</tr>
<tr>
<td><em>Semimembranosus deep 2 d</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>postmortem</td>
<td>1.86 (0.75)</td>
<td>2.02 (0.63)</td>
<td>0.19</td>
<td>0.39</td>
</tr>
<tr>
<td><em>Semimembranosus superficial</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 d postmortem</td>
<td>2.39</td>
<td>2.69</td>
<td>0.30</td>
<td>0.32</td>
</tr>
<tr>
<td><em>Semimembranosus deep 10 d</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>postmortem</td>
<td>3.46 (0.90)</td>
<td>3.54 (0.44)</td>
<td>0.18</td>
<td>0.67</td>
</tr>
<tr>
<td><em>Triceps brachii</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 d postmortem</td>
<td>1.68</td>
<td>1.58</td>
<td>0.28</td>
<td>0.71</td>
</tr>
<tr>
<td>10 d postmortem</td>
<td>1.85</td>
<td>1.97</td>
<td>0.17</td>
<td>0.49</td>
</tr>
</tbody>
</table>

*Indicates significant treatment effect.
**Least square means reported as ratio of 55 kDa intact desmin band (Figure 1).

1P-value representation between muscle locations.
Table 6. Effect of chilling on water holding capacity in cuts from the 
*Longissimus dorsi*, *Psoas major*, *Semimembranosus*, and *Triceps brachii*.

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Purge Loss¹</th>
<th>Blasted Sides</th>
<th>Conventional Chilled Sides</th>
<th>Chilled Sides</th>
<th>SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Longissimus dorsi</em></td>
<td>2.21</td>
<td>2.28</td>
<td>0.33</td>
<td>0.83</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Psoas major</em></td>
<td>0.48</td>
<td>0.74</td>
<td>0.11</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Semimembranosus</em></td>
<td>3.57</td>
<td>3.52</td>
<td>0.25</td>
<td>0.81</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Triceps brachii</em></td>
<td>1.83</td>
<td>1.82</td>
<td>0.13</td>
<td>0.92</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Cook Loss**²

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Cooked Loss²</th>
<th>Chilled Sides</th>
<th>Conventional Chilled Sides</th>
<th>Chilled Sides</th>
<th>SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Longissimus dorsi</em></td>
<td>22.37</td>
<td>24.24</td>
<td>0.81</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Psoas major</em></td>
<td>10.99</td>
<td>10.44</td>
<td>0.47</td>
<td>0.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Semimembranosus</em></td>
<td>21.92</td>
<td>21.13</td>
<td>0.55</td>
<td>0.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Triceps brachii</em></td>
<td>27.06</td>
<td>26.58</td>
<td>0.93</td>
<td>0.60</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Indicates significant effect of treatment (*P*<0.05). **Least square means of percent loss reported from BC (*n=40*) or CC (*n=40*) sides

¹% Purge Loss measures at time of sensory analysis (LM 10 d, PM 7 d, SM 8 d, TB 13 d) calculated as [(Initial weight-final weight)/initial weight]100

²Cooked to an internal temperature of 68°C; % Calculated as [(raw weight – cooked weight) / raw weight] x 100.
Table 7. Effect of chilling on sensory attributes in chops from the *Longissimus dorsi*.

<table>
<thead>
<tr>
<th></th>
<th>Conventional Chilled Sides</th>
<th>Blast Chilled Sides</th>
<th>SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marbling score$^1$</td>
<td>2.30</td>
<td>2.30</td>
<td>0.15</td>
<td>0.74</td>
</tr>
<tr>
<td>Color score$^2$</td>
<td>3.10</td>
<td>3.10</td>
<td>0.12</td>
<td>0.92</td>
</tr>
<tr>
<td>Hunter L$^3$</td>
<td>49.07</td>
<td>49.10</td>
<td>0.53</td>
<td>0.95</td>
</tr>
<tr>
<td>Hunter a$^3$</td>
<td>13.67</td>
<td>13.79</td>
<td>0.19</td>
<td>0.60</td>
</tr>
<tr>
<td>Hunter b$^3$</td>
<td>2.90</td>
<td>2.99</td>
<td>0.12</td>
<td>0.43</td>
</tr>
<tr>
<td>Star probe (kg)$^4$</td>
<td>5.45</td>
<td>5.69</td>
<td>0.19</td>
<td>0.21</td>
</tr>
<tr>
<td>Warner-Bratzler shear force (kg)$^5$</td>
<td>2.00</td>
<td>2.30</td>
<td>0.15</td>
<td>0.05</td>
</tr>
<tr>
<td>Juiciness$^6$</td>
<td>7.00</td>
<td>7.00</td>
<td>0.21</td>
<td>0.75</td>
</tr>
<tr>
<td>Tenderness$^6$</td>
<td>7.00</td>
<td>6.60</td>
<td>0.28</td>
<td>0.20</td>
</tr>
<tr>
<td>Chewiness$^6$</td>
<td>3.70</td>
<td>4.00</td>
<td>0.34</td>
<td>0.35</td>
</tr>
<tr>
<td>Flavor$^6$</td>
<td>3.60</td>
<td>3.60</td>
<td>0.15</td>
<td>0.94</td>
</tr>
<tr>
<td>Off-flavor$^6$</td>
<td>1.50</td>
<td>1.30</td>
<td>0.17</td>
<td>0.18</td>
</tr>
</tbody>
</table>

*Indicates significant effect of treatment ($P<0.05$).

**Least square means reported for each trait.

$^1$National Pork Board standards, 10-point scale (1=1.0% intramuscular fat; 10=10% intramuscular fat.

$^2$National Pork Board standards, 6-pont scale (1=pale pinkish gray to white; 6=dark purplish red).

$^3$Hunter L a b, D65 light source, 50 mm aperture, 0° observer.

$^4$Force required to compress sample to 20% of its original height.

$^5$Force required to shear 1.27 cm core.

$^6$Trained sensory analysis (n=4) samples scored on a ten point scale. Lower scores indicate less juiciness, tenderness, chewiness, flavor, and off-flavor.
Table 8. Effect of chilling on sensory attributes in roasts from the *Psoas major*.

<table>
<thead>
<tr>
<th></th>
<th>Conventional Chilled Sides</th>
<th>Blast Chilled Sides</th>
<th>SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color score&lt;sup&gt;1&lt;/sup&gt;</td>
<td>3.80</td>
<td>4.00</td>
<td>0.13</td>
<td>0.13</td>
</tr>
<tr>
<td>Hunter L&lt;sup&gt;2&lt;/sup&gt;</td>
<td>40.35</td>
<td>40.34</td>
<td>0.68</td>
<td>0.98</td>
</tr>
<tr>
<td>Hunter a&lt;sup&gt;2&lt;/sup&gt;</td>
<td>14.68</td>
<td>14.59</td>
<td>0.32</td>
<td>0.79</td>
</tr>
<tr>
<td>Hunter b&lt;sup&gt;2&lt;/sup&gt;</td>
<td>1.35</td>
<td>1.36</td>
<td>0.15</td>
<td>0.93</td>
</tr>
<tr>
<td>Star probe (kg)&lt;sup&gt;3&lt;/sup&gt;</td>
<td>2.40</td>
<td>2.39</td>
<td>0.09</td>
<td>0.91</td>
</tr>
<tr>
<td>Warner-Bratzler shear force (kg)&lt;sup&gt;3&lt;/sup&gt;</td>
<td>2.54</td>
<td>2.47</td>
<td>0.10</td>
<td>0.48</td>
</tr>
<tr>
<td>Juiciness&lt;sup&gt;5&lt;/sup&gt;</td>
<td>7.50</td>
<td>8.30</td>
<td>0.17</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Tenderness&lt;sup&gt;5&lt;/sup&gt;</td>
<td>7.90</td>
<td>8.60</td>
<td>0.25</td>
<td>0.005</td>
</tr>
<tr>
<td>Chewiness&lt;sup&gt;5&lt;/sup&gt;</td>
<td>2.80</td>
<td>2.10</td>
<td>0.20</td>
<td>0.002</td>
</tr>
<tr>
<td>Flavor&lt;sup&gt;5&lt;/sup&gt;</td>
<td>3.50</td>
<td>3.70</td>
<td>0.13</td>
<td>0.13</td>
</tr>
<tr>
<td>Off-flavor&lt;sup&gt;5&lt;/sup&gt;</td>
<td>1.90</td>
<td>1.90</td>
<td>0.15</td>
<td>0.93</td>
</tr>
</tbody>
</table>

*Indicates significant effect of treatment (*P*<0.05).
**Least square means reported for each trait.
<sup>1</sup>National Pork Board standards, 6-point scale (1=pale pinkish gray to white; 6=dark purplish red).
<sup>2</sup>Hunter L a b, D65 light source, 50 mm aperture, 0° observer.
<sup>3</sup>Force required to compress sample to 20% of its original height.
<sup>4</sup>Force required to shear 1.27 cm core
<sup>5</sup>Trained sensory analysis (n=4) samples scored on a ten point scale. Lower scores indicate less juiciness, tenderness, chewiness, flavor, and off-flavor.
Table 9. Effect of chilling on sensory attributes in chops from the *Semimembranosus*.

<table>
<thead>
<tr>
<th></th>
<th>Conventional Chilled Sides</th>
<th>Blast Chilled Sides</th>
<th>SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marbling score(^2)</td>
<td>2.30</td>
<td>2.40</td>
<td>0.12</td>
<td>0.31</td>
</tr>
<tr>
<td>Color score(^3)</td>
<td>3.00</td>
<td>3.20</td>
<td>0.09</td>
<td>0.05</td>
</tr>
<tr>
<td>Hunter L(^4)</td>
<td>41.76</td>
<td>41.20</td>
<td>0.64</td>
<td>0.39</td>
</tr>
<tr>
<td>Hunter a(^4)</td>
<td>16.35</td>
<td>16.02</td>
<td>0.16</td>
<td>0.05</td>
</tr>
<tr>
<td>Hunter b(^4)</td>
<td>3.11</td>
<td>2.93</td>
<td>0.13</td>
<td>0.17</td>
</tr>
<tr>
<td>Superficial star probe (kg)(^5)</td>
<td>5.79</td>
<td>5.71</td>
<td>0.17</td>
<td>0.63</td>
</tr>
<tr>
<td>Deep star probe (kg)(^5)</td>
<td>5.83 (0.81)(^1)</td>
<td>5.88 (0.35)(^1)</td>
<td>0.16</td>
<td>0.78</td>
</tr>
<tr>
<td>Superficial Warner-Bratzler shear force (kg)(^6)</td>
<td>4.28</td>
<td>4.07</td>
<td>0.29</td>
<td>0.50</td>
</tr>
<tr>
<td>Deep Warner-Bratzler shear force(^6)</td>
<td>4.21 (0.80)(^1)</td>
<td>4.38 (0.29)(^1)</td>
<td>0.20</td>
<td>0.53</td>
</tr>
<tr>
<td>Juiciness(^7)</td>
<td>6.90</td>
<td>6.90</td>
<td>0.10</td>
<td>0.80</td>
</tr>
<tr>
<td>Tenderness(^7)</td>
<td>6.10</td>
<td>5.90</td>
<td>0.20</td>
<td>0.38</td>
</tr>
<tr>
<td>Chewiness(^7)</td>
<td>4.80</td>
<td>5.10</td>
<td>0.06</td>
<td>0.34</td>
</tr>
<tr>
<td>Flavor(^7)</td>
<td>3.40</td>
<td>3.50</td>
<td>0.14</td>
<td>0.38</td>
</tr>
<tr>
<td>Off-flavor(^7)</td>
<td>1.40</td>
<td>1.30</td>
<td>0.28</td>
<td>0.07</td>
</tr>
</tbody>
</table>

\(^*\)Indicates significant effect of treatment (\(P<0.05\)).

**Least square means reported for each trait.

\(^1\)P-value representative between muscle locations. Fixed effects of TRT, harvest, harvest*TRT, muscle, and muscle*TRT with carcass serving as random effect used for analysis. Least square means for this model represented in table.

\(^2\)National Pork Board standards, 10-point scale (1=1.0% intramuscular fat; 10=10% intramuscular fat.

\(^3\)National Pork Board standards, 6-point scale (1=pale pinkish gray to white; 6=dark purplish red).

\(^4\)Hunter L a b, D65 light source, 50 mm aperture, 0° observer.

\(^5\)Force required to compress sample to 20% of its original height.

\(^6\)Force required to shear 1.27 cm core.

\(^7\)Trained sensory analysis (n=4) samples scored on a ten point scale. Lower scores indicate less juiciness, tenderness, chewiness, flavor, and off-flavor.
Table 10. Effect of chilling on sensory attributes in roasts from the *Triceps brachii*.

<table>
<thead>
<tr>
<th></th>
<th>Conventional Chilled Sides</th>
<th>Blast Chilled Sides</th>
<th>SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color score&lt;sup&gt;1&lt;/sup&gt;</td>
<td>2.90</td>
<td>2.90</td>
<td>0.10</td>
<td>0.62</td>
</tr>
<tr>
<td>Hunter L&lt;sup&gt;2&lt;/sup&gt;</td>
<td>41.79</td>
<td>41.70</td>
<td>0.42</td>
<td>0.82</td>
</tr>
<tr>
<td>Hunter a&lt;sup&gt;2&lt;/sup&gt;</td>
<td>16.03</td>
<td>16.02</td>
<td>0.13</td>
<td>0.95</td>
</tr>
<tr>
<td>Hunter b&lt;sup&gt;2&lt;/sup&gt;</td>
<td>4.35</td>
<td>4.35</td>
<td>0.12</td>
<td>0.99</td>
</tr>
<tr>
<td>Star Probe (kg)&lt;sup&gt;3&lt;/sup&gt;</td>
<td>4.93</td>
<td>5.00</td>
<td>0.15</td>
<td>0.64</td>
</tr>
<tr>
<td>Warner-Bratzler shear force (kg)&lt;sup&gt;4&lt;/sup&gt;</td>
<td>2.91</td>
<td>3.10</td>
<td>0.13</td>
<td>0.16</td>
</tr>
<tr>
<td>Juiciness&lt;sup&gt;5&lt;/sup&gt;</td>
<td>5.40</td>
<td>5.70</td>
<td>0.22</td>
<td>0.25</td>
</tr>
<tr>
<td>Tenderness&lt;sup&gt;5&lt;/sup&gt;</td>
<td>4.70</td>
<td>4.70</td>
<td>0.75</td>
<td>0.99</td>
</tr>
<tr>
<td>Chewiness&lt;sup&gt;5&lt;/sup&gt;</td>
<td>5.50</td>
<td>5.80</td>
<td>0.26</td>
<td>0.27</td>
</tr>
<tr>
<td>Flavor&lt;sup&gt;5&lt;/sup&gt;</td>
<td>4.00</td>
<td>3.90</td>
<td>0.10</td>
<td>0.18</td>
</tr>
<tr>
<td>Off-flavor&lt;sup&gt;4&lt;/sup&gt;</td>
<td>1.30</td>
<td>1.20</td>
<td>0.05</td>
<td>0.32</td>
</tr>
</tbody>
</table>

*Indicates significant effect of treatment (*P*<0.05).

**Least square means reported for each trait.

1National Pork Board standards, 6-point scale (1=pale pinkish gray to white; 6=dark purplish red).

2Hunter L a b, D65 light source, 50 mm aperture, 0° observer.

3Force required to compress sample to 20% of its original height.

4Force required to shear 1.27 cm core.

5Trained sensory analysis (n=4) samples scored on a ten point scale. Lower scores indicate less juiciness, tenderness, chewiness, flavor, and off-flavor.
Table 11. Effect of fat-free lean on sensory attributes

<table>
<thead>
<tr>
<th></th>
<th>LM&lt;sup&gt;13&lt;/sup&gt;</th>
<th>PM&lt;sup&gt;14&lt;/sup&gt;</th>
<th>SM&lt;sup&gt;15&lt;/sup&gt;</th>
<th>TB&lt;sup&gt;16&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>FFL&lt;sup&gt;P&lt;/sup&gt;</td>
<td>TRT&lt;sup&gt;P&lt;/sup&gt;</td>
<td>Effect</td>
<td>FFL&lt;sup&gt;P&lt;/sup&gt;</td>
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<tr>
<td>Enter chill treatment temperature&lt;sup&gt;1&lt;/sup&gt;</td>
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<td>-</td>
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</tr>
<tr>
<td>Exiting chill treatment temperature&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.70</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
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<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>4 h postmortem pH&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.43</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>22 h postmortem pH&lt;sup&gt;1&lt;/sup&gt;</td>
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<td>pH 30 h postmortem pH</td>
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<tr>
<td>Ultimate pH&lt;sup&gt;2&lt;/sup&gt;</td>
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<td>-</td>
<td>0.19</td>
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<tr>
<td>Purge loss&lt;sup&gt;3&lt;/sup&gt;</td>
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<td>-</td>
<td>-</td>
<td>0.39</td>
</tr>
<tr>
<td>Cook loss&lt;sup&gt;4&lt;/sup&gt;</td>
<td>0.36</td>
<td>-</td>
<td>-</td>
<td>0.78</td>
</tr>
<tr>
<td>Marbling score&lt;sup&gt;5&lt;/sup&gt;</td>
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<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Color score&lt;sup&gt;6&lt;/sup&gt;</td>
<td>0.27</td>
<td>-</td>
<td>-</td>
<td>0.33</td>
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</table>
Table 11. Effect of fat-free lean on sensory attributes continued.

<table>
<thead>
<tr>
<th></th>
<th>Hunter L</th>
<th>Hunter a</th>
<th>Hunter b</th>
<th>Star probe</th>
<th>Warner-Bratzler shear force</th>
<th>Juiciness</th>
<th>Tenderness</th>
<th>Chewiness</th>
<th>Flavor</th>
<th>Off-flavor</th>
<th>Sarcomere length</th>
<th>Degraded troponin-T 2 d postmortem</th>
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<tr>
<td></td>
<td>0.47</td>
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<td>-</td>
<td>0.79</td>
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<td>0.04</td>
<td>0.11</td>
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<td>0.01</td>
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</table>
Table 11. Effect of fat-free lean on sensory attributes continued.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Control</th>
<th>Treatment 1</th>
<th>Treatment 2</th>
<th>Treatment 3</th>
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<tbody>
<tr>
<td>Degraded troponin-T 10 d postmortem</td>
<td>0.001</td>
<td>0.46</td>
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<tr>
<td>Intact desmin 2 d postmortem</td>
<td>0.84</td>
<td>0.04</td>
<td>0.22</td>
<td>0.20</td>
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<tr>
<td>Intact desmin 10 d postmortem</td>
<td>0.03</td>
<td>0.23</td>
<td>0.25</td>
<td>0.89</td>
</tr>
<tr>
<td>76 kDa qutolyzed band</td>
<td>0.92</td>
<td>- - - -</td>
<td>- - - -</td>
<td>- - - -</td>
</tr>
<tr>
<td>Calpastatin activity</td>
<td>&lt;0.001</td>
<td>0.82</td>
<td>0.11</td>
<td>- - - -</td>
</tr>
</tbody>
</table>

*Indicates significant effect of treatment.

1 Measured at tenth rib in the Longissimus dorsi. Exiting chill temperature taken approximately 2 h postmortem in CC sides
2 pH measured on aged pork (Psoas major 7 Days, Semimembranosus 8 Days, Longissimus dorsi 10 Days, Triceps brachii 13 Days).
3 % Purge loss measured at sensory analysis (Psoas major 7 Days, Semimembranosus 8 Days, Longissimus dorsi 10 Days, Triceps brachii 13 Days), calculated as [(Initial weight - final weight)/initial weight].
4 % Cooked to an internal temperature of 68°C. % Calculated as [(raw weight-cooked weight/ raw weight]
5 National Pork Board standards, 10-point scale. (1=1% intramuscular fat; 10=10% intramuscular fat).
6 National Pork Board standards, 6-point scale. (1=pale pinkish gray; 6=dark purplish red).
7 Hunter L a b, D65 light source, 50 mm aperture, 0° Observer.
8 Force required to compress sample to 20% its original height.
9 Force required to shear 1.27 cm core.
10 Trained sensory analysis (n=4) samples scored on a ten point scale. Lower scores indicate less juiciness, tenderness, chewiness, flavor, and off-flavor.
11 Measurements recorded as µm.
12 Ratio of 30 kDa band degradation product see Figure 2.
13 Ratio of 55 kDa intact desmin band see Figure 1.
14 Longissimus dorsi
15 Psoas major
16 Semimembranosus
17 Triceps brachii
18 Semimembranosus superficial
19 Semimembranosus deep
Table 12. Effect of carcass weight on sensory attributes

<table>
<thead>
<tr>
<th></th>
<th>LM&lt;sup&gt;1&lt;/sup&gt;</th>
<th>PM&lt;sup&gt;1&lt;/sup&gt;</th>
<th>SM&lt;sup&gt;1&lt;/sup&gt;</th>
<th>TB&lt;sup&gt;1&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>HCW&lt;sup&gt;P&lt;/sup&gt;</td>
<td>TRT&lt;sup&gt;P&lt;/sup&gt;</td>
<td>Effect</td>
<td>HCW&lt;sup&gt;P&lt;/sup&gt;</td>
</tr>
<tr>
<td>Entering chill treatment temperature&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.49</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Exiting chill treatment temperature&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.23</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>Entering chill treatment pH&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.63</td>
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</tr>
<tr>
<td>4 h postmortem pH&lt;sup&gt;1&lt;/sup&gt;</td>
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<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>22 h postmortem pH&lt;sup&gt;1&lt;/sup&gt;</td>
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<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>30 h postmortem pH</td>
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<td>-</td>
<td>-</td>
<td>0.85</td>
</tr>
<tr>
<td>Ultimate pH&lt;sup&gt;2&lt;/sup&gt;</td>
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<td>-</td>
<td>-</td>
<td>0.89</td>
</tr>
<tr>
<td>Purge loss&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0.11</td>
<td>-</td>
<td>-</td>
<td>0.19</td>
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<tr>
<td>Cook loss&lt;sup&gt;4&lt;/sup&gt;</td>
<td>0.21</td>
<td>-</td>
<td>-</td>
<td>0.87</td>
</tr>
<tr>
<td>Marbling score&lt;sup&gt;5&lt;/sup&gt;</td>
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<td>Color score&lt;sup&gt;6&lt;/sup&gt;</td>
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<td>-</td>
<td>-</td>
<td>0.55</td>
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<tr>
<td>Hunter L&lt;sup&gt;7&lt;/sup&gt;</td>
<td>0.43</td>
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<td>-</td>
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Table 12. Effect of carcass weight on sensory attributes continued

<table>
<thead>
<tr>
<th></th>
<th>Hunter a&lt;sup&gt;7&lt;/sup&gt;</th>
<th>0.62</th>
<th>-</th>
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<th>0.67</th>
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<th>-</th>
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<td>-</td>
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<td>0.47</td>
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<td>-</td>
<td>0.96</td>
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<tr>
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<td>Star probe&lt;sup&gt;8&lt;/sup&gt;</td>
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<td>0.08</td>
<td>0.09</td>
<td>0.02</td>
<td>0.14</td>
<td>-0.06</td>
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<tr>
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<td>0.78</td>
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<tr>
<td>Tenderness&lt;sup&gt;10&lt;/sup&gt;</td>
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<td>0.18</td>
<td>0.23</td>
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<td>-</td>
<td>0.03</td>
<td>0.99</td>
<td>0.09</td>
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<tr>
<td>Chewiness&lt;sup&gt;10&lt;/sup&gt;</td>
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<td>0.05</td>
<td>0.26</td>
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</tr>
<tr>
<td>Flavor&lt;sup&gt;10&lt;/sup&gt;</td>
<td>0.02</td>
<td>0.94</td>
<td>0.06</td>
<td>0.40</td>
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<td>0.99</td>
<td>-0.06</td>
<td>0.91</td>
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<td>Off-flavor&lt;sup&gt;10&lt;/sup&gt;</td>
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<td>0.49</td>
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<td>0.07</td>
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<td>0.60</td>
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<td>Sarcomere length&lt;sup&gt;11&lt;/sup&gt;</td>
<td>&lt;.0001</td>
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<td>Degraded troponin-T 2 d Postmortem&lt;sup&gt;12&lt;/sup&gt;</td>
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<td>0.15</td>
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<tr>
<td>Degraded troponin-T 10 d postmortem&lt;sup&gt;12&lt;/sup&gt;</td>
<td>0.08</td>
<td>0.51</td>
<td>0.02</td>
<td>-</td>
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<tr>
<td>Intact desmin 2 d postmortem&lt;sup&gt;13&lt;/sup&gt;</td>
<td>0.50</td>
<td>-</td>
<td>-</td>
<td>0.18</td>
<td>-</td>
<td>-</td>
<td>0.81&lt;sub&gt;SMS17&lt;/sub&gt;</td>
<td>-</td>
<td>-</td>
<td>0.67&lt;sub&gt;SMD18&lt;/sub&gt;</td>
<td>-</td>
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<tr>
<td>Intact desmin 10 d postmortem&lt;sup&gt;13&lt;/sup&gt;</td>
<td>0.54</td>
<td>-</td>
<td>-</td>
<td>0.72</td>
<td>-</td>
<td>-</td>
<td>0.24&lt;sub&gt;SMS&lt;/sub&gt;</td>
<td>-</td>
<td>-</td>
<td>0.17&lt;sub&gt;SMD&lt;/sub&gt;</td>
<td>-</td>
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<td>&lt;.0001</td>
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Table 12. Effect of carcass weight on sensory attributes continued

<table>
<thead>
<tr>
<th>76 kDa autolyzed band µ-calpain 2 d postmortem</th>
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</thead>
<tbody>
<tr>
<td>Calpastatin activity (units/gram muscle)</td>
<td>0.43</td>
<td>-</td>
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*Indicates significant effect of treatment.

1. Measured at tenth rib in the *Longissimus dorsi*. Exiting chill temperature taken approximately 2 h postmortem in CC sides.
4. % Cooked to an internal temperature of 68°C. % Calculated as [(raw weight-cooked weight/ raw weight]
5. National Pork Board standards, 10-point scale. (1=1% intramuscular fat; 10=10% intramuscular fat).
6. National Pork Board standards, 6-point scale. (1=pale pinkish gray; 6=dark purplish red).
7. Hunter L a b, D65 light source, 50 mm aperture, 0° Observer.
8. Force required to compress sample to 20% its original height.
9. Force required to shear 1.27 cm core.
10. Trained sensory analysis (n=4) samples scored on a ten point scale. Lower scores indicate less juiciness, tenderness, chewiness, flavor, and off-flavor.
11. Measurements recorded as µm.
12. Ratio of 30 kDa band degradation product see Figure 2.
13. Ratio of 55 kDa intact desmin band see Figure 1.
14. *Longissimus dorsi*
15. *Psoas major*
16. *Semimembranosus*
17. *Triceps brachii*
18. *Semimembranosus superficial*
19. *Semimembranosus deep*
CHAPTER 4
GENERAL DISCUSSION AND CONCLUSION

The results of the current study support the conclusion that blast chilling alters fresh pork quality. This study was designed to determine the effect of blast chilling (BC) versus conventional chilling (CC) on various muscles that differ in fiber type and carcass location. The results demonstrate that the effect of blast chilling is not uniform across all muscles and some muscles may even benefit from the treatment. Roasts from the *Psoas major* (PM) from BC sides had improved sensory scores in tenderness (7.90 CC, 8.60 BC), juiciness (7.50 CC, 8.30 BC), and chewiness (2.80 CC, 2.10 BC). However, roasts from the PM of BC sides also had increased purge loss measured 7 d postmortem. The *Semimembranosus* (SM) from BC sides had improved color score (3.00 CC, 3.20 BC) and decreased Hunter a value (16.35 CC, 16.02 BC). Chops from the *Longissimus dorsi* (LM) in BC sides had increased cook loss (22.37 CC, 24.24 BC) and increased Warner-Bratzler shear force scores (2.00 kg CC, 2.30 kg BC). Chilling did not have an effect on sensory in roasts from the *Triceps brachii* (TB).

Sarcomere length, pH decline, and postmortem proteolysis have an impact on fresh pork quality (Iversen et al., 1995; Offer et al., 1991). The current study supports the conclusion that rapid chilling resulted in a slower rate of pH decline, but no difference in ultimate pH in the LM. This can be associated with rapid temperature decline in the LM of BC sides as measured exiting chilling treatment (21.8°C CC, 9.7°C BC), 4 (13.3°C CC, 3.8°C BC), 22 (4.2°C CC, 1.4°C BC) and 30 (0.4°C CC, -0.2°C BC) h postmortem. However no difference in calpastatin activity, µ-calpain autolysis, troponin-T
degradation, or desmin degradation in the LM on samples aged two days postmortem was reported. Treatment did not have an effect on proteolysis of desmin in the LM, PM, SMS, SMD, or the TB aged 2 and 10 d postmortem. No difference was found in sarcomere length in the LM.

The lack of difference between treatments on proteolysis is somewhat surprising given the results from sensory analysis. Results from the current study found a different response in development of fresh pork quality between BC and CC sides. These results are consistent with previous studies (Rybarczyk et al., 2015; Rosenvold et al., 2010; Shackelford et al., 2012). There seems to be an influence on the development of fresh pork quality during BC regimes that is not being detected through current evaluation procedures. Melody et al. (2004) reported protein degradation occurred as early as 45 minutes postmortem in pork. It is possible that with time points evaluated in the current study, true representation of the influence of protein degradation on sensory analysis is not represented.

Roasts from the PM of BC sides were found to be juicier, yet had increased purge loss. Chilling was not found to influence sarcomere length in LM in the current study. With the PM’s exterior location in the carcass, it likely to chill faster than other muscles evaluated. Results from the current study of ultimate pH and proteolysis do not necessarily explain quality variations. I speculate that these may be caused by ice crystal formation, sarcomere shortening, or rapid early postmortem proteolysis. However conclusions cannot be drawn from this study to support hypothesis of the effect of chilling on sarcomere shortening, ice crystallization, or proteolysis of addition proteins in the PM. Additional measurements of temperature, pH, sarcomere length, and time points
as well as protein degradation measurements (vinculin, titin, and nebulin) postmortem may help explain why these differences occurred between treatments.

The robust design of the current study allowed within carcass comparison to define the effect of rapid chilling on fresh pork quality. Carcasses were selected on the basis of fat free lean (FFL) and HCW to ensure the evaluation of treatment on fresh pork quality was an accurate representation of our sample population. While FFL and HCW did have a significant effect on fresh pork quality, when included in the model as covariates there was minimal effect on treatment.

Melody et al. (2004) and Christensen et al. (2004) found a difference in postmortem proteolysis between muscles based on fiber type. Current animal production industry practices focus on efficient production of fresh pork. These practices may be leading to increased development of type IIb fibers that age at a slower rate than type I fibers. It would be interesting to find if animals that have increased FFL have a different muscle fiber profile to determine if the true underlying reason sensory differences are detected between treatments is due to increased carcass size or due to animal selection for improved efficiency. Is the selection for improved growth in animals having an impact of fiber type across breeds? Or are there circumstances across breeds, genetic lines, or individual animals which may have a greater impact on fresh pork quality development?

Blast chilling had an impact on fresh pork quality between muscle groups. The results of this study defined, for the first time, how rapid chilling affects a number of different muscles. The true reason for differences in tenderness, juiciness, and chewiness in roasts from the PM and WBS in chops from the LD with BC is yet to be determined. It is well documented that alterations in postmortem muscle metabolism and muscle
structure will influence fresh pork quality. However, the current study found no difference in muscle structure as a result of proteolysis. In the LM of BC sides, pH decline was slowed. This may influence postmortem muscle structure modification. It is also well known that variations in fiber type will also influence resulting fresh pork quality. Future research could profile each muscle for fiber type to determine if there is a genetic influence on quality in addition to an effect of chilling in these cuts. Additional measurements of proteolysis, pH, and temperature decline at earlier time points may also be taken to better profile the changes of each muscle in each treatment. A study like this would be powerful to eliminate variations in each muscle and may provide additional insight into the effect of FFL on muscle fiber composition and resulting fresh pork quality.

Information gained on the impact of fiber type as a result of FFL or HCW could be an insightful tool to optimize the processing of pork carcasses. If a study was able to identify animals that would be more susceptible to increased purge loss, cook loss, or decreased tenderness as a result of a blast chilling treatment producers may be able to alter chilling methods to produce a more consistent product. Future project designs could include using a wide selection of carcasses with a much broader ranges of FFL and HCW variations. By selecting carcasses on this basis, it may be possible to identify exactly where quality defects may arise. Identification of differences in fiber types as a result of differences in FFL could also provide more information as to why the differences are occurring.

Much like the Melody et al. (2004) study, samples collected at varying time points from very early postmortem to late (10 + days) postmortem could give more
insight into the effects of chilling on rate of postmortem proteolysis throughout the aging process. However, rate of chilling is also important to the microbiological safety of the product. If a study such as the one proposed found that larger carcasses developed improved sensory quality traits as a result of a CC regime, questions could arise about the safety and shelf life of a product chilled at a slower rate.

There is still much to gain from additional studies. The true mechanisms that are influencing fresh pork quality have yet to be defined. To ensure success, meat producers need to ensure a consistent, quality, and safe product. This study supports the hypothesis that blast chilling is having a detrimental effect on fresh pork quality in the LM, yet the question of why remains.
LITERATURE CITED


ACKNOWLEDGEMENTS

There are few people who are lucky as I am to have the support and assistance I have had throughout my education, this has become apparent to me in the time I have spent at Iowa State University. The truly selfless nature of everyone at this institution have proven to me that I spent my time learning from the absolute best and brightest people I could have. First I do not think I could have gotten to this point in my career without the help and push from Ben Ruther. Every time I had doubt in my abilities or needed a little extra drive you kept me moving forward and restored confidence in myself.

To my mother and father, each of you has continued to nervously support me throughout all of my endeavors. I would not be the person I am today without the drive you have instilled in me. I am also extremely appreciative in your reluctant ability to let me spread my wings and learn my own lessons the hard way. While I cannot promise you that I will ever start listening to advice you give me, I can say that I wish I would have started listening long ago.

I am also extremely grateful for the support my friends have provided me outside of my schooling. Ben Hoskins, Jake White, Dylan Knudsen and David Knight I know that each of you are available at the drop of a hat to vent, distract, or celebrate any accomplishments I have made. Each of you has served as an extension of my family throughout the years. Through interactions with my close friends and family I have learned the importance of relationships and team building. This fundamental understanding has influenced my personal and professional development and will continue to drive me into the future.
My interactions on campus have been invaluable to me throughout my graduate career. I am extremely lucky to have been a part of the Lonergan Lab in my pursuit of a master’s degree. These individuals have not only ensured my success throughout my project, but have helped me throughout the common frustrations every student encounters in their career. Dr. Shannon Cruzen has been an invaluable wealth of knowledge as well as resource in assisting me to solving my complex problems with a simple “why don’t you do this”. Mr. Dr. Judson “Kyle” Grubbs has been an asset to bounce ideas off of and provide unique advice throughout my personal and academic development. I am truly grateful for Kyle’s open door whether or not he intended it to be open for me! I am also extremely grateful to Dr. Ed Steadham for all the assistance he provided me in the lab and the wealth of knowledge or “Steadisms” that I will be taking with me after I leave Iowa State.

Light hearted office antics have kept me on my toes and some days awake! For that I have to thank Megan Myers, Emily Usinger, Grant Sherrard, and Kelsey Carlson. I hope that each of you has learned as much from me that I have learned from you. My personal relationships I have built with you will undoubtedly keep the spirit of Iowa State alive when we all reach our professional careers. In particular I need to thank Kelsey Carlson for instilling a competitive drive in me in our short time spent together. I look forward to future interactions, conversation and challenges we may encounter down the road. My poster is still better.

I have had the opportunity to work with my committee members throughout my graduate and undergraduate career. Dr. Joe Sebranek and Dr. Ken Prusa have been invaluable resources as well as positive role models. Their genuine wonder and thirst for
knowledge have been a great influence on my educational and professional development. I have had the unique opportunity to take classes in product development from each of them. I will always admire their willingness to give even the craziest ideas a shot. I still can’t tell if they know whether or not something will work, or if they find each and every opportunity as a teachable moment. Each of you has taught me that there is always something new to learn and try no matter how long you might have spent doing it.

Finally I am indebted to Dr. Steven Lonergan and Dr. Elisabeth Huff-Lonergan. Odds are if you ask any recent Iowa State Meat Science graduate how they got into meat science, Steven and Elisabeth are the reason. I am absolutely no exception to this trend. Each of you have been a great asset into my degree and there is no way I would be where I am today without you. I am extremely grateful Elisabeth informed me of the opportunity to work with the Lonergan lab as an undergraduate on this project. This exposure has taught me so much about science, research, and myself.

There is without a question that my interactions with Steven over the past few years have forever changed my outlook on my future. He has challenged me, frustrated me, and taught me more than I think he will ever truly understand. It is obvious to me that this is not merely a job to Steven. Your dedication to your students is inspiring, and I hope to someday have the same impact on half of the people I reach out to as you have. You have taught me what it means to be an Iowa Stater and you are singly the reason why I will remain forever a Cyclone. I hope that while our paths may diverge for now, we will have future interactions. I know there a many lessons left to be learned from you, and I couldn’t have asked for a better mentor. Thank you.